ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2020 September 26; 12(9): 897-1049

Published by Baishideng Publishing Group Inc

World Journal of Stem Cells

Contents

Monthly Volume 12 Number 9 September 26, 2020

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

World Journal of Stem Cells

Monthly Volume 12 Number 9 September 26, 2020

Contents

ABOUT COVER

Editorial board member of *World Journal of Stem Cells*, Dr. Elisa Oltra is Distinguished Professor at the Catholic University of Valencia (UCV), Spain. Having received her Bachelor's and Master's degrees in Biochemistry at UCV in 1988 and her PhD degree in Cell and Molecular Biology at the University of Miami, FL, United States in 1999, she returned to UCV in 2009, where she became Director of the first official European Master's degree program in Biobanking. Her current research interests include the identification of molecular biomarkers of fibromyalgia and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and the study of immunomodulatory potential of mesenchymal stem cells. She has also served as Chair of the Biomarker subgroup ME/CFS Common Data Elements NINDS initiative at the National Institutes of Health (United States). (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells* (*WJSC*, *World J Stem Cells*) is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc*.

INDEXING/ABSTRACTING

The *WJSC* is now indexed in Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, BIOSIS Previews, PubMed, and PubMed Central. The 2020 Edition of Journal Citation Reports® cites the 2019 impact factor (IF) for *WJSC* as 3.231; IF without journal self cites: 3.128; Ranking: 18 among 29 journals in cell and tissue engineering; Quartile category: Q3; Ranking: 113 among 195 journals in cell biology; and Quartile category: Q3.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Carlo Ventura

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-0210/editorialboard.htm

PUBLICATION DATE
September 26, 2020

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 897-921

DOI: 10.4252/wjsc.v12.i9.897

ISSN 1948-0210 (online)

REVIEW

Dental stem cells: The role of biomaterials and scaffolds in developing novel therapeutic strategies

Cornelia Larissa Granz, Ali Gorji

ORCID number: Cornelia Larissa Granz 0000-0002-6284-0764; Ali Gorji 0000-0002-4557-3270.

Author contributions: Granz CL and Gorji A contributed to the conception and design of the study; Granz CL contributed to writing and drafting of the manuscript; Gorji A contributed to critical revision of the final draft of the manuscript; all authors approved the final version of the manuscript.

Conflict-of-interest statement: There is no conflict of interest.

Manuscript source: Invited manuscript

Received: May 4, 2020

Cornelia Larissa Granz, Ali Gorji, Epilepsy Research Center, Westfälische Wilhelms-Universität Münster, Münster 48149, Germany

Ali Gorji, Department of Neurosurgery and Department of Neurology, Westfälische Wilhelms-Universität, Münster 48149, Germany

Ali Gorji, Shefa Neuroscience Research Center, Khatam Alanbia Hospital, Tehran 1996836111, Iran

Ali Gorji, Neuroscience Research Center, Mashhad University of Medical Sciences, Mashhad 9177948564, Iran

Corresponding author: Ali Gorji, MD, Full Professor, Epilepsy Research Center, Westfälische Wilhelms-Universität Münster, Domagkstr 11, Münster 48149, Germany. gorjial@uni-muenster.de

Abstract

Dental stem cells (DSCs) are self-renewable cells that can be obtained easily from dental tissues, and are a desirable source of autologous stem cells. The use of DSCs for stem cell transplantation therapeutic approaches is attractive due to their simple isolation, high plasticity, immunomodulatory properties, and multipotential abilities. Using appropriate scaffolds loaded with favorable biomolecules, such as growth factors, and cytokines, can improve the proliferation, differentiation, migration, and functional capacity of DSCs and can optimize the cellular morphology to build tissue constructs for specific purposes. An enormous variety of scaffolds have been used for tissue engineering with DSCs. Of these, the scaffolds that particularly mimic tissue-specific micromilieu and loaded with biomolecules favorably regulate angiogenesis, cell-matrix interactions, degradation of extracellular matrix, organized matrix formation, and the mineralization abilities of DSCs in both *in vitro* and *in vivo* conditions. DSCs represent a promising cell source for tissue engineering, especially for tooth, bone, and neural tissue restoration. The purpose of the present review is to summarize the current developments in the major scaffolding approaches as crucial guidelines for tissue engineering using DSCs and compare their effects in tissue and organ regeneration.

Key Words: Cell transplantation; Regenerative medicine; Tissue engineering; Neural crest; Angiogenesis; Biomolecules

Peer-review started: May 4, 2020
First decision: May 24, 2020
Revised: June 5, 2020
Accepted: August 16, 2020
Article in press: August 16, 2020
Published online: September 26, 2020

P-Reviewer: Ding JX, Wakao H
S-Editor: Yan JP
L-Editor: Webster JR
P-Editor: Xing YX

Core Tip: Dental stem cells have been used for different types of cell transplantation therapies, including teeth, bone, and neural tissue regeneration. In planning for successful tissue engineering toward organ-specific regeneration, choosing an appropriate scaffold that mimics the extracellular matrix in native tissue and loaded with suitable biomolecules to boost dental stem cell functions is of utmost importance.

Citation: Granz CL, Gorji A. Dental stem cells: The role of biomaterials and scaffolds in developing novel therapeutic strategies. *World J Stem Cells* 2020; 12(9): 897-921
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/897.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.897

INTRODUCTION

Stem cells are undifferentiated cells with self-renewing and clonogenic capabilities, which can differentiate into various cell lineages. According to the basis of their origin, stem cells are categorized as embryonic, induced pluripotent stem cells (iPS), and adult (tissue-specific) stem cells[1-3]. Based on their differentiation potential, stem cells can be classified as totipotent (the ability to give rise to all types of cells), pluripotent (the potential of the cells to produce any type of cells in the organism), multipotent (the potential to give rise to cells of their tissue of origin), oligopotent (the potential to differentiate into only a few cell types), and unipotent (the ability to produce one cell type)[4]. Embryonic stem cells are pluripotent, whereas adult stem cells are limited to differentiating into various cell types of their original tissue (multipotent). iPS are pluripotent cells that originated from somatic differentiated cells after transduction. Adult stem cells exist in different tissues and organs, such as the bone marrow, blood vessels, peripheral blood, and skeletal muscles as well as the brain, heart, skin, intestine, liver, gonads, and teeth[5-7].

Human mesenchymal stem cells (MSCs), which are multipotent non-hematopoietic progenitor cells, have been isolated from both adult and fetal tissues, such as the bone marrow, adipose tissue, endometrium, bone, muscle, umbilical cord, blood, Wharton's jelly, and amniotic fluid as well as nervous and dental tissues[8]. Human MSCs have the potential to differentiate into both mesodermal (osteocytes, adipocytes, and chondrocytes) and non-mesodermal (endodermal and ectodermal) lineages (hepatocytes and neuronal cells)[9] with both anti- and pro-tumorigenic properties[10] as well as a limited risk of inflammatory reactions and uncontrolled growth[11]. The source of MSCs has a crucial role in the outcomes of stem cell-based tissue engineering[12]. Dental stem cells (DSCs) are neural crest-derived cells that can be obtained easily from dental tissues of both adults and children; therefore, they are a reliable, accessible source of autologous stem cells[13,14]. DSCs are undifferentiated cells that have non-limited self-renewal, multipotent differentiation potential, and colony-forming capacity[15]. DSCs can be isolated from the dental pulp of deciduous, natal, and permanent teeth, the periodontal ligament, the apical papilla, the dental follicle, and gingival tissue (Figure 1)[16,17]. One of the unique characteristics of DSCs is their ability to differentiate into mesodermal, ectodermal, and endodermal cell lineages[18]. DSCs from each source are capable of specifically differentiating into various distinct cells, including epithelial cells, odontoblasts, osteoblasts, chondroblasts, adipocytes, vascular cells, endotheliocytes, neuronal cells, glial cells, photoreceptor cells, and muscle cells[19,20]. Although all stem cells obtained from various sources are named DSCs in this study, their phenotype, differentiation potential (both in *in vitro* and *in vivo* conditions) and functional properties (such as biological response during differentiation and tissue repair) are different[21]. For instance, stem cells obtained from the apical papilla possess greater proliferation ability, express a higher variety of neural markers, and induce more uniform dentine-like tissues compared to dental pulp stem cells[22-24]. Furthermore, DSCs isolated from exfoliated deciduous teeth exert a higher capacity for osteogenic regeneration and a greater proliferation rate compared to dental pulp stem cells[25]. DSCs isolated from pulp tissues are the first and most frequent cells evaluated for their odontogenic, osteogenic, and neurogenic differentiation potentials[26]. This heterogeneity of DSCs is effectively modulated by the function of their microenvironment[27]. DSCs obtained from different sources exhibit

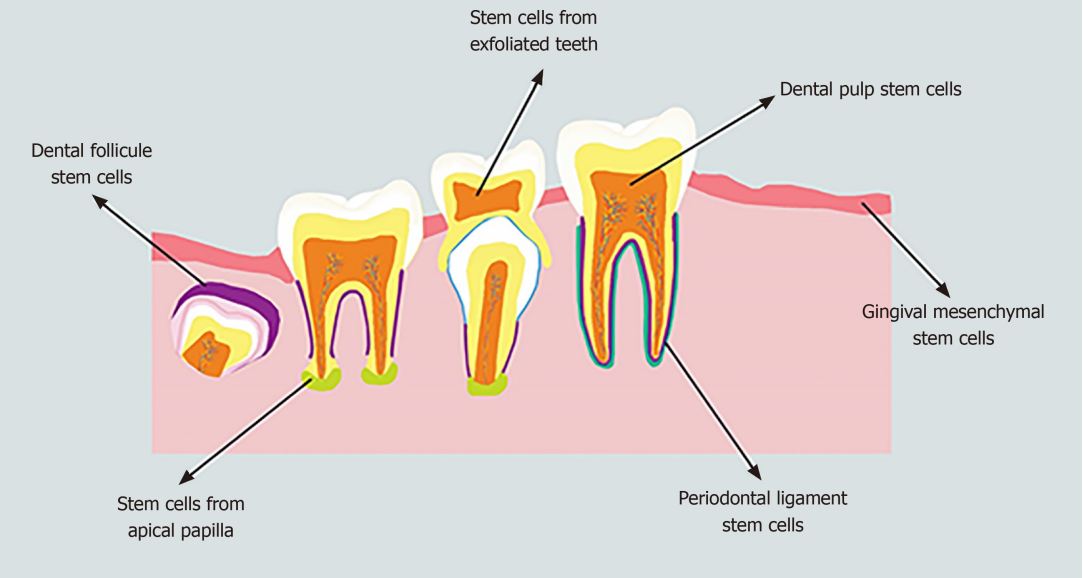


Figure 1 Schematic diagram of different sources where dental stem cells can be isolated. Various subpopulations of dental stem cells (DSCs) can be classified according to their tissue of origin. DSCs can be derived from the dental pulp stem cells, exfoliated deciduous teeth, periodontal ligament, dental follicle, apical papilla, and gingival tissue.

various patterns of cell surface markers (Table 1)[28-31].

DSCs secrete numerous immunomodulatory mediators, such as interleukin (IL)-6, IL-10, IL-1β, interferon-γ, and tumor necrosis factor-α as well as transforming growth factor-beta (TGF-β), hepatocyte growth factor, and vascular endothelial growth factor (VEGF)[32], and do not express the major histocompatibility complex class II antigen[33], which suggests their potential in the regulation of immune responses to promote tissue regeneration[34]. DSCs from different sources may exert their immunomodulatory properties through the suppression of T-cell proliferation and lymphocyte activity as well as the activation of T-cell apoptosis[34,35].

The fate of the stem cells (proliferation and differentiation) is regulated *via* a combination of *intrinsic* and *extrinsic* mechanisms. Intrinsic mechanisms consist of various transcription factors expressed by the cells. Extrinsic mechanisms are signals provided by the dynamic microenvironment (or "niche"), including the extracellular matrix (ECM), signaling molecules (such as growth factors and hormones), and neighboring cells[36,37]. The microenvironment, which is a three-dimensional (3D) structure surrounded by specific cells and ECM, protects stem cells from inappropriate differentiation, cell damage, and apoptosis and governs tissue maintenance, regeneration, and repair[38,39]. In addition to providing a physical microenvironment for cells, the ECM gives the tissue its mechanical properties (elasticity and rigidity), provides bioactive molecules and cues to residing cells, and establishes an environment to facilitate tissue remodeling in response to dynamic processes, such as wound healing[40]. Furthermore, the ECM is produced and arranged by tissue-resident cells and secreted into the surrounding environment to provide support to the stem cells with its bioactive compounds[41]. Stem cell behaviors are reciprocally regulated by the ECM and signals from the surrounding cells and molecules. Furthermore, inorganic ions, such as calcium and magnesium, as well as metabolic products, such as oxygen drive metabolites, and maintain stem cell fate[42].

The nature of the stem cell microenvironment differs in various tissues. In teeth, a particular microenvironment exists at specific anatomic sites that regulates the behavior of DSCs[39]. Two different stem cell microenvironments have been identified in teeth; (1) the pulp cell-rich zone; and (2) the perivascular and perineurium of the dental pulp. The pulp is composed of four distinct zones; an outermost layer containing the odontoblasts, a cell-free zone (zone of Weil) with no cells and rich in the ECM, the cell-rich zone contains stem/progenitor cells, and the pulp core. Dental pulp tissue is populated by odontoblasts, fibroblasts, dendritic cells, macrophages, and progenitor cells, whereas the pulp core contains dental pulp cells, vessels, nerves, and ECM[43]. The induction of odontoblasts, the biological cells of neural crest origin that survive throughout life, occurs during tooth development. However, under appropriate conditions, DSCs can differentiate into pre-odontoblasts and later secretory odontoblasts, which actively participate in reactionary dentinogenesis[44].

Table 1 Dental stem cells obtained from different sources exhibit various patterns of cell surface markers

Markers	DPSCs	SCEDT	PLSCs	DFSCs	SCAP
Nestin	+	+	+	+	
Vimentin	+	+			+
SOX2	+		+	+	+
SOX10	+		+		
Stro-1	+	+	+	+	+
Oct-4	+	+	+	+	+
EphB	+				
Nanog	+	+	+		+
CD10			+	+	
CD13	+	+	+	+	+
CD14	+	+	+		
CD19	+				
CD24	+				+
CD25				+	
CD29	+	+	+	+	+
CD34	+	+	+	+	+
CD44	+	+	+	+	+
CD45	+	+	+	+	+
CD49					+
CD53				+	
CD59	+		+	+	
CD73	+	+	+	+	+
CD90	+	+	+	+	+
CD105	+	+	+	+	+
CD106					+
CD117	+				
CD146	+	+		+	+
CD150					+
CD166		+			+
CD271	+		+		
SSEA-3		+			
SSEA-4		+			
TWIST-1	+				
c-myc		+		+	
Notch				+	+
3G5	+				
Klf-4			+		
FlK1	+	+			

DPSCs: Dental pulp stem cells; SCEDT: Stem cells obtained from exfoliated deciduous teeth; PLSCs: Periodontal ligament stem cells; DFSCs: Dental follicle stem cells; SCAP: Stem cells obtained from apical papilla.

Odontoblasts produce the main part of the ECM components of dentin and are involved in dentin mineralization[45]. The dentin ECM consists of collagen (approximately 90%; Type I, III, and V), proteoglycans (such as chondroitin sulfate and heparan sulfate), growth factors [such as TGF-β and bone morphogenetic protein (BMP)], and enzymes (such as matrix metallopeptidase 1, 2, 3, 9, and 20)[46]. In the dental pulp, DSCs also reside in perivascular and perineurium regions[47], which can be identified by aldehyde dehydrogenase-1 expression[48]. The EphB/ephrin-B signaling pathway reciprocally modulates the attachment and migration of DSCs originated from the perivascular niche *via* the mitogen-activated protein kinase pathway and phosphorylation of Src family tyrosine kinases[49].

Pointing to the importance of ECM in maintaining homeostasis for proliferation and differentiation of DSCs, several studies have indicated that reconstruction of the appropriate microenvironment and boosting its interaction with stem cells are essential steps to successful cell therapy[50]. The application of DSCs in stem cell therapeutic approaches is attractive due to their simple isolation and efficient administration[51]. The multi-lineage capacity of DSCs differentiation to various tissues and organs suggests their greater ability than other adult stem cell populations for the treatment of different diseases[52]. There is an enormous amount of evidence to indicate that DSCs have great potential for therapeutic cell approaches in various diseases, including liver disease, diabetes, myocardial infarction, ophthalmologic diseases, muscular dystrophy, Alzheimer's disease, Parkinson's disease, cerebral ischemia, and spinal cord injury[53]. Furthermore, several studies have explored the potential of DSCs in the treatment of caries, periodontal disease, oral and maxillofacial defects, and alveolar bone atrophy[54,55]. DSCs possess strong immunomodulatory abilities, which suggest that they are a favorable cell source for cell transplantation therapy in inflammatory disorders[33]. It has been shown that DSCs are more beneficial for axonal regeneration than bone marrow stem cells due to their greater release of neurotrophic factors[56]. Despite these extensive efforts, several essential parameters still need to be optimized for the clinical use of DSCs in cell transplantation therapy. One of the key challenges is the lack of an appropriate stem cell microenvironment, which leads to short-term survival of DSCs after implantation. To increase cell viability, transplanted cells require particular 3D structures with specific ECM components that protect DSCs from cell damage, maintain the stem cell homeostasis, and promote mutual biological information transfer between stem cells and the ECM[50,57,58]. A large number of investigations have been carried out to reconstruct the stem cell microenvironment to strengthen the viability, proliferation, and appropriate differentiation of the transplanted cells for successful cell therapy[59]. In this context, ECM scaffolds can form a desirable microenvironment for DSCs, which serve as a more favorable template for tissue repair and reconstruction[60,61]. In this review, we provide a critical overview of the role of different biomaterials used to deliver DSCs to damaged tissue and their applications to improve, restore, and maintain tissue or organ reconstruction.

DIFFERENT TYPES OF SCAFFOLD FORMULATIONS

The general concept of tissue bioengineering involves three essential components; identification of suitable stem cells, development of appropriate scaffolds, and induction of potent signals to repair or regenerate human cells, tissues, or organs[62]. Biomaterials are essential components for the construction of scaffolds. Tissue bioengineering combines scaffolds with various types of stem cells to reconstruct damaged tissues (Figure 2). The application of appropriate scaffolds could improve DSCs proliferation, differentiation, adhesion, and migration, which may promote their ability to repair the injured tissues and regenerate functional organs[63]. Acellular tissues, as well as natural and synthetic biomaterials, can be used as the primary source for generating scaffolds[64]. Acellular tissue matrices, such as an acellular adipose matrix, are derived from animal or human tissues with all cells eliminated during manufacture[65]. In addition, the amniotic membrane has been suggested as a suitable biological scaffold for the proliferation and transplantation of DSCs[66]. Natural biomaterials consist of proteins (collagen, gelatin, fibrin, and silk) and polysaccharides (agarose, alginate, hyaluronan, polylactic acid, and chitosan) which tend to be biocompatible due to their cellular adhesion sites, such as Arg-Gly-Asp binding sequences, and the ability to degrade without releasing toxic substances. However, due to the variability of materials, limited mechanical properties, the risk of transmitting pathogens, and provoking immune reactions, their formulations need to be promoted for stem cell culture[67,68]. Synthetic biomaterials, including polymer-based

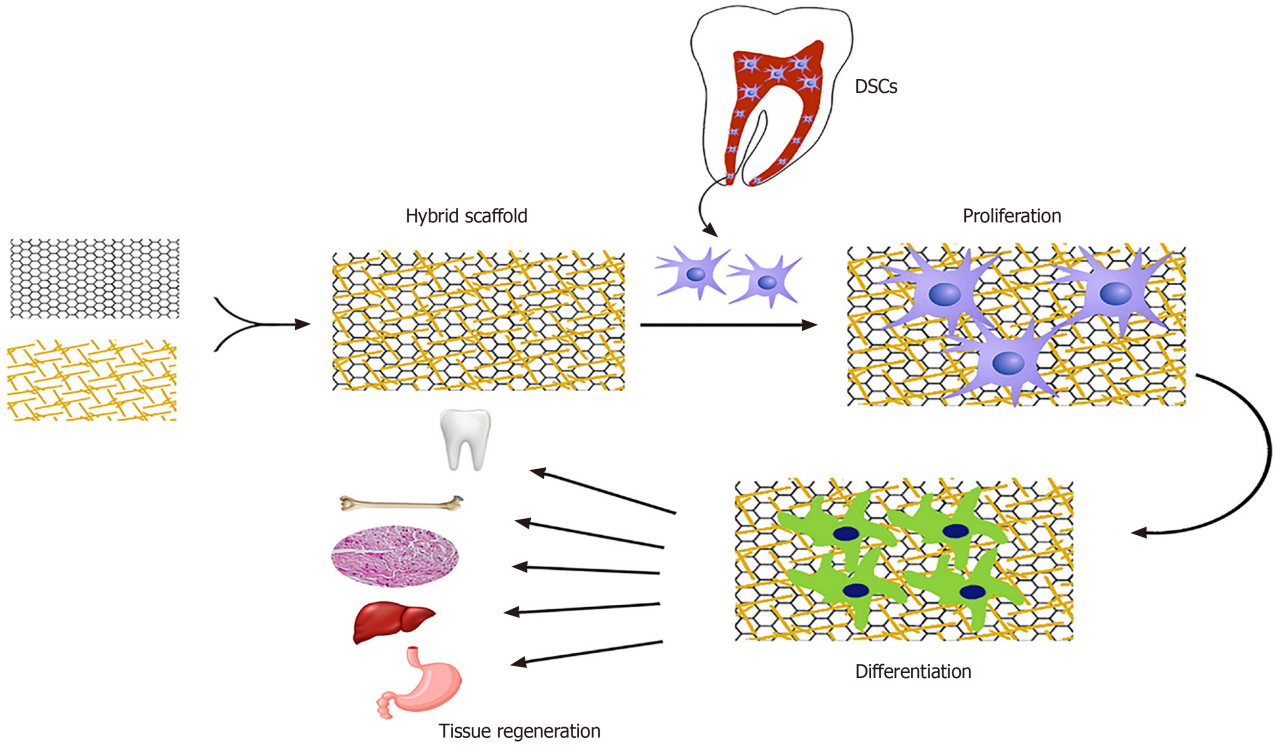


Figure 2 Schematic diagram of seeding dental stem cells on the hybrid scaffold, differentiation capability, and potential clinical applications for the regeneration of different tissues. DSCs: Dental stem cells.

biomaterials (such as polycaprolactone, polylactic acid, poly-lactic-co-glycolic acid, polyglycolide, poly-e-caprolactone, and poly-ethylene glycol) and ceramic-based biomaterials (such as hydroxyapatite, bioactive glass, and calcium phosphate) display a better mechanical property, reproducibility, and electrical conductivity as well as a lower degradation rate[68-70]. Furthermore, synthetic biomaterials possess the possibility of optimizing the chemical and physical properties of a scaffold for a particular application[71]. Hydrogels, which are networks of hydrophilic polymers, can be manufactured from natural biomaterials (such as collagen, fibrin, proteoglycans, and hyaluronic acid) or synthetic polymers (such as self-assembly peptide molecules or poly-ethylene glycol)[37,72,73]. Hydrogels provide tissue-like microenvironments with particular cellular signals, desirable biocompatibility, semi-permeable membranes, and cell delivery vehicles[74]. Furthermore, a wide range of nanocomposite biomaterials has been assembled by merging nanomaterials within the polymeric matrix to promote the efficiency of bioactive scaffolds[75].

The desirable properties of a scaffold for stem cell transplantation are biocompatibility, biodegradability, mimic the 3D biological microenvironment, incorporation of different ECM, pore size, stability, electrical conductivity, porosity, non-immunogenicity, interconnectivity, safety (low or non-toxic) and alignment[70,76,77]. Various fabrication techniques have been developed to produce different scaffolds, such as emulsion freeze-drying, electrospinning, thermally-induced phase separation, solvent casting/particular leaching, computer-aided design/computer-aided manufacturing, melt molding, rapid prototyping (3D printing, selective laser sintering, stereolithography, and fused deposition modeling), nanofiber self-assembly, and photolithography[78,79]. The basic tissue engineering procedures consist of appropriate scaffold manufacture, hydrogel matrix support, and patterning design. The combination of these approaches promotes the development of the desired complex, both in tissue structure and function[20,80].

Scaffold-based cultures are conventionally applied in two-dimensional (2D) systems. Although 2D systems are a valuable medium for the investigation of basic cell biology and preclinical drug testing, data generated in these systems are insufficient to translate into *in vivo* experimental studies[81]. Furthermore, inappropriate cell-to-cell and cell-to-ECM contacts, reduction of polarization, and alteration of key signaling pathways modulate stem cell differentiation ability[81,82]. Thus, several 3D scaffold-based cultures have been developed. Although existing 3D cultures are not without limitations, they enhance cell viability, growth, differentiation, and migration

and improve cellular communications[82]. Combining DSCs with suitable scaffolds offers a promising strategy for cell delivery and transplantation. Two main approaches for this combination are cell-based and cell-free tissue engineering. In the cell-based approach, stem cells are seeded and cultured onto the scaffold *in vitro* to produce the desired tissue before transplantation[83]. In the cell-free approach, a bioactive scaffold with growth and differentiation factors is embedded in the respective tissues, induces the homing of resident stem cells, and promotes their proliferation and differentiation[84].

Furthermore, the environmental cues, such as various growth factors/morphogens, markedly affect the behavior of DSCs seeded in scaffolds and are vital to the success of regenerative therapies[85,86]. Several proteins, such as BMP, sialoprotein, fibronectin, and osteopontin, are able to coat various types of biomaterials and promote the behaviors of DSCs[87]. Pre-treatment of biomaterials with the abovementioned proteins could enhance adhesion, differentiation, proliferation, migration, and function of DSCs and improve the formation of new tissues[87,88].

DSCS IN COMBINATION WITH SCAFFOLDS FOR TISSUE RESTORATION

DSCs represent an auspicious cell source for tissue engineering, particularly for tooth, bone, and neural tissue reconstruction. A vast number of these investigations point to the importance of various scaffolds to design effective tissue engineering approaches (Table 2).

TOOTH RECONSTRUCTION

The therapeutic role of DSCs in combination with various scaffolds has been extensively investigated in restoring tooth damage or loss due to caries, periodontal disease, trauma, or genetic disorders[89]. The procedure of dentin formation consists of odontoblastic deposition, vascularization, and neuron formation[20]. Among multiple approaches to promote dentin formation and teeth tissue regeneration, the application of DSCs with a synthetic pre-designed and optimized scaffold is the most accepted technique for tooth regeneration[90]. The appropriate scaffold can be implemented with DSCs and growth factors to induce the generation of dental tissues, which can integrate with the adjacent tissues[16]. Scaffolds developed from either synthetic or natural biomaterials have been used for tooth reconstruction. Natural materials, such as collagen[91], chitosan/gelatin[92], silk protein[93], alginate[94], hyaluronic acid[95] as well as synthetic polymers, such as polyglycolate/poly-l-lactate[96], polycaprolactone-poly glycolic acid[97], polylactic acid-co-polyglycolic acid[98], polycaprolactone /gelatin/ nano- hydroxyapatite[99], nano-hydroxyapatite/collagen/poly-l-lactide[100] and poly-ethyl methacrylate-co-hydroxyethyl acrylate[101] were used as scaffold materials for dental restoration and regeneration. Several investigations have indicated the regeneration of vascularized pulp-like tissue after subcutaneous implantation of tooth slices containing DSCs accompanied by an appropriate scaffold, particularly in the presence of growth factors such as dentin matrix protein[102,103]. In several experiments, a combination of the abovementioned scaffolds was used to promote cell differentiation, vascularization, and safety as well as to reduce immunological and ectopic complications. The development of a vascularized dentin/pulp tissue in a subcutaneously transplanted human root canal containing a poly-lactic-co-glycolic acid scaffold seeded with DSCs has been reported[104]. A scaffold consists of a pulp-specific ECM (an acellular ECM within the hydrogel) and an endothelial ECM (collagen-chitosan hydrogels) to promote odontogenic differentiation of DSCs and induce extensive vascularization in an *in vivo* model of a tooth root slice[105]. Comparing collagen and gelatin with chitosan, it has been stated that chitosan exerts weaker support for human DSCs growth and differentiation[106]. In addition, 3D nano-fibrous gelatin/silica bioactive glass hybrid scaffolds provide a suitable microenvironment that mimics the architecture and composition of a natural dental micromilieu and enhances the growth and differentiation of human DSCs[107]. Furthermore, the administration of human DSCs associated with acellular dental pulp resulted in pulp-like tissue structures and the maintenance of ECM[108]. DSCs seeded in 3D scaffold-free stem-cell sheet-derived pellets promote odontogenic differentiation[109].

The application of combined DSCs with the ECM scaffold can be used for root canal therapy. It has been shown that DSCs are able to differentiate into functional odontoblasts with angiogenic potential[110]. Implantation of a 3D scaffold by shaping

Table 2 A vast number of these investigations indicate the importance of various scaffolds to design effective tissue engineering approaches

Scaffold	Growth factors/bioactive molecules	Experimental model	Target tissue	Ref.
Silicon	-	*In vitro*	Teeth	[168]
Collagen sponge	SCF	*In vitro* and *in vivo* (mice)	Teeth	[126]
Collagen type I and type III	SDF-1	*In vivo* (dogs)	Teeth	[91]
Collagen type-I and N-acetic acid	SDF-1, bFGF, BMP-7	*In vitro* and *in vivo* (rats)	Teeth	[120]
Collagen/chitsosan	-	*In vitro*	-	[129]
Collagen-polyvinylpyrrolidone sponge	-	Case report	Teeth	[144]
Silk fibroin	SDF-1	*In vitro* and *in vivo* (mice)	Teeth	[123]
Acellular dental pulp ECM	-	*In vivo* (mice)	Teeth	[108]
Intrafibrillar-silicified collagen	-	*In vitro* and *in vivo* (mice)	Teeth	[169]
Matrigel	bFGF-2, TGF-β1	*In vitro*	-	[121]
Peptide hydrogel	VEGF, TGF-β1, FGF-1	*In vivo* (mice)	Teeth	[118]
Gelatin methacrylate hydrogel	-	*In vivo* (rats)	Teeth	[133]
PuraMatrix™	VEGF	*In vivo* (mice)	Teeth	[134]
Poly-ε-caprolactone and hydroxyapatite	SDF-1, BMP-7	*In vitro* and *in vivo* (rats)	Teeth	[124]
Polycaprolactone-poly-glycolic acid	BMP-7	*In vitro* and *in vivo* (mice)	Teeth	[97]
Thermoresponsive hydrogel	-	*In vitro* and *in vivo* (mice)	Teeth	[111]
DL-lactide/co-polymer of L-lactide /DL-lactide, and hydroxyapatite tricalcium phosphate	BMP-2	*In vitro* and *in vivo* (mice)	Teeth	[132]
3D hydroxyapatite scaffolds containing peptide hydrogels	-	*In vivo* (mice)	Teeth	[112]
Poly-lactic-co-glycolic acid	-	*In vitro* and *in vivo* (mice)	Teeth	[104]
Beta-tricalcium phosphate scaffold	BMP-2	*In vitro* and *in vivo* (mice)	Teeth	[131]
Collagen sponge	-	Clinical trial	Bone	[153]
Collagen sponge	-	Clinical trial	Bone	[151]
Collagen sponge	-	Clinical trial	Bone	[152]
Chitosan/gelatin	BMP-2	*In vitro* and *in vivo* (mice)	Bone	[92]
Arginine-glycine-aspartic acid	-	*In vitro* and *in vivo* (mice)	Bone	[141]
Granular 3D chitosan	-	*In vitro*	Neural tissue	[193]
Matrigel	BMP-9	*In vitro* and *in vivo* (mice)	Bone	[180]
3D nano-fibrous gelatin/silica bioactive glass hybrid	-	*In vitro*	Teeth	[107]
3D gel collagen matrix	BMP-2	*In vitro* and *in vivo* (rats)	Bone	[177]
Poly-ε-caprolactone biphasic calcium phosphate	-	*In vitro* and *in vivo* (rabbit)	Bone	[160]
Glass nanoparticles/chitosan-gelatin	-	*In vitro* and *in vivo* (rats)	Bone	[159]
3D poly-lactide	Extracellular vesicles	*In vitro* and *in vivo* (rats)	Bone	[179]
2D monolayer culture/3D poly lactic-co-glycolic	-	*In vitro*	Bone	[174]
Dense collagen gel or acellular	-	*In vivo* (rats)	Bone	[150]
Calcium phosphate cement functionalized with iron oxide nanoparticles	-	*In vitro*	Bone	[166]
Poly-lactic-co-glycolic acid	-	*In vitro* and *in vivo* (rats)	Bone	[164]
Hydroxyapatite-collagen sponge	-	Clinical trial	Bone	[146]

3D porous chitosan	bFGF	*In vitro*	Neural tissue	[201]
Fibrin and collagen	-	*In vivo* (rats)	Sciatic nerves	[9]
Collagen	Tetracycline	*In vivo* (mice)	Sciatic nerves	[206]
Collagen	-	*In vitro* and *in vivo* (rats)	Sciatic nerves	[210]
3D alginate/hyaluronic acid	NGF	*In vitro* and *in vivo* (mice)	Peripheral nerves	[207]
Collagen sponge (DSCs condition medium)	-	*In vitro* and *in vivo* (rats)	Facial nerves	[211]
3D bio-printing of scaffold-free nervous tissue	-	*In vitro* and *in vivo* (rats)	Facial nerves	[208]
Chitosan	-	*In vitro* and *in vivo* (rats)	Spinal cord	[198]
Aligned electrospun poly-ε-caprolactone/ poly-lactide-co-glycolic acid	-	*In vitro* and *in vivo* (rats)	Spinal cord	[202]

bFGF: Basic fibroblast growth factor; BMP: Bone morphogenetic protein; NGF: Nerve growth factor; SDF: Stromal cell-derived factor; SCF: Stem cell factor; TGF-β: Transforming growth factor beta; VEGF: Vascular endothelial growth factor; DSCs: Dental stem cells; BMP: Bone morphogenetic protein; ECM: Extracellular matrix.

sheet-like aggregates of DSCs with a thermos-responsive hydrogel into the human tooth root canal generates pulp-like tissues with rich neovascularization without adding growth factors[111]. Furthermore, transplantation of human DSCs with 3D hydroxyapatite scaffolds containing peptide hydrogels resulted in vascular ingrowth, osteodentin deposition, and pulp tissue formation in immunocompromised mice[112]. Using bioengineered methods, it has been shown that it is possible to achieve functional teeth with entire roots[113]. The nanofiber hydrogel PuraMatrix is a synthetic matrix that is used to create a biocompatible, biodegradable, and non-toxic 3D environment for a variety of cells[114]. DSCs injected with PuraMatrix into full-length human root canals differentiate into functional odontoblasts; pointing to a novel strategy to facilitate root formation in damaged teeth[115]. Several *in vitro* and *in vivo* studies revealed that the addition of various signaling molecules and growth factors [such as granulocyte colony-stimulating factor (G-CSF), stromal cell-derived factor (SDF), basic fibroblast growth factor (bFGF), and VEGF] to different scaffolds (both natural and synthetic) enhances the regeneration of intra-canal pulp-like tissues *via* the promotion of dentine formation, mineralization, neovascularization, and innervation[116]. DSCs transplanted with SDF-1 or G-CSF on a collagen scaffold promote pulp reconstruction in an animal pulpitis model[91,117]. Autologous DSCs transplanted into a root canal with collagen types I and III associated with SDF-1 after pulpectomy in dogs significantly increased the expression of angiogenic and neurotrophic factors, indicating the potent trophic effects of the combined scaffold and chemokine on neo-vascularization during pulp regeneration[91]. In addition, DSCs seeded into peptide hydrogel loaded with FGF-1, TGF-β1, and VEGF differentiated into odontoblasts-like cells and formed a vascularized dental pulp-like tissue inside the dentin cylinder[118]. Moreover, TGF-β2 increased the odontogenic differentiation of DSCs isolated from the apical papilla[119]. DSCs isolated from adult human tooth pulp and seeded on the surfaces of 3D collagen gel cylinders exhibited significantly increased cellular recruitment when applied with SDF-1α, bFGF, or BMP-7[120]. Encapsulating TGF-β1 and FGF-2 in a biodegradable polymer of lactide and glycolide microspheres provides the controlled release of growth factors to human pulp cells[121]. Furthermore, scaffold composition plays a key role in determining whether the application of signaling molecules or growth factors is needed. Various growth factors, such as SDF, FGF, TGF-β1, VEGF, and BMP were loaded on different scaffolds, such as peptide hydrogel, collagen, gelatin hydrogel, and alginate hydrogel, to enhance endodontic regeneration of DSCs[122]. In addition, a silk fibroin scaffold loaded with bFGF has been described as a promising scaffold for the proliferation and differentiation of DSCs *in vitro*[123]. Implantation of DSCs with poly-ε-caprolactone and hydroxyapatite in association with SDF-1 and BMP-7 generated tooth-like structures (putative periodontal ligament and new bone formation) in the mandibular incisor extraction socket[124]. In addition to SDF-1, stem cell factor (SCF), a potent chemokine, enhances the mobilization and trafficking of stem cells[125]. SCF promoted neovascularization and new collagen fiber formation after subcutaneous implantation of DSCs with a collagen sponge scaffold in mice. Furthermore, SCF improved DSCs migration, proliferation, and chemotaxis *in vitro*, possibly *via* the upregulation of ERK and AKT phosphorylation[126].

Sialoprotein is a dominant non-collagenous protein in dentin, which plays a role in the induction of dental pulp cell differentiation into odontoblast-like cells and is essential for dental pulp stem cell identity and fate[127,128]. Subcutaneous implantation of DSCs seeded on a 3D scaffold containing an acellular ECM embedded in a collagen/chitosan scaffold led to the production of dental pulp-like tissue and the expression of dentin sialoprotein in nude mice[129]. The application of DCSs combined with treated dentin matrix, a biological scaffold, has been suggested as a suitable therapeutic approach for the reconstruction of the tooth root[130]. Immortalized DSCs exhibited potent odontogenic differentiation ability and secreted dentin sialophosphoprotein when seeded in a beta-tricalcium phosphate scaffold and BMP-2 in nude mice[131]. Among three different scaffolds (DL-lactide, co-polymer of L-lactide and DL-lactide, and hydroxyapatite tricalcium phosphate), a copolymer of L-lactide and DL-lactide showed the highest odontogenic regenerative capacity after the addition of DSCs and BMP-2[132].

A few studies have indicated that using a co-culture of DSCs with other stem cells improves neovascularization. The co-culture of DSCs and human umbilical vein endothelial cells with gelatin methacrylate xenogeneic hydrogel resulted in the neovascularization of mouse dental pulp[133]. Transplantation of DSCs and human umbilical vein endothelial cells with VEGF seeded into PuraMatrix significantly enhanced vascularization and mineralization of mouse vascularized pulp-like tissue and osteodentin[134]. In addition, using silk fibroin scaffolds promoted the ability of human DSCs in attracting vessels, which leads to the improvement of healing and regeneration of damaged tissues[135]. Transplantation of DSCs with a tooth fragment/silk fibroin scaffold loaded with SDF-1 resulted in the generation of pulp-like tissues with vascularity, organized fibrous matrix formation, and dentin formation in nude mice[136].

BONE RECONSTRUCTION

An enormous number of studies have been carried out to investigate the role of various scaffolds on the bone regeneration capacity of DSCs[137]. The osteogenic differentiation ability of DSCs, mostly isolated from dental pulp or periodontal ligament, has been well demonstrated in both *in vivo* and *in vitro* studies[138]. DSCs originating from dental pulp, dental follicle, gingival tissue, and periodontal ligament exert different osteogenic capacity[139], which can be modulated by various types of biomaterial scaffolds[140]. For instance, an *in vivo* investigation has shown that DSCs from the periodontal ligament encapsulated in an arginine-glycine-aspartic acid tripeptide scaffold exhibit a greater ability to repair bone defects by promoting the formation of mineralized tissue compared to gingival MSCs[141]. In addition, DSCs derived from the dental pulp exhibit great neovascularization potential while differentiating into osteoblasts, which subsequently promote bone restoration[142].

The most common scaffolds used to seed DSCs (particularly isolated from human dental pulp or exfoliated deciduous teeth) for bone tissue engineering in both experimental studies and clinical trials are collagen sponge membrane and hydroxyapatite/tri-calcium phosphate granules ceramic[143]. DSCs seeded in collagen sponge scaffolds exhibit strong restoration ability in human mandible bone defects[144]. The application of DSCs seeded onto a collagen-polyvinylpyrrolidone sponge scaffold in the left lower premolar region of a patient with periodontal disease increased bone density and decreased tooth mobility, periodontal pocket depth, and the bone defect area[145]. Using DSCs with a hydroxyapatite-collagen sponge scaffold to fill the alveolar defect in 6 patients with cleft lip and palate resulted in satisfactory bone regeneration[146]. A three-year clinical study revealed that the bone tissue regenerated following the application of human DSCs seeded on collagen scaffolds was uniformly vascularized and compact[147]. However, this study revealed that the new bone developed at the implantation sites was compact and different from the normal spongy alveolar bone in the mandibles[147]. In contrast, no ectopic bone formation was observed when DSCs were seeded on hydroxyapatite–tri-calcium phosphate scaffolds[148]. Furthermore, it should be noted that for any successful cell transplantation approach, the optimal number of DSCs is essential. It has been demonstrated that dense culture conditions improve the mineralized nodule formation of DSCs and promote osteogenic-lineage commitment, possibly *via* the integrin signaling pathway[149]. DSCs seeded in dense collagen gel scaffolds exert a higher beneficial effect on the craniofacial bone healing process compared to acellular scaffolds[150].

DSCs isolated from the dental follicle and the periapical papilla have been considered for the regeneration of alveolar bone and were successfully assessed in a few preclinical pilot studies. The application of dissociative dental pulp with a collagen sponge scaffold in patients with deep intrabony defects due to chronic periodontitis led to the effective restoration of defects with significant stability of the gingival margin[151]. In addition, the application of DSCs seeded onto collagen sponge in the deep intrabony defects of 29 patients suffering from chronic periodontitis significantly improved clinical outcomes of the periodontal regeneration process[152]. Another clinical trial has shown that using DSCs in combination with the collagen sponge scaffold in 6 patients resulted in a well-differentiated bone with Haversian system formation in the tooth extraction site[153].

Scaffold composition and surface properties play a key role in the osteogenic differentiation of DSCs and the process of bone tissue regeneration[142,154]. Significantly greater mineralization occurred when DSCs were seeded into a collagen type I matrix[155]. Furthermore, DSCs seeded on hyaluronic acid, fibrin, and polyesteramide type-C exhibit higher mineralization compared to standard tissue culture polystyrene[156].

Ceramic scaffolds, such as tri-calcium phosphate, hydroxyapatite, bioactive glass biphasic calcium phosphate, and calcium silicate, have chemical and structural similarities to the native bone and are commonly used as scaffolds to enhance bone regeneration and restoration of DSCs[157]. The addition of tricalcium phosphate to the composition of the other scaffolds enhances the differentiation of DSCs into osteoblast-like cells[158]. Chitosan/gelatin scaffolds significantly increased DSCs viability and differentiation as well as the formation of hydroxyapatite-rich nanocrystalline calcium phosphate in immunocompromised mice, particularly when cells were pre-treated with recombinant human BMP-2[92]. Potent bone formation was observed in the defect area of rat femoral bone after application of DSCs seeded in bioactive glass nanoparticles/chitosan-gelatin bionanocomposite compared to mesoporous bioactive glass nanospheres[159]. A combination of poly-ε-caprolactone biphasic calcium phosphate with DSCs increased the newly formed bone regeneration of calvarial defects in rabbit models[160]. Furthermore, a combination of poly-lactic-co-glycolic acid with ceramics is usually used to enhance biomimetic potential and promote bone regeneration[161]. An *in vitro* study has revealed that human dental pulp SCs adhesion and proliferation, as well as their differentiation toward the osteogenic lineage, are significantly improved when seeded in hydroxyapatite (a member of the calcium phosphate-based bioceramics) and poly-lactide-co-glycolide[162]. Implantation of human DSCs seeded in beta-tri-calcium phosphate scaffolds exerted an anti-inflammatory effect and restored periodontal hard tissue defects[163]. Greater bone regeneration was also reported when human DSCs were seeded on poly-lactic-co-glycolic acid[164] and α- calcium sulfate hemihydrate/amorphous calcium phosphate[165] scaffolds. Calcium phosphate cement functionalized with iron oxide nanoparticles also exhibits a potent effect on the spreading, osteogenic differentiation, and bone mineral synthesis of DSCs, possibly *via* activation of the extracellular signal-related kinases WNT/β-catenin pathway[166].

Different forms of silicon, particularly the orthosilicic acid form, promote osteoblast proliferation and differentiation, the mineralization process, and collagen production through enhancement of the precipitation of apatite from calcium and phosphate-containing solutions[167]. Semicarbazide-treated porous silicon exerted an appropriate scaffold for DSCs adhesion and *in vivo* cell therapy, whereas silanization with aminopropyltriethoxysilane-treated porous silicon has been suggested as a favorable scaffold for a long-term *in vitro* culture system for DSCs proliferation and differentiation[168]. Intrafibrillar-silicified collagen scaffolds markedly improved the proliferation, osteogenic differentiation, and mineralization capacity of human DSCs compared to non-silicified collagen scaffolds[169]. A novel biocompatible nano-engineered osteoinductive and elastomeric scaffold fabricated from a porous nanocomposite of poly-glycerol sebacate and nanosilicates enhanced the physical integrity and mechanical strength of the cellular microenvironment for *in vitro* osteogenic differentiation and bone regeneration without persistent scaffold-related inflammation *in vivo*[170].

Hybrid composites are also used as promising biomaterials for bone regeneration. It has been suggested that four different scaffold materials, including porous hydroxyapatite alone or combined with three polymers polylactic-co-glycolic acid, alginate, and ethylene vinylacetate/ethylene vinylversatate, are suitable for DSCs osteogenic differentiation[171]. Electrospun nano-ECM nanofibers with fluorapatite scaffolds enhance the growth, differentiation, and mineralization of DSCs[172], possibly mediated *via* modulation of the FGF and VEGF signaling pathways[173]. Comparing the

behavior of DSCs seeded on a 2D monolayer culture or 3D poly lactic-co-glycolic scaffold, it has been shown that DSCs exerted proper adherence and enhanced osteogenic differentiation on the 3D scaffold cultures[174]. Furthermore, it has been suggested that DSCs seeded in hydrogel scaffolds have greater potential for odontogenic differentiation than cells embedded in collagen-I hydrogel scaffolds[175]. Various layer-by-layer-modified gelatin sponge scaffolds increased the adhesion and proliferation of DSCs and enhanced their potential for bone tissue regeneration[176].

Several differentiation factors, such as BMP, were used to potentiate DSCs bone formation capacity. A 3D gel-based heparin-conjugated collagen matrix combined with recombinant human BMP-2 improved DSCs differentiation and seeding efficiency *in vitro* and promoted the osteogenic differentiation of these stem cells to form ectopic bone formation in a rat model[177]. Exfoliated human DSCs significantly increased the expression of BMP-2 and 7, bone and cartilage formation markers, when seeded in carbonate apatite scaffold in an *in vivo* alveolar bone remodeling model in rats[178]. DSCs isolated from human gingival tissues seeded onto 3D poly-lactide scaffolds enriched with extracellular vesicles, small membrane vesicles containing various bioactive molecules, exhibited potent osteogenic inductivity *in vitro* and showed a marked improvement in bone healing of rat calvaria bone tissue *in vivo*[179]. On the other hand, some biomaterial scaffolds may facilitate biomolecule-induced tissue formation. For instance, 3D matrigel scaffold enriched with DSCs led to enhanced BMP-9-induced osteogenesis and mineralization in ectopic bones in nude mice[180].

Although the majority of studies rely on the application of DSCs alone, several studies have employed co-culture systems (DSCs in combination with other cells) intending to promote bone regeneration, particularly in 3D scaffolds[181]. Human DSCs and amniotic fluid stem cells seeded onto fibroin scaffolds resulted in pronounced bone repair associated with neovascularization in critical-size rat cranial bone defects[182]. The co-cultured constructs of DSCs and endothelial cells seeded in 3D polycaprolactone blended with poly-L/D-lactide revealed a significantly higher up-regulation of genes related to osteogenesis and angiogenesis[183].

NEUROLOGICAL DISORDERS

DSCs derived from dental pulp and oral mucosa display high expression of various neural crest-related and developmental genes[184]. DSCs can be differentiated into the neuron-, Schwann-, glia-, and oligodendrocyte-like cells[185]. Due to the high proliferative capacity and propensity to differentiate into neural stem cells, DSCs are attractive candidates for developing a human neuronal lineage for the treatment of various disorders[186]. The role of DSCs in cell transplantation therapy of traumatic and hypoxic-ischemic injuries of the central or peripheral nervous system as well as neurodegenerative diseases has been extensively investigated[187]. DSCs are promising sources for cellular transplantation-based therapeutic strategies for neurological disorders[188]. The seeding of DCSs into different scaffolds promotes cell viability and differentiation towards neuronal-like cells[189,190]. Scaffolds can be designed to provide biological growth factors for neuronal tissues and to accurately adjust the diffusion rate of these essential biomolecules and enzymes[187].

The application of combined DSCs with various scaffolds promotes the function of injured neural tissues and reduces the inflammatory responses. The most common scaffolds applied for neural tissue regeneration and repair include chitosan, heparin-poloxamer, silicone tubes, poly-ε-caprolactone/poly-lactide-co-glycolic acid, and electrospun neuro-supportive scaffolds[191]. Different scaffolds were used to enhance neural differentiation and promote their neuronal characteristics. DSCs can be seeded in the biodegradable electrospun neuro-supportive scaffold, which is amended by different 3D coatings, for enhanced *in vitro* and *in vivo* recovery of neuronal damage[192]. The granular 3D chitosan scaffolds provide an appropriate microenvironment for attachment, proliferation, and neural differentiation of DSCs[193]. Furthermore, a 3D floating sphere culture system has been shown to provide a suitable micromilieu for human DSCs to retain their neuronal characteristics compared to myogenic and osteogenic properties[194]. Using an acellular ECM scaffold has been shown to promote DSCs to obtain a neuronal-like organization, including a central body associated with long cytoplasmic extensions that follow the underlying fibers, with high cell-matrix adhesion properties[195]. Some scaffolds can support the neurotrophic release of DSCs for the subsequent survival and differentiation of neural stem cells as well as neural cells. For instance, DSCs promoted the survival and

differentiation of adult murine neural stem cells on ethyl acrylate and hydroxyethyl acrylate copolymer scaffold through the enhancement of neurotrophic factor secretion[196].

Transplantation of DSCs with a chitosan scaffold markedly enhanced the recovery of motor function and suppressed inflammatory responses, possibly *via* the secretion of neurotrophic factors, such as glial cell-derived neurotrophic factor and brain-derived neurotrophic factor, in experimental models of spinal cord injury. Furthermore, the combination of DSCs with scaffolds inhibited cell injury and death through the reduction of caspase activity[197]. A significant functional recovery of hind-limb locomotor activities has also been observed following the transplantation of DSCs seeded in chitosan scaffolds in a spinal cord injury animal model[198]. Solubilized forms of acellular ECM from dentine, bone, and spinal cord have discrete structural, mechanical, and functional properties. Human DSCs exhibited a strong positive response to spinal cord ECM hydrogels by the greater expression of neural lineage markers. This ECM scaffold markedly enhanced the differentiation of DSCs to a neural lineage; indicating the importance of site-specific tissues in the promotion of stem cell behavior for constructive spinal cord regeneration[24]. A combination of DSCs with heparin-poloxamer, a desirable thermosensitive hydrogel for *in vivo* applications, loaded with various growth factors, such as bFGF and nerve growth factor (NGF), markedly promoted functional recovery, cellular regeneration, and tissue repair in a rat model of spinal cord injury[187], possibly *via* modulation of the MAPK/ERK, PI3K/Akt and JAK/STAT3 signaling pathways[199]. Indeed, both bFGF and NGF play an essential role in the neural differentiation of DSCs[200]. Chitosan scaffolds in combination with bFGF exerted a synergistic facilitating effect on DSCs differentiation to neural cells, possibly *via* activation of the ERK signaling pathway[201]. It has been shown that DSCs can proliferate efficiently on an aligned electrospun poly ε-caprolactone/poly lactide-co-glycolic acid scaffold and restore defects in rat spinal cord. Furthermore, these cells contribute to remyelination by the expression of oligodendrogenic lineage markers[202].

Multiple studies have assessed the effects of DSCs with various scaffolds on various experimental models of peripheral nerve injury. DSCs seeded into a polylactic-glycolic acid scaffold significantly improved the regeneration of injured facial nerve and promoted functional recovery compared to autografts[203]. Schwann-like cells derived from DSCs and grown in collagen scaffolds facilitated axonal outgrowth and myelination in both 2D and 3D *in vitro* models of peripheral nerve injury[204,205]. Furthermore, oligodendrocyte progenitor cells induced by differentiation of human DSCs *via* gene transfection in combination with collagen or collagen and fibrin scaffolds improved axonal outgrowth and myelination in an animal sciatic nerve injury model[9,206]. Human DSCs isolated from the periodontal ligament and gingival tissues and encapsulated in 3D alginate/hyaluronic acid scaffolds in the presence of NGF improved the proliferation and differentiation of DSCs toward the formation of neural tissues[207]. DSCs seeded on poly-lactic-co-glycolic acid collagen enhanced the interconnections of injured axons in a model of facial nerve injury[203]. When cultured under either 2D- or 3D-collagen scaffolds, human DSCs originating from gingival tissue have shown a greater capability of differentiating into neurons and Schwann-like cells in a 3D collagen scaffold compared to the 2D culture system. Furthermore, these cells with a 3D scaffold improved regeneration and functional recovery of neural tissues in rat facial nerve defects[208]. It has been shown that collagen scaffolds in the presence of different growth factors, such as bFGF, exhibited favorable mechanical properties and improved facial nerve regeneration[209]. Human DSCs expressing STRO-1, c-Kit, and CD34 markers and seeded in a collagen scaffold engrafted into rat sciatic nerve defects improved axonal regeneration from proximal to distal stumps[210]. Interestingly, the administration of serum-free conditioned medium from DSCs plunged in a collagen sponge into the gap caused by rat facial nerve transection, induced axonal regrowth and restored the neurological deficits[211].

THE IMPACT OF DIFFERENT SCAFFOLDS USED IN DSCS CELL THERAPY

Using an appropriate scaffold can promote the proliferation, differentiation, migration, and functional capacity of DSCs and can optimize and preserve the cellular morphology to build tissue constructs for a specific purpose[63]. Although the application of DSCs alone could yield promising outcomes in cell replacement therapy in particular conditions[212], an appropriate scaffold provides a viable

microenvironment to boost the development of DSCs towards new tissue formation, especially in tissues or organs with extensive defects[213]. The optimal number of DSCs is essential to develop tissue and organ substitutes and to restore organ function[149]. In addition to establishing definitive protocols for DSCs preparation, appropriate carrier scaffolds play a crucial role to increase the number of cells for implantation (Figure 3)[214].

A crucial and challenging demand for an appropriate scaffold design is recapitulating the dynamic nature of the native tissue[215]. Although each polymer scaffold has its pros and cons and favorable tissue engineering applications, collagen and fibrin, alone or by forming hybrid scaffolds, provide an adequate pulp connective tissue formation associated with marked vascularization, particularly when loaded with active biomolecules[117,118,216]. Collagen is the main component of the ECM and is expressed widely in bone, teeth, and the brain. A collagen scaffold provides excellent biocompatibility and controllable biodegradability, particularly for bone tissue engineering[217,218]. However, collagen has poor mechanical, chemical, and thermal stability and degrades fast at an uncontrolled rate. Fibrin is a non-toxic biomaterial scaffold that can attach various biological surfaces to regenerate tissues, such as bone and nervous tissues, with a low inflammatory response[219]. However, low mechanical stiffness of fibrin scaffolds limits tissue diffusion and direct implantation of cells to the damaged tissues[220,221]. Different bioceramic scaffolds exhibit excellent biocompatibility and osteoconductivity due to their chemical and structural similarity to native bone, which is characterized by high mechanical stiffness and low elasticity[158,222]. Furthermore, bioceramic scaffolds improve stem cell differentiation and osteogenesis[222]. The main disadvantages of bioceramic scaffolds are brittleness and slow biodegradation in the crystalline phase[218]. Soft polymers with highly aqueous hydrogels, such as collagen, share a resemblance to neural tissues, play an important role as a possible internal filler for neural conduits and increase the quality of peripheral nerve regeneration[223].

Furthermore, the scaffold should be porous and spongy to be able to deliver sufficient DSCs to injured tissues and to allow the stream of ECM and the formation of neovascularization[165,166]. However, some of the currently available biomaterials do not fully imitate the essential functions of natural ECM and fail to provide an appropriate scaffold[224]. Among the different biomaterials, the self-assembly of monodisperse cells into 2D or 3D complex structures that produce more extracellular matrix and promote intercellular communication possess the characteristics of the ideal approach[225]. Although both 2D and 3D cell culture systems provide appropriate methods for stem cell replacement transplantation, 3D systems seem to be more effective at mimicking the ECM in native tissues[226,227]. In general, 3D culture systems have been shown to be more beneficial in providing a template for the reconstruction of defects and cell-to-cell interactions as well as for improving cell adhesion, proliferation, ECM generation, maintenance of cell polarity, and restoration of various tissues[228,229]. In addition, 3D scaffolds enhance the sensitivity of stem cells towards drugs and biomolecules[230]. The optimization of 3D scaffold pore sizes may lead to better tissue regeneration through the enhancement of mechanical strength[231]. The dimension of the defect is a key factor in selecting a scaffold for tissue or organ regeneration[232]. For instance, in the reconstruction of cleft lip and palate, the amount of bone formation may not be enough to fill the bone defect[233], a problem that may be solved by the application of 3D cell culture systems[234]. In this regard, higher osteogenic differentiation of DSCs and MSCs has been observed in 3D than in 2D cell culture[174,235].

In addition to an appropriate scaffold, using bioactive molecules, such as growth and angiogenic factors, has been suggested as a promising strategy for the improvement of DSCs transplantation. Bioactive molecules, such as VEGF, have a short half-life and need to be encapsulated in degradable materials to regulate their release and promote their effects[236]. Scaffolds provide a purposeful approach for better incorporation between stem cells and biomolecules to improve tissue regeneration[26]. The interpolation of active biomolecules with the scaffold is essential for their transport into the injured tissues and for their efficacy to promote the colonization of DSCs and their matrix deposition[123]. DSCs and various scaffolds transplanted together with bioactive molecules, such as G-CSF, BMP, and bFGF, can fill the entire pulp or bone defect as well as develop new dentin or bone formation[92,93,118]. Biomolecules, such as SDF-1, SCF, and G-CSF, help to summon DSCs and enhance the number of cells in the implantation site, and other factors, such as VEGF, can enhance the formation of new blood vessels in regenerative tissues[26,237].

On the contrary, a few investigations have suggested that transplantation of DSCs without scaffold may have more beneficial effects on tissue regeneration. To prevent the inflammatory response, immune rejection, or infections, a few studies have

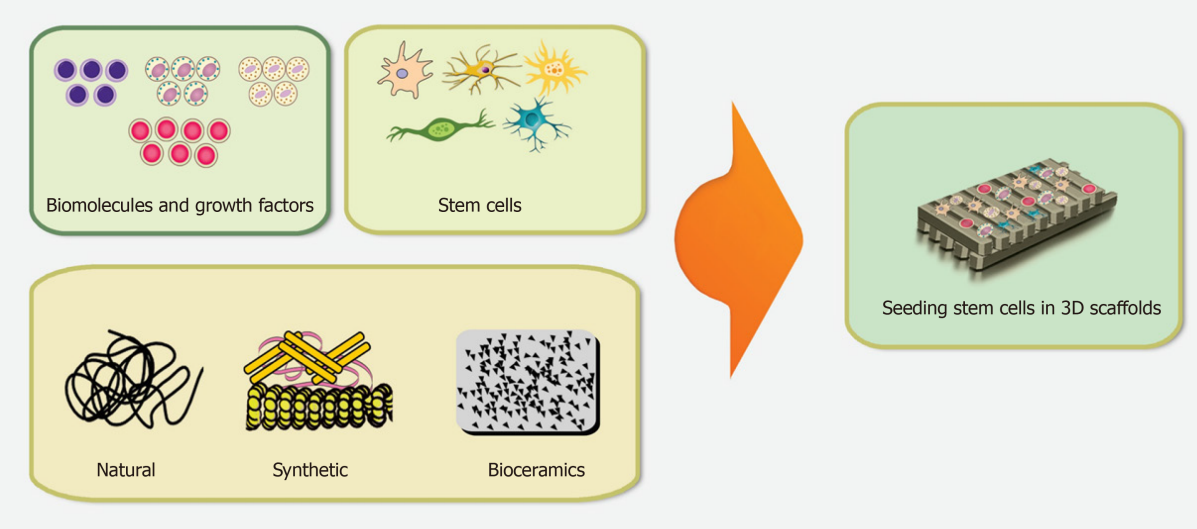


Figure 3 Schematic overview of the optimal biomimetic environment for application in tissue engineering. Stem cells require appropriate scaffold materials as well as biomolecules and growth factors to achieve optimal therapeutic effects.

indicated that transplantation of stem cells without scaffolds (such as 3D stem cell spheroids) may be an alternative option for DSCs transplantation[238]. Transplantation of DSCs without a scaffold for injured tooth tissues in 26 patients led to the regeneration of 3D pulp tissue which contained blood vessels and sensory nerves 12 months after therapy[239]. Despite these studies, it seems that the simple injection of competent DSCs inside organ defects is poorly regenerative[123].

A few decades of intense basic studies and clinical trials on DSCs are essential to translate knowledge gained on these cells into the implementation of defined and reproducible therapeutic approaches to cure or alleviate diseases. In addition to the application of an ideal scaffold, the success of cell transplantation therapy using DSCs also relied greatly on designing methodologies for isolation and purification, a sufficient number of stem cells, and effective and safe differentiation into different lineages[240,241]. The development of an accurate immunomodulatory strategy for injectable and implantable biomaterials is of particular importance to facilitate the grafting of DSCs at inflamed sites[242,243].

CONCLUSION

This study describes the main scaffolds, both natural and synthetic, used in DSCs transplantation and evaluated the advantages and disadvantages of various types of scaffolds. Most of the existing studies concerning the development of novel therapeutic approaches for restoration of damaged tissues have been limited to *in vitro* and *in vivo* DSCs testing, with a small number of clinical trials. Although the co-application of biomolecules with an appropriate scaffold seems to be crucial for effective cell transplantation therapy with DSCs, there is still much to learn about the dynamics of these molecules as well as their interactions with the ECM and DSCs to allow planning of appropriate therapeutic approaches. Further advances in tissue engineering need to focus on innovative combinations of biopolymers and biomolecules to promote the capability of DSCs for novel and effective therapeutic approaches (Figure 3).

REFERENCES

1 **Caplan AI**. Are All Adult Stem Cells The Same? *Regen Eng Transl Med* 2015; **1**: 4-10 [DOI: 10.1007/s40883-015-0001-4]
2 **Khorraminejad-Shirazi M**, Dorvash M, Estedlal A, Hoveidaei AH, Mazloomrezaei M, Mosaddeghi P. Aging: A cell source limiting factor in tissue engineering. *World J Stem Cells* 2019; **11**: 787-802 [PMID: 31692986 DOI: 10.4252/wjsc.v11.i10.787]
3 **Liu G**, David BT, Trawczynski M, Fessler RG. Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, and Applications. *Stem Cell Rev Rep* 2020; **16**: 3-32 [PMID: 31760627 DOI:

10.1007/s12015-019-09935-x]
4 **Xu M**, He J, Zhang C, Xu J, Wang Y. Strategies for derivation of endothelial lineages from human stem cells. *Stem Cell Res Ther* 2019; **10**: 200 [PMID: 31286997 DOI: 10.1186/s13287-019-1274-1]
5 **Mahla RS**. Stem Cells Applications in Regenerative Medicine and Disease Therapeutics. *Int J Cell Biol* 2016; **2016**: 6940283 [PMID: 27516776 DOI: 10.1155/2016/6940283]
6 **Morsczeck C**, Reichert TE. Dental stem cells in tooth regeneration and repair in the future. *Expert Opin Biol Ther* 2018; **18**: 187-196 [PMID: 29110535 DOI: 10.1080/14712598.2018.1402004]
7 **Ghasemi S**, Aligholi H, Koulivand PH, Jafarian M, Hosseini Ravandi H, Khaleghi Ghadiri M, Gorji A. Generation of motor neurons from human amygdala-derived neural stem-like cells. *Iran J Basic Med Sci* 2018; **21**: 1155-1160 [PMID: 30483389 DOI: 10.22038/IJBMS.2018.29587.7146]
8 **Orbay H**, Tobita M, Mizuno H. Mesenchymal stem cells isolated from adipose and other tissues: basic biological properties and clinical applications. *Stem Cells Int* 2012; **2012**: 461718 [PMID: 22666271 DOI: 10.1155/2012/461718]
9 **Ullah I**, Subbarao RB, Rho GJ. Human mesenchymal stem cells - current trends and future prospective. *Biosci Rep* 2015; **35** [PMID: 25797907 DOI: 10.1042/BSR20150025]
10 **Lee MW**, Ryu S, Kim DS, Lee JW, Sung KW, Koo HH, Yoo KH. Mesenchymal stem cells in suppression or progression of hematologic malignancy: current status and challenges. *Leukemia* 2019; **33**: 597-611 [PMID: 30705410 DOI: 10.1038/s41375-018-0373-9]
11 **Musiał-Wysocka A**, Kot M, Majka M. The Pros and Cons of Mesenchymal Stem Cell-Based Therapies. *Cell Transplant* 2019; **28**: 801-812 [PMID: 31018669 DOI: 10.1177/0963689719837897]
12 **Brown PT**, Handorf AM, Jeon WB, Li WJ. Stem cell-based tissue engineering approaches for musculoskeletal regeneration. *Curr Pharm Des* 2013; **19**: 3429-3445 [PMID: 23432679 DOI: 10.2174/1381612811319990350]
13 **Kerkis I**, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, Caplan AI, Cerruti HF. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. *Cells Tissues Organs* 2006; **184**: 105-116 [PMID: 17409736 DOI: 10.1159/000099617]
14 **Sharpe PT**. Dental mesenchymal stem cells. *Development* 2016; **143**: 2273-2280 [PMID: 27381225 DOI: 10.1242/dev.134189]
15 **Bianco P**, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell* 2008; **2**: 313-319 [PMID: 18397751 DOI: 10.1016/j.stem.2008.03.002]
16 **Volponi AA**, Sharpe PT. The tooth -- a treasure chest of stem cells. *Br Dent J* 2013; **215**: 353-358 [PMID: 24113958 DOI: 10.1038/sj.bdj.2013.959]
17 **Grawish ME**. Gingival-derived mesenchymal stem cells: An endless resource for regenerative dentistry. *World J Stem Cells* 2018; **10**: 116-118 [PMID: 30310530 DOI: 10.4252/wjsc.v10.i9.116]
18 **Nakamura S**, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. *J Endod* 2009; **35**: 1536-1542 [PMID: 19840643 DOI: 10.1016/j.joen.2009.07.024]
19 **Huang GT**, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res* 2009; **88**: 792-806 [PMID: 19767575 DOI: 10.1177/0022034509340867]
20 **Yang X**, Li L, Xiao L, Zhang D. Recycle the dental fairy's package: overview of dental pulp stem cells. *Stem Cell Res Ther* 2018; **9**: 347 [PMID: 30545418 DOI: 10.1186/s13287-018-1094-8]
21 **Martens W**, Bronckaers A, Politis C, Jacobs R, Lambrichts I. Dental stem cells and their promising role in neural regeneration: an update. *Clin Oral Investig* 2013; **17**: 1969-1983 [PMID: 23846214 DOI: 10.1007/s00784-013-1030-3]
22 **Sonoyama W**, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* 2008; **34**: 166-171 [PMID: 18215674 DOI: 10.1016/j.joen.2007.11.021]
23 **Wang W**, Dang M, Zhang Z, Hu J, Eyster TW, Ni L, Ma PX. Dentin regeneration by stem cells of apical papilla on injectable nanofibrous microspheres and stimulated by controlled BMP-2 release. *Acta Biomater* 2016; **36**: 63-72 [PMID: 26971664 DOI: 10.1016/j.actbio.2016.03.015]
24 **Viswanath A**, Vanacker J, Germain L, Leprince JG, Diogenes A, Shakesheff KM, White LJ, des Rieux A. Extracellular matrix-derived hydrogels for dental stem cell delivery. *J Biomed Mater Res A* 2017; **105**: 319-328 [PMID: 27636889 DOI: 10.1002/jbm.a.35901]
25 **Wang X**, Sha XJ, Li GH, Yang FS, Ji K, Wen LY, Liu SY, Chen L, Ding Y, Xuan K. Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. *Arch Oral Biol* 2012; **57**: 1231-1240 [PMID: 22455989 DOI: 10.1016/j.archoralbio.2012.02.014]
26 **Bakhtiar H**, Mazidi S A, Mohammadi Asl S, Ellini MR, Moshiri A, Nekoofar MH, Dummer PMH. The role of stem cell therapy in regeneration of dentine-pulp complex: a systematic review. *Prog Biomater* 2018; **7**: 249-268 [PMID: 30267369 DOI: 10.1007/s40204-018-0100-7]
27 **da Silva Meirelles L**, Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. *Stem Cells* 2008; **26**: 2287-2299 [PMID: 18566331 DOI: 10.1634/stemcells.2007-1122]
28 **Rodríguez-Lozano FJ**, Bueno C, Insausti CL, Meseguer L, Ramírez MC, Blanquer M, Marín N, Martínez S, Moraleda JM. Mesenchymal stem cells derived from dental tissues. *Int Endod J* 2011; **44**: 800-806 [PMID: 21477154 DOI: 10.1111/j.1365-2591.2011.01877.x]
29 **Pisciotta A**, Bertoni L, Vallarola A, Bertani G, Mecugni D, Carnevale G. Neural crest derived stem cells from dental pulp and tooth-associated stem cells for peripheral nerve regeneration. *Neural Regen Res* 2020; **15**: 373-381 [PMID: 31571644 DOI: 10.4103/1673-5374.266043]
30 **Sonoyama W**, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S, Shi S. Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PLoS One* 2006; **1**: e79 [PMID: 17183711 DOI: 10.1371/journal.pone.0000079]
31 **Jeon BG**, Kang EJ, Kumar BM, Maeng GH, Ock SA, Kwack DO, Park BW, Rho GJ. Comparative analysis of telomere length, telomerase and reverse transcriptase activity in human dental stem cells. *Cell Transplant*

2011; **20**: 1693-1705 [PMID: 21396170 DOI: 10.3727/096368911X565001]
32 **Demircan PC**, Sariboyaci AE, Unal ZS, Gacar G, Subasi C, Karaoz E. Immunoregulatory effects of human dental pulp-derived stem cells on T cells: comparison of transwell co-culture and mixed lymphocyte reaction systems. *Cytotherapy* 2011; **13**: 1205-1220 [PMID: 21905956 DOI: 10.3109/14653249.2011.605351]
33 **Li Z**, Jiang CM, An S, Cheng Q, Huang YF, Wang YT, Gou YC, Xiao L, Yu WJ, Wang J. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells. *Oral Dis* 2014; **20**: 25-34 [PMID: 23463961 DOI: 10.1111/odi.12086]
34 **Andrukhov O**, Behm C, Blufstein A, Rausch-Fan X. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: Implication in disease and tissue regeneration. *World J Stem Cells* 2019; **11**: 604-617 [PMID: 31616538 DOI: 10.4252/wjsc.v11.i9.604]
35 **Shin C**, Kim M, Han JA, Choi B, Hwang D, Do Y, Yun JH. Human periodontal ligament stem cells suppress T-cell proliferation via down-regulation of non-classical major histocompatibility complex-like glycoprotein CD1b on dendritic cells. *J Periodontal Res* 2017; **52**: 135-146 [PMID: 27021598 DOI: 10.1111/jre.12378]
36 **Watt FM**, Huck WT. Role of the extracellular matrix in regulating stem cell fate. *Nat Rev Mol Cell Biol* 2013; **14**: 467-473 [PMID: 23839578 DOI: 10.1038/nrm3620]
37 **Sahab-Negah S**, Hajali V, Moradi HR, Gorji A. The Impact of Estradiol on Neurogenesis and Cognitive Functions in Alzheimer's Disease. *Cell Mol Neurobiol* 2020; **40**: 283-299 [PMID: 31502112 DOI: 10.1007/s10571-019-00733-0]
38 **Moore KA**, Lemischka IR. Stem cells and their niches. *Science* 2006; **311**: 1880-1885 [PMID: 16574858 DOI: 10.1126/science.1110542]
39 **Mitsiadis TA**, Feki A, Papaccio G, Catón J. Dental pulp stem cells, niches, and notch signaling in tooth injury. *Adv Dent Res* 2011; **23**: 275-279 [PMID: 21677078 DOI: 10.1177/0022034511405386]
40 **Chan BP**, Leong KW. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *Eur Spine J* 2008; **17** Suppl 4: 467-479 [PMID: 19005702 DOI: 10.1007/s00586-008-0745-3]
41 **Yi S**, Ding F, Gong L, Gu X. Extracellular Matrix Scaffolds for Tissue Engineering and Regenerative Medicine. *Curr Stem Cell Res Ther* 2017; **12**: 233-246 [PMID: 27593448 DOI: 10.2174/1574888X11666160905092513]
42 **Uccelli A**, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; **8**: 726-736 [PMID: 19172693 DOI: 10.1038/nri2395]
43 **Marrelli M**, Codispoti B, Shelton RM, Scheven BA, Cooper PR, Tatullo M, Paduano F. Dental Pulp Stem Cell Mechanoresponsiveness: Effects of Mechanical Stimuli on Dental Pulp Stem Cell Behavior. *Front Physiol* 2018; **9**: 1685 [PMID: 30534086 DOI: 10.3389/fphys.2018.01685]
44 **Larmas M**. Pre-odontoblasts, odontoblasts, or "odontocytes". *J Dent Res* 2008; **87**: 198; author reply 199 [PMID: 18296601 DOI: 10.1177/154405910808700310]
45 **Goldberg M**, Six N, Chaussain C, DenBesten P, Veis A, Poliard A. Dentin extracellular matrix molecules implanted into exposed pulps generate reparative dentin: a novel strategy in regenerative dentistry. *J Dent Res* 2009; **88**: 396-399 [PMID: 19493881 DOI: 10.1177/0022034509337101]
46 **Kawashima N**, Okiji T. Odontoblasts: Specialized hard-tissue-forming cells in the dentin-pulp complex. *Congenit Anom (Kyoto)* 2016; **56**: 144-153 [PMID: 27131345 DOI: 10.1111/cga.12169]
47 **Shi S**, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* 2003; **18**: 696-704 [PMID: 12674330 DOI: 10.1359/jbmr.2003.18.4.696]
48 **Machado CV**, Passos ST, Campos TM, Bernardi L, Vilas-Bôas DS, Nör JE, Telles PD, Nascimento IL. The dental pulp stem cell niche based on aldehyde dehydrogenase 1 expression. *Int Endod J* 2016; **49**: 755-763 [PMID: 26198909 DOI: 10.1111/iej.12511]
49 **Stokowski A**, Shi S, Sun T, Bartold PM, Koblar SA, Gronthos S. EphB/ephrin-B interaction mediates adult stem cell attachment, spreading, and migration: implications for dental tissue repair. *Stem Cells* 2007; **25**: 156-164 [PMID: 17204606 DOI: 10.1634/stemcells.2006-0373]
50 **Wan PX**, Wang BW, Wang ZC. Importance of the stem cell microenvironment for ophthalmological cell-based therapy. *World J Stem Cells* 2015; **7**: 448-460 [PMID: 25815128 DOI: 10.4252/wjsc.v7.i2.448]
51 **Bonaventura G**, Incontro S, Iemmolo R, La Cognata V, Barbagallo I, Costanzo E, Barcellona ML, Pellitteri R, Cavallaro S. Dental mesenchymal stem cells and neuro-regeneration: a focus on spinal cord injury. *Cell Tissue Res* 2020; **379**: 421-428 [PMID: 31776822 DOI: 10.1007/s00441-019-03109-4]
52 **Ferro F**, Spelat R, Baheney CS. Dental pulp stem cell (DPSC) isolation, characterization, and differentiation. *Methods Mol Biol* 2014; **1210**: 91-115 [PMID: 25173163 DOI: 10.1007/978-1-4939-1435-7_8]
53 **Yamada Y**, Nakamura-Yamada S, Kusano K, Baba S. Clinical Potential and Current Progress of Dental Pulp Stem Cells for Various Systemic Diseases in Regenerative Medicine: A Concise Review. *Int J Mol Sci* 2019; **20** [PMID: 30845639 DOI: 10.3390/ijms20051132]
54 **Onizuka S**, Iwata T. Application of Periodontal Ligament-Derived Multipotent Mesenchymal Stromal Cell Sheets for Periodontal Regeneration. *Int J Mol Sci* 2019; **20** [PMID: 31181666 DOI: 10.3390/ijms20112796]
55 **Spagnuolo G**, Codispoti B, Marrelli M, Rengo C, Rengo S, Tatullo M. Commitment of Oral-Derived Stem Cells in Dental and Maxillofacial Applications. *Dent J (Basel)* 2018; **6** [PMID: 30551556 DOI: 10.3390/dj6040072]
56 **Mead B**, Logan A, Berry M, Leadbeater W, Scheven BA. Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. *Invest Ophthalmol Vis Sci* 2013; **54**: 7544-7556 [PMID: 24150755 DOI: 10.1167/iovs.13-13045]
57 **Peerani R**, Zandstra PW. Enabling stem cell therapies through synthetic stem cell-niche engineering. *J Clin Invest* 2010; **120**: 60-70 [PMID: 20051637 DOI: 10.1172/JCI41158]
58 **Pera MF**, Tam PP. Extrinsic regulation of pluripotent stem cells. *Nature* 2010; **465**: 713-720 [PMID: 20535200 DOI: 10.1038/nature09228]
59 **Wilems T**, Vardhan S, Wu S, Sakiyama-Elbert S. The influence of microenvironment and extracellular

matrix molecules in driving neural stem cell fate within biomaterials. *Brain Res Bull* 2019; **148**: 25-33 [PMID: 30898579 DOI: 10.1016/j.brainresbull.2019.03.004]
60 **Conde MC**, Chisini LA, Demarco FF, Nör JE, Casagrande L, Tarquinio SB. Stem cell-based pulp tissue engineering: variables enrolled in translation from the bench to the bedside, a systematic review of literature. *Int Endod J* 2016; **49**: 543-550 [PMID: 26101143 DOI: 10.1111/iej.12489]
61 **Kanjevac T**, Gustafson C, Ivanovska A, Ravanetti F, Cacchioli A, Bosnakovski D. Inflammatory Cytokines and Biodegradable Scaffolds in Dental Mesenchymal Stem Cells Priming. *Curr Stem Cell Res Ther* 2019; **14**: 320-326 [PMID: 30608044 DOI: 10.2174/1574888X14666190103170109]
62 **La Noce M**, Paino F, Spina A, Naddeo P, Montella R, Desiderio V, De Rosa A, Papaccio G, Tirino V, Laino L. Dental pulp stem cells: state of the art and suggestions for a true translation of research into therapy. *J Dent* 2014; **42**: 761-768 [PMID: 24589847 DOI: 10.1016/j.jdent.2014.02.018]
63 **Tsutsui TW**. Dental Pulp Stem Cells: Advances to Applications. *Stem Cells Cloning* 2020; **13**: 33-42 [PMID: 32104005 DOI: 10.2147/SCCAA.S166759]
64 **He Y**, Lu F. Development of Synthetic and Natural Materials for Tissue Engineering Applications Using Adipose Stem Cells. *Stem Cells Int* 2016; **2016**: 5786257 [PMID: 26977158 DOI: 10.1155/2016/5786257]
65 **Sano H**, Orbay H, Terashi H, Hyakusoku H, Ogawa R. Acellular adipose matrix as a natural scaffold for tissue engineering. *J Plast Reconstr Aesthet Surg* 2014; **67**: 99-106 [PMID: 24035153 DOI: 10.1016/j.bjps.2013.08.006]
66 **Monteiro BG**, Loureiro RR, Cristovam PC, Covre JL, Gomes JÁP, Kerkis I. Amniotic membrane as a biological scaffold for dental pulp stem cell transplantation in ocular surface reconstruction. *Arq Bras Oftalmol* 2019; **82**: 32-37 [PMID: 30652765 DOI: 10.5935/0004-2749.20190009]
67 **Edgar L**, McNamara K, Wong T, Tamburrini R, Katari R, Orlando G. Heterogeneity of Scaffold Biomaterials in Tissue Engineering. *Materials (Basel)* 2016; 9 [PMID: 28773457 DOI: 10.3390/ma9050332]
68 **Willerth SM**, Sakiyama-Elbert SE. Combining stem cells and biomaterial scaffolds for constructing tissues and cell delivery. *Stem J* 2008 [PMID: 20614610 DOI: 10.3824/stembook.1.1.1]
69 **Alaribe FN**, Manoto SL, Motaung SCKM. Scaffolds from biomaterials: advantages and limitations in bone and tissue engineering. *Biologia* 2016; **71**: 353-366 [DOI: 10.1515/biolog-2016-0056]
70 **Zarrintaj P**, Bakhshandeh B, Saeb MR, Sefat F, Rezaeian I, Ganjali MR, Ramakrishna S, Mozafari M. Oligoaniline-based conductive biomaterials for tissue engineering. *Acta Biomater* 2018; **72**: 16-34 [PMID: 29625254 DOI: 10.1016/j.actbio.2018.03.042]
71 **FitzGerald JF**, Kumar AS. Biologic versus Synthetic Mesh Reinforcement: What are the Pros and Cons? *Clin Colon Rectal Surg* 2014; **27**: 140-148 [PMID: 26106284 DOI: 10.1055/s-0034-1394155]
72 **Wang C**, Feng N, Chang F, Wang J, Yuan B, Cheng Y, Liu H, Yu J, Zou J, Ding J, Chen X. Injectable Cholesterol-Enhanced Stereocomplex Polylactide Thermogel Loading Chondrocytes for Optimized Cartilage Regeneration. *Adv Healthc Mater* 2019; **8**: e1900312 [PMID: 31094096 DOI: 10.1002/adhm.201900312]
73 **Van Vlierberghe S**, Dubruel P, Schacht E. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. *Biomacromolecules* 2011; **12**: 1387-1408 [PMID: 21388145 DOI: 10.1021/bm200083n]
74 **Choe G**, Park J, Park H, Lee JY. Hydrogel Biomaterials for Stem Cell Microencapsulation. *Polymers (Basel)* 2018; **10** [PMID: 30960922 DOI: 10.3390/polym10090997]
75 **Gaharwar AK**, Peppas NA, Khademhosseini A. Nanocomposite hydrogels for biomedical applications. *Biotechnol Bioeng* 2014; **111**: 441-453 [PMID: 24264728 DOI: 10.1002/bit.25160]
76 **Sahab Negah S**, Oliazadeh P, Jahanbazi Jahan-Abad A, Eshaghabadi A, Samini F, Ghasemi S, Asghari A, Gorji A. Transplantation of human meningioma stem cells loaded on a self-assembling peptide nanoscaffold containing IKVAV improves traumatic brain injury in rats. *Acta Biomater* 2019; **92**: 132-144 [PMID: 31075516 DOI: 10.1016/j.actbio.2019.05.010]
77 **Sahab Negah S**, Khooei A, Samini F, Gorji A. Laminin-derived Ile-Lys-Val-ala-Val: a promising bioactive peptide in neural tissue engineering in traumatic brain injury. *Cell Tissue Res* 2018; **371**: 223-236 [PMID: 29082446 DOI: 10.1007/s00441-017-2717-6]
78 **Janik H**, Marzec M. A review: fabrication of porous polyurethane scaffolds. *Mater Sci Eng C Mater Biol Appl* 2015; **48**: 586-591 [PMID: 25579961 DOI: 10.1016/j.msec.2014.12.037]
79 **Raeisdasteh Hokmabad V**, Davaran S, Ramazani A, Salehi R. Design and fabrication of porous biodegradable scaffolds: a strategy for tissue engineering. *J Biomater Sci Polym Ed* 2017; **28**: 1797-1825 [PMID: 28707508 DOI: 10.1080/09205063.2017.1354674]
80 **Mantha S**, Pillai S, Khayambashi P, Upadhyay A, Zhang Y, Tao O, Pham HM, Tran SD. Smart Hydrogels in Tissue Engineering and Regenerative Medicine. *Materials (Basel)* 2019; **12** [PMID: 31614735 DOI: 10.3390/ma12203323]
81 **Kapałczyńska M**, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, Ibbs M, Bliźniak R, Łuczewski Ł, Lamperska K. 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. *Arch Med Sci* 2018; **14**: 910-919 [PMID: 30002710 DOI: 10.5114/aoms.2016.63743]
82 **Meng X**, Leslie P, Zhang Y, Dong J. Stem cells in a three-dimensional scaffold environment. *Springerplus* 2014; **3**: 80 [PMID: 24570851 DOI: 10.1186/2193-1801-3-80]
83 **Huang BJ**, Hu JC, Athanasiou KA. Cell-based tissue engineering strategies used in the clinical repair of articular cartilage. *Biomaterials* 2016; **98**: 1-22 [PMID: 27177218 DOI: 10.1016/j.biomaterials.2016.04.018]
84 **Galler KM**, Eidt A, Schmalz G. Cell-free approaches for dental pulp tissue engineering. *J Endod* 2014; **40**: S41-S45 [PMID: 24698692 DOI: 10.1016/j.joen.2014.01.014]
85 **Alsberg E**, von Recum HA, Mahoney MJ. Environmental cues to guide stem cell fate decision for tissue engineering applications. *Expert Opin Biol Ther* 2006; **6**: 847-866 [PMID: 16918253 DOI: 10.1517/14712598.6.9.847]
86 **Demarco FF**, Casagrande L, Zhang Z, Dong Z, Tarquinio SB, Zeitlin BD, Shi S, Smith AJ, Nör JE. Effects of morphogen and scaffold porogen on the differentiation of dental pulp stem cells. *J Endod* 2010; **36**: 1805-1811 [PMID: 20951292 DOI: 10.1016/j.joen.2010.08.031]

87 **Chatakun P**, Núñez-Toldrà R, Díaz López EJ, Gil-Recio C, Martínez-Sarrà E, Hernández-Alfaro F, Ferrés-Padró E, Giner-Tarrida L, Atari M. The effect of five proteins on stem cells used for osteoblast differentiation and proliferation: a current review of the literature. *Cell Mol Life Sci* 2014; **71**: 113-142 [PMID: 23568025 DOI: 10.1007/s00018-013-1326-0]

88 **Zhang J**, Lu X, Feng G, Gu Z, Sun Y, Bao G, Xu G, Lu Y, Chen J, Xu L, Feng X, Cui Z. Chitosan scaffolds induce human dental pulp stem cells to neural differentiation: potential roles for spinal cord injury therapy. *Cell Tissue Res* 2016; **366**: 129-142 [PMID: 27147262 DOI: 10.1007/s00441-016-2402-1]

89 **Jazayeri HE**, Lee SM, Kuhn L, Fahimipour F, Tahriri M, Tayebi L. Polymeric scaffolds for dental pulp tissue engineering: A review. *Dent Mater* 2020; **36**: e47-e58 [PMID: 31791734 DOI: 10.1016/j.dental.2019.11.005]

90 **Zhang L**, Morsi Y, Wang Y, Li Y, Ramakrishna S. Review scaffold design and stem cells for tooth regeneration. *Jpn Dent Sci Rev* 2013; **49**: 14-26 [DOI: 10.1016/j.jdsr.2012.09.001]

91 **Iohara K**, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, Matsushita K, Nakamura H, Nakashima M. Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. *Tissue Eng Part A* 2011; **17**: 1911-1920 [PMID: 21417716 DOI: 10.1089/ten.tea.2010.0615]

92 **Bakopoulou A**, Georgopoulou Grivas I, Bekiari C, Prymak O, Loza K, Epple M, Papadopoulos GC, Koidis P, Chatzinikolaidou M. Dental pulp stem cells in chitosan/gelatin scaffolds for enhanced orofacial bone regeneration. *Dent Mater* 2019; **35**: 310-327 [PMID: 30527589 DOI: 10.1016/j.dental.2018.11.025]

93 **Xu WP**, Zhang W, Asrican R, Kim HJ, Kaplan DL, Yelick PC. Accurately shaped tooth bud cell-derived mineralized tissue formation on silk scaffolds. *Tissue Eng Part A* 2008; **14**: 549-557 [PMID: 18352829 DOI: 10.1089/tea.2007.0227]

94 **Hori Y**, Winans AM, Irvine DJ. Modular injectable matrices based on alginate solution/microsphere mixtures that gel in situ and co-deliver immunomodulatory factors. *Acta Biomater* 2009; **5**: 969-982 [PMID: 19117820 DOI: 10.1016/j.actbio.2008.11.019]

95 **Ahmadian E**, Eftekhari A, Dizaj SM, Sharifi S, Mokhtarpour M, Nasibova AN, Khalilov R, Samiei M. The effect of hyaluronic acid hydrogels on dental pulp stem cells behavior. *Int J Biol Macromol* 2019; **140**: 245-254 [PMID: 31419560 DOI: 10.1016/j.ijbiomac.2019.08.119]

96 **Wang X**, Li G, Liu Y, Yu W, Sun Q. Biocompatibility of biological material polylactic acid with stem cells from human exfoliated deciduous teeth. *Biomed Rep* 2017; **6**: 519-524 [PMID: 28515910 DOI: 10.3892/br.2017.881]

97 **Park CH**, Rios HF, Jin Q, Bland ME, Flanagan CL, Hollister SJ, Giannobile WV. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. *Biomaterials* 2010; **31**: 5945-5952 [PMID: 20471083 DOI: 10.1016/j.biomaterials.2010.04.027]

98 **Maspero FA**, Ruffieux K, Müller B, Wintermantel E. Resorbable defect analog PLGA scaffolds using CO2 as solvent: structural characterization. *J Biomed Mater Res* 2002; **62**: 89-98 [PMID: 12124790 DOI: 10.1002/jbm.10212]

99 **Yang X**, Yang F, Walboomers XF, Bian Z, Fan M, Jansen JA. The performance of dental pulp stem cells on nanofibrous PCL/gelatin/nHA scaffolds. *J Biomed Mater Res A* 2010; **93**: 247-257 [PMID: 19557787 DOI: 10.1002/jbm.a.32535]

100 **Liu HC**, E LL, Wang DS, Su F, Wu X, Shi ZP, Lv Y, Wang JZ. Reconstruction of alveolar bone defects using bone morphogenetic protein 2 mediated rabbit dental pulp stem cells seeded on nano-hydroxyapatite/collagen/poly(L-lactide). *Tissue Eng Part A* 2011; **17**: 2417-2433 [PMID: 21563858 DOI: 10.1089/ten.tea.2010.0620]

101 **Lluch AV**, Fernández AC, Ferrer GG, Pradas MM. Bioactive scaffolds mimicking natural dentin structure. *J Biomed Mater Res B Appl Biomater* 2009; **90**: 182-194 [PMID: 19072987 DOI: 10.1002/jbm.b.31272]

102 **Prescott RS**, Alsanea R, Fayad MI, Johnson BR, Wenckus CS, Hao J, John AS, George A. In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. *J Endod* 2008; **34**: 421-426 [PMID: 18358888 DOI: 10.1016/j.joen.2008.02.005]

103 **Bento LW**, Zhang Z, Imai A, Nör F, Dong Z, Shi S, Araujo FB, Nör JE. Endothelial differentiation of SHED requires MEK1/ERK signaling. *J Dent Res* 2013; **92**: 51-57 [PMID: 23114032 DOI: 10.1177/0022034512466263]

104 **Huang W**, Shi X, Ren L, Du C, Wang Y. PHBV microspheres--PLGA matrix composite scaffold for bone tissue engineering. *Biomaterials* 2010; **31**: 4278-4285 [PMID: 20199806 DOI: 10.1016/j.biomaterials.2010.01.059]

105 **Huang CC**, Narayanan R, Warshawsky N, Ravindran S. Dual ECM Biomimetic Scaffolds for Dental Pulp Regenerative Applications. *Front Physiol* 2018; **9**: 495 [PMID: 29887803 DOI: 10.3389/fphys.2018.00495]

106 **Kim NR**, Lee DH, Chung PH, Yang HC. Distinct differentiation properties of human dental pulp cells on collagen, gelatin, and chitosan scaffolds. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; **108**: e94-100 [PMID: 19836718 DOI: 10.1016/j.tripleo.2009.07.031]

107 **Qu T**, Liu X. Nano-Structured Gelatin/Bioactive Glass Hybrid Scaffolds for the Enhancement of Odontogenic Differentiation of Human Dental Pulp Stem Cells. *J Mater Chem B* 2013; **1**: 4764-4772 [PMID: 24098854 DOI: 10.1039/C3TB21002B]

108 **Hu L**, Gao Z, Xu J, Zhu Z, Fan Z, Zhang C, Wang J, Wang S. Decellularized Swine Dental Pulp as a Bioscaffold for Pulp Regeneration. *Biomed Res Int* 2017; **2017**: 9342714 [PMID: 29387727 DOI: 10.1155/2017/9342714]

109 **Na S**, Zhang H, Huang F, Wang W, Ding Y, Li D, Jin Y. Regeneration of dental pulp/dentine complex with a three-dimensional and scaffold-free stem-cell sheet-derived pellet. *J Tissue Eng Regen Med* 2016; **10**: 261-270 [PMID: 23365018 DOI: 10.1002/term.1686]

110 **Liu M**, Zhao L, Hu J, Wang L, Li N, Wu D, Shi X, Yuan M, Hu W, Wang X. Endothelial cells and endothelin-1 promote the odontogenic differentiation of dental pulp stem cells. *Mol Med Rep* 2018; **18**: 893-901 [PMID: 29845193 DOI: 10.3892/mmr.2018.9033]

111 **Itoh Y**, Sasaki JI, Hashimoto M, Katata C, Hayashi M, Imazato S. Pulp Regeneration by 3-dimensional Dental Pulp Stem Cell Constructs. *J Dent Res* 2018; **97**: 1137-1143 [PMID: 29702010 DOI:

10.1177/0022034518772260]
112 **Lambrichts I**, Driesen RB, Dillen Y, Gervois P, Ratajczak J, Vangansewinkel T, Wolfs E, Bronckaers A, Hilkens P. Dental Pulp Stem Cells: Their Potential in Reinnervation and Angiogenesis by Using Scaffolds. *J Endod* 2017; **43**: S12-S16 [PMID: 28781091 DOI: 10.1016/j.joen.2017.06.001]
113 **Oshima M**, Tsuji T. Functional tooth regenerative therapy: tooth tissue regeneration and whole-tooth replacement. *Odontology* 2014; **102**: 123-136 [PMID: 25052182 DOI: 10.1007/s10266-014-0168-z]
114 **Jahanbazi Jahan-Abad A**, Sahab Negah S, Hosseini Ravandi H, Ghasemi S, Borhani-Haghighi M, Stummer W, Gorji A, Khaleghi Ghadiri M. Human Neural Stem/Progenitor Cells Derived From Epileptic Human Brain in a Self-Assembling Peptide Nanoscaffold Improve Traumatic Brain Injury in Rats. *Mol Neurobiol* 2018; **55**: 9122-9138 [PMID: 29651746 DOI: 10.1007/s12035-018-1050-8]
115 **Rosa V**, Zhang Z, Grande RH, Nör JE. Dental pulp tissue engineering in full-length human root canals. *J Dent Res* 2013; **92**: 970-975 [PMID: 24056227 DOI: 10.1177/0022034513505772]
116 **Eramo S**, Natali A, Pinna R, Milia E. Dental pulp regeneration via cell homing. *Int Endod J* 2018; **51**: 405-419 [PMID: 29047120 DOI: 10.1111/iej.12868]
117 **Iohara K**, Murakami M, Takeuchi N, Osako Y, Ito M, Ishizaka R, Utunomiya S, Nakamura H, Matsushita K, Nakashima M. A novel combinatorial therapy with pulp stem cells and granulocyte colony-stimulating factor for total pulp regeneration. *Stem Cells Transl Med* 2013; **2**: 521-533 [PMID: 23761108 DOI: 10.5966/sctm.2012-0132]
118 **Galler KM**, D'Souza RN, Federlin M, Cavender AC, Hartgerink JD, Hecker S, Schmalz G. Dentin conditioning codetermines cell fate in regenerative endodontics. *J Endod* 2011; **37**: 1536-1541 [PMID: 22000458 DOI: 10.1016/j.joen.2011.08.027]
119 **Yu S**, Li J, Zhao Y, Li X, Ge L. Comparative Secretome Analysis of Mesenchymal Stem Cells From Dental Apical Papilla and Bone Marrow During Early Odonto/Osteogenic Differentiation: Potential Role of Transforming Growth Factor-β2. *Front Physiol* 2020; **11**: 41 [PMID: 32210829 DOI: 10.3389/fphys.2020.00041]
120 **Suzuki T**, Lee CH, Chen M, Zhao W, Fu SY, Qi JJ, Chotkowski G, Eisig SB, Wong A, Mao JJ. Induced migration of dental pulp stem cells for in vivo pulp regeneration. *J Dent Res* 2011; **90**: 1013-1018 [PMID: 21586666 DOI: 10.1177/0022034511408426]
121 **Mathieu S**, Jeanneau C, Sheibat-Othman N, Kalaji N, Fessi H, About I. Usefulness of controlled release of growth factors in investigating the early events of dentin-pulp regeneration. *J Endod* 2013; **39**: 228-235 [PMID: 23321236 DOI: 10.1016/j.joen.2012.11.007]
122 **Zein N**, Harmouch E, Lutz JC, Fernandez De Grado G, Kuchler-Bopp S, Clauss F, Offner D, Hua G, Benkirane-Jessel N, Fioretti F. Polymer-Based Instructive Scaffolds for Endodontic Regeneration. *Materials (Basel)* 2019; **12** [PMID: 31344822 DOI: 10.3390/ma12152347]
123 **Yang JW**, Zhang YF, Sun ZY, Song GT, Chen Z. Dental pulp tissue engineering with bFGF-incorporated silk fibroin scaffolds. *J Biomater Appl* 2015; **30**: 221-229 [PMID: 25791684 DOI: 10.1177/0885328215577296]
124 **Kim K**, Lee CH, Kim BK, Mao JJ. Anatomically shaped tooth and periodontal regeneration by cell homing. *J Dent Res* 2010; **89**: 842-847 [PMID: 20448245 DOI: 10.1177/0022034510370803]
125 **Swenson ES**, Kuwahara R, Krause DS, Theise ND. Physiological variations of stem cell factor and stromal-derived factor-1 in murine models of liver injury and regeneration. *Liver Int* 2008; **28**: 308-318 [PMID: 18290773 DOI: 10.1111/j.1478-3231.2007.01659.x]
126 **Pan S**, Dangaria S, Gopinathan G, Yan X, Lu X, Kolokythas A, Niu Y, Luan X. SCF promotes dental pulp progenitor migration, neovascularization, and collagen remodeling - potential applications as a homing factor in dental pulp regeneration. *Stem Cell Rev Rep* 2013; **9**: 655-667 [PMID: 23703692 DOI: 10.1007/s12015-013-9442-7]
127 **Guo S**, Lim D, Dong Z, Saunders TL, Ma PX, Marcelo CL, Ritchie HH. Dentin sialophosphoprotein: a regulatory protein for dental pulp stem cell identity and fate. *Stem Cells Dev* 2014; **23**: 2883-2894 [PMID: 25027178 DOI: 10.1089/scd.2014.0066]
128 **Ritchie H**. The functional significance of dentin sialoprotein-phosphophoryn and dentin sialoprotein. *Int J Oral Sci* 2018; **10**: 31 [PMID: 30393383 DOI: 10.1038/s41368-018-0035-9]
129 **Ravindran S**, Zhang Y, Huang CC, George A. Odontogenic induction of dental stem cells by extracellular matrix-inspired three-dimensional scaffold. *Tissue Eng Part A* 2014; **20**: 92-102 [PMID: 23859633 DOI: 10.1089/ten.tea.2013.0192]
130 **Yang B**, Chen G, Li J, Zou Q, Xie D, Chen Y, Wang H, Zheng X, Long J, Tang W, Guo W, Tian W. Tooth root regeneration using dental follicle cell sheets in combination with a dentin matrix - based scaffold. *Biomaterials* 2012; **33**: 2449-2461 [PMID: 22192537 DOI: 10.1016/j.biomaterials.2011.11.074]
131 **Li X**, Wang L, Su Q, Ye L, Zhou X, Song D, Huang D. Highly Proliferative Immortalized Human Dental Pulp Cells Retain the Odontogenic Phenotype when Combined with a Beta-Tricalcium Phosphate Scaffold and BMP2. *Stem Cells Int* 2020; **2020**: 4534128 [PMID: 32148517 DOI: 10.1155/2020/4534128]
132 **Atalayin C**, Tezel H, Dagci T, Karabay Yavasoglu NU, Oktem G, Kose T. In vivo performance of different scaffolds for dental pulp stem cells induced for odontogenic differentiation. *Braz Oral Res* 2016; **30**: e120 [PMID: 27901202 DOI: 10.1590/1807-3107bor-2016.vol30.0120]
133 **Khayat A**, Monteiro N, Smith EE, Pagni S, Zhang W, Khademhosseini A, Yelick PC. GelMA-Encapsulated hDPSCs and HUVECs for Dental Pulp Regeneration. *J Dent Res* 2017; **96**: 192-199 [PMID: 28106508 DOI: 10.1177/0022034516682005]
134 **Dissanayaka WL**, Hargreaves KM, Jin L, Samaranayake LP, Zhang C. The interplay of dental pulp stem cells and endothelial cells in an injectable peptide hydrogel on angiogenesis and pulp regeneration in vivo. *Tissue Eng Part A* 2015; **21**: 550-563 [PMID: 25203774 DOI: 10.1089/ten.tea.2014.0154]
135 **Woloszyk A**, Buschmann J, Waschkies C, Stadlinger B, Mitsiadis TA. Human Dental Pulp Stem Cells and Gingival Fibroblasts Seeded into Silk Fibroin Scaffolds Have the Same Ability in Attracting Vessels. *Front Physiol* 2016; **7**: 140 [PMID: 27148078 DOI: 10.3389/fphys.2016.00140]
136 **Yang JW**, Zhang YF, Wan CY, Sun ZY, Nie S, Jian SJ, Zhang L, Song GT, Chen Z. Autophagy in SDF-1α-mediated DPSC migration and pulp regeneration. *Biomaterials* 2015; **44**: 11-23 [PMID: 25617122 DOI: 10.1016/j.biomaterials.2014.12.006]

137 **Ercal P**, Pekozer GG. A Current Overview of Scaffold-Based Bone Regeneration Strategies with Dental Stem Cells. *Adv Exp Med Biol* 2020 [PMID: 32185698 DOI: 10.1007/5584_2020_505]

138 **Monterubbianesi R**, Bencun M, Pagella P, Woloszyk A, Orsini G, Mitsiadis TA. A comparative in vitro study of the osteogenic and adipogenic potential of human dental pulp stem cells, gingival fibroblasts and foreskin fibroblasts. *Sci Rep* 2019; **9**: 1761 [PMID: 30741963 DOI: 10.1038/s41598-018-37981-x]

139 **Zhang Y**, Xing Y, Jia L, Ji Y, Zhao B, Wen Y, Xu X. An In Vitro Comparative Study of Multisource Derived Human Mesenchymal Stem Cells for Bone Tissue Engineering. *Stem Cells Dev* 2018; **27**: 1634-1645 [PMID: 30234437 DOI: 10.1089/scd.2018.0119]

140 **Moshaverinia A**, Chen C, Akiyama K, Xu X, Chee WW, Schricker SR, Shi S. Encapsulated dental-derived mesenchymal stem cells in an injectable and biodegradable scaffold for applications in bone tissue engineering. *J Biomed Mater Res A* 2013; **101**: 3285-3294 [PMID: 23983201 DOI: 10.1002/jbm.a.34546]

141 **Han Y**, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal Stem Cells for Regenerative Medicine. *Cells* 2019; **8** [PMID: 31412678 DOI: 10.3390/cells8080886]

142 **Moshaverinia A**, Chen C, Xu X, Akiyama K, Ansari S, Zadeh HH, Shi S. Bone regeneration potential of stem cells derived from periodontal ligament or gingival tissue sources encapsulated in RGD-modified alginate scaffold. *Tissue Eng Part A* 2014; **20**: 611-621 [PMID: 24070211 DOI: 10.1089/ten.tea.2013.0229]

143 **Leyendecker Junior A**, Gomes Pinheiro CC, Lazzaretti Fernandes T, Franco Bueno D. The use of human dental pulp stem cells for in vivo bone tissue engineering: A systematic review. *J Tissue Eng* 2018; **9**: 2041731417752766 [PMID: 29375756 DOI: 10.1177/2041731417752766]

144 **d'Aquino R**, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur Cell Mater* 2009; **18**: 75-83 [PMID: 19908196 DOI: 10.22203/ecm.v018a07]

145 **Hernández-Monjaraz B**, Santiago-Osorio E, Ledesma-Martínez E, Alcauter-Zavala A, Mendoza-Núñez VM. Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: A case report. *J Int Med Res* 2018; **46**: 2983-2993 [PMID: 29911458 DOI: 10.1177/0300060518773244]

146 **Tanikawa DYS**, Pinheiro CCG, Almeida MCA, Oliveira CRGCM, Coudry RA, Rocha DL, Bueno DF. Deciduous Dental Pulp Stem Cells for Maxillary Alveolar Reconstruction in Cleft Lip and Palate Patients. *Stem Cells Int* 2020; **2020**: 6234167 [PMID: 32256610 DOI: 10.1155/2020/6234167]

147 **Giuliani A**, Manescu A, Langer M, Rustichelli F, Desiderio V, Paino F, De Rosa A, Laino L, d'Aquino R, Tirino V, Papaccio G. Three years after transplants in human mandibles, histological and in-line holotomography revealed that stem cells regenerated a compact rather than a spongy bone: biological and clinical implications. *Stem Cells Transl Med* 2013; **2**: 316-324 [PMID: 23502599 DOI: 10.5966/sctm.2012-0136]

148 **Zhang W**, Walboomers XF, van Osch GJ, van den Dolder J, Jansen JA. Hard tissue formation in a porous HA/TCP ceramic scaffold loaded with stromal cells derived from dental pulp and bone marrow. *Tissue Eng Part A* 2008; **14**: 285-294 [PMID: 18333781 DOI: 10.1089/tea.2007.0146]

149 **Noda S**, Kawashima N, Yamamoto M, Hashimoto K, Nara K, Sekiya I, Okiji T. Effect of cell culture density on dental pulp-derived mesenchymal stem cells with reference to osteogenic differentiation. *Sci Rep* 2019; **9**: 5430 [PMID: 30931957 DOI: 10.1038/s41598-019-41741-w]

150 **Chamieh F**, Collignon AM, Coyac BR, Lesieur J, Ribes S, Sadoine J, Llorens A, Nicoletti A, Letourneur D, Colombier ML, Nazhat SN, Bouchard P, Chaussain C, Rochefort GY. Accelerated craniofacial bone regeneration through dense collagen gel scaffolds seeded with dental pulp stem cells. *Sci Rep* 2016; **6**: 38814 [PMID: 27934940 DOI: 10.1038/srep38814]

151 **Aimetti M**, Ferrarotti F, Gamba MN, Giraudi M, Romano F. Regenerative Treatment of Periodontal Intrabony Defects Using Autologous Dental Pulp Stem Cells: A 1-Year Follow-Up Case Series. *Int J Periodontics Restorative Dent* 2018; **38**: 51-58 [PMID: 29240205 DOI: 10.11607/prd.3425]

152 **Ferrarotti F**, Romano F, Gamba MN, Quirico A, Giraudi M, Audagna M, Aimetti M. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: A randomized controlled clinical trial. *J Clin Periodontol* 2018; **45**: 841-850 [PMID: 29779220 DOI: 10.1111/jcpe.12931]

153 **Monti M**, Graziano A, Rizzo S, Perotti C, Del Fante C, d'Aquino R, Redi CA, Rodriguez Y Baena R. In Vitro and In Vivo Differentiation of Progenitor Stem Cells Obtained After Mechanical Digestion of Human Dental Pulp. *J Cell Physiol* 2017; **232**: 548-555 [PMID: 27277190 DOI: 10.1002/jcp.25452]

154 **Graziano A**, d'Aquino R, Laino G, Papaccio G. Dental pulp stem cells: a promising tool for bone regeneration. *Stem Cell Rev* 2008; **4**: 21-26 [PMID: 18300003 DOI: 10.1007/s12015-008-9013-5]

155 **Fang TJ**, Wang DH, Wang CY, Poongodi R, Liou NH, Liu JC, Hsu ML, Hong PD, Yang SF, Liu ML. Osteogenic prospective of deriving human dental stem cells in collagen matrix boost. *J Mater Sci Mater Med* 2017; **28**: 192 [PMID: 29143185 DOI: 10.1007/s10856-017-6001-9]

156 **Apel C**, Buttler P, Salber J, Dhanasingh A, Neuss S. Differential mineralization of human dental pulp stem cells on diverse polymers. *Biomed Tech (Berl)* 2018; **63**: 261-269 [PMID: 28157689 DOI: 10.1515/bmt-2016-0141]

157 **Roseti L**, Parisi V, Petretta M, Cavallo C, Desando G, Bartolotti I, Grigolo B. Scaffolds for Bone Tissue Engineering: State of the art and new perspectives. *Mater Sci Eng C Mater Biol Appl* 2017; **78**: 1246-1262 [PMID: 28575964 DOI: 10.1016/j.msec.2017.05.017]

158 **Liao SS**, Cui FZ. In vitro and in vivo degradation of mineralized collagen-based composite scaffold: nanohydroxyapatite/collagen/poly(L-lactide). *Tissue Eng* 2004; **10**: 73-80 [PMID: 15009932 DOI: 10.1089/107632704322791718]

159 **Covarrubias C**, Cádiz M, Maureira M, Celhay I, Cuadra F, von Marttens A. Bionanocomposite scaffolds based on chitosan-gelatin and nanodimensional bioactive glass particles: In vitro properties and in vivo bone regeneration. *J Biomater Appl* 2018; **32**: 1155-1163 [PMID: 29451421 DOI: 10.1177/0885328218759042]

160 **Wongsupa N**, Nuntanaranont T, Kamolmattayakul S, Thuaksuban N. Assessment of bone regeneration of a tissue-engineered bone complex using human dental pulp stem cells/poly(ε-caprolactone)-biphasic calcium phosphate scaffold constructs in rabbit calvarial defects. *J Mater Sci Mater Med* 2017; **28**: 77 [PMID: 28386853 DOI: 10.1007/s10856-017-5883-x]

161 **Pan Z**, Ding J. Poly(lactide-co-glycolide) porous scaffolds for tissue engineering and regenerative medicine. *Interface Focus* 2012; **2**: 366-377 [PMID: 23741612 DOI: 10.1098/rsfs.2011.0123]

162 **Jiménez NT**, Carlos Munévar J, González JM, Infante C, Lara SJP. In vitro response of dental pulp stem cells in 3D scaffolds: A regenerative bone material. *Heliyon* 2018; **4**: e00775 [PMID: 30263971 DOI: 10.1016/j.heliyon.2018.e00775]

163 **Li Y**, Zhao S, Nan X, Wei H, Shi J, Li A, Gou J. Repair of human periodontal bone defects by autologous grafting stem cells derived from inflammatory dental pulp tissues. *Stem Cell Res Ther* 2016; **7**: 141 [PMID: 27655627 DOI: 10.1186/s13287-016-0404-2]

164 **Xavier Acasigua GA**, Bernardi L, Braghirolli DI, Filho MS, Pranke P, Medeiros Fossati AC. Nanofiber scaffolds support bone regeneration associated with pulp stem cells. *Curr Stem Cell Res Ther* 2014; **9**: 330-337 [PMID: 24588088 DOI: 10.2174/1574888X09666140228123911]

165 **Kuo TF**, Lee SY, Wu HD, Poma M, Wu YW, Yang JC. An in vivo swine study for xeno-grafts of calcium sulfate-based bone grafts with human dental pulp stem cells (hDPSCs). *Mater Sci Eng C Mater Biol Appl* 2015; **50**: 19-23 [PMID: 25746240 DOI: 10.1016/j.msec.2015.01.092]

166 **Xia Y**, Guo Y, Yang Z, Chen H, Ren K, Weir MD, Chow LC, Reynolds MA, Zhang F, Gu N, Xu HHK. Iron oxide nanoparticle-calcium phosphate cement enhanced the osteogenic activities of stem cells through WNT/β-catenin signaling. *Mater Sci Eng C Mater Biol Appl* 2019; **104**: 109955 [PMID: 31500064 DOI: 10.1016/j.msec.2019.109955]

167 **Reffitt DM**, Ogston N, Jugdaohsingh R, Cheung HF, Evans BA, Thompson RP, Powell JJ, Hampson GN. Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro. *Bone* 2003; **32**: 127-135 [PMID: 12633784 DOI: 10.1016/s8756-3282(02)00950-x]

168 **Collart-Dutilleul PY**, Secret E, Panayotov I, Deville de Périère D, Martín-Palma RJ, Torres-Costa V, Martin M, Gergely C, Durand JO, Cunin F, Cuisinier FJ. Adhesion and proliferation of human mesenchymal stem cells from dental pulp on porous silicon scaffolds. *ACS Appl Mater Interfaces* 2014; **6**: 1719-1728 [PMID: 24428409 DOI: 10.1021/am4046316]

169 **Niu LN**, Sun JQ, Li QH, Jiao K, Shen LJ, Wu D, Tay F, Chen JH. Intrafibrillar-silicified collagen scaffolds enhance the osteogenic capacity of human dental pulp stem cells. *J Dent* 2014; **42**: 839-849 [PMID: 24705068 DOI: 10.1016/j.jdent.2014.03.016]

170 **Kerativitayanan P**, Tatullo M, Khariton M, Joshi P, Perniconi B, Gaharwar AK. Nanoengineered osteoinductive and elastomeric scaffolds for bone tissue engineering. *ACS Biomater Sci Eng* 2017; **3**: 590-600 [DOI: 10.1021/acsbiomaterials.7b00029]

171 **Karadzic I**, Vucic V, Jokanovic V, Debeljak-Martacic J, Markovic D, Petrovic S, Glibetic M. Effects of novel hydroxyapatite-based 3D biomaterials on proliferation and osteoblastic differentiation of mesenchymal stem cells. *J Biomed Mater Res A* 2015; **103**: 350-357 [PMID: 24665062 DOI: 10.1002/jbm.a.35180]

172 **Guo T**, Li Y, Cao G, Zhang Z, Chang S, Czajka-Jakubowska A, Nör JE, Clarkson BH, Liu J. Fluorapatite-modified scaffold on dental pulp stem cell mineralization. *J Dent Res* 2014; **93**: 1290-1295 [PMID: 25139361 DOI: 10.1177/0022034514547914]

173 **Clark D**, Wang X, Chang S, Czajka-Jakubowska A, Clarkson BH, Liu J. VEGF promotes osteogenic differentiation of ASCs on ordered fluorapatite surfaces. *J Biomed Mater Res A* 2015; **103**: 639-645 [PMID: 24797761 DOI: 10.1002/jbm.a.35215]

174 **Jafar H**, Abuarqoub D, Ababneh N, Hasan M, Al-Sotari S, Aslam N, Kailani M, Ammoush M, Shraideh Z, Awidi A. hPL promotes osteogenic differentiation of stem cells in 3D scaffolds. *PLoS One* 2019; **14**: e0215667 [PMID: 31063489 DOI: 10.1371/journal.pone.0215667]

175 **Paduano F**, Marrelli M, White LJ, Shakesheff KM, Tatullo M. Odontogenic Differentiation of Human Dental Pulp Stem Cells on Hydrogel Scaffolds Derived from Decellularized Bone Extracellular Matrix and Collagen Type I. *PLoS One* 2016; **11**: e0148225 [PMID: 26882351 DOI: 10.1371/journal.pone.0148225]

176 **Fu Q**, Ren H, Zheng C, Zhuang C, Wu T, Qin J, Wang Z, Chen Y, Qi N. Improved osteogenic differentiation of human dental pulp stem cells in a layer-by-layer-modified gelatin scaffold. *J Biomater Appl* 2018; **33**: 477-487 [PMID: 30217134 DOI: 10.1177/0885328218799162]

177 **Fahimipour F**, Dashtimoghadam E, Mahdi Hasani-Sadrabadi M, Vargas J, Vashaee D, Lobner DC, Jafarzadeh Kashi TS, Ghasemzadeh B, Tayebi L. Enhancing cell seeding and osteogenesis of MSCs on 3D printed scaffolds through injectable BMP2 immobilized ECM-Mimetic gel. *Dent Mater* 2019; **35**: 990-1006 [PMID: 31027908 DOI: 10.1016/j.dental.2019.04.004]

178 **Prahasanti C**, Nugraha AP, Saskianti T, Suardita K, Riawan W, Ernawati DS. Exfoliated Human Deciduous Tooth Stem Cells Incorporating Carbonate Apatite Scaffold Enhance BMP-2, BMP-7 and Attenuate MMP-8 Expression During Initial Alveolar Bone Remodeling in Wistar Rats (*Rattus norvegicus*). *Clin Cosmet Investig Dent* 2020; **12**: 79-85 [PMID: 32273773 DOI: 10.2147/CCIDE.S245678]

179 **Diomede F**, D'Aurora M, Gugliandolo A, Merciaro I, Ettorre V, Bramanti A, Piattelli A, Gatta V, Mazzon E, Fontana A, Trubiani O. A novel role in skeletal segment regeneration of extracellular vesicles released from periodontal-ligament stem cells. *Int J Nanomedicine* 2018; **13**: 3805-3825 [PMID: 29988728 DOI: 10.2147/IJN.S162836]

180 **Fu T**, Liang P, Song J, Wang J, Zhou P, Tang Y, Li J, Huang E. Matrigel Scaffolding Enhances BMP9-induced Bone Formation in Dental Follicle Stem/Precursor Cells. *Int J Med Sci* 2019; **16**: 567-575 [PMID: 31171908 DOI: 10.7150/ijms.30801]

181 **Polo-Corrales L**, Latorre-Esteves M, Ramirez-Vick JE. Scaffold design for bone regeneration. *J Nanosci Nanotechnol* 2014; **14**: 15-56 [PMID: 24730250 DOI: 10.1166/jnn.2014.9127]

182 **Riccio M**, Maraldi T, Pisciotta A, La Sala GB, Ferrari A, Bruzzesi G, Motta A, Migliaresi C, De Pol A. Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. *Tissue Eng Part A* 2012; **18**: 1006-1013 [PMID: 22166080 DOI: 10.1089/ten.tea.2011.0542]

183 **Jin GZ**, Kim HW. Co-culture of Human Dental Pulp Stem Cells and Endothelial Cells Using Porous Biopolymer Microcarriers: A Feasibility Study for Bone Tissue Engineering. *Tissue Eng Regen Med* 2017; **14**: 393-401 [PMID: 30603495 DOI: 10.1007/s13770-017-0061-2]

184 **Abe S**, Yamaguchi S, Sato Y, Harada K. Sphere-Derived Multipotent Progenitor Cells Obtained From

Human Oral Mucosa Are Enriched in Neural Crest Cells. *Stem Cells Transl Med* 2016; **5**: 117-128 [PMID: 26582909 DOI: 10.5966/sctm.2015-0111]

185 **Anitua E**, Troya M, Zalduendo M. Progress in the use of dental pulp stem cells in regenerative medicine. *Cytotherapy* 2018; **20**: 479-498 [PMID: 29449086 DOI: 10.1016/j.jcyt.2017.12.011]

186 **Tatullo M**, Codispoti B, Pacifici A, Palmieri F, Marrelli M, Pacifici L, Paduano F. Potential Use of Human Periapical Cyst-Mesenchymal Stem Cells (hPCy-MSCs) as a Novel Stem Cell Source for Regenerative Medicine Applications. *Front Cell Dev Biol* 2017; **5**: 103 [PMID: 29259970 DOI: 10.3389/fcell.2017.00103]

187 **Luo L**, He Y, Wang X, Key B, Lee BH, Li H, Ye Q. Potential Roles of Dental Pulp Stem Cells in Neural Regeneration and Repair. *Stem Cells Int* 2018; **2018**: 1731289 [PMID: 29853908 DOI: 10.1155/2018/1731289]

188 **Sakai K**, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Sakamoto K, Tauchi R, Wakao N, Imagama S, Hibi H, Kadomatsu K, Ishiguro N, Ueda M. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. *J Clin Invest* 2012; **122**: 80-90 [PMID: 22133879 DOI: 10.1172/JCI59251]

189 **Chang CC**, Chang KC, Tsai SJ, Chang HH, Lin CP. Neurogenic differentiation of dental pulp stem cells to neuron-like cells in dopaminergic and motor neuronal inductive media. *J Formos Med Assoc* 2014; **113**: 956-965 [PMID: 25438878 DOI: 10.1016/j.jfma.2014.09.003]

190 **Mayo V**, Sawatari Y, Huang CY, Garcia-Godoy F. Neural crest-derived dental stem cells--where we are and where we are going. *J Dent* 2014; **42**: 1043-1051 [PMID: 24769107 DOI: 10.1016/j.jdent.2014.04.007]

191 **Wang D**, Wang Y, Tian W, Pan J. Advances of tooth-derived stem cells in neural diseases treatments and nerve tissue regeneration. *Cell Prolif* 2019; **52**: e12572 [PMID: 30714230 DOI: 10.1111/cpr.12572]

192 **Das S**, Bellare JR. Dental Pulp Stem Cells in Customized 3D Nanofibrous Scaffolds for Regeneration of Peripheral Nervous System. *Methods Mol Biol* 2020; **2125**: 157-166 [PMID: 30294747 DOI: 10.1007/7651_2018_194]

193 **Feng X**, Lu X, Huang D, Xing J, Feng G, Jin G, Yi X, Li L, Lu Y, Nie D, Chen X, Zhang L, Gu Z, Zhang X. 3D porous chitosan scaffolds suit survival and neural differentiation of dental pulp stem cells. *Cell Mol Neurobiol* 2014; **34**: 859-870 [PMID: 24789753 DOI: 10.1007/s10571-014-0063-8]

194 **Pisciotta A**, Bertoni L, Riccio M, Mapelli J, Bigiani A, La Noce M, Orciani M, de Pol A, Carnevale G. Use of a 3D Floating Sphere Culture System to Maintain the Neural Crest-Related Properties of Human Dental Pulp Stem Cells. *Front Physiol* 2018; **9**: 547 [PMID: 29892229 DOI: 10.3389/fphys.2018.00547]

195 **Laudani S**, La Cognata V, Iemmolo R, Bonaventura G, Villaggio G, Saccone S, Barcellona ML, Cavallaro S, Sinatra F. Effect of a Bone Marrow-Derived Extracellular Matrix on Cell Adhesion and Neural Induction of Dental Pulp Stem Cells. *Front Cell Dev Biol* 2020; **8**: 100 [PMID: 32211401 DOI: 10.3389/fcell.2020.00100]

196 **Soria JM**, Sancho-Tello M, Esparza MA, Mirabet V, Bagan JV, Monleón M, Carda C. Biomaterials coated by dental pulp cells as substrate for neural stem cell differentiation. *J Biomed Mater Res A* 2011; **97**: 85-92 [PMID: 21319296 DOI: 10.1002/jbm.a.33032]

197 **Zhang F**, Song J, Zhang H, Huang E, Song D, Tollemar V, Wang J, Wang J, Mohammed M, Wei Q, Fan J, Liao J, Zou Y, Liu F, Hu X, Qu X, Chen L, Yu X, Luu HH, Lee MJ, He TC, Ji P. Wnt and BMP Signaling Crosstalk in Regulating Dental Stem Cells: Implications in Dental Tissue Engineering. *Genes Dis* 2016; **3**: 263-276 [PMID: 28491933 DOI: 10.1016/j.gendis.2016.09.004]

198 **Jung J**, Kim JW, Moon HJ, Hong JY, Hyun JK. Characterization of Neurogenic Potential of Dental Pulp Stem Cells Cultured in Xeno/Serum-Free Condition: In Vitro and In Vivo Assessment. *Stem Cells Int* 2016; **2016**: 6921097 [PMID: 27688776 DOI: 10.1155/2016/6921097]

199 **Li R**, Li Y, Wu Y, Zhao Y, Chen H, Yuan Y, Xu K, Zhang H, Lu Y, Wang J, Li X, Jia X, Xiao J. Heparin-Poloxamer Thermosensitive Hydrogel Loaded with bFGF and NGF Enhances Peripheral Nerve Regeneration in Diabetic Rats. *Biomaterials* 2018; **168**: 24-37 [PMID: 29609091 DOI: 10.1016/j.biomaterials.2018.03.044]

200 **Zhang J**, Lian M, Cao P, Bao G, Xu G, Sun Y, Wang L, Chen J, Wang Y, Feng G, Cui Z. Effects of Nerve Growth Factor and Basic Fibroblast Growth Factor Promote Human Dental Pulp Stem Cells to Neural Differentiation. *Neurochem Res* 2017; **42**: 1015-1025 [PMID: 28005222 DOI: 10.1007/s11064-016-2134-3]

201 **Zheng K**, Feng G, Zhang J, Xing J, Huang D, Lian M, Zhang W, Wu W, Hu Y, Lu X, Feng X. Basic fibroblast growth factor promotes human dental pulp stem cells cultured in 3D porous chitosan scaffolds to neural differentiation. *Int J Neurosci* 2020; 1-9 [PMID: 32186218 DOI: 10.1080/00207454.2020.1744592]

202 **Li X**, Yang C, Li L, Xiong J, Xie L, Yang B, Yu M, Feng L, Jiang Z, Guo W, Tian W. A therapeutic strategy for spinal cord defect: human dental follicle cells combined with aligned PCL/PLGA electrospun material. *Biomed Res Int* 2015; **2015**: 197183 [PMID: 25695050 DOI: 10.1155/2015/197183]

203 **Sasaki R**, Aoki S, Yamato M, Uchiyama H, Wada K, Ogiuchi H, Okano T, Ando T. PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. *J Tissue Eng Regen Med* 2011; **5**: 823-830 [PMID: 22002926 DOI: 10.1002/term.387]

204 **Martens W**, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, Phillips J, Lambrichts I. Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. *FASEB J* 2014; **28**: 1634-1643 [PMID: 24352035 DOI: 10.1096/fj.13-243980]

205 **Sanen K**, Martens W, Georgiou M, Ameloot M, Lambrichts I, Phillips J. Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair? *J Tissue Eng Regen Med* 2017; **11**: 3362-3372 [PMID: 28052540 DOI: 10.1002/term.2249]

206 **Askari N**, Yaghoobi MM, Shamsara M, Esmaeili-Mahani S. Tetracycline-regulated expression of OLIG2 gene in human dental pulp stem cells lead to mouse sciatic nerve regeneration upon transplantation. *Neuroscience* 2015; **305**: 197-208 [PMID: 26254831 DOI: 10.1016/j.neuroscience.2015.07.088]

207 **Ansari S**, Diniz IM, Chen C, Sarrion P, Tamayol A, Wu BM, Moshaverinia A. Human Periodontal Ligament- and Gingiva-derived Mesenchymal Stem Cells Promote Nerve Regeneration When Encapsulated in Alginate/Hyaluronic Acid 3D Scaffold. *Adv Healthc Mater* 2017; **6** [PMID: 29076281 DOI: 10.1002/adhm.201700670]

208 **Zhang Q**, Nguyen PD, Shi S, Burrell JC, Cullen DK, Le AD. 3D bio-printed scaffold-free nerve constructs with human gingiva-derived mesenchymal stem cells promote rat facial nerve regeneration. *Sci Rep* 2018; **8**: 6634 [PMID: 29700345 DOI: 10.1038/s41598-018-24888-w]

209 **Cui Y**, Lu C, Meng D, Xiao Z, Hou X, Ding W, Kou D, Yao Y, Chen B, Zhang Z, Li J, Pan J, Dai J. Collagen scaffolds modified with CNTF and bFGF promote facial nerve regeneration in minipigs. *Biomaterials* 2014; **35**: 7819-7827 [PMID: 24930851 DOI: 10.1016/j.biomaterials.2014.05.065]

210 **Carnevale G**, Pisciotta A, Riccio M, Bertoni L, De Biasi S, Gibellini L, Zordani A, Cavallini GM, La Sala GB, Bruzzesi G, Ferrari A, Cossarizza A, de Pol A. Human dental pulp stem cells expressing STRO-1, c-kit and CD34 markers in peripheral nerve regeneration. *J Tissue Eng Regen Med* 2018; **12**: e774-e785 [PMID: 27943583 DOI: 10.1002/term.2378]

211 **Kano F**, Matsubara K, Ueda M, Hibi H, Yamamoto A. Secreted Ectodomain of Sialic Acid-Binding Ig-Like Lectin-9 and Monocyte Chemoattractant Protein-1 Synergistically Regenerate Transected Rat Peripheral Nerves by Altering Macrophage Polarity. *Stem Cells* 2017; **35**: 641-653 [PMID: 27862629 DOI: 10.1002/stem.2534]

212 **Kilkenny C**, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* 2010; **8**: e1000412 [PMID: 20613859 DOI: 10.1371/journal.pbio.1000412]

213 **Amghar-Maach S**, Gay-Escoda C, Sánchez-Garcés MÁ. Regeneration of periodontal bone defects with dental pulp stem cells grafting: Systematic Review. *J Clin Exp Dent* 2019; **11**: e373-e381 [PMID: 31110618 DOI: 10.4317/jced.55574]

214 **Egusa H**, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry--Part II: Clinical applications. *J Prosthodont Res* 2012; **56**: 229-248 [PMID: 23137671 DOI: 10.1016/j.jpor.2012.10.001]

215 **Ahadian S**, Khademhosseini A. Smart scaffolds in tissue regeneration. *Regen Biomater* 2018; **5**: 125-128 [PMID: 29977595 DOI: 10.1093/rb/rby007]

216 **Khan R**, Khan MH. Use of collagen as a biomaterial: An update. *J Indian Soc Periodontol* 2013; **17**: 539-542 [PMID: 24174741 DOI: 10.4103/0972-124X.118333]

217 **Thavornyutikarn B**, Chantarapanich N, Sitthiseripratip K, Thouas GA, Chen Q. Bone tissue engineering scaffolding: computer-aided scaffolding techniques. *Prog Biomater* 2014; **3**: 61-102 [PMID: 26798575 DOI: 10.1007/s40204-014-0026-7]

218 **Ahmed TA**, Dare EV, Hincke M. Fibrin: a versatile scaffold for tissue engineering applications. *Tissue Eng Part B Rev* 2008; **14**: 199-215 [PMID: 18544016 DOI: 10.1089/ten.teb.2007.0435]

219 **Jockenhoevel S**, Zund G, Hoerstrup SP, Chalabi K, Sachweh JS, Demircan L, Messmer BJ, Turina M. Fibrin gel -- advantages of a new scaffold in cardiovascular tissue engineering. *Eur J Cardiothorac Surg* 2001; **19**: 424-430 [PMID: 11306307 DOI: 10.1016/s1010-7940(01)00624-8]

220 **Aper T**, Teebken OE, Steinhoff G, Haverich A. Use of a fibrin preparation in the engineering of a vascular graft model. *Eur J Vasc Endovasc Surg* 2004; **28**: 296-302 [PMID: 15288634 DOI: 10.1016/j.ejvs.2004.05.016]

221 **Chocholata P**, Kulda V, Babuska V. Fabrication of Scaffolds for Bone-Tissue Regeneration. *Materials (Basel)* 2019; **12** [PMID: 30769821 DOI: 10.3390/ma12040568]

222 **Lee KY**, Mooney DJ. Hydrogels for tissue engineering. *Chem Rev* 2001; **101**: 1869-1879 [PMID: 11710233 DOI: 10.1021/cr000108x]

223 **Boni R**, Ali A, Shavandi A, Clarkson AN. Current and novel polymeric biomaterials for neural tissue engineering. *J Biomed Sci* 2018; **25**: 90 [PMID: 30572957 DOI: 10.1186/s12929-018-0491-8]

224 **Zhai Q**, Dong Z, Wang W, Li B, Jin Y. Dental stem cell and dental tissue regeneration. *Front Med* 2019; **13**: 152-159 [PMID: 29971640 DOI: 10.1007/s11684-018-0628-x]

225 **Geckil H**, Xu F, Zhang X, Moon S, Demirci U. Engineering hydrogels as extracellular matrix mimics. *Nanomedicine (Lond)* 2010; **5**: 469-484 [PMID: 20394538 DOI: 10.2217/nnm.10.12]

226 **Huh D**, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. *Trends Cell Biol* 2011; **21**: 745-754 [PMID: 22033488 DOI: 10.1016/j.tcb.2011.09.005]

227 **Ito Y**, Tanabe Y, Sugawara K, Koshino M, Takahashi T, Tanigaki K, Aoki H, Chen M. Three-dimensional porous graphene networks expand graphene-based electronic device applications. *Phys Chem Chem Phys* 2018; **20**: 6024-6033 [PMID: 29300402 DOI: 10.1039/c7cp07667c]

228 **Nikolova MP**, Chavali MS. Recent advances in biomaterials for 3D scaffolds: A review. *Bioact Mater* 2019; **4**: 271-292 [PMID: 31709311 DOI: 10.1016/j.bioactmat.2019.10.005]

229 **Jensen C**, Teng Y. Is It Time to Start Transitioning From 2D to 3D Cell Culture? *Front Mol Biosci* 2020; **7**: 33 [PMID: 32211418 DOI: 10.3389/fmolb.2020.00033]

230 **Bachmann A**, Moll M, Gottwald E, Nies C, Zantl R, Wagner H, Burkhardt B, Sánchez JJ, Ladurner R, Thasler W, Damm G, Nussler AK. 3D Cultivation Techniques for Primary Human Hepatocytes. *Microarrays (Basel)* 2015; **4**: 64-83 [PMID: 27600213 DOI: 10.3390/microarrays4010064]

231 **Liu H**, Cheng Y, Chen J, Chang F, Wang J, Ding J, Chen X. Component effect of stem cell-loaded thermosensitive polypeptide hydrogels on cartilage repair. *Acta Biomater* 2018; **73**: 103-111 [PMID: 29684624 DOI: 10.1016/j.actbio.2018.04.035]

232 **Awadeen MA**, Al-Belasy FA, Ameen LE, Helal ME, Grawish ME. Early therapeutic effect of platelet-rich fibrin combined with allogeneic bone marrow-derived stem cells on rats' critical-sized mandibular defects. *World J Stem Cells* 2020; **12**: 55-69 [PMID: 32110275 DOI: 10.4252/wjsc.v12.i1.55]

233 **Seo BM**, Sonoyama W, Yamaza T, Coppe C, Kikuiri T, Akiyama K, Lee JS, Shi S. SHED repair critical-size calvarial defects in mice. *Oral Dis* 2008; **14**: 428-434 [PMID: 18938268 DOI: 10.1111/j.1601-0825.2007.01396.x]

234 **Rosales-Ibanez R**, Mateo NC, Rodriguez-Lorenzo L, Amairany RN, María FS. Potential Benefits from 3D Printing and Dental pulp stem cells in cleft palate treatments: an in vivo model study. *Biomed J Sci Tech Res* 2019; **16**: 11950-11953 [DOI: 10.26717/BJSTR.2019.16.002831]

235 **Sankar S**, Sharma CS, Rath SN. Enhanced osteodifferentiation of MSC spheroids on patterned electrospun fiber mats - An advanced 3D double strategy for bone tissue regeneration. *Mater Sci Eng C Mater Biol Appl* 2019; **94**: 703-712 [PMID: 30423757 DOI: 10.1016/j.msec.2018.10.025]

236 **Mahon E**, Salvati A, Baldelli Bombelli F, Lynch I, Dawson KA. Designing the nanoparticle-biomolecule interface for "targeting and therapeutic delivery". *J Control Release* 2012; **161**: 164-174 [PMID: 22516097 DOI: 10.1016/j.jconrel.2012.04.009]

237 **Ruangsawasdi N**, Zehnder M, Patcas R, Ghayor C, Siegenthaler B, Gjoksi B, Weber FE. Effects of Stem Cell Factor on Cell Homing During Functional Pulp Regeneration in Human Immature Teeth. *Tissue Eng Part A* 2017; **23**: 115-123 [PMID: 27762658 DOI: 10.1089/ten.tea.2016.0227]

238 **Bu NU**, Lee HS, Lee BN, Hwang YC, Kim SY, Chang SW, Choi KK, Kim DS, Jang JH. In Vitro Characterization of Dental Pulp Stem Cells Cultured in Two Microsphere-Forming Culture Plates. *J Clin Med* 2020; **9** [PMID: 31963371 DOI: 10.3390/jcm9010242]

239 **Xuan K**, Li B, Guo H, Sun W, Kou X, He X, Zhang Y, Sun J, Liu A, Liao L, Liu S, Liu W, Hu C, Shi S, Jin Y. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. *Sci Transl Med* 2018; **10** [PMID: 30135248 DOI: 10.1126/scitranslmed.aaf3227]

240 **De Luca M**, Aiuti A, Cossu G, Parmar M, Pellegrini G, Robey PG. Advances in stem cell research and therapeutic development. *Nat Cell Biol* 2019; **21**: 801-811 [PMID: 31209293 DOI: 10.1038/s41556-019-0344-z]

241 **Zakrzewski W**, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem Cell Res Ther* 2019; **10**: 68 [PMID: 30808416 DOI: 10.1186/s13287-019-1165-5]

242 **Yu Y**, Wu RX, Yin Y, Chen FM. Directing immunomodulation using biomaterials for endogenous regeneration. *J Mater Chem B* 2016; **4**: 569-584 [PMID: 32262939 DOI: 10.1039/c5tb02199e]

243 **Leach DG**, Young S, Hartgerink JD. Advances in immunotherapy delivery from implantable and injectable biomaterials. *Acta Biomater* 2019; **88**: 15-31 [PMID: 30771535 DOI: 10.1016/j.actbio.2019.02.016]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 922-937

DOI: 10.4252/wjsc.v12.i9.922

ISSN 1948-0210 (online)

REVIEW

Inflammatory niche: Mesenchymal stromal cell priming by soluble mediators

Aleksandra Jauković, Tamara Kukolj, Hristina Obradović, Ivana Okić-Đorđević, Slavko Mojsilović, Diana Bugarski

ORCID number: Aleksandra Jauković 0000-0003-2686-7481; Tamara Kukolj 0000-0002- 3174-4358; Hristina Obradović 0000-0003-4626-7184; Ivana Okić-Đorđević 0000-0003-3552-1546; Slavko Mojsilović 0000-0002-4399-6720; Diana Bugarski 0000-0002-2629-5471.

Author contributions: Jauković A conceived of, designed, and wrote the manuscript; Kukolj T contributed to the article's conception and writing; Obradović H performed the literature review and writing; Okić-Đorđević I contributed to the literature review and article writing; Mojsilović S performed the literature analysis and article editing; Bugarski D made critical revisions to the manuscript; all authors read and approved the final version of the manuscript.

Supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, No. 451-03-68/2020-14/200015.

Conflict-of-interest statement: The authors declare no conflict of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external

Aleksandra Jauković, Tamara Kukolj, Hristina Obradović, Ivana Okić-Đorđević, Slavko Mojsilović, Diana Bugarski, Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, Belgrade 11129, Serbia

Corresponding author: Aleksandra Jauković, PhD, Associate Professor, Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, Dr. Subotića 4, PO BOX 102, Belgrade 11129, Serbia. aleksandra@imi.bg.ac.rs

Abstract

Mesenchymal stromal/stem cells (MSCs) are adult stem cells of stromal origin that possess self-renewal capacity and the ability to differentiate into multiple mesodermal cell lineages. They play a critical role in tissue homeostasis and wound healing, as well as in regulating the inflammatory microenvironment through interactions with immune cells. Hence, MSCs have garnered great attention as promising candidates for tissue regeneration and cell therapy. Because the inflammatory niche plays a key role in triggering the reparative and immunomodulatory functions of MSCs, priming of MSCs with bioactive molecules has been proposed as a way to foster the therapeutic potential of these cells. In this paper, we review how soluble mediators of the inflammatory niche (cytokines and alarmins) influence the regenerative and immunomodulatory capacity of MSCs, highlighting the major advantages and concerns regarding the therapeutic potential of these inflammatory primed MSCs. The data summarized in this review may provide a significant starting point for future research on priming MSCs and establishing standardized methods for the application of preconditioned MSCs in cell therapy.

Key Words: Mesenchymal stem cells; Pro-inflammatory cytokines; Alarmins; Priming; Boosting the therapeutic potential

Core Tip: The inflammatory niche plays a key role in triggering the reparative and immunomodulatory functions of mesenchymal stromal/stem cells (MSCs). This paper summarizes the data on how soluble factors in the inflammatory microenvironment, including pro-inflammatory cytokines secreted by immune cells and alarmins released by

Manuscript source: Invited manuscript

Received: April 14, 2020
Peer-review started: April 14, 2020
First decision: April 29, 2020
Revised: May 13, 2020
Accepted: September 1, 2020
Article in press: September 1, 2020
Published online: September 26, 2020

P-Reviewer: Farahzadi R
S-Editor: Liu M
L-Editor: A
P-Editor: Xing YX

damaged cells, affect MSCs' ability to regenerate tissue and modulate the inflammatory response. We also analyze data from *in vitro* and *in vivo* studies, which highlight the influence of these factors on the therapeutic potential of MSCs, thus providing an important background for the development of preconditioning strategies that might improve the outcomes of MSC-based cell therapies.

Citation: Jauković A, Kukolj T, Obradović H, Okić-Đorđević I, Mojsilović S, Bugarski D. Inflammatory niche: Mesenchymal stromal cell priming by soluble mediators. *World J Stem Cells* 2020; 12(9): 922-937
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/922.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.922

INTRODUCTION

Inflammation is a localized immunologic response of the tissue elicited by harmful stimuli, including pathogens, irritants, or physical injury. This complex and protective response plays a fundamental role in the regulation of tissue repair, serving to eliminate harmful stimuli and begin the healing process[1]. In fact, inflammation is considered an important initial phase, followed by cell proliferation and extracellular matrix remodeling. These phases overlap over time and each of them represents a sequence of dynamic cellular and biochemical events, contributing to tissue regeneration through the collaboration of many cell types and their soluble products[2]. Immune cells, together with blood vessels, various stromal cells, extracellular matrix components, and a plethora of secreted soluble mediators, comprise an inflammatory microenvironment capable of inducing different responses of cells within injured tissue[3].

Soluble mediators released from injured/necrotic cells or damaged microvasculature lead to enhanced endothelium permeability and infiltration of neutrophils and macrophages. Among these mediators are endogenous danger signals, known as alarmins, which are rapidly released by dying necrotic cells upon tissue damage and play an important role in promoting and enhancing the immune response[4-6]. To date, the best-characterized alarmins are the interleukin (IL)-1 family of cytokines (IL-1α and IL-33), high-mobility group protein B1 (HMGB1), S100 proteins, and heat shock proteins (Hsps)[4,7]. In addition, during the inflammatory process, the phagocytosis of necrotic cells by resident/recruited neutrophils and macrophages induces the release of various inflammatory factors, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IL-1β, IL-17, and chemokines[8].

Aside from numerous soluble mediators, tissue injury mediated by immunity or infection involves an even greater number of various immune cells, including B cells, CD4+ and CD8+ T cells, and natural killer cells. While all immune cells play key roles in wound healing through the eradication of damaged tissue and invading pathogens, their excessive activation can actually aggravate the injury. Therefore, a comprehensive understanding of inflammatory niche elements might contribute to the development of novel therapeutic strategies for the treatment of inflammatory-associated diseases, as well as conditions of failed tissue regeneration.

One of the cellular compartments participating in the inflammatory niche represents mesenchymal stromal/stem cells (MSCs). MSCs are stem cells of stromal origin that possess self-renewal capacity and the ability to differentiate into three mesodermal cell lineages, including osteocytes, chondrocytes, and adipocytes[9]. Considering their critical role in tissue homeostasis and wound healing, MSCs have garnered great attention as promising candidates for tissue regeneration. Although first isolated from the bone marrow (BM)[10], MSCs may be obtained from various fetal and adult tissues, such as the umbilical cord (UC), peripheral blood, adipose tissue (AT), and skin and dental tissues[11,12]. According to the minimum criteria proposed by the International Society for Cellular Therapy, MSCs originating from different tissues are evidenced by the property of plastic adherence *in vitro* and expression of various non-specific surface molecules, such as cluster of differentiation (CD)105, CD90, CD73, and CD29, in parallel with trilineage differentiation potential[13]. However, the term MSC has recently been considered inappropriate, as it has become clear that MSCs from different tissues are not the same, especially with respect to their differentiation capacities[14,15], whereas their multipotent differentiation potential has not been

confirmed in *in vivo* conditions. Therefore, Caplan[17] recently proposed this term to stand for medicinal signaling cells[16], indicating the correlation of the therapeutic benefits of MSCs with the secretion of various bioactive molecules.

Many studies have demonstrated that MSCs contribute to tissue repair by accumulating at sites of tissue damage and inflammation, where together with resident MSCs, they exert reparative effects in two ways. One way is to replace damaged cells through differentiation, and another is related to the ability of MSCs to strongly influence the microenvironment by releasing bioactive factors and interacting with multiple cell types[18,19]. Indeed, poorly immunogenic MSCs that weakly express major histocompatibility complex (MHC) class I and lack MHC class II play a critical role in regulating the inflammatory microenvironment through interactions with immune cells such as T cells, B cells, natural killer cells, and dendritic cells[20-22]. As a result of these interactions, MSCs suppress lymphocyte proliferation and maturation of monocytes into dendritic cells, while stimulating the generation of regulatory T cells (Tregs) and M2 macrophages[23,24].

The major role in the crosstalk between MSCs and immune cells has been ascribed to soluble factors, which upon release by activated immune cells, significantly affect MSCs paracrine activity, conversely influencing immune cells. In particular, the immunosuppressive activity of MSCs has been related to the production of indoleamine-2,3-dioxygenase (IDO), nitric oxide (NO), prostaglandin-E2 (PGE2), IL-10, transforming growth factor (TGF)-β, and TNFα-stimulated gene-6[25].

Inflammatory priming of MSCs

It is believed that the inflammatory niche plays a key role in triggering the reparative function of MSCs. Namely, studies have demonstrated that the immunosuppressive potential of MSCs is not inherently expressed but requires priming by inflammatory factors, including IFN-γ, TNF-α, or IL-1β[18,26]. Moreover, it has been found that MSCs can polarize into MSC type 1 with a pro-inflammatory profile or MSC type 2 with an immunosuppressive phenotype, depending on the inflammatory condition[23,27]. On the other hand, the inflammatory microenvironment influences the differentiation potential of resident and recruited MSCs, significantly impairing their regenerative capacity. In addition, several studies have shown that MSCs of different tissue origin may exert differential sensitivity to inflammatory conditions[28,29]. These data point to the critical importance of interactions between MSCs and inflammatory factors for the outcome of wound healing.

Indeed, the regenerative potential of transplanted MSCs is affected by inflammatory conditions[30], indicating the strong influence of the recipient's inflammatory status on the efficacy of MSC-based therapies. Interestingly, to reduce the heterogeneity of MSCs and generate more homogenous therapeutic products, MSC priming with cytokines has been proposed[31]. The application of bioactive molecules in this context has been considered a supplemental molecular signal used to foster the therapeutic potential of MSCs and contribute to establishing a favorable microenvironment for tissue repair.

The complex cytokine network has been considered a critical part of the inflammatory microenvironment, where the pleiotropic properties of pro-inflammatory cytokines play a decisive role in the healing process and tissue regeneration. TNF-α, IFN-γ, IL-1, IL-17, and IL-6 are the most common inflammatory cytokines in this complex network[32]. Moreover, another significant constituent of the inflammatory niche considers alarmins, such as IL-1α and IL-33, HMGB1, S100 proteins, and Hsps, which can promote the immune response, thereby supporting host defense and tissue repair[7]. Here, we review how these soluble mediators of the inflammatory niche influence the regenerative and immunomodulatory potential of MSCs, highlighting the major advantages and concerns regarding the therapeutic potential of inflammatory primed MSCs.

MSCs priming with pro-inflammatory cytokines

IFN-γ priming: One of the most studied inflammatory priming mediators is IFN-γ, which is a key player in cellular immunity regulation, heightening immune responses in infection and cancer. However, very little is known about the effects of IFN-γ on the regenerative potential of MSCs. Namely, Croitoru-Lamoury *et al*[33] demonstrated that IFN-γ exerts significant antiproliferative effects on mouse and human BM-MSCs (Figure 1) through IDO induction and production of downstream tryptophan metabolites, such as kynurenine, which can potentiate the suppressive effects on cell proliferation in an autocrine manner. This was the first study that linked IFN-γ-induced IDO with the control of MSC differentiation potential, as evidenced by the inhibition of both osteogenic and adipogenic marker expression in IFN-γ-primed BM-

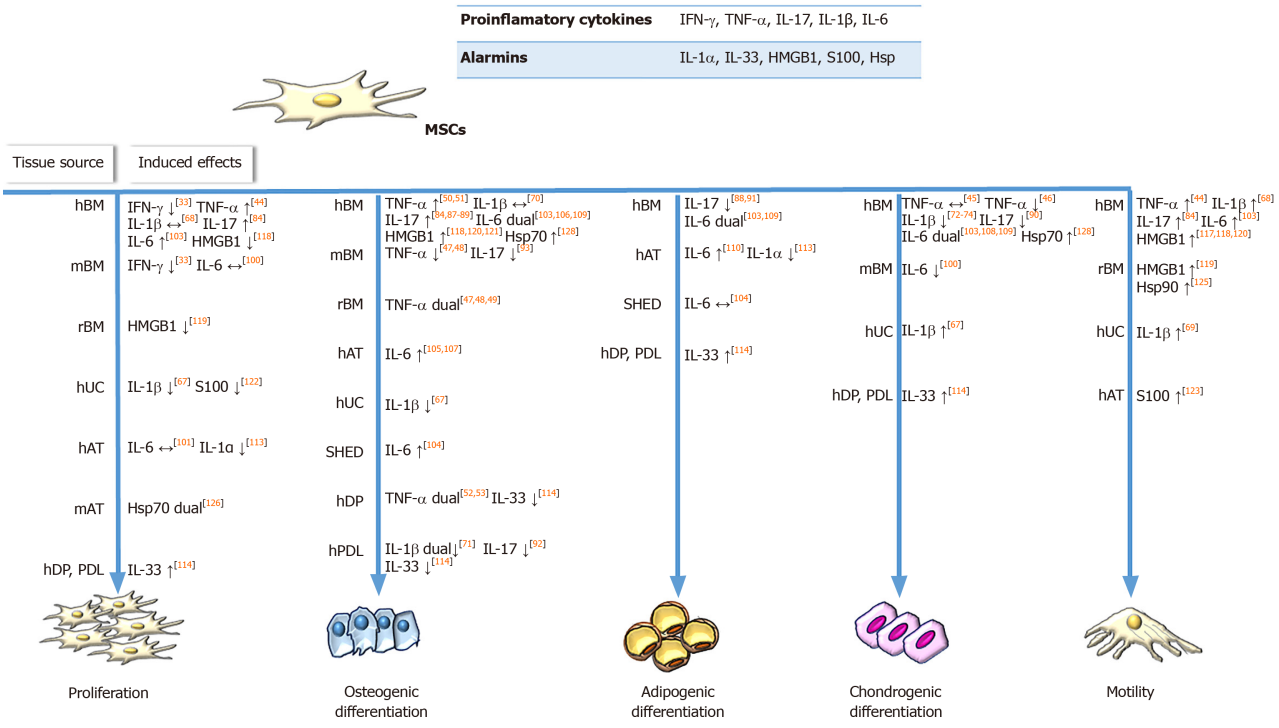


Figure 1 Influence of inflammatory priming on regenerative features of mesenchymal stromal/stem cells under *in vitro* conditions. Soluble factors in the inflammatory microenvironment, including pro-inflammatory cytokines secreted by immune cells and alarmins released by damaged cells, influence mesenchymal stromal/stem cells' (MSCs) ability to regenerate tissue by affecting their proliferation, migration and differentiation potential (osteogenic, chondrogenic, adipogenic). When applied *in vitro*, indicated inflammatory factors differently affect the regenerative properties of MSCs depending on the species or tissue origin of MSCs. Symbols: ↑ and ↓ represent stimulatory or inhibitory activity of priming factor, respectively; ↔ indicates no effects of priming factor. The term 'dual' indicates the data where priming factor dually affected MSCs function depending on applied concentration or MSCs donor age. The numbers in square brackets indicate the references. IFN-γ: Interferon gamma; TNF-α: Tumor Necrosis Factor alpha; IL: Interleukin; HMGB1: High mobility group box 1; Hsp: Heat shock proteins; MSCs: Mesenchymal stromal/stem cells; h: Human; WJ: Warthon jelly; BM: Bone marrow; r: Rat; UC: Umbilical cord; AT: Adipose tissue; DP: Dental pulp; PDL: Periodontal ligament; SHED: Exfoliated deciduous teeth.

MSCs upon induction of differentiation.

Numerous studies have demonstrated that the priming of MSCs with IFN-γ can enhance the immunosuppressive property of these cells by IDO stimulation[34]. In addition, exposure to IFN-γ has been shown to induce UC Wharton's jelly (WJ)-MSCs to express other immunosuppressive factors, such as human leukocyte antigen G5, as well as C-X-C motif chemokine ligand (CXCL)9, CXCL10, and CXCL11[35]. Even more, IFN-γ-primed WJ-MSCs secrete more IL-6 and IL-10 upon co-culture with activated lymphocytes, increasing the percentage of Tregs, while decreasing the frequency of T helper (Th)17 cells (Table 1).

Another mechanism underlying the inhibitory effects of IFN-γ-primed BM-MSCs on T-cell effector functions is the upregulation of programmed death-ligand 1 (referred to as PD-L1)[36] (Table 1). However, in the context of potential MSCs' therapeutic use, the findings from several studies which have demonstrated that MSCs priming with IFN-γ led to upregulation of class I and class II human leukocyte antigen (referred to as HLA) molecules should be considered, as they indicated more immunogenic MSCs profile that is linked to a higher susceptibility to host immune cells recognition[37,38]. Noteworthy, a recent study found that priming with IFN-γ did not increase HLA class II expression on senescent BM-MSCs but upregulated this molecule on early passage BM-MSCs, suggesting that IFN-γ priming effects can also be influenced by cell aging[39].

Interestingly, *in vivo* experiments have demonstrated that IFN-γ affects the therapeutic efficacy of MSCs in a dose-dependent manner. Namely, when low concentrations of IFN-γ were used for murine BM-MSCs priming, the therapeutic effects of MSCs on experimental autoimmune encephalomyelitis in mice were completely inhibited, as demonstrated by the increased secretion of pro-inflammatory chemokine CCL2 and higher expression of MHC molecules class I and II[38]. BM-MSCs primed with higher concentrations of IFN-γ prior to use in murine models of colitis reportedly increase MSC therapeutic efficacy, as demonstrated by the significantly attenuated development and/or reduced symptoms of colitis[40]. These effects were

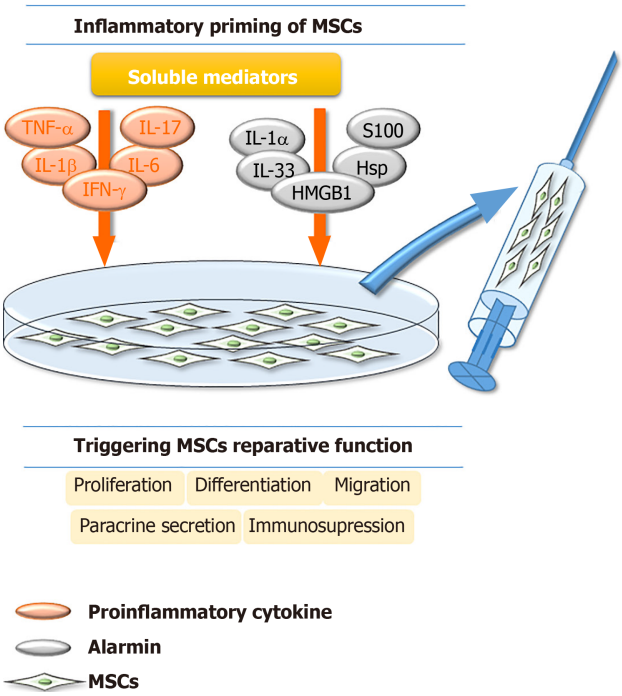
Table 1 Influence of inflammatory priming on immunomodulatory features of mesenchymal stromal/stem cells under *in vitro* conditions

	Priming factor	MSCs source	Immunomodulatory effects of inflammatory priming	Suggested mechanism	Ref.
Pro-inflammatory cytokines	IFN-γ	hWJ-MSCs	↓ Th1 and Th17 cells proliferation	↑ CXCL9, CXCL10, CXCL11, ICAM-1, VCAM-1, IDO1 and HLA-G5 gene expression	Wang *et al*[35], 2016
			↑ Th2 and Tregs (CD4+, CD25+, CD127dim/− cells) percentage	↓ IFNγ, TNFα but ↑ IL-10 and IL-6 secretion in co-culture with PB-MNCs	
		hBM-MSCs	↓ T-cell effector functions and Th1 cytokine (IFN-γ, TNF-α, and IL-2) production	↑ B7H1 and B7DC (ligands for PD1) expression	Chinnadurai *et al*[36], 2014
	IFN-γ and TNF-α	hBM-MSCs	↓ T cell proliferation	↑ IDO activity	François *et al*[55], 2012
			↑ Monocyte differentiation into M2 (IL-10-secreting CD206+) immunosuppressive macrophages		
			↓ CD3/CD28-induced T-cell proliferation	↓ Potency to trigger increased IFN-γ and IL-2 synthesis by activated T cells	Cuerquis *et al*[59], 2014
	TNF-α/IFN-γ and IL-β	rBM-MSCs	↓ Proliferation of syngeneic lymphocytes	↑ NO production	Murphy *et al*[80], 2019
	IL-17	hBM-MSCs	↓ PHA-stimulated T-cell proliferation	↑ IL-6 gene expression	Sivanathan *et al*[94], 2015
			↓ Expression of CD25 on CD4+ effector T cells	Treg-mediated IL-2 deprivation	
			↓ T cell effector function and Th1 cytokines (IFN-γ, TNF-α, IL-2) secretion		
			↑ Formation of Tregs (CD4+ CD25 high CD127 low FoxP3+)	Cell-contacts and ↑ PGE2 and TGF-β expression	
	IFN-γ, TNF-α and IL-17	mBM-MSC	↓ T-cell proliferation	↑ iNOS expression	Han *et al*[95], 2014
	IFN-γ, TNF-α and IL-6	hAT-MSC	↓ Proliferation of PHA or MLR activated PB-MNCs	↑ IDO expression	Crop *et al*[111], 2010
Alarmins	IL-1α	hBM-MSCs	↓ IL-6, TNF-α and ↑ IL-10 secretion in LPS-activated mouse microglial BV2 cells	↑ G-CSF secretion	Redondo-Castro *et al*[78], 2017
	HMGB1	hBM-MSC	Unaffected inhibition of Con A-induced lymphocyte proliferation	/	Meng *et al*[118], 2008

Symbols: ↑ and ↓ represent stimulatory or inhibitory activity of priming factor, respectively. IFN-γ: Interferon gamma; TNF-α: Tumor Necrosis Factor alpha; IL: Interleukin; HMGB1: High mobility group box 1; MSCs: Mesenchymal stromal/stem cells; h: Human; WJ: Warthon jelly's; BM: Bone marrow; r:rat; AT: Adipose tissue; Th: T helper; PHA: Phytohaemagglutinin; MLR: Mixed lymphocyte reaction; LPS: Lipopolysaccharide; Con A: Concanavalin A; CXCL: C-X-C motif chemokine ligand; ICAM-1: Intercellular adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule 1; IDO: Indoleamine-pyrrole 2,3-dioxygenase; HLA-G5: Human leukocyte antigen-G molecules; PB-MNCs: Peripheral blood mononuclear cells; B7H1: B7 homolog 1; PD1: Programmed cell death protein 1; NO: Nitric oxide; PGE2: Prostaglandin E□; TGF-β: Transforming growth factor beta; iNOS: Inducible NO synthase; G-CSF: Granulocyte - colony stimulating factor.

found to be related to the increased migration of IFN-γ-primed MSCs along with their enhanced capacity to inhibit Th1 inflammatory responses, all of which contributed to decreased mucosal damage. In addition, Polchert *et al*[41] showed that IFN-γ-primed mouse BM-MSCs suppress graft *vs* host disease more efficiently than non-primed MSCs depending on the magnitude of IFN-γ exposure (Figure 2). By contrast, a recent study showed that infusion of thawed IFN-γ-primed human MSCs failed to improve retinal damage in a murine model of retinal disease[42].

TNF-α priming: TNF-α is a pleiotropic cytokine involved in systemic inflammation, which also affects the metabolism, growth, and differentiation potential of various cell types. Regarding the regenerative potential of TNF-α-treated MSCs, it has been



Improved therapeutic efficiency		
Priming factor/MSCs	**Model**	**Reference**
TNF-α/IL-β- primed rat BM-MSCs	Rat cornea transplant model in rats	Murphy *et al*[82], 2019
IL-1β- primed human UC-MSCs	DSS-induced colitis in mice	Fan *et al*[83], 2012; Yu *et al*[84], 2019
IL-17- primed mouse BM-MSCs	Skin transplantation model in mice	Ma *et al*[83], 2018
IFN-γ- primed murine BM-MSCs	Murine models of colitis	Duijvestein *et al*[41], 2011
IFN-γ- primed mouse BM-MSCs	Graft versus host disease model in mice	Polchert *et al*[42], 2008
TNFα- primed mouse BM-MSCs	Experimental allergic conjunctivitis model in mice	Su *et al*[61], 2015
TNF-α- primed human BM-MSCs	Model of mouse myocardial infarction	Lee *et al*[63], 2009
S100A8/A9- primed human AT-MSCs	Excisional wound healing model in mice	Basu *et al*[127], 2018

Figure 2 Improved *in vivo* therapeutic potential of inflammatory primed mesenchymal stromal/stem cells. The inflammatory niche mediators, including proinflammatory cytokines and alarmins, play a key role in triggering the reparative functions of mesenchymal stromal/stem cells, influencing their paracrine secretion, proliferation, differentiation, migration and immunomodulatory potential. When applied *in vivo*, the improved therapeutic efficiency of inflammatory primed MSCs has been detected in various experimental models. The numbers in square brackets indicate the references. TNF-α: Tumor Necrosis Factor alpha; IL: Interleukin; IFN-γ: Interferon gamma; HMGB1: High mobility group box 1; Hsp: Heat shock proteins; BM: Bone marrow; MSCs: Mesenchymal stromal/stem cells; UC: Umbilical cord; AT: Adipose tissue; DSS: Dextran sulfate sodium.

determined that this cytokine promotes the proliferation of human synovial MSCs and BM-MSCs[43,44], while not affecting their clonogenic potential. Moreover, the involvement of the nuclear factor-kappa B (NF-κB) signaling pathway is implicated in TNF-α-stimulated invasion and proliferation of BM-MSCs[44] (Figure 1). Furthermore, the preconditioning of MSCs with TNF-α differentially regulates their chondrogenic differentiation depending on the tissue source and donor age (Figure 1). Namely, while TNF-α does not affect the chondrogenic capacity of human synovial MSCs and BM-MSCs[43,45], Wheiling *et al*[46] revealed that it inhibited the chondrogenesis of human BM-MSCs isolated from elderly donors. In addition, several studies have indicated that TNF-α alters the osteogenic differentiation of MSCs in dose-, tissue source-, and species-specific manners (Figure 1). Regarding rodent MSCs, several studies have found that TNF-α inhibits the osteogenic differentiation of MSCs[47,48], while continuous delivery of TNF-α stimulates the osteogenic differentiation of rat BM-MSCs seeded onto three-dimensional biodegradable scaffolds[49]. The enhanced osteogenic potential has also been evidenced for human BM-MSCs treated with TNF-α[50,51]. However, it dually affects dental pulp stem cells, promoting their osteogenic differentiation through the Wnt/β-catenin signaling pathway, while suppressing the osteogenesis of these cells at high concentrations[52,53].

In the context of the stronger immunomodulatory capacity of TNF-α-primed MSCs, studies have demonstrated increased secretion of the immunosuppressive molecules PGE2 and IDO, chemokine IL-8, CXCL5, CXCL6, and certain growth factors such as hepatocyte growth factor, insulin-like growth factor 1, and vascular endothelial growth factor (VEGF)[54-57] (Table 1). Moreover, it has been revealed that TNF-α priming of rat UC-MSCs suppresses the inflammatory milieu by increasing TGF-β and IL-10 expression[58]. Because the immunosuppressive effects of TNF-α are less pronounced compared to IFN-γ priming in WJ-MSCs[28], several studies have investigated the combined effects of TNF-α and IFN-γ on the immunomodulatory potential of MSCs. Indeed, when human BM-MSCs were subjected to combined pretreatment with TNF-α plus IFN-γ, more effective inhibition of CD3/CD28-induced T-cell proliferation was observed compared to non-primed MSCs[59] (Table 1). Also, combined TNF-α and IFN-γ preconditioning was shown to increase IDO activity in BM-MSCs, resulting in monocyte differentiation into M2 immunosuppressive

macrophages, which further inhibited T-cell proliferation *via* IL-10 secretion[55].

Regarding the beneficial effects of MSC preconditioning with TNF-α, an *in vivo* study performed by Su *et al*[60] showed that the culture medium of TNFα-primed mouse BM-MSCs reduced experimental allergic conjunctivitis through multiple cyclooxygenase-2-dependent antiallergic mechanisms. In addition, the culture medium of TNF-primed human AT-MSCs has been shown to accelerate cutaneous wound closure, angiogenesis, and infiltration of immune cells in a rat excisional wound model *via* IL-6 and IL-8 secretion[61]. Also, in a model of mouse myocardial infarction, improved cardiac function related to decreased inflammatory responses and reduced infarct size has been documented in animals receiving TNF-α-primed human BM-MSCs[62]. By contrast, TNF priming reverses the immunosuppressive effects of mouse MSCs on T-cell proliferation, resulting in the failure of MSC treatment of murine collagen-induced arthritis[63].

IL-1β priming: IL-1β is the key mediator of inflammatory responses, which contributes to the host-defense response facilitating activation of innate immune cells[64]. Regarding the effects of IL-1β priming on MSCs proliferation and differentiation, heterogeneous results have been reported (Figure 1). Namely, while IL-1β preconditioning increases equine AT-MSCs and human synovium-derived MSCs proliferation[65,66], exposure of UC-MSCs to IL-1β results in suppressed proliferation[67]. Moreover, functional analyses of human BM-MSCs have revealed that treatment with IL-1β does not affect the proliferation of these cells, and promotes their migration and adhesion to extracellular matrix components[68]. Also, a study by Guo *et al*[69] demonstrated that IL-1β promoted UC-MSCs transendothelial migration through the CXCR3-CXCL9 axis, indicating the beneficial effects on MSC homing to target sites.

Many studies have described the modulatory effects of IL-1β stimulation on the osteogenic differentiation of MSCs, with conflicting results depending on the MSC tissue origin as well as IL-1β concentration (Figure 1). Sonomoto *et al*[70] demonstrated the ability of IL-1β to induce the osteogenic differentiation of human BM-MSCs *via* the Wnt pathway. Increased osteogenesis has also been found for equine AT-MSCs and human UC-MSCs treated with IL-1β[65,67]. On the other hand, depending on the concentration, IL-1β exerts dual effects on the osteogenesis of periodontal ligament-MSCs, since low doses of IL-1β promote osteogenesis by activating the bone morphogenetic protein (referred to as BMP)/Smad signaling pathway, while higher doses of the cytokine impede osteogenesis[71]. IL-1β treatment also inhibits the chondrogenesis of MSCs from the femoral intramedullary canal in a dose-dependent manner *via* NF-κB activation[46]. In accordance with these findings, decreased chondrogenic differentiation has been reported for BM-MSCs and synovial fluid-MSCs treated with IL-1β[72-75]. However, Hingert *et al*[76] showed that pretreatment of BM-MSCs with IL-1β followed by BMP-3 stimulation in a three-dimensional *in vitro* hydrogel model resulted in high proteoglycan accumulation and SRY-box transcription factor 9 expression, suggesting that IL-1β may be the causative factor.

Several studies have indicated changes in the secretory profile of MSCs primed with IL-1β, as well as the significance of IL-1β priming in combination with other factors. Regarding gingival-MSCs, IL-1β preconditioning induces the expression of TGF-β and matrix metalloproteinase agonists[77], while in human BM-MSCs, IL-1β increases granulocyte colony-stimulating factor (referred to as G-CSF)[78], IL-6, VEGF, CXCL1, and CCL2 chemokines[79]. The immunosuppressive activities of rat BM-MSCs were shown to be significantly promoted after preconditioning with TNF-α or IFN-γ in combination with IL-β, as the decreased proliferation of syngeneic lymphocytes *in vitro* was demonstrated[80] (Table 1). These effects were confirmed by *in vivo* experiments in a rat cornea transplant model, where after transplantation of syngeneic MSCs primed with TNF-α/IL-β enhanced graft survival (up to 70%) was observed compared to unprimed MSCs (up to 50%). In addition, the increased number of Tregs and reduced expression of pro-inflammatory cytokines in the draining lymph node of these animals were found, whereas there was an increased number of regulatory monocyte/macrophage cells and Tregs in the lungs and spleen[80].

Administration of IL-β-primed human UC-MSCs in mice with dextran sulfate sodium-induced colitis also increases the number of Tregs and Th2 cells, while reducing Th1 and Th17 cells in the spleen and mesenteric lymph nodes[81]. A recent study by Yu *et al*[82] emphasized the role of PGE2 and IDO induction in the observed immunosuppressive effects of umbilical cord blood-MSCs primed with IL-1β and IFN-γ in the same disease model. Moreover, another recent study showed that the culture medium of IL-1β-primed AT-MSCs increased the phagocytic capacity of neutrophils, which may contribute to inflammation resolution, removal of tissue debris, and support of tissue repair in joint pathology[83]. Overall, the results indicating the

immunosuppressive phenotype of IL-1β-primed MSCs strongly suggest that this cytokine might promote the therapeutic efficacy of MSCs in disorders related to an exaggerated immune response (Figure 2).

IL-17 priming: IL-17 is another pro-inflammatory cytokine that plays a pivotal role in linking the immune and hematopoietic systems, while also contributing to the pathogenesis of numerous autoimmune and inflammatory diseases. However, the effects that this cytokine exerts on MSCs are still not fully understood. To date, it has been shown that IL-17 stimulates the proliferation of mouse and human BM-MSCs, as well as the migration of human BM-MSCs and trans-endothelial migration of peripheral blood MSCs[84-86] (Figure 1). Regarding the differentiation potential, published results have shown that IL-17 priming enhances osteogenic[84,87-89], but inhibits chondrogenic[90] and adipogenic[88,91] differentiation in human BM-MSCs. Moreover, IL-17 can decrease the osteogenic differentiation of periodontal ligament-MSCs through extracellular signal-regulated protein kinases 1 and 2 (referred to as ERK1/2), and c-Jun N-terminal kinase mitogen-activated protein kinases[92]. Research related to the effects of IL-17 on the differentiation potential of mouse BM-MSCs has led to conflicting results, as one study found that IL-17 did not affect the differentiation potential of MSCs towards osteoblasts[85], whereas another showed suppressed osteogenic differentiation of these cells mediated by IκB kinase and NF-κB[93].

Regarding the immunomodulatory activity of MSCs, IL-17 priming enhances the immunosuppressive features of MSCs. While IL-17 has no impact on MSC markers and the low immunogenic phenotype of human BM-MSCs, IL-17-primed MSCs suppress T-cell proliferation and inhibit CD25 expression and expression of Th1 cytokines, including IFN-γ, TNF-α, and IL-2[94]. Moreover, a study showed that mouse MSCs pretreated with IFN-γ and TNF-α in combination with IL-17 significantly reduced T-cell proliferation *via* the inducible nitric oxide synthase (referred to as iNOS) pathway[95] (Table 1). The same study confirmed the immunosuppressive activity of BM-MSCs primed with IL-17 *in vivo* in a mouse model of concanavalin A-induced liver injury. However, another study showed that IL-17 significantly reduced the suppressive capacity of olfactory ecto-MSCs on CD4+ T cells, mainly through the downregulation of suppressive factors (PD-L1, iNOS, IL-10, and TGF-β)[96]. The positive effect of IL-17 on the immunomodulatory features of MSCs has been confirmed in *in vivo* studies with different animal models. Namely, in a study in which mouse BM-MSCs were treated with IL-17 prior to their use in ischemia-reperfusion acute kidney injury, a significant decrease in IL-6, TNF-α, and IFN-γ levels and higher spleen and kidney Treg levels were shown compared to mice that received non-primed MSCs[97]. In another work, IL-17-primed mouse BM-MSCs used in a skin transplantation model were found to increase the Treg subpopulation as well as IL-10 and TGF-β levels, significantly prolonging graft survival[98].

IL-6 priming: Pleiotropic effects on immune regulation, hematopoiesis, and tissue regeneration are exerted by another inflammatory cytokine, IL-6[99]. Preconditioning MSCs with IL-6 has been shown to influence their behaviors in different manners depending on the tissue origin of the MSCs (Figure 1). Namely, a few studies have reported conflicting data showing that IL-6 has no effect on the proliferation of human AT-MSCs and mouse BM-MSCs[100,101], whereas in other studies, IL-6 increased the proliferation of human placenta-derived MSCs and BM-MSCs[102,103]. Moreover, the stimulating effect of IL-6 on BM-MSCs growth and *in vitro* wound healing is mediated by ERK1/2 activation[103]. It has also been reported that IL-6 differentially influences stem cell differentiation (Figure 1). Priming with IL-6 under osteogenic induction conditions has been shown to enhance mineralization and alkaline phosphatase expression in human BM-MSCs, AT-MSCs, and stem cells from human exfoliated deciduous teeth (called SHEDs)[104-107]. However, studies using lower concentrations of IL-6 have shown no effect on osteogenic differentiation potency of human BM-MSCs[103]. Although IL-6 inhibits the chondrogenic differentiation of human BM-MSCs when added during differentiation induction[103], concomitant supplementation with IL-6 and soluble IL-6 receptor contributes to the enhanced chondrogenesis of this type of MSC[108]. Treatment with IL-6 during or prior to adipogenic differentiation induction reduces the adipogenesis capacity of human BM-MSCs[103], while other studies have reported no or positive effects of IL-6 on the adipogenic ability of human BM-MSCs, AT-MSCs, and SHEDs[104,109,110].

Even less is known about the immunomodulatory potential of IL-6-preconditioned MSCs. In this context, few studies have analyzed IL-6 effects in combination with other pro-inflammatory cytokines. Namely, altered immunological status has been reported

for AT-MSCs primed with a combination of IFN-γ, TNF-α, and IL-6 (Table 1), as shown by the upregulated expression of HLA class I and class II, as well as CD40[111], indicating a potentially more immunogenic phenotype. In addition, although the same priming conditions have no effect on AT-MSCs differentiation capacity, they enhance their immunosuppressive activity mainly through increased IDO expression. Another study demonstrated that human AT-MSCs and BM-MSCs primed with another combination of pro-inflammatory cytokines, IL-1, IL-6, and IL-23, exerted increased differentiation potential towards osteogenic and adipogenic lineages, while their morphology, immunophenotype (except upregulated CD45), and costimulatory molecule expression were similar to non-primed cells. Also, primed MSCs showed increased TGF-β and decreased IL-4 production, while their suppressive effect on T-cell proliferation was comparable to controls[112] Although these findings suggest that priming with IL-6 might promote the therapeutic efficacy of MSCs in the treatment of various inflammatory and autoimmune disorders, no study investigating the *in vivo* therapeutic potential of MSCs preconditioned with IL-6 (alone or combined with other cytokines) has been performed to date.

MSCs priming with alarmins

Alarmins are constitutively expressed inside cells and exert various functions under physiological conditions. Upon tissue damage induced by pathogens or physical/chemical injuries, dying necrotic cells passively and rapidly release alarmins outside of cells to promote the immune response and support tissue repair[4,5,6]. In addition to these activities, alarmins are involved in many other processes, such as cellular homeostasis, wound healing, and tumor development[6,7]. The best-characterized alarmins, such as IL-1α, IL-33, HMGB1, S100 proteins, and Hsps, will be discussed here in the context of their effects on MSC biology and potential role in tissue repair.

IL-1α and IL-33 are both dual-function cytokines that are localized in the nucleus under homeostatic conditions, where they function as transcription factors. Data on their extracellular effects on MSCs are very elusive. It has been demonstrated that IL-1α stimulates the expression of trophic factor G-CSF *via* IL-1 receptor type 1 signaling in human BM-MSCs. In addition, the conditioned medium of IL-1α-primed BM-MSCs was shown to inhibit the secretion of inflammatory and apoptotic markers in lipopolysaccharide-activated mouse microglial BV2 cells and increase secretion of the anti-inflammatory IL-10 cytokine[78], suggesting that MSCs priming with IL-1α favors their immunosuppressive activities (Table 1). Another recent study showed that IL-1α decreased the proliferative and adipogenic differentiation capacity of AT-MSCs, whereby adipogenesis was inhibited predominantly during the early phase of differentiation *via* NF-κB and ERK1/2 pathways with subsequent stimulation of pro-inflammatory cytokines, such as IL-8, IL-6, CCL2, and IL-1β, during adipogenic differentiation of AT-MSCs[113] (Figure 1). Since the effects of IL-1α are conspicuous at the beginning of the differentiation process, it is important to further examine IL-1α priming in the context of MSC differentiation. It was recently demonstrated that without changing MSC marker expression[114,115], IL-33 treatment has the potential to modify the regenerative[114] and immunomodulatory characteristics of MSCs[115]. Our recent study demonstrated that IL-33 treatment reduced periodontal ligament-MSCs and dental pulp MSCs osteogenesis but supported their proliferation, clonogenicity, and stemness (Figure 1). Both MSC types primed with IL-33 maintained their differentiation capacity, while increased alkaline phosphatase activity was also observed, indicating that IL-33 may contribute to the preservation of the dental stem cell pool[114]. Research conducted by Terraza *et al*[115] demonstrated that IL-33 with IFN-γ stimulated the high expression of IL-6, TGF-β, and iNOS in mouse BM-MSCs. Despite the scarce data on IL-1α and IL-33 priming of MSCs, overall data indicate that preconditioning with these molecules should be additionally explored as an MSC priming strategy.

Another nuclear alarmin is HMGB1, a non-histone DNA-binding protein involved in the maintenance of the chromatin structure and gene expression regulation[116]. The knowledge on the effects of released HMGB1 on MSCs functions is still contradictory, as its stimulatory[117] as well as inhibitory[118,119] actions on MSCs proliferation have been reported. The promoted migratory capacity of MSCs primed with HMGB1 has also been demonstrated[117-119], indicating its beneficial effects for MSC functional adjustment in therapeutic use. In the presence of HMGB1, the osteogenic differentiation of MSCs is also induced[118,120,121] (Figure 1). Moreover, HMGB1 stimulates the secretion of various cytokines by MSCs including macrophage CSF, eotaxin-3, epidermal growth factor receptor, VEGF, angiopoietin-2, CCL-5, urokinase plasminogen activator receptor, and macrophage migration inhibitory factor, which

may be associated with the induced osteogenic differentiation under HMGB1 influence[121]. Furthermore, in rat BM-MSCs, along with promoted MSC migration, HMGB1 stimulates VEGF-induced differentiation to endothelial cells but decreases their proliferation and platelet-derived growth factor-induced differentiation to smooth muscle cells[119]. These findings indicate that HMGB1 priming could be a significant factor in tissue engineering for MSC-guided differentiation. Regarding immunomodulatory functions, it has been reported that HMGB1 priming has no effect on BM-MSCs ability to inhibit the proliferation of concanavalin A-stimulated lymphocytes *in vitro*[118] (Table 1), but additional research is needed to confirm these functions.

Unlike IL-1α, IL-33 and HMGB1 alarmins, S100 proteins, and Hsps are located in the cytoplasm during homeostasis[4]. The effect of extracellular S100A6 has been investigated on MSCs derived from WJ of the UC, and the results have shown the ability of this molecule to increase cellular adhesion and reduce their proliferation capacity by interacting with integrin β1[122] (Figure 1). Regardless, pretreatment of human AT-MSCs with S100A8/A9 and their subsequent application to wounds induced in C57BL/6 mice significantly improve wound healing due to transcriptome expression profile changes related to the enhanced protective MSCs phenotype[123]. Regarding the Hsp protein family, it has been demonstrated that Hsp90 increases viability and protects rat BM-MSCs against apoptosis, simultaneously increasing the paracrine effect of MSCs[124]. Another study showed that Hsp90α promotes rat MSCs migration, possibly mediated by the increased secretion of MMPs, SDF-1/CXCR4, and vascular cell adhesion protein 1[125] (Figure 1). Interestingly, the dual effects of Hsp70 have been demonstrated depending on the age of the MSCs. Namely, in a study by Andreeva *et al*[126], Hsp70 increased the growth of aged but not young mouse AT-MSCs (Figure 1), suggesting the potential beneficial effects of Hsp70 priming. Moreover, the important role of Hsp70 in the osteogenesis of human MSCs is demonstrated by increased alkaline phosphatase activity and MSC mineralization[127,128].

CONCLUSION

As MSCs are crucial cellular components for tissue repair, it is essential to understand how the inflammatory microenvironment modulates the functionality of these cells. The beneficial effects of MSCs have been demonstrated, but due to the large heterogeneity detected within MSC populations, the success of their application in clinical trials has been limited. Moreover, it is believed that the inflammatory niche is indispensable for triggering MSC activity in an appropriate manner. Therefore, preconditioning methods have been applied to enhance and/or adjust MSCs functionality, including their regenerative and immunomodulatory status. To date, studies of the MSCs response to soluble factors featuring the inflammatory niche have been mostly focused on the effects provoked during their presence, pointing to the necessity of further exploring the durability of these changes. In this work, we collected data on the therapeutic potential of MSCs treated with pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1β, IL-17 and IL-6) and alarmins (IL-1α, IL-33, HMGB1, S100 proteins, and Hsps) that are predominantly released at the site of the damaged tissue.

The reviewed data strongly indicate that all aforementioned factors possess the ability to modify the regenerative and immunomodulatory activities of MSCs, and the effects of these factors depend on the MSC tissue and species origin, as well as on donor age and cellular aging (senescence) status. In addition, different effects have been reported depending on the priming factor concentration and their selected combinations, as well as on the disease model, indicating that all of these aspects together should be carefully considered in relation to specific application requirements. Importantly, the effects of primed MSCs have been demonstrated in various animal wound and disease models, suggesting the validity of priming approaches for MSC therapy. Indeed, priming MSCs with certain inflammatory factors, such as TNF-α, IL-β, IFN-γ or S100A8/A9, contribute to the suppression of graft *vs* host disease and colitis, as well as to improved corneal and skin graft survival, mediated by their dominant immunosuppressive activity (Figure 2). Together, the data summarized in this paper provide a significant starting point for future research on priming MSCs and set future directions for establishing standardized methods for the application of preconditioned MSCs in cell therapy.

REFERENCES

1 **Hall SW**, Cooke A. Autoimmunity and inflammation: murine models and translational studies. *Mamm Genome* 2011; **22**: 377-389 [PMID: 21688192 DOI: 10.1007/s00335-011-9338-2]
2 **Reinke JM**, Sorg H. Wound repair and regeneration. *Eur Surg Res* 2012; **49**: 35-43 [PMID: 22797712 DOI: 10.1159/000339613]
3 **Julier Z**, Park AJ, Briquez PS, Martino MM. Promoting tissue regeneration by modulating the immune system. *Acta Biomater* 2017; **53**: 13-28 [PMID: 28119112 DOI: 10.1016/j.actbio.2017.01.056]
4 **Chan JK**, Roth J, Oppenheim JJ, Tracey KJ, Vogl T, Feldmann M, Horwood N, Nanchahal J. Alarmins: awaiting a clinical response. *J Clin Invest* 2012; **122**: 2711-2719 [PMID: 22850880 DOI: 10.1172/JCI62423]
5 **Rider P**, Voronov E, Dinarello CA, Apte RN, Cohen I. Alarmins: Feel the Stress. *J Immunol* 2017; **198**: 1395-1402 [PMID: 28167650 DOI: 10.4049/jimmunol.1601342]
6 **Kapurniotu A**, Gokce O, Bernhagen J. The Multitasking Potential of Alarmins and Atypical Chemokines. *Front Med (Lausanne)* 2019; **6**: 3 [PMID: 30729111 DOI: 10.3389/fmed.2019.00003]
7 **Yang**, Han Z, Oppenheim JJ. Alarmins and immunity. *Immunol Rev* 2017; **280**: 41-56 [PMID: 29027222 DOI: 10.1111/imr.12577]
8 **Eming SA**, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol* 2007; **127**: 514-525 [PMID: 17299434 DOI: 10.1038/sj.jid.5700701]
9 **Klimczak A**, Kozlowska U. Mesenchymal Stromal Cells and Tissue-Specific Progenitor Cells: Their Role in Tissue Homeostasis. *Stem Cells Int* 2016; **2016**: 4285215 [PMID: 26823669 DOI: 10.1155/2016/4285215]
10 **Pittenger MF**, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; **284**: 143-147 [PMID: 10102814 DOI: 10.1126/science.284.5411.143]
11 **Hass R**, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal* 2011; **9**: 12 [PMID: 21569606 DOI: 10.1186/1478-811X-9-12]
12 **Marquez-Curtis LA**, Janowska-Wieczorek A, McGann LE, Elliott JA. Mesenchymal stromal cells derived from various tissues: Biological, clinical and cryopreservation aspects. *Cryobiology* 2015; **71**: 181-197 [PMID: 26186998 DOI: 10.1016/j.cryobiol.2015.07.003]
13 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]
14 **Bianco P**, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell* 2008; **2**: 313-319 [PMID: 18397751 DOI: 10.1016/j.stem.2008.03.002]
15 **Sacchetti B**, Funari A, Remoli C, Giannicola G, Kogler G, Liedtke S, Cossu G, Serafini M, Sampaolesi M, Tagliafico E, Tenedini E, Saggio I, Robey PG, Riminucci M, Bianco P. No Identical "Mesenchymal Stem Cells" at Different Times and Sites: Human Committed Progenitors of Distinct Origin and Differentiation Potential Are Incorporated as Adventitial Cells in Microvessels. *Stem Cell Reports* 2016; **6**: 897-913 [PMID: 27304917 DOI: 10.1016/j.stemcr.2016.05.011]
16 **Caplan AI**. Mesenchymal Stem Cells: Time to Change the Name! *Stem Cells Transl Med* 2017; **6**: 1445-1451 [PMID: 28452204 DOI: 10.1002/sctm.17-0051]
17 **Liang X**, Ding Y, Zhang Y, Tse HF, Lian Q. Paracrine mechanisms of mesenchymal stem cell-based therapy: current status and perspectives. *Cell Transplant* 2014; **23**: 1045-1059 [PMID: 23676629 DOI: 10.3727/096368913X667709]
18 **Shi Y**, Hu G, Su J, Li W, Chen Q, Shou P, Xu C, Chen X, Huang Y, Zhu Z, Huang X, Han X, Xie N, Ren G. Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. *Cell Res* 2010; **20**: 510-518 [PMID: 20368733 DOI: 10.1038/cr.2010.44]
19 **Fayyad-Kazan M**, Fayyad-Kazan H, Lagneaux L, Najar M. The potential of mesenchymal stromal cells in immunotherapy. *Immunotherapy* 2016; **8**: 839-842 [PMID: 27381681 DOI: 10.2217/imt-2016-0037]
20 **Corcione A**, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006; **107**: 367-372 [PMID: 16141348 DOI: 10.1182/blood-2005-07-2657]
21 **Castro-Manrreza ME**, Montesinos JJ. Immunoregulation by mesenchymal stem cells: biological aspects and clinical applications. *J Immunol Res* 2015; **2015**: 394917 [PMID: 25961059 DOI: 10.1155/2015/394917]
22 **Espagnolle N**, Balguerie A, Arnaud E, Sensebé L, Varin A. CD54-Mediated Interaction with Pro-inflammatory Macrophages Increases the Immunosuppressive Function of Human Mesenchymal Stromal Cells. *Stem Cell Reports* 2017; **8**: 961-976 [PMID: 28330617 DOI: 10.1016/j.stemcr.2017.02.008]
23 **Keating A**. Mesenchymal stromal cells: new directions. *Cell Stem Cell* 2012; **10**: 709-716 [PMID: 22704511 DOI: 10.1016/j.stem.2012.05.015]
24 **Wang M**, Yuan Q, Xie L. Mesenchymal Stem Cell-Based Immunomodulation: Properties and Clinical Application. *Stem Cells Int* 2018; **2018**: 3057624 [PMID: 30013600 DOI: 10.1155/2018/3057624]
25 **Noronha NC**, Mizukami A, Caliári-Oliveira C, Cominal JG, Rocha JLM, Covas DT, Swiech K, Malmegrim KCR. Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. *Stem Cell Res Ther* 2019; **10**: 131 [PMID: 31046833 DOI: 10.1186/s13287-019-1224-y]
26 **Ren G**, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008; **2**: 141-150 [PMID: 18371435 DOI: 10.1016/j.stem.2007.11.014]
27 **Prockop DJ**, Oh JY. Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. *Mol Ther* 2012; **20**: 14-20 [PMID: 22008910 DOI: 10.1038/mt.2011.211]
28 **Prasanna SJ**, Gopalakrishnan D, Shankar SR, Vasandan AB. Pro-inflammatory cytokines, IFNgamma and

TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. *PLoS One* 2010; **5**: e9016 [PMID: 20126406 DOI: 10.1371/journal.pone.0009016]
29 **Najar M**, Raicevic G, Fayyad-Kazan H, De Bruyn C, Bron D, Toungouz M, Lagneaux L. Immune-related antigens, surface molecules and regulatory factors in human-derived mesenchymal stromal cells: the expression and impact of inflammatory priming. *Stem Cell Rev Rep* 2012; **8**: 1188-1198 [PMID: 22983809 DOI: 10.1007/s12015-012-9408-1]
30 **Sui BD**, Hu CH, Liu AQ, Zheng CX, Xuan K, Jin Y. Stem cell-based bone regeneration in diseased microenvironments: Challenges and solutions. *Biomaterials* 2019; **196**: 18-30 [PMID: 29122279 DOI: 10.1016/j.biomaterials.2017.10.046]
31 **Galipeau J**, Krampera M, Barrett J, Dazzi F, Deans RJ, DeBruijn J, Dominici M, Fibbe WE, Gee AP, Gimble JM, Hematti P, Koh MB, LeBlanc K, Martin I, McNiece IK, Mendicino M, Oh S, Ortiz L, Phinney DG, Planat V, Shi Y, Stroncek DF, Viswanathan S, Weiss DJ, Sensebe L. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. *Cytotherapy* 2016; **18**: 151-159 [PMID: 26724220 DOI: 10.1016/j.jcyt.2015.11.008]
32 **Turner MD**, Nedjai B, Hurst T, Pennington DJ. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta* 2014; **1843**: 2563-2582 [PMID: 24892271 DOI: 10.1016/j.bbamcr.2014.05.014]
33 **Croitoru-Lamoury J**, Lamoury FM, Caristo M, Suzuki K, Walker D, Takikawa O, Taylor R, Brew BJ. Interferon-γ regulates the proliferation and differentiation of mesenchymal stem cells via activation of indoleamine 2,3 dioxygenase (IDO). *PLoS One* 2011; **6**: e14698 [PMID: 21359206 DOI: 10.1371/journal.pone.0014698]
34 **Shi Y**, Su J, Roberts AI, Shou P, Rabson AB, Ren G. How mesenchymal stem cells interact with tissue immune responses. *Trends Immunol* 2012; **33**: 136-143 [PMID: 22227317 DOI: 10.1016/j.it.2011.11.004]
35 **Wang Q**, Yang Q, Wang Z, Tong H, Ma L, Zhang Y, Shan F, Meng Y, Yuan Z. Comparative analysis of human mesenchymal stem cells from fetal-bone marrow, adipose tissue, and Warton's jelly as sources of cell immunomodulatory therapy. *Hum Vaccin Immunother* 2016; **12**: 85-96 [PMID: 26186552 DOI: 10.1080/21645515.2015.1030549]
36 **Chinnadurai R**, Copland IB, Patel SR, Galipeau J. IDO-independent suppression of T cell effector function by IFN-γ-licensed human mesenchymal stromal cells. *J Immunol* 2014; **192**: 1491-1501 [PMID: 24403533 DOI: 10.4049/jimmunol.1301828]
37 **Noone C**, Kihm A, English K, O'Dea S, Mahon BP. IFN-γ stimulated human umbilical-tissue-derived cells potently suppress NK activation and resist NK-mediated cytotoxicity in vitro. *Stem Cells Dev* 2013; **22**: 3003-3014 [PMID: 23795941 DOI: 10.1089/scd.2013.0028]
38 **Rafei M**, Birman E, Forner K, Galipeau J. Allogeneic mesenchymal stem cells for treatment of experimental autoimmune encephalomyelitis. *Mol Ther* 2009; **17**: 1799-1803 [PMID: 19602999 DOI: 10.1038/mt.2009.157]
39 **Chinnadurai R**, Rajan D, Ng S, McCullough K, Arafat D, Waller EK, Anderson LJ, Gibson G, Galipeau J. Immune dysfunctionality of replicative senescent mesenchymal stromal cells is corrected by IFNγ priming. *Blood Adv* 2017; **1**: 628-643 [PMID: 28713871 DOI: 10.1182/bloodadvances.2017006205]
40 **Duijvestein M**, Wildenberg ME, Welling MM, Hennink S, Molendijk I, van Zuylen VL, Bosse T, Vos AC, de Jonge-Muller ES, Roelofs H, van der Weerd L, Verspaget HW, Fibbe WE, te Velde AA, van den Brink GR, Hommes DW. Pretreatment with interferon-γ enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. *Stem Cells* 2011; **29**: 1549-1558 [PMID: 21898680 DOI: 10.1002/stem.698]
41 **Polchert D**, Sobinsky J, Douglas G, Kidd M, Moadsiri A, Reina E, Genrich K, Mehrotra S, Setty S, Smith B, Bartholomew A. IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft *vs* host disease. *Eur J Immunol* 2008; **38**: 1745-1755 [PMID: 18493986 DOI: 10.1002/eji.200738129]
42 **Burand AJ**, Gramlich OW, Brown AJ, Ankrum JA. Function of Cryopreserved Mesenchymal Stromal Cells With and Without Interferon-γ Prelicensing is Context Dependent. *Stem Cells* 2017; **35**: 1437-1439 [PMID: 27758056 DOI: 10.1002/stem.2528]
43 **Shioda M**, Muneta T, Tsuji K, Mizuno M, Komori K, Koga H, Sekiya I. TNFα promotes proliferation of human synovial MSCs while maintaining chondrogenic potential. *PLoS One* 2017; **12**: e0177771 [PMID: 28542363 DOI: 10.1371/journal.pone.0177771]
44 **Böcker W**, Docheva D, Prall WC, Egea V, Pappou E, Rossmann O, Popov C, Mutschler W, Ries C, Schieker M. IKK-2 is required for TNF-alpha-induced invasion and proliferation of human mesenchymal stem cells. *J Mol Med (Berl)* 2008; **86**: 1183-1192 [PMID: 18600306 DOI: 10.1007/s00109-008-0378-3]
45 **Jagielski M**, Wolf J, Marzahn U, Völker A, Lemke M, Meier C, Ertel W, Godkin O, Arens S, Schulze-Tanzil G. The influence of IL-10 and TNFα on chondrogenesis of human mesenchymal stromal cells in three-dimensional cultures. *Int J Mol Sci* 2014; **15**: 15821-15844 [PMID: 25207597 DOI: 10.3390/ijms150915821]
46 **Wehling N**, Palmer GD, Pilapil C, Liu F, Wells JW, Müller PE, Evans CH, Porter RM. Interleukin-1beta and tumor necrosis factor alpha inhibit chondrogenesis by human mesenchymal stem cells through NF-kappaB-dependent pathways. *Arthritis Rheum* 2009; **60**: 801-812 [PMID: 19248089 DOI: 10.1002/art.24352]
47 **Lacey DC**, Simmons PJ, Graves SE, Hamilton JA. Proinflammatory cytokines inhibit osteogenic differentiation from stem cells: implications for bone repair during inflammation. *Osteoarthritis Cartilage* 2009; **17**: 735-742 [PMID: 19136283 DOI: 10.1016/j.joca.2008.11.011]
48 **Gilbert L**, He X, Farmer P, Rubin J, Drissi H, van Wijnen AJ, Lian JB, Stein GS, Nanes MS. Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. *J Biol Chem* 2002; **277**: 2695-2701 [PMID: 11723115 DOI: 10.1074/jbc.M106339200]
49 **Mountziaris PM**, Tzouanas SN, Mikos AG. Dose effect of tumor necrosis factor-alpha on *in vitro* osteogenic differentiation of mesenchymal stem cells on biodegradable polymeric microfiber scaffolds. *Biomaterials* 2010; **31**: 1666-1675 [PMID: 19963268 DOI: 10.1016/j.biomaterials.2009.11.058]
50 **Croes M**, Oner FC, Kruyt MC, Blokhuis TJ, Bastian O, Dhert WJ, Alblas J. Proinflammatory Mediators

Enhance the Osteogenesis of Human Mesenchymal Stem Cells after Lineage Commitment. *PLoS One* 2015; **10**: e0132781 [PMID: 26176237 DOI: 10.1371/journal.pone.0132781]

51 **Hess K**, Ushmorov A, Fiedler J, Brenner RE, Wirth T. TNFalpha promotes osteogenic differentiation of human mesenchymal stem cells by triggering the NF-kappaB signaling pathway. *Bone* 2009; **45**: 367-376 [PMID: 19414075 DOI: 10.1016/j.bone.2009.04.252]

52 **Qin Z**, Fang Z, Zhao L, Chen J, Li Y, Liu G. High dose of TNF-α suppressed osteogenic differentiation of human dental pulp stem cells by activating the Wnt/β-catenin signaling. *J Mol Histol* 2015; **46**: 409-420 [PMID: 26115593 DOI: 10.1007/s10735-015-9630-7]

53 **Feng G**, Zheng K, Song D, Xu K, Huang D, Zhang Y, Cao P, Shen S, Zhang J, Feng X, Zhang D. SIRT1 was involved in TNF-α-promoted osteogenic differentiation of human DPSCs through Wnt/β-catenin signal. *In Vitro Cell Dev Biol Anim* 2016; **52**: 1001-1011 [PMID: 27530621 DOI: 10.1007/s11626-016-0070-9]

54 **Lee MJ**, Kim J, Kim MY, Bae YS, Ryu SH, Lee TG, Kim JH. Proteomic analysis of tumor necrosis factor-alpha-induced secretome of human adipose tissue-derived mesenchymal stem cells. *J Proteome Res* 2010; **9**: 1754-1762 [PMID: 20184379 DOI: 10.1021/pr900898n]

55 **François M**, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. *Mol Ther* 2012; **20**: 187-195 [PMID: 21934657 DOI: 10.1038/mt.2011.189]

56 **Zhang A**, Wang Y, Ye Z, Xie H, Zhou L, Zheng S. Mechanism of TNF-α-induced migration and hepatocyte growth factor production in human mesenchymal stem cells. *J Cell Biochem* 2010; **111**: 469-475 [PMID: 20533298 DOI: 10.1002/jcb.22729]

57 **Wang M**, Crisostomo PR, Herring C, Meldrum KK, Meldrum DR. Human progenitor cells from bone marrow or adipose tissue produce VEGF, HGF, and IGF-I in response to TNF by a p38 MAPK-dependent mechanism. *Am J Physiol Regul Integr Comp Physiol* 2006; **291**: R880-R884 [PMID: 16728464 DOI: 10.1152/ajpregu.00280.2006]

58 **Putra A**, Ridwan FB, Putridewi AI, Kustiyah AR, Wirastuti K, Sadyah NAC, Rosdiana I, Munir D. The Role of TNF-α induced MSCs on Suppressive Inflammation by Increasing TGF-β and IL-10. *Open Access Maced J Med Sci* 2018; **6**: 1779-1783 [PMID: 30455748 DOI: 10.3889/oamjms.2018.404]

59 **Cuerquis J**, Romieu-Mourez R, François M, Routy JP, Young YK, Zhao J, Eliopoulos N. Human mesenchymal stromal cells transiently increase cytokine production by activated T cells before suppressing T-cell proliferation: effect of interferon-γ and tumor necrosis factor-α stimulation. *Cytotherapy* 2014; **16**: 191-202 [PMID: 24438900 DOI: 10.1016/j.jcyt.2013.11.008]

60 **Su W**, Wan Q, Huang J, Han L, Chen X, Chen G, Olsen N, Zheng SG, Liang D. Culture medium from TNF-α-stimulated mesenchymal stem cells attenuates allergic conjunctivitis through multiple antiallergic mechanisms. *J Allergy Clin Immunol* 2015; **136**: 423-32.e8 [PMID: 25652765 DOI: 10.1016/j.jaci.2014.12.1926]

61 **Heo SC**, Jeon ES, Lee IH, Kim HS, Kim MB, Kim JH. Tumor necrosis factor-α-activated human adipose tissue-derived mesenchymal stem cells accelerate cutaneous wound healing through paracrine mechanisms. *J Invest Dermatol* 2011; **131**: 1559-1567 [PMID: 21451545 DOI: 10.1038/jid.2011.64]

62 **Lee RH**, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell* 2009; **5**: 54-63 [PMID: 19570514 DOI: 10.1016/j.stem.2009.05.003]

63 **Djouad F**, Fritz V, Apparailly F, Louis-Plence P, Bony C, Sany J, Jorgensen C, Noël D. Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis. *Arthritis Rheum* 2005; **52**: 1595-1603 [PMID: 15880818 DOI: 10.1002/art.21012]

64 **Lopez-Castejon G**, Brough D. Understanding the mechanism of IL-1β secretion. *Cytokine Growth Factor Rev* 2011; **22**: 189-195 [PMID: 22019906 DOI: 10.1016/j.cytogfr.2011.10.001]

65 **Brandt L**, Schubert S, Scheibe P, Brehm W, Franzen J, Gross C, Burk J. Tenogenic Properties of Mesenchymal Progenitor Cells Are Compromised in an Inflammatory Environment. *Int J Mol Sci* 2018; **19** [PMID: 30154348 DOI: 10.3390/ijms19092549]

66 **Matsumura E**, Tsuji K, Komori K, Koga H, Sekiya I, Muneta T. Pretreatment with IL-1β enhances proliferation and chondrogenic potential of synovium-derived mesenchymal stem cells. *Cytotherapy* 2017; **19**: 181-193 [PMID: 27979606 DOI: 10.1016/j.jcyt.2016.11.004]

67 **Yang C**, Chen Y, Li F, You M, Zhong L, Li W, Zhang B, Chen Q. The biological changes of umbilical cord mesenchymal stem cells in inflammatory environment induced by different cytokines. *Mol Cell Biochem* 2018; **446**: 171-184 [PMID: 29356988 DOI: 10.1007/s11010-018-3284-1]

68 **Carrero R**, Cerrada I, Lledó E, Dopazo J, García-García F, Rubio MP, Trigueros C, Dorronsoro A, Ruiz-Sauri A, Montero JA, Sepúlveda P. IL1β induces mesenchymal stem cells migration and leucocyte chemotaxis through NF-κB. *Stem Cell Rev Rep* 2012; **8**: 905-916 [PMID: 22467443 DOI: 10.1007/s12015-012-9364-9]

69 **Guo YC**, Chiu YH, Chen CP, Wang HS. Interleukin-1β induces CXCR3-mediated chemotaxis to promote umbilical cord mesenchymal stem cell transendothelial migration. *Stem Cell Res Ther* 2018; **9**: 281 [PMID: 30359318 DOI: 10.1186/s13287-018-1032-9]

70 **Sonomoto K**, Yamaoka K, Oshita K, Fukuyo S, Zhang X, Nakano K, Okada Y, Tanaka Y. Interleukin-1β induces differentiation of human mesenchymal stem cells into osteoblasts via the Wnt-5a/receptor tyrosine kinase-like orphan receptor 2 pathway. *Arthritis Rheum* 2012; **64**: 3355-3363 [PMID: 22674197 DOI: 10.1002/art.34555]

71 **Mao CY**, Wang YG, Zhang X, Zheng XY, Tang TT, Lu EY. Double-edged-sword effect of IL-1β on the osteogenesis of periodontal ligament stem cells via crosstalk between the NF-κB, MAPK and BMP/Smad signaling pathways. *Cell Death Dis* 2016; **7**: e2296 [PMID: 27415426 DOI: 10.1038/cddis.2016.204]

72 **Felka T**, Schäfer R, Schewe B, Benz K, Aicher WK. Hypoxia reduces the inhibitory effect of IL-1beta on chondrogenic differentiation of FCS-free expanded MSC. *Osteoarthritis Cartilage* 2009; **17**: 1368-1376 [PMID: 19463979 DOI: 10.1016/j.joca.2009.04.023]

73 **Gao B**, Gao W, Wu Z, Zhou T, Qiu X, Wang X, Lian C, Peng Y, Liang A, Qiu J, Zhu Y, Xu C, Li Y, Su P,

Huang D. Melatonin rescued interleukin 1β-impaired chondrogenesis of human mesenchymal stem cells. *Stem Cell Res Ther* 2018; **9**: 162 [PMID: 29898779 DOI: 10.1186/s13287-018-0892-3]

74 **Majumdar MK**, Wang E, Morris EA. BMP-2 and BMP-9 promotes chondrogenic differentiation of human multipotential mesenchymal cells and overcomes the inhibitory effect of IL-1. *J Cell Physiol* 2001; **189**: 275-284 [PMID: 11748585 DOI: 10.1002/jcp.10025]

75 **Liu W**, Sun Y, He Y, Zhang H, Zheng Y, Yao Y, Zhang Z. IL-1β impedes the chondrogenic differentiation of synovial fluid mesenchymal stem cells in the human temporomandibular joint. *Int J Mol Med* 2017; **39**: 317-326 [PMID: 28000839 DOI: 10.3892/ijmm.2016.2832]

76 **Hingert D**, Barreto Henriksson H, Brisby H. Human Mesenchymal Stem Cells Pretreated with Interleukin-1β and Stimulated with Bone Morphogenetic Growth Factor-3 Enhance Chondrogenesis. *Tissue Eng Part A* 2018; **24**: 775-785 [PMID: 28978269 DOI: 10.1089/ten.TEA.2017.0087]

77 **Magne B**, Dedier M, Nivet M, Coulomb B, Banzet S, Lataillade JJ, Trouillas M. IL-1β-Primed Mesenchymal Stromal Cells Improve Epidermal Substitute Engraftment and Wound Healing via Matrix Metalloproteinases and Transforming Growth Factor-β1. *J Invest Dermatol* 2020; **140**: 688-698.e21 [PMID: 31513805 DOI: 10.1016/j.jid.2019.07.721]

78 **Redondo-Castro E**, Cunningham C, Miller J, Martuscelli L, Aoulad-Ali S, Rothwell NJ, Kielty CM, Allan SM, Pinteaux E. Interleukin-1 primes human mesenchymal stem cells towards an anti-inflammatory and pro-trophic phenotype in vitro. *Stem Cell Res Ther* 2017; **8**: 79 [PMID: 28412968 DOI: 10.1186/s13287-017-0531-4]

79 **Amann EM**, Groß A, Rojewski MT, Kestler HA, Kalbitz M, Brenner RE, Huber-Lang M, Schrezenmeier H. Inflammatory response of mesenchymal stromal cells after *in vivo* exposure with selected trauma-related factors and polytrauma serum. *PLoS One* 2019; **14**: e0216862 [PMID: 31086407 DOI: 10.1371/journal.pone.0216862]

80 **Murphy N**, Treacy O, Lynch K, Morcos M, Lohan P, Howard L, Fahy G, Griffin MD, Ryan AE, Ritter T. TNF-α/IL-1β-licensed mesenchymal stromal cells promote corneal allograft survival *via* myeloid cell-mediated induction of Foxp3^{+} regulatory T cells in the lung. *FASEB J* 2019; **33**: 9404-9421 [PMID: 31108041 DOI: 10.1096/fj.201900047R]

81 **Fan H**, Zhao G, Liu L, Liu F, Gong W, Liu X, Yang L, Wang J, Hou Y. Pre-treatment with IL-1β enhances the efficacy of MSC transplantation in DSS-induced colitis. *Cell Mol Immunol* 2012; **9**: 473-481 [PMID: 23085948 DOI: 10.1038/cmi.2012.40]

82 **Yu Y**, Yoo SM, Park HH, Baek SY, Kim YJ, Lee S, Kim YL, Seo KW, Kang KS. Preconditioning with interleukin-1 beta and interferon-gamma enhances the efficacy of human umbilical cord blood-derived mesenchymal stem cells-based therapy via enhancing prostaglandin E2 secretion and indoleamine 2,3-dioxygenase activity in dextran sulfate sodium-induced colitis. *J Tissue Eng Regen Med* 2019; **13**: 1792-1804 [PMID: 31293088 DOI: 10.1002/term.2930]

83 **van Dalen SCM**, Blom AB, Walgreen B, Slöetjes AW, Helsen MMA, Geven EJW, Ter Huurne M, Vogl T, Roth J, van de Loo FAJ, Koenders MI, Casteilla L, van der Kraan PM, van den Bosch MHJ, van Lent PLEM. IL-1β-Mediated Activation of Adipose-Derived Mesenchymal Stromal Cells Results in PMN Reallocation and Enhanced Phagocytosis: A Possible Mechanism for the Reduction of Osteoarthritis Pathology. *Front Immunol* 2019; **10**: 1075 [PMID: 31191517 DOI: 10.3389/fimmu.2019.01075]

84 **Huang H**, Kim HJ, Chang EJ, Lee ZH, Hwang SJ, Kim HM, Lee Y, Kim HH. IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. *Cell Death Differ* 2009; **16**: 1332-1343 [PMID: 19543237 DOI: 10.1038/cdd.2009.74]

85 **Mojsilović S**, Krstić A, Ilić V, Okić Djordjević I I, Kocić J, Trivanović D, Santibañez JF, JovčićG, Bugarski D. IL-17 and FGF signaling involved in mouse mesenchymal stem cell proliferation. *Cell Tissue Res* 2011; **346**: 305-316 [PMID: 22160457 DOI: 10.1007/s00441-011-1284-5]

86 **Krstić J**, Obradović H, Jauković A, Okić Djordjević I, Trivanović D, Kukolj T, Mojsilović S, Ilić V, Santibañez JF, Bugarski D. Urokinase type plasminogen activator mediates Interleukin-17-induced peripheral blood mesenchymal stem cell motility and transendothelial migration. *Biochim Biophys Acta* 2015; **1853**: 431-444 [PMID: 25433194 DOI: 10.1016/j.bbamcr.2014.11.025]

87 **Croes M**, Öner FC, van Neerven D, Sabir E, Kruyt MC, Blokhuis TJ, Dhert WJA, Alblas J. Proinflammatory T cells and IL-17 stimulate osteoblast differentiation. *Bone* 2016; **84**: 262-270 [PMID: 26780388 DOI: 10.1016/j.bone.2016.01.010]

88 **Noh M**. Interleukin-17A increases leptin production in human bone marrow mesenchymal stem cells. *Biochem Pharmacol* 2012; **83**: 661-670 [PMID: 22197587 DOI: 10.1016/j.bcp.2011.12.010]

89 **Osta B**, Lavocat F, Eljaafari A, Miossec P. Effects of Interleukin-17A on Osteogenic Differentiation of Isolated Human Mesenchymal Stem Cells. *Front Immunol* 2014; **5**: 425 [PMID: 25228904 DOI: 10.3389/fimmu.2014.00425]

90 **Kondo M**, Yamaoka K, Sonomoto K, Fukuyo S, Oshita K, Okada Y, Tanaka Y. IL-17 inhibits chondrogenic differentiation of human mesenchymal stem cells. *PLoS One* 2013; **8**: e79463 [PMID: 24260226 DOI: 10.1371/journal.pone.0079463]

91 **Shin JH**, Shin DW, Noh M. Interleukin-17A inhibits adipocyte differentiation in human mesenchymal stem cells and regulates pro-inflammatory responses in adipocytes. *Biochem Pharmacol* 2009; **77**: 1835-1844 [PMID: 19428338 DOI: 10.1016/j.bcp.2009.03.008]

92 **Djordjević IO**, Kukolj T, Krstić J, Trivanović D, Obradović H, Santibañez JF, Mojsilović S, Ilić V, Bugarski D, Jauković A. The inhibition of periodontal ligament stem cells osteogenic differentiation by IL-17 is mediated via MAPKs. *Int J Biochem Cell Biol* 2016; **71**: 92-101 [PMID: 26718973 DOI: 10.1016/j.biocel.2015.12.007]

93 **Chang J**, Liu F, Lee M, Wu B, Ting K, Zara JN, Soo C, Al Hezaimi K, Zou W, Chen X, Mooney DJ, Wang CY. NF-κB inhibits osteogenic differentiation of mesenchymal stem cells by promoting β-catenin degradation. *Proc Natl Acad Sci USA* 2013; **110**: 9469-9474 [PMID: 23690607 DOI: 10.1073/pnas.1300532110]

94 **Sivanathan KN**, Rojas-Canales DM, Hope CM, Krishnan R, Carroll RP, Gronthos S, Grey ST, Coates PT. Interleukin-17A-Induced Human Mesenchymal Stem Cells Are Superior Modulators of Immunological Function. *Stem Cells* 2015; **33**: 2850-2863 [PMID: 26037953 DOI: 10.1002/stem.2075]

95 **Han X**, Yang Q, Lin L, Xu C, Zheng C, Chen X, Han Y, Li M, Cao W, Cao K, Chen Q, Xu G, Zhang Y, Zhang J, Schneider RJ, Qian Y, Wang Y, Brewer G, Shi Y. Interleukin-17 enhances immunosuppression by mesenchymal stem cells. *Cell Death Differ* 2014; **21**: 1758-1768 [PMID: 25034782 DOI: 10.1038/cdd.2014.85]

96 **Tian J**, Rui K, Tang X, Wang W, Ma J, Tian X, Wang Y, Xu H, Lu L, Wang S. IL-17 down-regulates the immunosuppressive capacity of olfactory ecto-mesenchymal stem cells in murine collagen-induced arthritis. *Oncotarget* 2016; **7**: 42953-42962 [PMID: 27356747 DOI: 10.18632/oncotarget.10261]

97 **Bai M**, Zhang L, Fu B, Bai J, Zhang Y, Cai G, Bai X, Feng Z, Sun S, Chen X. IL-17A improves the efficacy of mesenchymal stem cells in ischemic-reperfusion renal injury by increasing Treg percentages by the COX-2/PGE2 pathway. *Kidney Int* 2018; **93**: 814-825 [PMID: 29132705 DOI: 10.1016/j.kint.2017.08.030]

98 **Ma T**, Wang X, Jiao Y, Wang H, Qi Y, Gong H, Zhang L, Jiang D. Interleukin 17 (IL-17)-Induced Mesenchymal Stem Cells Prolong the Survival of Allogeneic Skin Grafts. *Ann Transplant* 2018; **23**: 615-621 [PMID: 30166501 DOI: 10.12659/AOT.909381]

99 **Rincon M**. Interleukin-6: from an inflammatory marker to a target for inflammatory diseases. *Trends Immunol* 2012; **33**: 571-577 [PMID: 22883707 DOI: 10.1016/j.it.2012.07.003]

100 **Wei H**, Shen G, Deng X, Lou D, Sun B, Wu H, Long L, Ding T, Zhao J. The role of IL-6 in bone marrow (BM)-derived mesenchymal stem cells (MSCs) proliferation and chondrogenesis. *Cell Tissue Bank* 2013; **14**: 699-706 [PMID: 23322270 DOI: 10.1007/s10561-012-9354-9]

101 **Song HY**, Jeon ES, Jung JS, Kim JH. Oncostatin M induces proliferation of human adipose tissue-derived mesenchymal stem cells. *Int J Biochem Cell Biol* 2005; **37**: 2357-2365 [PMID: 15979922 DOI: 10.1016/j.biocel.2005.05.007]

102 **Li D**, Wang GY, Dong BH, Zhang YC, Wang YX, Sun BC. Biological characteristics of human placental mesenchymal stem cells and their proliferative response to various cytokines. *Cells Tissues Organs* 2007; **186**: 169-179 [PMID: 17630477 DOI: 10.1159/000105674]

103 **Pricola KL**, Kuhn NZ, Haleem-Smith H, Song Y, Tuan RS. Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. *J Cell Biochem* 2009; **108**: 577-588 [PMID: 19650110 DOI: 10.1002/jcb.22289]

104 **Nowwarote N**, Sukarawan W, Kanjana K, Pavasant P, Fournier BPJ, Osathanon T. Interleukin 6 promotes an *in vitro* mineral deposition by stem cells isolated from human exfoliated deciduous teeth. *R Soc Open Sci* 2018; **5**: 180864 [PMID: 30473835 DOI: 10.1098/rsos.180864]

105 **Fukuyo S**, Yamaoka K, Sonomoto K, Oshita K, Okada Y, Saito K, Yoshida Y, Kanazawa T, Minami Y, Tanaka Y. IL-6-accelerated calcification by induction of ROR2 in human adipose tissue-derived mesenchymal stem cells is STAT3 dependent. *Rheumatology (Oxford)* 2014; **53**: 1282-1290 [PMID: 24599911 DOI: 10.1093/rheumatology/ket496]

106 **Xie Z**, Tang S, Ye G, Wang P, Li J, Liu W, Li M, Wang S, Wu X, Cen S, Zheng G, Ma M, Wu Y, Shen H. Interleukin-6/interleukin-6 receptor complex promotes osteogenic differentiation of bone marrow-derived mesenchymal stem cells. *Stem Cell Res Ther* 2018; **9**: 13 [PMID: 29357923 DOI: 10.1186/s13287-017-0766-0]

107 **Bastidas-Coral AP**, Bakker AD, Zandieh-Doulabi B, Kleverlaan CJ, Bravenboer N, Forouzanfar T, Klein-Nulend J. Cytokines TNF-α, IL-6, IL-17F, and IL-4 Differentially Affect Osteogenic Differentiation of Human Adipose Stem Cells. *Stem Cells Int* 2016; **2016**: 1318256 [PMID: 27667999 DOI: 10.1155/2016/1318256]

108 **Kondo M**, Yamaoka K, Sakata K, Sonomoto K, Lin L, Nakano K, Tanaka Y. Contribution of the Interleukin-6/STAT-3 Signaling Pathway to Chondrogenic Differentiation of Human Mesenchymal Stem Cells. *Arthritis Rheumatol* 2015; **67**: 1250-1260 [PMID: 25604648 DOI: 10.1002/art.39036]

109 **Huang RL**, Sun Y, Ho CK, Liu K, Tang QQ, Xie Y, Li Q. IL-6 potentiates BMP-2-induced osteogenesis and adipogenesis via two different BMPR1A-mediated pathways. *Cell Death Dis* 2018; **9**: 144 [PMID: 29396550 DOI: 10.1038/s41419-017-0126-0]

110 **Mrácek T**, Cannon B, Houstek J. IL-1 and LPS but not IL-6 inhibit differentiation and downregulate PPAR gamma in brown adipocytes. *Cytokine* 2004; **26**: 9-15 [PMID: 15016406 DOI: 10.1016/j.cyto.2003.12.001]

111 **Crop MJ**, Baan CC, Korevaar SS, Ijzermans JN, Pescatori M, Stubbs AP, van Ijcken WF, Dahlke MH, Eggenhofer E, Weimar W, Hoogduijn MJ. Inflammatory conditions affect gene expression and function of human adipose tissue-derived mesenchymal stem cells. *Clin Exp Immunol* 2010; **162**: 474-486 [PMID: 20846162 DOI: 10.1111/j.1365-2249.2010.04256.x]

112 **Pourgholaminejad A**, Aghdami N, Baharvand H, Moazzeni SM. The effect of pro-inflammatory cytokines on immunophenotype, differentiation capacity and immunomodulatory functions of human mesenchymal stem cells. *Cytokine* 2016; **85**: 51-60 [PMID: 27288632 DOI: 10.1016/j.cyto.2016.06.003]

113 **Sun X**, Zou T, Zuo C, Zhang M, Shi B, Jiang Z, Cui H, Liao X, Li X, Tang Y, Liu Y, Liu X. IL-1α inhibits proliferation and adipogenic differentiation of human adipose-derived mesenchymal stem cells through NF-κB- and ERK1/2-mediated proinflammatory cytokines. *Cell Biol Int* 2018; **42**: 794-803 [PMID: 29288588 DOI: 10.1002/cbin.10932]

114 **Kukolj T**, Trivanović D, Mojsilović S, Okić Djordjević I, Obradović H, Krstić J, Jauković A, Bugarski D. IL-33 guides osteogenesis and increases proliferation and pluripotency marker expression in dental stem cells. *Cell Prolif* 2019; **52**: e12533 [PMID: 30430681 DOI: 10.1111/cpr.12533]

115 **Terraza C**, Fuentes R, Pino-Lagos K. IFN-γ and IL-33 modulate mesenchymal stem cells function targeting Th1/Th17 axis in a murine skin transplantation model. *Cytokine* 2018; **111**: 317-324 [PMID: 30269028 DOI: 10.1016/j.cyto.2018.09.013]

116 **Martinotti S**, Patrone M, Ranzato E. Emerging roles for HMGB1 protein in immunity, inflammation, and cancer. *Immunotargets Ther* 2015; **4**: 101-109 [PMID: 27471716 DOI: 10.2147/ITT.S58064]

117 **Lotfi R**, Eisenbacher J, Solgi G, Fuchs K, Yildiz T, Nienhaus C, Rojewski MT, Schrezenmeier H. Human mesenchymal stem cells respond to native but not oxidized damage associated molecular pattern molecules from necrotic (tumor) material. *Eur J Immunol* 2011; **41**: 2021-2028 [PMID: 21538978 DOI: 10.1002/eji.201041324]

118 **Meng E**, Guo Z, Wang H, Jin J, Wang J, Wang H, Wu C, Wang L. High mobility group box 1 protein

inhibits the proliferation of human mesenchymal stem cells and promotes their migration and differentiation along osteoblastic pathway. *Stem Cells Dev* 2008; **17**: 805-813 [PMID: 18715162 DOI: 10.1089/scd.2007.0276]

119 **Meng X**, Chen M, Su W, Tao X, Sun M, Zou X, Ying R, Wei W, Wang B. The differentiation of mesenchymal stem cells to vascular cells regulated by the HMGB1/RAGE axis: its application in cell therapy for transplant arteriosclerosis. *Stem Cell Res Ther* 2018; **9**: 85 [PMID: 29615103 DOI: 10.1186/s13287-018-0827-z]

120 **Lin F**, Zhang W, Xue D, Zhu T, Li J, Chen E, Yao X, Pan Z. Signaling pathways involved in the effects of HMGB1 on mesenchymal stem cell migration and osteoblastic differentiation. *Int J Mol Med* 2016; **37**: 789-797 [PMID: 26846297 DOI: 10.3892/ijmm.2016.2479]

121 **Feng L**, Xue D, Chen E, Zhang W, Gao X, Yu J, Feng Y, Pan Z. HMGB1 promotes the secretion of multiple cytokines and potentiates the osteogenic differentiation of mesenchymal stem cells through the Ras/MAPK signaling pathway. *Exp Ther Med* 2016; **12**: 3941-3947 [PMID: 28105126 DOI: 10.3892/etm.2016.3857]

122 **Jurewicz E**, Góral A, Filipek A. S100A6 is secreted from Wharton's jelly mesenchymal stem cells and interacts with integrin β1. *Int J Biochem Cell Biol* 2014; **55**: 298-303 [PMID: 25256682 DOI: 10.1016/j.biocel.2014.09.015]

123 **Basu A**, Munir S, Mulaw MA, Singh K, Crisan D, Sindrilaru A, Treiber N, Wlaschek M, Huber-Lang M, Gebhard F, Scharffetter-Kochanek K. A Novel S100A8/A9 Induced Fingerprint of Mesenchymal Stem Cells associated with Enhanced Wound Healing. *Sci Rep* 2018; **8**: 6205 [PMID: 29670130 DOI: 10.1038/s41598-018-24425-9]

124 **Gao F**, Hu XY, Xie XJ, Xu QY, Wang YP, Liu XB, Xiang MX, Sun Y, Wang JA. Heat shock protein 90 protects rat mesenchymal stem cells against hypoxia and serum deprivation-induced apoptosis via the PI3K/Akt and ERK1/2 pathways. *J Zhejiang Univ Sci B* 2010; **11**: 608-617 [PMID: 20669351 DOI: 10.1631/jzus.B1001007]

125 **Gao F**, Hu X, Xie X, Liu X, Wang J. Heat shock protein 90 stimulates rat mesenchymal stem cell migration via PI3K/Akt and ERK1/2 pathways. *Cell Biochem Biophys* 2015; **71**: 481-489 [PMID: 25287672 DOI: 10.1007/s12013-014-0228-6]

126 **Andreeva NV**, Zatsepina OG, Garbuz DG, Evgen'ev MB, Belyavsky AV. Recombinant HSP70 and mild heat shock stimulate growth of aged mesenchymal stem cells. *Cell Stress Chaperones* 2016; **21**: 727-733 [PMID: 27091568 DOI: 10.1007/s12192-016-0691-7]

127 **Chen E**, Xue D, Zhang W, Lin F, Pan Z. Extracellular heat shock protein 70 promotes osteogenesis of human mesenchymal stem cells through activation of the ERK signaling pathway. *FEBS Lett* 2015; **589**: 4088-4096 [PMID: 26608032 DOI: 10.1016/j.febslet.2015.11.021]

128 **Li C**, Sunderic K, Nicoll SB, Wang S. Downregulation of Heat Shock Protein 70 Impairs Osteogenic and Chondrogenic Differentiation in Human Mesenchymal Stem Cells. *Sci Rep* 2018; **8**: 553 [PMID: 29323151 DOI: 10.1038/s41598-017-18541-1]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 938-951

DOI: 10.4252/wjsc.v12.i9.938

ISSN 1948-0210 (online)

REVIEW

Role of the CXCR4-SDF1-HMGB1 pathway in the directional migration of cells and regeneration of affected organs

Nazmul Haque, Ismail M Fareez, Liew Fong Fong, Chanchal Mandal, Noor Hayaty Abu Kasim, Kranthi Raja Kacharaju, Pratiwi Soesilawati

ORCID number: Nazmul Haque 0000-0002-8191-8993; Ismail M Fareez 0000-0001-6993-5339; Liew Fong Fong 0000-0001-9165-2343; Chanchal Mandal 0000-0003-2865-2847; Noor Hayaty Abu Kasim 0000-0002-8889-842X; Kranthi Raja Kacharaju 0000-0002-2057-9473; Pratiwi Soesilawati 0000-0002-1141-5645.

Author contributions: Fareez IM and Fong LF contributed equally to this work; Haque N contributed to the conception and design of the study; Mandel C, Kacharaju KR and Soesilawati P drafted and wrote the article; Haque N, Fareez IM, Fong LF and Abu Kasim NH contributed to the writing of the manuscript, made critical revisions related to relevant intellectual content of the manuscript; Haque N and Abu Kasim NH approved the final version of the article.

Supported by MAHSA University Grant, No. RP158-05/19.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Nazmul Haque, Ismail M Fareez, Liew Fong Fong, Department of Oral Biology and Biomedical Sciences, Faculty of Dentistry, MAHSA University, Selangor 42610, Malaysia

Chanchal Mandal, Biotechnology and Genetic Engineering Discipline, Life Science, Khulna University, Khulna 9208, Bangladesh

Noor Hayaty Abu Kasim, Faculty of Dentistry, University Kebangsaan Malaysia, Kuala Lumpur 50300, Malaysia

Noor Hayaty Abu Kasim, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 411007, Indonesia

Kranthi Raja Kacharaju, Department of Conservative Dentistry, Faculty of Dentistry MAHSA University, Selangor 42610, Malaysia

Pratiwi Soesilawati, Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya 60115, Indonesia

Corresponding author: Nazmul Haque, PhD, Senior Lecturer, Department of Oral Biology and Biomedical Sciences, Faculty of Dentistry, MAHSA University, Jalan SP 2, Bandar Saujana Putra, Selangor 42610, Malaysia. nazmul@mahsa.edu.my; tanna.bge@gmail.com

Abstract

In recent years, several studies have reported positive outcomes of cell-based therapies despite insufficient engraftment of transplanted cells. These findings have created a huge interest in the regenerative potential of paracrine factors released from transplanted stem or progenitor cells. Interestingly, this notion has also led scientists to question the role of proteins in the secretome produced by cells, tissues or organisms under certain conditions or at a particular time of regenerative therapy. Further studies have revealed that the secretomes derived from different cell types contain paracrine factors that could help to prevent apoptosis and induce proliferation of cells residing within the tissues of affected organs. This could also facilitate the migration of immune, progenitor and stem cells within the body to the site of inflammation. Of these different paracrine factors present within the secretome, researchers have given proper consideration to stromal cell-derived factor-1 (SDF1) that plays a vital role in tissue-specific migration of the cells needed for regeneration. Recently researchers recognized that SDF1 could facilitate site-specific migration of cells by regulating SDF1-

Manuscript source: Invited manuscript

Received: March 3, 2020
Peer-review started: March 3, 2020
First decision: June 7, 2020
Revised: June 18, 2020
Accepted: July 18, 2020
Article in press: July 18, 2020
Published online: September 26, 2020

P-Reviewer: Huang YC, Politi L, Tang JM
S-Editor: Yan JP
L-Editor: Webster JR
P-Editor: Xing YX

CXCR4 and/or HMGB1-SDF1-CXCR4 pathways which is vital for tissue regeneration. Hence in this study, we have attempted to describe the role of different types of cells within the body in facilitating regeneration while emphasizing the HMGB1-SDF1-CXCR4 pathway that orchestrates the migration of cells to the site where regeneration is needed.

Key Words: C-X-C motif chemokine 12; Mesenchymal stem cells; Monocytes; Neutrophils; Peripheral blood mononuclear cells; Receptor for advanced glycation end products

Core tip: In the last few decades, cell-based regenerative therapy has received considerable attention for the treatment of degenerative diseases or the regeneration of injured organs. However, poor cell retention is considered a major drawback associated with the short-term regenerative benefits. Furthermore, the short-term regenerative benefits are linked to paracrine factors secreted by the transplanted stem cells. To improve regenerative outcomes, researchers have identified the role of stromal cell-derived factor-1 (SDF1) as a key chemotactic factor that can facilitate site-specific migration and retention of transplanted cells, and stem or progenitor cells within the body by activating the SDF1-CXCR4 or HMGB1-SFD1-CXCR4 pathways.

Citation: Haque N, Fareez IM, Fong LF, Mandal C, Abu Kasim NH, Kacharaju KR, Soesilawati P. Role of the CXCR4-SDF1-HMGB1 pathway in the directional migration of cells and regeneration of affected organs. *World J Stem Cells* 2020; 12(9): 938-951
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/938.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.938

INTRODUCTION

Over the past decades, non-communicable diseases, especially degenerative diseases are becoming more prevalent worldwide, which also contributes to major morbidity. During the past two decades, stem cell-based regenerative therapy has been considered hopeful in addressing the unmet needs of treating degenerative diseases[1].

Among the different tools of regenerative medicine "stem cells" are considered the most promising due to their self-renewal capability and multi-differentiation potential. However, recent studies have shown that the positive outcomes of different types of stem cell-based regenerative therapies are not directly correlated to the engraftment of transplanted cells[2,3]. These findings have created a huge interest in the regenerative potential of paracrine factors and have led scientists to reveal the regenerative potential of proteins in the secretomes. Further studies have revealed the mitogenic, angiogenic, anti-apoptotic, anti-scarring and chemoattractant features of secretomes or cell culture supernatants that make them a potential tool for regenerative therapy[1,4]. Furthermore, the regenerative potential of the secretome from adult stem cells[5], freshly isolated healthy peripheral blood mononuclear cells (PBMC)[6] and apoptosis-induced PBMC[7,8] has been acknowledged by several researchers. Secretomes from stem and progenitor cells have been found to be favorable for regenerating tissues or treating several disorders including neuronal disorders[9], vascular diseases[10] and cutaneous wounds[11]. The growing evidence on the role of paracrine factors (cytokines, chemokines and growth factors) in the regeneration of affected organs has led to the introduction of cell culture supernatants or secretomes as a new therapeutic tool of regenerative medicine.

Regeneration is a complex process and several types of cells namely lymphocytes, monocytes, neutrophils, endothelial progenitor cells (EPCs), hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and tissue resident stem cells are involved in the process of regeneration. Recent studies reported that stromal cell-derived factor-1α (SDF1α) present in the secretome increases proliferation, viability, migration and homing of stem and progenitor cells; helps lymphoid tissue development and differentiation; and inhibits apoptosis of cells[4,12]. All these features are highly important for regeneration of damaged organs[13]. Furthermore, SDF1 and C-X-C-X-C

chemokine receptor type 4 (CXCR-4) play a vital role in the high mobility group box 1 (HMGB1)-mediated inflammatory cell recruitment to the site of damaged tissue which is also vital for tissue repair and regeneration[14]. Hence, in this review we have attempted to describe the role of different types of cells in regeneration while emphasizing activation of the HMGB1-SDF1-CXCR4 pathway which is considered a key pathway that regulates the directional migration of all cells and facilitates the process of regeneration.

PBMC IN REGENERATION

PBMC are widely used in preclinical research and applications in vaccine trials, source of biomarkers in various infectious and chronic diseases, and are a useful tool in studying various aspects of pathology and biology *in vitro*[15]. In addition, PBMC are an easily accessible source of different types of adult stem and progenitor cells, such as HSCs, MSCs, osteoclast precursor cells, and EPCs[16]. Due to the content of different types of adult stem cells, in a favorable microenvironment the potential to differentiate into several tissue specific cells including mature blood cells, endothelial cells, hepatocytes, cardiomyocytes, smooth muscle cells, epithelial cells, neural cells, osteoblasts, osteoclasts, and myofibroblasts has been shown[16-18]. Furthermore, compared to bone marrow (BM) or other multipotent cells sources, the isolation of PBMC is less invasive. However, a series of standard procedures for PBMC collection, isolation, cryopreservation and preparation are crucial for their use in cell-based regenerative therapy[15]. PBMC contain terminally differentiated immune cells, namely monocytes and lymphocytes that also a play vital role in tissue remodeling and regeneration[19-21].

Monocytes

Monocytes that contribute approximately 4%-10% of leukocytes in our bloodstream are highly plastic in nature[22]. Monocytes and macrophages are the largest types of white blood cells and are involved in inflammation and elimination of harmful foreign substances[23,24]. As part of the innate immunity they are involved in tissue homeostasis and facilitate wound healing by removing apoptotic and necrotic cells[24].

In regenerative tissues, macrophages are highly plastic and play a decisive role in tissue repair and regeneration[25]. In response to injury and subsequent healing, macrophages are capable of polarization towards a spectrum of phenotypes. Based on the environmental cues and molecular mediators, these cells will differentiate into either pro-inflammatory type I macrophage (M1) or anti-inflammatory type II macrophage (M2) phenotypes[25-27].

Studies have reported that M1 macrophages infiltrate tissues at the earlier stages of acute injury to promote the clearance of necrotic cells or tissue debris. Moreover, following activation M1 macrophages secrete a wide range of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-12, IL-18, IL-23, tumor necrosis factor (TNF)-α, monocyte chemoattractant protein (MCP)-1 and macrophage inflammatory protein (MIP)-1[28]. Whereas, M2 macrophages appear within the injured tissue at later stages and release high amounts of anti-inflammatory paracrine factors such as IL-10 and transforming growth factor-β (TGF-β)[25,29]. They are also capable of secreting extracellular matrix (ECM) remodeling components such as fibronectin, osteopontin, fibrin cross-linker transglutaminase and promote tissue healing[30,31]. Although there are controversies regarding the sequential presence of the two different macrophages within tissues, this is because of the dynamic shift in macrophage polarization or the recruitment of new monocytes which do not invalidate the role of macrophages in tissue regeneration.

Lymphocytes

Among the different types of lymphocytes, the regulatory T-cells (Treg) are involved in the repair and regeneration of affected tissues and organ systems. Following injury, Treg are recruited to the site to regulate inflammation and modulate the process of regeneration[32]. Following the initiation of inflammation, Treg inhibit recruitment of neutrophils by secreting IL-10 which in turn helps to minimize the secretion of inflammatory cytokines namely IL-1β, IL-6, interferon (IFN)-γ, and TNF-α. Moreover, Treg induce apoptosis of neutrophils and clear debris by activation of M1 macrophages. In addition, they play a vital role in macrophage polarization towards the M2 phenotype by secreting anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 which eventually support tissue repair and regeneration[32,33]. However, the

regenerative function of Treg follows a tissue specific manner. For instance, in skeletal muscle, Treg infiltrate the tissues in response to IL-33 following activation of the M1 population and removal of necrotic tissues by these cells. Following infiltration, Treg inhibit M1-mediated inflammation and shift the polarization of macrophages towards the M2 population[32]. Whereas, in heart tissues, recruitment of the higher number of Treg induces polarization of macrophages towards the M2 phenotype that help to inhibit inflammation, excessive matrix degradation, and adverse remodeling which eventually reduce ventricular ruptures and increases the rate of survival[34].

PERIPHERAL BLOOD POLYMORPHONUCLEAR CELLS IN REGENERATION

Neutrophils are the most abundantly found white blood cells in the human peripheral circulation and contribute approximately 50%-70% to all circulatory white blood cells. They are the first leukocyte population recruited to the site of injury and regulate the process of tissue regeneration positively or negatively[32]. However, the role of neutrophils in the process of regeneration is microenvironment-dependent and context-specific.

For example, in the case of skeletal muscle injury, neutrophils impair the restoration and function of muscles by releasing hypochlorous acid, nicotinamide adenine dinucleotide phosphate oxidase, and other cytokines[32,35,36]. Downregulation of lung regeneration following ischemia-reperfusion by neutrophils has also been reported[37]. It is noteworthy that neutrophils have also shown positive effects on the repair of lung epithelium and nerve cells[38,39].

MESENCHYMAL AND OTHER TISSUE-SPECIFIC STEM CELLS IN REGENERATION

Apart from HSCs, other adult stem cells or tissue-specific progenitor cells such as MSCs, EPCs, mammary stem cells, intestinal stem cells, and neural stem cells are found in adult tissues[40]. Tissue-specific stem cells maintain tissue homeostasis, while MSCs can differentiate into a variety of cell types. MSCs, in particular, have promising cell sources, as they can be harvested from various sources, such as BM, umbilical cord (UC), adipose tissue, and dental tissues[41-43]. Unlike embryonic stem cells (ES cells or ESCs), which are pluripotent, MSCs are multipotent cells which possess limited differentiation potential. Nevertheless, their potential to differentiate into osteoblasts and osteocytes is very well known. There is also accumulating evidence regarding their robust potential in tissue healing and regenerative medicine, in both preclinical and clinical studies[44-46]. According to a recent PubMed search conducted on November 2019, there were 110 MSC-based human clinical trials exploring the safety and efficacy of stem cells for tissue healing and the treatment of degenerative diseases. However, most of these trials were phase I and phase II, or a mixture of phase I/II studies. Whereas, phase III or phase II/III trials which investigate the long-term safety of MSC-based therapies prior to full establishment of MSCs in clinical practice are poorly documented. Thus, BM-derived MSCs (BMSCs) have been the most studied stem cells in cell therapy and tissue repair for the last 5 years, due to their multi-lineage differentiation potential[47].

However, it is worthwhile noting that different MSC populations exhibit tissue-specific characteristics such as the expression of specific cell surface markers and transcription factors. In response to injury signals, these MSCs can potentially migrate from their niche to reach target tissues through vessel walls in the peripheral circulation[48]. Many studies have been conducted to investigate both the chemical and mechanical factors that influence the homing mechanism and engraftment of MSCs into local areas of damaged sites. The chemical factors that affect the trafficking process are the presence of a variety of chemokines, growth factors and cytokines, whereas the mechanical factors involved in the process include ECM stiffness, vascular cyclic stretching and hemodynamic forces or shear stress on the vessel walls[49]. These factors make up the vital characteristics of MSCs and result in their promising effect in tissue healing and differentiation.

The first characteristic of MSCs is their multi-lineage differentiation potential. MSCs are capable of differentiating into several mesoderm lineages, including adipogenic, osteogenic, chondrogenic and myogenic lineages, depending on the multitude of

stimuli and inhibitors present in the tissue microenvironment[50]. The microenvironment plays an important role in the activation or downregulation of transcription factors that regulate the expression of genes responsible for the induction and progression of tissue-specific differentiation[51]. MSCs can also generate neural cells in the ectodermal layer, and hepatic cells and pancreatic cells in the endodermal layer[52]. The study by Chen *et al*[53] was among the first to explore the ability of MSCs to differentiate into functional islet-like cells that might play an important role in the future treatment of diabetes. MSCs cultured in stiff scaffolds can easily differentiate into osteoblasts, and showed the potential for myogenic, adipogenic and neurogenic differentiation, respectively, but with a decrease in elasticity. Recently, Jung *et al*[54] demonstrated that ECM proteins in 3D composites were able to trigger differentiation of BMSCs into mesodermal lineages with enhanced adipogenic differentiation and IL-6 expression compared to that in 2D ECM proteins.

Secondly, MSCs are capable of dynamic interactions with their microenvironment and secrete a wide variety of paracrine factors that are required for tissue recovery or wound healing. Several studies refuted the hypothesis that direct trans-differentiation or cell fusion of MSCs was the principal mechanism underlying their therapeutic action in tissue regeneration[55]. Indeed, MSCs transplantation regulated released factors in experimental models of tissue injury, which was largely associated with suppression of immune and inflammatory reactions, inhibition of apoptosis, and enhancement of cell proliferation and angiogenesis, thereby promoting regeneration of the tissue[56]. Apart of MSCs-mediated secretion of these paracrine and autocrine factors, extracellular vesicles such as exosomes and microvesicles may also regulate these functional roles[57,58]. The MSC-mediated factors released at high levels include the following: (1) Growth factors and their receptors [*i.e.*, granulocyte-macrophage colony-stimulating factor (GM-CSF), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-binding proteins (IGFBP3, IGFBP4, IGFBP7) and bone morphogenetic protein 2 (BMP-2)]; (2) Extracellular matrix remodelers/mediators [*i.e.*, periostin, fibronectin, collagen, TIMP metallopeptidase inhibitor 2 (TIMP-2), metalloproteinase inhibitors, and decorin]; and (3) Immune system signaling regulatory proteins (*i.e.*, TGF-β, MCP-1, IL-6, and IL-8)[49,59]. Various studies have demonstrated that the released pro-inflammatory cytokines up-regulate the efficacy of MSC-mediated immunomodulation and functional improvement in microvascular injury[60], inflammatory liver disease[61], osteoarthritis[62], spinal cord injury[63], brain cancer[64], ischemic limb regeneration[65], and asthmatic[66] models. Taken together, it is well accepted that the combination of MSCs with these trophic factors can modulate their behavior during inflammation and tissue injury. Research should now focus on the strategies to manipulate and modulate the secretion of these molecules in the infused or implanted MSCs microenvironment to enhance their functional role[1].

Finally, MSCs exhibit immunomodulatory properties[67-69]. The immunomodulatory properties of MSCs proved effective in treating various immune disorders in both *in vivo* and human studies. MSCs modulate the functions of almost all cells of both the innate and adaptive immune systems and induce an anti-inflammatory phenotype[59]. MSCs interact with a variety of immune cells and have the capacity to inhibit the excessive response of B cells, T cells, macrophages, dendritic cells, and natural killer cells[68]. Nevertheless, the underlying molecular and cellular mechanisms behind MSC-mediated immunomodulation have not been fully elucidated. MSCs have been shown to modulate the immune response by secreting soluble factors [*e.g.*, IL-6, M-CSF, IL-10, TGF-β, HGF, and prostaglandin E2 (PGE2)] in the presence of adhesion molecules [*i.e.*, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and lymphocyte function-associated antigen (LFA)-3][70-72]. Through a synergy of cell contact-dependent mechanisms and these soluble factors, MSCs are able to initiate the T-cell interactions that play a prominent role in their immunomodulatory potential[71,73]. Furthermore, anti-inflammatory monocytes/macrophages and Tregs are also important in MSC-mediated immunosuppression[69,74]. Studies also linked the low immunogenic properties of MSCs to the lower level of expression of major histocompatibility complex (MHC) class I antigens, and lack of MHC class II and co-stimulatory molecules such as CD80, CD86, and CD40[75,76]. Although the immunomodulatory effect of MSCs is hypothesized to be *via* MSC-secreted cytokines in many studies, most studies documented that MSCs act differently depending on the local microenvironment and the presence of inflammatory cytokines during the pre-treatment of MSCs. An understanding of the immune suppressive role of MSCs would enhance prospective clinical applications of these cells.

Thus, the fate of MSCs is vastly influenced by their environment which includes mechanical or physical stimulation, growth factors, cell density, and cell-cell

attachment or interactions. However, this multipotency of MSCs could also be due to another reason which has been widely discussed. In fact, a debate is currently ongoing regarding the 'stem cell' status of MSCs[77]. It is postulated that MSCs are purely specific adult stem cells, which contradicts findings that MSCs are a diverse mixture of many specific lineage progenitor cells. However, these shortcomings provide a good reason for the continuous research on MSCs in stem-cell based therapy.

CELL MIGRATION IS ESSENTIAL FOR TISSUE REGENERATION

Progenitors and MSCs migrate and initiate the homing mechanism in response to inflammatory signaling molecules and corresponding receptors around the injured tissue. MSCs are therapeutically capable of reaching and homing to sites of inflammation by various routes such as intravenous (IV), intra-arterial (IA), intraparenchymal, intracoronary (IC) local administration and into the subarachnoid and epidural spaces[48]. From the systemic circulation, MSCs migrate specifically to damaged tissue sites and exert their functional effects locally under a variety of pathologic conditions. Luger *et al*[78] demonstrated that intravenously administered fluorescent and radiolabeled MSCs homed to regions of myocardial injury to suppress the progressive deterioration in left ventricular function and adverse remodeling in mice, and it is thought to be a feasible and effective therapeutic strategy for the treatment of patients with large infarcts and ischemic cardiomyopathy. MSCs homing involves various chemokines and their receptors (*i.e.*, SDF1, CCL5, CXCR4, CXCR5, CXCR6, CCR2, CCR3, and CCR4), matrix metalloproteinases (MMPs) [MMP-2 and membrane type 1 MMP (MT1-MMP)], receptor tyrosine kinase dependent growth factors [*e.g.*, hepatocyte growth factor-Mesenchymal Epithelial Transition Factor (c-Met) proto-oncogene/receptor tyrosine kinase (HGF/c-Met) axes, platelet-derived growth factor (PDGF) and insulin-like growth factor 1 (IGF-1)] and some other adhesion molecules (*i.e.*, integrin β1, integrin α4, and VCAM)[79-82]. These homing signals are released by injured cells and/or respondent immune cells. Besides these homing signals, other molecules are implicated in different steps of the homing process such as PGE2 and hematopoietic cell E-/L-selectin ligand (HCELL) that are functionally involved in cell migration to the injured tissue[83]. These factors could be a feasible strategy to facilitate therapeutic delivery of MSCs to targeted injured tissue.

Of the different chemokines and chemokine-mediated pathways, the SDF1-CXCR4 and HMGB1-SDF1-CXCR4 axis have received considerable attention due to their potential in-site specific directional migration of stem and progenitor cells. The role of HMGB1-SDF1-CXCR4 in regeneration of injured tissues or organs is discussed further below.

HMGB1-SDF1-CXCR4 AXIS IN FACILITATING TISSUE-SPECIFIC MIGRATION

HMGB1 in orchestrating the process of migration and regeneration

HMGB1 protein is a highly conserved non-histone nuclear protein that binds to DNA and regulates the expression of genes and the chromosomal architecture[84]. Extracellular HMGB1 is actively secreted from activated or stressed immune cells, while passively secreted from necrotic tissues[85,86]. Following secretion into the extracellular space, HMGB1 exerts chemotactic activity or acts as a damage-associated molecular pattern molecule[87]. Indeed, the overall signaling mechanism by HMGB1 interacting with target cells needs to be elucidated for future therapeutic intervention[88].

Wound healing is a complex process that involves the ECM, cytokines, growth factors and several types of cells. The steps involved in the process of wound healing include hemostasis, inflammation, cell migration and proliferation, wound contraction, and remodeling[89,90]. During the inflammatory phase, vasodilation followed by early vasoconstriction which is mediated by histamine, leukotrienes, and prostaglandins, increases capillary permeability and cell migration into the wound site[91]. Neutrophils are the first among the infiltrated cells to the site of injury followed by monocytes and lymphocytes. Initiation of leukocyte migration is mediated by several autocrine and paracrine factors. In addition, proteases are involved in the elimination of denatured ECM components. Following infiltration into the site of injury, monocytes transform into macrophages and clear debris from the area, release

cytokines and growth factors, such as FGF, TGF-β, PDGF, and EGF that help to initiate the formation of granulation tissue[92]. HMGB1 also acts as an important chemotactic factor that regulates the directional migration of monocytes and neutrophils[93]. Following injury or inflammation, HMGB1 is released into the extracellular space and triggers the secretion of TNF, IL-1α, IL-6, and IL-8 from monocytes, macrophages and neutrophils[94-96].

Upon interaction of HMGB1 with the advanced glycation products (RAGE), toll-like receptor (TLR) 2, TLR4, and TLR9, activate pro-inflammatory responses thereby facilitating cell migration and the release of pro-inflammatory cytokines (Figure 1)[97-99]. In 2018, Xue *et al*[100] showed that the HMGB1/RAGE axis mediated migration of neural stem cells (NSCs) by the formation of filopodia which was further linked to the activation of RAGE/Rac and CDC42 or the RAGE/MAPK signaling cascade.

SDF1 in upregulation of the HMGB1-SDF1-CXCR4 axis

SDF1α, known as C-X-C motif chemokine 12 (CXCL12), is a chemotactic factor encoded by the CXCL12 gene on chromosome 10[101]. Studies have reported the therapeutic potential of the SDF1-CXCR4 axis in tissue regeneration. SDF1 is capable of activation, mobilization, homing and retention of HSCs, MSCs and several progenitor cells[80,102-104]. SDF1 is able to bind to CXCR4 and CXCR7. However, the SDF1-CXCR4 axis induces the homing process by regulating the cellular secretion and cell adhesion molecules, while SDF1-CXCR7 is involved in angiogenesis and tumor development[105].

Principally, the binding of chemokine SDF1 to the chemokine receptor CXCR4 plays an important role in homeostatic regulation of leukocyte trafficking, hematopoiesis, organogenesis, cell differentiation and tissue regeneration in response to other molecules that are involved in triggering inflammation[14,106]. The mechanism of MSCs mobilization mediated by HMGB1 is analogous to the recruitment of inflammatory cells to injured tissues for leukocyte trafficking and homing (Figure 1). As mentioned above, HMGB1 acts as a damage-associated molecular pattern which is released either from necrotic cells or by secretion from activated immune cells, hepatocytes, enterocytes, and possibly several other types of cells under distress[107]. Stress conditions that promote HMGB1 secretion include hypoxia[108], lethal irradiation[109], treatment with specific antitumor drugs[110] or through regulation of autophagy[111]. HMGB1-induced cell migration requires both IκB kinase (IKK)-β and IKKα-dependent nuclear factor-κB (NF-κB) activation. IKKβ-mediated activation of NF-κB maintains expression of RAGE, while continuous production of SDF1 is ensured by IKKα-dependent NF-κB activation[112,113]. Moreover, HMGB1 induces both physical and functional interactions between molecules that prevent the degradation of SDF1[114].

In 2012, Kew *et al*[115] proposed that the SDF1-CXCR4 axis works as a co-receptor signal for RAGE receptor-dependent HMGB1 migration responses. Furthermore, SDF1 binding to CXCR4 can also induce CXCR4-TCR heterodimerization, which in turn can enhance gene transcription, cytokine production, increased calcium ion concentrations, and could facilitate cell migration. However, it is possible that the SDF1-CXCR4 axis might have other indirect effects on the regulation of cell migration such as enhancing HMGB1 binding to RAGE, which require further investigation.

TLR and RAGE dependent or independent activation of the HMGB1-SDF1-CXCR4 axis

There is ample evidence of the capability of stem cells to regulate numerous growth factors, cytokines and chemokines. Chemokines, specifically, regulate cell locomotion and integrin function by binding to seven transmembrane domain receptors coupled to G-protein-coupled receptors (GPCR)s, which are heterotrimeric GTP-binding proteins, that are differentially expressed in various cell types[48,107]. In addition, there is always a need to assess the consequences of the combined activity of these chemokines and other inflammatory molecules to control appropriate tissue distribution of distinct leukocyte subsets under normal and pathological conditions. One of the interesting insights in stem cell research is the effect of such paracrine factors in the HMGB1-SDF1-CXCR4 signaling pathway during tissue regeneration.

Extracellular HMGB1 can interact with different molecules to dictate their biologic effects. The role of HMGB1 as a chemokine or cytokine is determined by its oxidative state (Figure 1). The role of extracellular HMGB1 to promote cell migration was first reported in smooth muscle cells in 2010[116]. Similar involvement was also reported in different cell types in the same year by Rauvala and Rouhiainen[117]. Studies showed that HMGB1-induced cell migration requires the formation of a heterocomplex with

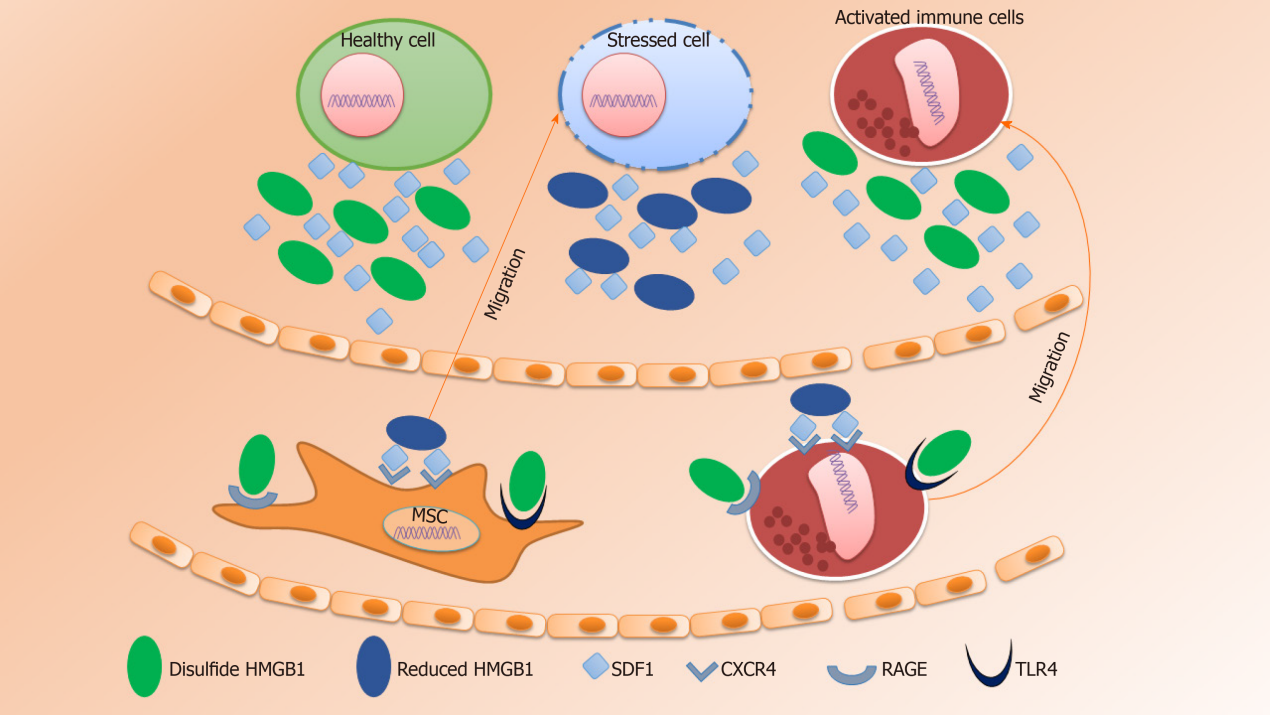


Figure 1 Schematic diagram of the dynamic HMGB1–CXCL12–SDF1 axis for accelerated tissue regeneration. Disulfide (oxidized) HMGB1 usually binds to RAGE and TLR and regulates the expression of genes with pro- or anti-inflammatory properties and partial chemotactic properties. Whereas, fully reduced HMGB1 released from necrotic or stressed cells forms a heterocomplex with SDF1 secreted from activated immune cells or from cells within the injured tissues. Later, this heterocomplex binds to the CXCR4 receptors on the cells and facilitates site-specific migration[123-125]. MSC: Mesenchymal stem cells.

SDF1 and further binding with CXCR4, and not with RAGE, TLR2, or TLR4. Furthermore, it was also reported that HMGB1 does not affect migration by other chemokines such as CXCL8, CCL2, CCL7, CCL19, and CCL21[14].

The bonding chemistry between SDF1 and HMGB1 was analyzed by NMR chemical shift mapping and revealed that most of the amino acids present in SDF1 have the ability to bind HMGB1 or its individual HMG boxes[14]. Each HMGB1 molecule has two HMG boxes and thereby can attach two SDF1 molecules at a time. Interestingly, the first few N-terminal residues of the SDF1 molecule do not attach to the HMGB1 molecule and remain free. These free residues can access deep inside the CXCR4 transmembrane domain to initiate signaling cascades[118]. As the HMGB1-SDF1 heterocomplex can present two SDF1 ligand molecules to dimers of the CXCR4 receptor, this heterocomplex would be more efficient than SDF1 alone in inducing cellular migration[119]. Alternatively, the HMGB1-SDF1 heterocomplex may help to unlock the CXCR4 binding site to promote SDF1 binding, or help lock in SDF1 into the CXCR4 transmembrane domain by providing direct HMGB1-CXCR4 contacts.

HMGB1 induces changes in SDF1 residues that are responsible for the activation of CXCR4, the SDF1 receptor. An analysis using fluorescence resonance energy transfer (FRET) demonstrated that there are different conformational rearrangements of CXCR4 homodimers triggered by SDF1 alone or in complex with HMGB1[14]. It has also been hypothesized that the formation of a heterocomplex between HMGB1 and SDF1 acts through CXCR4 which promotes the recruitment of monocytes to the injury site[14]. The interaction of locally produced SDF1 and its receptor CXCR4 expressed on the surface of MSCs plays an important role in the homing of transplanted cells. The binding of SDF1 to both CXCR4 and CXCR7 is also responsible for the production of paracrine mediators, including VEGF, IGF-1, β-FGF and HGF that exert mitogenic, pro-angiogenic, anti-apoptotic, and anti-inflammatory effects[120]. Hypoxia has been shown to enhance the expression of both SDF1 receptors, CXCR4 and CXCR7, in MSCs. Liu *et al*[121] demonstrated that SDF1α is upregulated in ischemic kidneys during reduced oxygen tension. Hypoxia induces expression of CXCR4 and CXCR7 while promoting the role of both SDF1 receptors for enhanced migration, adhesion and survival of hypoxia preconditioned (HP)-MSCs and thus improves homing of systemically delivered MSCs to the ischemic kidney. In addition, in normal culture-expanded MSCs, CXCR4 expression will alleviate progressively and thus could affect its ability to migrate toward the SDF1 gradient in the ischemic tissue.

The intracellular signaling cascades have not yet been clearly demonstrated. In the case of SDF1-CXCR4 bonding, activation and coordination of focal adhesion kinase (FAK) and phosphoinositide 3-kinase (PI3K) were reported in the migration of human dental pulp stem cells[122]. Subsequently, increased β-catenin expression by phosphorylation of protein kinase B (Akt) at ser473 that inhibits the activation of glycogen synthase kinase 3 beta (GSK3β) was also reported. All these results indicate that the SDF1-CXCR4 axis activates the FAK/PI3K/Akt and GSK3β/β-catenin pathways that could facilitate the migration of human dental pulp stem cells. Whereas in the HMGB1-SDF1-CXCR4 axis, elevated extracellular signal-regulated kinase (ERK) phosphorylation and Ca^{2+} release from stores were reported[14]. Elevated ERK phosphorylation was observed in the presence of the SDF1-HMGB1 heterocomplex but not observed in the presence of SDF1 and HMGB1 alone. In the presence of HMGB1 a suboptimal SDF1 concentration was reported with a rapid increase in intracellular Ca^{2+}.

CONCLUSION

Until now, the migration and retention of transplanted cells have been considered a major drawback of cell-based regenerative therapy. SDF1 and its receptor CXCR4 play an important role in maintaining homeostasis by facilitating the homing of progenitor or other adult multipotent stem cells in the BM and regulating their mobilization into peripheral tissues during injury or stress. Studies have shown the potential of the SDF1-CXCR4 axis and/or HMGB1-SDF1-CXCR4 signaling pathways in regulating the process of directional migration followed by retention which are vital for the regeneration of injured tissues or organs. In addition, these pathways could play a major role in regulating the inflammatory conditions at the site of injury. Further studies concentrating on these pathways could make cell-based regenerative therapy more efficient and fruitful.

REFERENCES

1 **Haque N**, Abdullah BJJ, BJJ, Kasim NHA. Secretome: Pharmaceuticals for Cell-Free Regenerative Therapy. In: Pham PV. Stem Cell Drugs - A New Generation of Biopharmaceuticals. Cham: Springer International Publishing; 2018: 17-35 [DOI: 10.1007/978-3-319-99328-7_2]

2 **Crivelli B**, Chlapanidas T, Perteghella S, Lucarelli E, Pascucci L, Brini AT, Ferrero I, Marazzi M, Pessina A, Torre ML; Italian Mesenchymal Stem Cell Group (GISM). Mesenchymal stem/stromal cell extracellular vesicles: From active principle to next generation drug delivery system. *J Control Release* 2017; **262**: 104-117 [PMID: 28736264 DOI: 10.1016/j.jconrel.2017.07.023]

3 **Boido M**, Ghibaudi M, Gentile P, Favaro E, Fusaro R, Tonda-Turo C. Chitosan-based hydrogel to support the paracrine activity of mesenchymal stem cells in spinal cord injury treatment. *Sci Rep* 2019; **9**: 6402 [PMID: 31024032 DOI: 10.1038/s41598-019-42848-w]

4 **Haque N**, Kasim NHA, Kassim NLA, Rahman MT. Autologous serum supplement favours in vitro regenerative paracrine factors synthesis. *Cell Prolif* 2017; **50**: e12354 [PMID: 28682474 DOI: 10.1111/cpr.12354]

5 **Madrigal M**, Rao KS, Riordan NH. A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods. *J Transl Med* 2014; **12**: 260 [PMID: 25304688 DOI: 10.1186/s12967-014-0260-8]

6 **Hoetzenecker K**, Zimmermann M, Hoetzenecker W, Schweiger T, Kollmann D, Mildner M, Hegedus B, Mitterbauer A, Hacker S, Birner P, Gabriel C, Gyöngyösi M, Blyszczuk P, Eriksson U, Ankersmit HJ. Mononuclear cell secretome protects from experimental autoimmune myocarditis. *Eur Heart J* 2015; **36**: 676-685 [PMID: 23321350 DOI: 10.1093/eurheartj/ehs459]

7 **Hoetzenecker K**, Assinger A, Lichtenauer M, Mildner M, Schweiger T, Starlinger P, Jakab A, Berényi E, Pavo N, Zimmermann M, Gabriel C, Plass C, Gyöngyösi M, Volf I, Ankersmit HJ. Secretome of apoptotic peripheral blood cells (APOSEC) attenuates microvascular obstruction in a porcine closed chest reperfused acute myocardial infarction model: role of platelet aggregation and vasodilation. *Basic Res Cardiol* 2012; **107**: 292 [PMID: 22899170 DOI: 10.1007/s00395-012-0292-2]

8 **Altmann P**, Mildner M, Haider T, Traxler D, Beer L, Ristl R, Golabi B, Gabriel C, Leutmezer F, Ankersmit HJ. Secretomes of apoptotic mononuclear cells ameliorate neurological damage in rats with focal ischemia. *F1000Res* 2014; **3**: 131 [PMID: 25383184 DOI: 10.12688/f1000research.4219.2]

9 **Pires AO**, Neves-Carvalho A, Sousa N, Salgado AJ. The Secretome of Bone Marrow and Wharton Jelly Derived Mesenchymal Stem Cells Induces Differentiation and Neurite Outgrowth in SH-SY5Y Cells. *Stem Cells Int* 2014; **2014**: 438352 [PMID: 25132857 DOI: 10.1155/2014/438352]

10 **Dao M**, Tate CC, McGrogan M, Case CC. Comparing the angiogenic potency of naïve marrow stromal cells and Notch-transfected marrow stromal cells. *J Transl Med* 2013; **11**: 81 [PMID: 23531336 DOI: 10.1186/1479-5876-11-81]

11 **Mildner M**, Hacker S, Haider T, Gschwandtner M, Werba G, Barresi C, Zimmermann M, Golabi B, Tschachler E, Ankersmit HJ. Secretome of peripheral blood mononuclear cells enhances wound healing.

PLoS One 2013; **8**: e60103 [PMID: 23533667 DOI: 10.1371/journal.pone.0060103]
12 **Zhang M**, Mal N, Kiedrowski M, Chacko M, Askari AT, Popovic ZB, Koc ON, Penn MS. SDF-1 expression by mesenchymal stem cells results in trophic support of cardiac myocytes after myocardial infarction. *FASEB J* 2007; **21**: 3197-3207 [PMID: 17496162 DOI: 10.1096/fj.06-6558com]
13 **Abduelmula A**, Huang R, Pu Q, Tamamura H, Morosan-Puopolo G, Brand-Saberi B. SDF-1 controls the muscle and blood vessel formation of the somite. *Int J Dev Biol* 2016; **60**: 29-38 [PMID: 26934287 DOI: 10.1387/ijdb.150132rh]
14 **Schiraldi M**, Raucci A, Muñoz LM, Livoti E, Celona B, Venereau E, Apuzzo T, De Marchis F, Pedotti M, Bachi A, Thelen M, Varani L, Mellado M, Proudfoot A, Bianchi ME, Uguccioni M. HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. *J Exp Med* 2012; **209**: 551-563 [PMID: 22370717 DOI: 10.1084/jem.20111739]
15 **Betsou F**, Gaignaux A, Ammerlaan W, Norris PJ, Stone M. Biospecimen Science of Blood for Peripheral Blood Mononuclear Cell (PBMC) Functional Applications. *Curr Pathobiol Rep* 2019; **7**: 17-27 [DOI: 10.1007/s40139-019-00192-8]
16 **Zhang M**, Huang B. The multi-differentiation potential of peripheral blood mononuclear cells. *Stem Cell Res Ther* 2012; **3**: 48 [PMID: 23200034 DOI: 10.1186/scrt139]
17 **Hopper N**, Wardale J, Brooks R, Power J, Rushton N, Henson F. Peripheral Blood Mononuclear Cells Enhance Cartilage Repair in in vivo Osteochondral Defect Model. *PLoS One* 2015; **10**: e0133937 [PMID: 26252391 DOI: 10.1371/journal.pone.0133937]
18 **Xian B**, Zhang Y, Peng Y, Huang J, Li W, Wang W, Zhang M, Li K, Zhang H, Zhao M, Liu X, Huang B. Adult Human Peripheral Blood Mononuclear Cells Are Capable of Producing Neurocyte or Photoreceptor-Like Cells That Survive in Mouse Eyes After Preinduction With Neonatal Retina. *Stem Cells Transl Med* 2016; **5**: 1515-1524 [PMID: 27458266 DOI: 10.5966/sctm.2015-0395]
19 **Wang X**, Balaji S, Steen EH, Li H, Rae MM, Blum AJ, Miao Q, Butte MJ, Bollyky PL, Keswani SG. T Lymphocytes Attenuate Dermal Scarring by Regulating Inflammation, Neovascularization, and Extracellular Matrix Remodeling. *Adv Wound Care (New Rochelle)* 2019; **8**: 527-537 [PMID: 31637099 DOI: 10.1089/wound.2019.0981]
20 **Wynn TA**, Vannella KM. Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* 2016; **44**: 450-462 [PMID: 26982353 DOI: 10.1016/j.immuni.2016.02.015]
21 **Ogle ME**, Segar CE, Sridhar S, Botchwey EA. Monocytes and macrophages in tissue repair: Implications for immunoregenerative biomaterial design. *Exp Biol Med (Maywood)* 2016; **241**: 1084-1097 [PMID: 27229903 DOI: 10.1177/1535370216650293]
22 **Yang J**, Zhang L, Yu C, Yang XF, Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. *Biomark Res* 2014; **2**: 1 [PMID: 24398220 DOI: 10.1186/2050-7771-2-1]
23 **Chiu S**, Bharat A. Role of monocytes and macrophages in regulating immune response following lung transplantation. *Curr Opin Organ Transplant* 2016; **21**: 239-245 [PMID: 26977996 DOI: 10.1097/MOT.0000000000000313]
24 **Gordon S**, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005; **5**: 953-964 [PMID: 16322748 DOI: 10.1038/nri1733]
25 **Atri C**, Guerfali FZ, Laouini D. Role of Human Macrophage Polarization in Inflammation during Infectious Diseases. *Int J Mol Sci* 2018; **19**: 1801 [PMID: 29921749 DOI: 10.3390/ijms19061801]
26 **Mosser DM**, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008; **8**: 958-969 [PMID: 19029990 DOI: 10.1038/nri2448]
27 **Gu B**, Kaneko T, Zaw SYM, Sone PP, Murano H, Sueyama Y, Zaw ZCT, Okiji T. Macrophage populations show an M1-to-M2 transition in an experimental model of coronal pulp tissue engineering with mesenchymal stem cells. *Int Endod J* 2019; **52**: 504-514 [PMID: 30387178 DOI: 10.1111/iej.13033]
28 **McWhorter FY**, Davis CT, Liu WF. Physical and mechanical regulation of macrophage phenotype and function. *Cell Mol Life Sci* 2015; **72**: 1303-1316 [PMID: 25504084 DOI: 10.1007/s00018-014-1796-8]
29 **Rigamonti E**, Zordan P, Sciorati C, Rovere-Querini P, Brunelli S. Macrophage plasticity in skeletal muscle repair. *Biomed Res Int* 2014; **2014**: 560629 [PMID: 24860823 DOI: 10.1155/2014/560629]
30 **Gratchev A**, Kzhyshkowska J, Köthe K, Muller-Molinet I, Kannookadan S, Utikal J, Goerdt S. Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. *Immunobiology* 2006; **211**: 473-486 [PMID: 16920487 DOI: 10.1016/j.imbio.2006.05.017]
31 **Krzyszczyk P**, Schloss R, Palmer A, Berthiaume F. The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes. *Front Physiol* 2018; **9**: 419 [PMID: 29765329 DOI: 10.3389/fphys.2018.00419]
32 **Li J**, Tan J, Martino MM, Lui KO. Regulatory T-Cells: Potential Regulator of Tissue Repair and Regeneration. *Front Immunol* 2018; **9**: 585 [PMID: 29662491 DOI: 10.3389/fimmu.2018.00585]
33 **Tiemessen MM**, Jagger AL, Evans HG, van Herwijnen MJ, John S, Taams LS. CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc Natl Acad Sci USA* 2007; **104**: 19446-19451 [PMID: 18042719 DOI: 10.1073/pnas.0706832104]
34 **Weirather J**, Hofmann UD, Beyersdorf N, Ramos GC, Vogel B, Frey A, Ertl G, Kerkau T, Frantz S. Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res* 2014; **115**: 55-67 [PMID: 24786398 DOI: 10.1161/CIRCRESAHA.115.303895]
35 **Toumi H**, F'guyer S, Best TM. The role of neutrophils in injury and repair following muscle stretch. *J Anat* 2006; **208**: 459-470 [PMID: 16637872 DOI: 10.1111/j.1469-7580.2006.00543.x]
36 **Pizza FX**, Peterson JM, Baas JH, Koh TJ. Neutrophils contribute to muscle injury and impair its resolution after lengthening contractions in mice. *J Physiol* 2005; **562**: 899-913 [PMID: 15550464 DOI: 10.1113/jphysiol.2004.073965]
37 **Yang Z**, Sharma AK, Linden J, Kron IL, Laubach VE. CD4+ T lymphocytes mediate acute pulmonary ischemia-reperfusion injury. *J Thorac Cardiovasc Surg* 2009; **137**: 695-702; discussion 702 [PMID: 19258091 DOI: 10.1016/j.jtcvs.2008.10.044]
38 **Lindborg JA**, Mack M, Zigmond RE. Neutrophils Are Critical for Myelin Removal in a Peripheral Nerve

Injury Model of Wallerian Degeneration. *J Neurosci* 2017; **37**: 10258-10277 [PMID: 28912156 DOI: 10.1523/JNEUROSCI.2085-17.2017]
39 **Zemans RL**, Briones N, Campbell M, McClendon J, Young SK, Suzuki T, Yang IV, De Langhe S, Reynolds SD, Mason RJ, Kahn M, Henson PM, Colgan SP, Downey GP. Neutrophil transmigration triggers repair of the lung epithelium via beta-catenin signaling. *Proc Natl Acad Sci USA* 2011; **108**: 15990-15995 [PMID: 21880956 DOI: 10.1073/pnas.1110144108]
40 **Saez B**, Yusuf RZ, Scadden DT. Harnessing the Biology of Stem Cells' Niche. Biology and Engineering of Stem Cell Niches: Elsevier; 2017: 15-31 [DOI: 10.1016/B978-0-12-802734-9.00002-0]
41 **Mushahary D**, Spittler A, Kasper C, Weber V, Charwat V. Isolation, cultivation, and characterization of human mesenchymal stem cells. *Cytometry A* 2018; **93**: 19-31 [PMID: 29072818 DOI: 10.1002/cyto.a.23242]
42 **Haque N**, Abu Kasim NH. Pooled Human Serum Increases Regenerative Potential of In Vitro Expanded Stem Cells from Human Extracted Deciduous Teeth. *Adv Exp Med Biol* 2018; **1083**: 29-44 [PMID: 28730381 DOI: 10.1007/5584_2017_74]
43 **Haque N**, Widera D, Abu Kasim NH. Stem Cells from Human Extracted Deciduous Teeth Expanded in Foetal Bovine and Human Sera Express Different Paracrine Factors After Exposure to Freshly Prepared Human Serum. *Adv Exp Med Biol* 2019; **1084**: 175-186 [PMID: 30771186 DOI: 10.1007/5584_2018_299]
44 **Grayson WL**, Bunnell BA, Martin E, Frazier T, Hung BP, Gimble JM. Stromal cells and stem cells in clinical bone regeneration. *Nat Rev Endocrinol* 2015; **11**: 140-150 [PMID: 25560703 DOI: 10.1038/nrendo.2014.234]
45 **Trounson A**, McDonald C. Stem Cell Therapies in Clinical Trials: Progress and Challenges. *Cell Stem Cell* 2015; **17**: 11-22 [PMID: 26140604 DOI: 10.1016/j.stem.2015.06.007]
46 **Gu W**, Hong X, Potter C, Qu A, Xu Q. Mesenchymal stem cells and vascular regeneration. *Microcirculation* 2017; 24 [PMID: 27681821 DOI: 10.1111/micc.12324]
47 **Shi Z**, Wang Q, Jiang D. Extracellular vesicles from bone marrow-derived multipotent mesenchymal stromal cells regulate inflammation and enhance tendon healing. *J Transl Med* 2019; **17**: 211 [PMID: 31238964 DOI: 10.1186/s12967-019-1960-x]
48 **Nitzsche F**, Müller C, Lukomska B, Jolkkonen J, Deten A, Boltze J. Concise Review: MSC Adhesion Cascade-Insights into Homing and Transendothelial Migration. *Stem Cells* 2017; **35**: 1446-1460 [PMID: 28316123 DOI: 10.1002/stem.2614]
49 **Fu X**, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal Stem Cell Migration and Tissue Repair. *Cells* 2019; **8**: 784 [PMID: 31357692 DOI: 10.3390/cells8080784]
50 **Visweswaran M**, Pohl S, Arfuso F, Newsholme P, Dilley R, Pervaiz S, Dharmarajan A. Multi-lineage differentiation of mesenchymal stem cells - To Wnt, or not Wnt. *Int J Biochem Cell Biol* 2015; **68**: 139-147 [PMID: 26410622 DOI: 10.1016/j.biocel.2015.09.008]
51 **Almalki SG**, Llamas Valle Y, Agrawal DK. MMP-2 and MMP-14 Silencing Inhibits VEGFR2 Cleavage and Induces the Differentiation of Porcine Adipose-Derived Mesenchymal Stem Cells to Endothelial Cells. *Stem Cells Transl Med* 2017; **6**: 1385-1398 [PMID: 28213979 DOI: 10.1002/sctm.16-0329]
52 **Zhang Y**, Khan D, Delling J, Tobiasch E. Mechanisms underlying the osteo- and adipo-differentiation of human mesenchymal stem cells. *ScientificWorldJournal* 2012; **2012**: 793823 [PMID: 22500143 DOI: 10.1100/2012/793823]
53 **Chen LB**, Jiang XB, Yang L. Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells. *World J Gastroenterol* 2004; **10**: 3016-3020 [PMID: 15378785 DOI: 10.3748/wjg.v10.i20.3016]
54 **Jung JP**, Bache-Wiig MK, Provenzano PP, Ogle BM. Heterogeneous Differentiation of Human Mesenchymal Stem Cells in 3D Extracellular Matrix Composites. *Biores Open Access* 2016; **5**: 37-48 [PMID: 26862471 DOI: 10.1089/biores.2015.0044]
55 **Gruh I**, Martin U. Transdifferentiation of stem cells: a critical view. *Adv Biochem Eng Biotechnol* 2009; **114**: 73-106 [PMID: 19343303 DOI: 10.1007/10_2008_49]
56 **Bassi ÊJ**, de Almeida DC, Moraes-Vieira PM, Câmara NO. Exploring the role of soluble factors associated with immune regulatory properties of mesenchymal stem cells. *Stem Cell Rev Rep* 2012; **8**: 329-342 [PMID: 21881832 DOI: 10.1007/s12015-011-9311-1]
57 **Merino-González C**, Zuñiga FA, Escudero C, Ormazabal V, Reyes C, Nova-Lamperti E, Salomón C, Aguayo C. Mesenchymal Stem Cell-Derived Extracellular Vesicles Promote Angiogenesis: Potencial Clinical Application. *Front Physiol* 2016; **7**: 24 [PMID: 26903875 DOI: 10.3389/fphys.2016.00024]
58 **Lee Y**, El Andaloussi S, Wood MJ. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. *Hum Mol Genet* 2012; **21**: R125-R134 [PMID: 22872698 DOI: 10.1093/hmg/dds317]
59 **Haque N**, Khan IM, Abu Kasim NH. Survival and immunomodulation of stem cells from human extracted deciduous teeth expanded in pooled human and foetal bovine sera. *Cytokine* 2019; **120**: 144-154 [PMID: 31071675 DOI: 10.1016/j.cyto.2019.04.018]
60 **Chang PY**, Zhang BY, Cui S, Qu C, Shao LH, Xu TK, Qu YQ, Dong LH, Wang J. MSC-derived cytokines repair radiation-induced intra-villi microvascular injury. *Oncotarget* 2017; **8**: 87821-87836 [PMID: 29152123 DOI: 10.18632/oncotarget.21236]
61 **de Witte SFH**, Merino AM, Franquesa M, Strini T, van Zoggel JAA, Korevaar SS, Luk F, Gargesha M, O'Flynn L, Roy D, Elliman SJ, Newsome PN, Baan CC, Hoogduijn MJ. Cytokine treatment optimises the immunotherapeutic effects of umbilical cord-derived MSC for treatment of inflammatory liver disease. *Stem Cell Res Ther* 2017; **8**: 140 [PMID: 28595619 DOI: 10.1186/s13287-017-0590-6]
62 **Toh WS**, Lai RC, Hui JHP, Lim SK. MSC exosome as a cell-free MSC therapy for cartilage regeneration: Implications for osteoarthritis treatment. *Semin Cell Dev Biol* 2017; **67**: 56-64 [PMID: 27871993 DOI: 10.1016/j.semcdb.2016.11.008]
63 **Cantinieaux D**, Quertainmont R, Blacher S, Rossi L, Wanet T, Noël A, Brook G, Schoenen J, Franzen R. Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats: an original strategy to avoid cell transplantation. *PLoS One* 2013; **8**: e69515 [PMID: 24013448 DOI: 10.1371/journal.pone.0069515]
64 **Motaln H**, Turnsek TL. Cytokines play a key role in communication between mesenchymal stem cells and

brain cancer cells. *Protein Pept Lett* 2015; **22**: 322-331 [PMID: 25642990 DOI: 10.2174/0929866522666150131123808]

65 **Bhang SH**, Cho SW, Lim JM, Kang JM, Lee TJ, Yang HS, Song YS, Park MH, Kim HS, Yoo KJ, Jang Y, Langer R, Anderson DG, Kim BS. Locally delivered growth factor enhances the angiogenic efficacy of adipose-derived stromal cells transplanted to ischemic limbs. *Stem Cells* 2009; **27**: 1976-1986 [PMID: 19544425 DOI: 10.1002/stem.115]

66 **Zhang LB**, He M. Effect of mesenchymal stromal (stem) cell (MSC) transplantation in asthmatic animal models: A systematic review and meta-analysis. *Pulm Pharmacol Ther* 2019; **54**: 39-52 [PMID: 30496803 DOI: 10.1016/j.pupt.2018.11.007]

67 **Yaochite JN**, de Lima KW, Caliari-Oliveira C, Palma PV, Couri CE, Simões BP, Covas DT, Voltarelli JC, Oliveira MC, Donadi EA, Malmegrim KC. Multipotent mesenchymal stromal cells from patients with newly diagnosed type 1 diabetes mellitus exhibit preserved in vitro and in vivo immunomodulatory properties. *Stem Cell Res Ther* 2016; **7**: 14 [PMID: 26781648 DOI: 10.1186/s13287-015-0261-4]

68 **Palomares Cabeza V**, Hoogduijn MJ, Kraaijeveld R, Franquesa M, Witte-Bouma J, Wolvius EB, Farrell E, Brama PAJ. Pediatric Mesenchymal Stem Cells Exhibit Immunomodulatory Properties Toward Allogeneic T and B Cells Under Inflammatory Conditions. *Front Bioeng Biotechnol* 2019; **7**: 142 [PMID: 31245368 DOI: 10.3389/fbioe.2019.00142]

69 **Haque N**, Ramasamy TS, Kasim NHA. Mechanisms of Mesenchymal Stem Cells for Autoimmune Disease Treatment. In: Pham PV. Stem Cell Transplantation for Autoimmune Diseases and Inflammation. Cham: Springer International Publishing; 2019: 27-44 [DOI: 10.1007/978-3-030-23421-8_2]

70 **Chen X**, Wang S, Cao W. Mesenchymal stem cell-mediated immunomodulation in cell therapy of neurodegenerative diseases. *Cell Immunol* 2018; **326**: 8-14 [PMID: 28778534 DOI: 10.1016/j.cellimm.2017.06.006]

71 **Gebler A**, Zabel O, Seliger B. The immunomodulatory capacity of mesenchymal stem cells. *Trends Mol Med* 2012; **18**: 128-134 [PMID: 22118960 DOI: 10.1016/j.molmed.2011.10.004]

72 **Coulson-Thomas VJ**, Coulson-Thomas YM, Gesteira TF, Kao WW. Extrinsic and Intrinsic Mechanisms by Which Mesenchymal Stem Cells Suppress the Immune System. *Ocul Surf* 2016; **14**: 121-134 [PMID: 26804815 DOI: 10.1016/j.jtos.2015.11.004]

73 **Najar M**, Raicevic G, Fayyad-Kazan H, Bron D, Toungouz M, Lagneaux L. Mesenchymal stromal cells and immunomodulation: A gathering of regulatory immune cells. *Cytotherapy* 2016; **18**: 160-171 [PMID: 26794710 DOI: 10.1016/j.jcyt.2015.10.011]

74 **Dazzi F**, Lopes L, Weng L. Mesenchymal stromal cells: a key player in 'innate tolerance'? *Immunology* 2012; **137**: 206-213 [PMID: 22804624 DOI: 10.1111/j.1365-2567.2012.03621.x]

75 **Krampera M**, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood* 2003; **101**: 3722-3729 [PMID: 12506037 DOI: 10.1182/blood-2002-07-2104]

76 **Romieu-Mourez R**, François M, Boivin MN, Stagg J, Galipeau J. Regulation of MHC class II expression and antigen processing in murine and human mesenchymal stromal cells by IFN-gamma, TGF-beta, and cell density. *J Immunol* 2007; **179**: 1549-1558 [PMID: 17641021 DOI: 10.4049/jimmunol.179.3.1549]

77 **Bhartiya D**. The need to revisit the definition of mesenchymal and adult stem cells based on their functional attributes. *Stem Cell Res Ther* 2018; **9**: 78 [PMID: 29587828 DOI: 10.1186/s13287-018-0833-1]

78 **Luger D**, Lipinski MJ, Westman PC, Glover DK, Dimastromatteo J, Frias JC, Albelda MT, Sikora S, Kharazi A, Vertelov G, Waksman R, Epstein SE. Intravenously Delivered Mesenchymal Stem Cells: Systemic Anti-Inflammatory Effects Improve Left Ventricular Dysfunction in Acute Myocardial Infarction and Ischemic Cardiomyopathy. *Circ Res* 2017; **120**: 1598-1613 [PMID: 28232595 DOI: 10.1161/CIRCRESAHA.117.310599]

79 **Chen W**, Zhou D, Li YR, Liu JX, Wang XM, Zhu F, Xu KL. [Effect of Lentiviral Vector-Mediated CXCR4 Gene Overexpression on Mesenchymal Stem Cell Homing Capacity]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2018; **26**: 1543-1547 [PMID: 30295281 DOI: 10.7534/j.issn.1009-2137.2018.05.047]

80 **Jin W**, Liang X, Brooks A, Futrega K, Liu X, Doran MR, Simpson MJ, Roberts MS, Wang H. Modelling of the SDF-1/CXCR4 regulated *in vivo* homing of therapeutic mesenchymal stem/stromal cells in mice. *PeerJ* 2018; **6**: e6072 [PMID: 30564525 DOI: 10.7717/peerj.6072]

81 **Sordi V**. Mesenchymal stem cell homing capacity. *Transplantation* 2009; **87**: S42-S45 [PMID: 19424004 DOI: 10.1097/TP.0b013e3181a28533]

82 **Hocking AM**. The Role of Chemokines in Mesenchymal Stem Cell Homing to Wounds. *Adv Wound Care (New Rochelle)* 2015; **4**: 623-630 [PMID: 26543676 DOI: 10.1089/wound.2014.0579]

83 **Sackstein R**. The biology of CD44 and HCELL in hematopoiesis: the 'step 2-bypass pathway' and other emerging perspectives. *Curr Opin Hematol* 2011; **18**: 239-248 [PMID: 21546828 DOI: 10.1097/MOH.0b013e3283476140]

84 **Martinotti S**, Patrone M, Ranzato E. Emerging roles for HMGB1 protein in immunity, inflammation, and cancer. *Immunotargets Ther* 2015; **4**: 101-109 [PMID: 27471716 DOI: 10.2147/ITT.S58064]

85 **Yang H**, Wang H, Chavan SS, Andersson U. High Mobility Group Box Protein 1 (HMGB1): The Prototypical Endogenous Danger Molecule. *Mol Med* 2015; **21** Suppl 1: S6-S12 [PMID: 26605648 DOI: 10.2119/molmed.2015.00087]

86 **Raucci A**, Di Maggio S, Scavello F, D'Ambrosio A, Bianchi ME, Capogrossi MC. The Janus face of HMGB1 in heart disease: a necessary update. *Cell Mol Life Sci* 2019; **76**: 211-229 [PMID: 30306212 DOI: 10.1007/s00018-018-2930-9]

87 **Kohno T**, Anzai T, Naito K, Miyasho T, Okamoto M, Yokota H, Yamada S, Maekawa Y, Takahashi T, Yoshikawa T, Ishizaka A, Ogawa S. Role of high-mobility group box 1 protein in post-infarction healing process and left ventricular remodelling. *Cardiovasc Res* 2009; **81**: 565-573 [PMID: 18984601 DOI: 10.1093/cvr/cvn291]

88 **Andersson U**, Erlandsson-Harris H, Yang H, Tracey KJ. HMGB1 as a DNA-binding cytokine. *J Leukoc Biol* 2002; **72**: 1084-1091 [PMID: 12488489]

89 **Frangogiannis NG**. Fibroblast-Extracellular Matrix Interactions in Tissue Fibrosis. *Curr Pathobiol Rep* 2016; **4**: 11-18 [PMID: 27171595 DOI: 10.1007/s40139-016-0099-1]

90 **Klingberg F**, Hinz B, White ES. The myofibroblast matrix: implications for tissue repair and fibrosis. *J Pathol* 2013; **229**: 298-309 [PMID: 22996908 DOI: 10.1002/path.4104]
91 **Zaja-Milatovic S**, Richmond A. CXC chemokines and their receptors: a case for a significant biological role in cutaneous wound healing. *Histol Histopathol* 2008; **23**: 1399-1407 [PMID: 18785122 DOI: 10.14670/HH-23.1399]
92 **Behm B**, Babilas P, Landthaler M, Schreml S. Cytokines, chemokines and growth factors in wound healing. *J Eur Acad Dermatol Venereol* 2012; **26**: 812-820 [PMID: 22211801 DOI: 10.1111/j.1468-3083.2011.04415.x]
93 **Wang H**, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999; **285**: 248-251 [PMID: 10398600 DOI: 10.1126/science.285.5425.248]
94 **Andersson U**, Wang H, Palmblad K, Aveberger AC, Bloom O, Erlandsson-Harris H, Janson A, Kokkola R, Zhang M, Yang H, Tracey KJ. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. *J Exp Med* 2000; **192**: 565-570 [PMID: 10952726 DOI: 10.1084/jem.192.4.565]
95 **Kokkola R**, Andersson A, Mullins G, Ostberg T, Treutiger CJ, Arnold B, Nawroth P, Andersson U, Harris RA, Harris HE. RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. *Scand J Immunol* 2005; **61**: 1-9 [PMID: 15644117 DOI: 10.1111/j.0300-9475.2005.01534.x]
96 **Park JS**, Arcaroli J, Yum HK, Yang H, Wang H, Yang KY, Choe KH, Strassheim D, Pitts TM, Tracey KJ, Abraham E. Activation of gene expression in human neutrophils by high mobility group box 1 protein. *Am J Physiol Cell Physiol* 2003; **284**: C870-C879 [PMID: 12620891 DOI: 10.1152/ajpcell.00322.2002]
97 **Palumbo R**, De Marchis F, Pusterla T, Conti A, Alessio M, Bianchi ME. Src family kinases are necessary for cell migration induced by extracellular HMGB1. *J Leukoc Biol* 2009; **86**: 617-623 [PMID: 19401391 DOI: 10.1189/jlb.0908581]
98 **Palumbo R**, Galvez BG, Pusterla T, De Marchis F, Cossu G, Marcu KB, Bianchi ME. Cells migrating to sites of tissue damage in response to the danger signal HMGB1 require NF-kappaB activation. *J Cell Biol* 2007; **179**: 33-40 [PMID: 17923528 DOI: 10.1083/jcb.200704015]
99 **Yang J**, Chen L, Yang J, Ding J, Rong H, Dong W, Li X. High mobility group box-1 induces migration of vascular smooth muscle cells via TLR4-dependent PI3K/Akt pathway activation. *Mol Biol Rep* 2012; **39**: 3361-3367 [PMID: 21698364 DOI: 10.1007/s11033-011-1106-6]
100 **Xue X**, Chen X, Fan W, Wang G, Zhang L, Chen Z, Liu P, Liu M, Zhao J. High-mobility group box 1 facilitates migration of neural stem cells via receptor for advanced glycation end products signaling pathway. *Sci Rep* 2018; **8**: 4513 [PMID: 29540727 DOI: 10.1038/s41598-018-22672-4]
101 **Shirozu M**, Nakano T, Inazawa J, Tashiro K, Tada H, Shinohara T, Honjo T. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. *Genomics* 1995; **28**: 495-500 [PMID: 7490086 DOI: 10.1006/geno.1995.1180]
102 **Grunewald M**, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Jung S, Chimenti S, Landsman L, Abramovitch R, Keshet E. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 2006; **124**: 175-189 [PMID: 16413490 DOI: 10.1016/j.cell.2005.10.036]
103 **Ratajczak MZ**, Kim CH, Abdel-Latif A, Schneider G, Kucia M, Morris AJ, Laughlin MJ, Ratajczak J. A novel perspective on stem cell homing and mobilization: review on bioactive lipids as potent chemoattractants and cationic peptides as underappreciated modulators of responsiveness to SDF-1 gradients. *Leukemia* 2012; **26**: 63-72 [PMID: 21886175 DOI: 10.1038/leu.2011.242]
104 **Cheng M**, Qin G. Progenitor cell mobilization and recruitment: SDF-1, CXCR4, α4-integrin, and c-kit. *Prog Mol Biol Transl Sci* 2012; **111**: 243-264 [PMID: 22917234 DOI: 10.1016/B978-0-12-398459-3.00011-3]
105 **Burns JM**, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold ME, Sunshine MJ, Littman DR, Kuo CJ, Wei K, McMaster BE, Wright K, Howard MC, Schall TJ. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med* 2006; **203**: 2201-2213 [PMID: 16940167 DOI: 10.1084/jem.20052144]
106 **Ponte AL**, Marais E, Gallay N, Langonné A, Delorme B, Hérault O, Charbord P, Domenech J. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells* 2007; **25**: 1737-1745 [PMID: 17395768 DOI: 10.1634/stemcells.2007-0054]
107 **Gauley J**, Pisetsky DS. The translocation of HMGB1 during cell activation and cell death. *Autoimmunity* 2009; **42**: 299-301 [PMID: 19811282 DOI: 10.1080/08916930902831522]
108 **Tang Z**, Jiang M, Ou-Yang Z, Wu H, Dong S, Hei M. High mobility group box 1 protein (HMGB1) as biomarker in hypoxia-induced persistent pulmonary hypertension of the newborn: a clinical and *in vivo* pilot study. *Int J Med Sci* 2019; **16**: 1123-1131 [PMID: 31523175 DOI: 10.7150/ijms.34344]
109 **Zhao Y**, Yi J, Tao L, Huang G, Chu X, Song H, Chen L. Wnt signaling induces radioresistance through upregulating HMGB1 in esophageal squamous cell carcinoma. *Cell Death Dis* 2018; **9**: 433 [PMID: 29567990 DOI: 10.1038/s41419-018-0466-4]
110 **Yadav SS**, Kumar M, Varshney A, Yadava PK. KLF4 sensitizes the colon cancer cell HCT-15 to cisplatin by altering the expression of HMGB1 and hTERT. *Life Sci* 2019; **220**: 169-176 [PMID: 30716337 DOI: 10.1016/j.lfs.2019.02.005]
111 **Foglio E**, Pellegrini L, Germani A, Russo MA, Limana F. HMGB1-mediated apoptosis and autophagy in ischemic heart diseases. *Vasc Biol* 2019; **1**: H89-H96 [DOI: 10.1530/VB-19-0013]
112 **Penzo M**, Habiel DM, Ramadass M, Kew RR, Marcu KB. Cell migration to CXCL12 requires simultaneous IKKα and IKKβ-dependent NF-κB signaling. *Biochim Biophys Acta* 2014; **1843**: 1796-1804 [PMID: 24747690 DOI: 10.1016/j.bbamcr.2014.04.011]
113 **Penzo M**, Molteni R, Suda T, Samaniego S, Raucci A, Habiel DM, Miller F, Jiang HP, Li J, Pardi R, Palumbo R, Olivotto E, Kew RR, Bianchi ME, Marcu KB. Inhibitor of NF-kappa B kinases alpha and beta are both essential for high mobility group box 1-mediated chemotaxis [corrected]. *J Immunol* 2010; **184**: 4497-4509 [PMID: 20231695 DOI: 10.4049/jimmunol.0903131]

114 **Lolmede K**, Campana L, Vezzoli M, Bosurgi L, Tonlorenzi R, Clementi E, Bianchi ME, Cossu G, Manfredi AA, Brunelli S, Rovere-Querini P. Inflammatory and alternatively activated human macrophages attract vessel-associated stem cells, relying on separate HMGB1- and MMP-9-dependent pathways. *J Leukoc Biol* 2009; **85**: 779-787 [PMID: 19197071 DOI: 10.1189/jlb.0908579]

115 **Kew RR**, Penzo M, Habiel DM, Marcu KB. The IKKα-dependent NF-κB p52/RelB noncanonical pathway is essential to sustain a CXCL12 autocrine loop in cells migrating in response to HMGB1. *J Immunol* 2012; **188**: 2380-2386 [PMID: 22287708 DOI: 10.4049/jimmunol.1102454]

116 **Degryse B**, Bonaldi T, Scaffidi P, Müller S, Resnati M, Sanvito F, Arrigoni G, Bianchi ME. The high mobility group (HMG) boxes of the nuclear protein HMG1 induce chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. *J Cell Biol* 2001; **152**: 1197-1206 [PMID: 11257120 DOI: 10.1083/jcb.152.6.1197]

117 **Rauvala H**, Rouhiainen A. Physiological and pathophysiological outcomes of the interactions of HMGB1 with cell surface receptors. *Biochim Biophys Acta* 2010; **1799**: 164-170 [PMID: 19914413 DOI: 10.1016/j.bbagrm.2009.11.012]

118 **Kofuku Y**, Yoshiura C, Ueda T, Terasawa H, Hirai T, Tominaga S, Hirose M, Maeda Y, Takahashi H, Terashima Y, Matsushima K, Shimada I. Structural basis of the interaction between chemokine stromal cell-derived factor-1/CXCL12 and its G-protein-coupled receptor CXCR4. *J Biol Chem* 2009; **284**: 35240-35250 [PMID: 19837984 DOI: 10.1074/jbc.M109.024851]

119 **Venereau E**, Schiraldi M, Uguccioni M, Bianchi ME. HMGB1 and leukocyte migration during trauma and sterile inflammation. *Mol Immunol* 2013; **55**: 76-82 [PMID: 23207101 DOI: 10.1016/j.molimm.2012.10.037]

120 **Lee G**, Espirito Santo AI, Zwingenberger S, Cai L, Vogl T, Feldmann M, Horwood NJ, Chan JK, Nanchahal J. Fully reduced HMGB1 accelerates the regeneration of multiple tissues by transitioning stem cells to G_{Alert}. *Proc Natl Acad Sci USA* 2018; **115**: E4463-E4472 [PMID: 29674451 DOI: 10.1073/pnas.1802893115]

121 **Liu H**, Liu S, Li Y, Wang X, Xue W, Ge G, Luo X. The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. *PLoS One* 2012; **7**: e34608 [PMID: 22511954 DOI: 10.1371/journal.pone.0034608]

122 **Li M**, Sun X, Ma L, Jin L, Zhang W, Xiao M, Yu Q. SDF-1/CXCR4 axis induces human dental pulp stem cell migration through FAK/PI3K/Akt and GSK3β/β-catenin pathways. *Sci Rep* 2017; **7**: 40161 [PMID: 28067275 DOI: 10.1038/srep40161]

123 **Lau TT**, Wang DA. Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine. *Expert Opin Biol Ther* 2011; **11**: 189-197 [PMID: 21219236 DOI: 10.1517/14712598.2011.546338]

124 **Vénéreau E**, Ceriotti C, Bianchi ME. DAMPs from Cell Death to New Life. *Front Immunol* 2015; **6**: 422 [PMID: 26347745 DOI: 10.3389/fimmu.2015.00422]

125 **Zhao W**, Jin K, Li J, Qiu X, Li S. Delivery of stromal cell-derived factor 1α for *in situ* tissue regeneration. *J Biol Eng* 2017; **11**: 22 [PMID: 28670340 DOI: 10.1186/s13036-017-0058-3]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 952-965

DOI: 10.4252/wjsc.v12.i9.952

ISSN 1948-0210 (online)

REVIEW

Mechanotransduction of stem cells for tendon repair

Hao-Nan Wang, Yong-Can Huang, Guo-Xin Ni

ORCID number: Hao-Nan Wang 0000-0003-3590-7633; Yong-Can Huang 0000-0001-8548-8233; Guo-Xin Ni 0000-0001-9181-8155.

Author contributions: Wang HN and Huang YC contributed equally to this manuscript; Wang HN and Huang YC wrote the manuscript; Huang YC and Ni GX edited the manuscript and provided feedback.

Supported by National Natural Science Foundation of China, No. 81871848 and No. 81702171; and Shenzhen Double Chain Project for Innovation and Development Industry, No. 201806081018272960.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Hao-Nan Wang, Guo-Xin Ni, School of Sport Medicine and Rehabilitation, Beijing Sport University, Beijing 100084, China

Yong-Can Huang, Shenzhen Engineering Laboratory of Orthopaedic Regenerative Technologies, Department of Spine Surgery, Peking University Shenzhen Hospital, Shenzhen 518036, Guangdong Province, China

Yong-Can Huang, National and Local Joint Engineering Research Center of Orthopaedic Biomaterials, Peking University Shenzhen Hospital, Shenzhen 518036, Guangdong Province, China

Corresponding author: Guo-Xin Ni, PhD, Professor, School of Sport Medicine and Rehabilitation, Beijing Sport University, No. 48 Xinxi Road, Haidian District, Beijing 100084, China. niguoxin@bsu.edu.cn

Abstract

Tendon is a mechanosensitive tissue that transmits force from muscle to bone. Physiological loading contributes to maintaining the homeostasis and adaptation of tendon, but aberrant loading may lead to injury or failed repair. It is shown that stem cells respond to mechanical loading and play an essential role in both acute and chronic injuries, as well as in tendon repair. In the process of mechanotransduction, mechanical loading is detected by mechanosensors that regulate cell differentiation and proliferation *via* several signaling pathways. In order to better understand the stem-cell response to mechanical stimulation and the potential mechanism of the tendon repair process, in this review, we summarize the source and role of endogenous and exogenous stem cells active in tendon repair, describe the mechanical response of stem cells, and finally, highlight the mechanotransduction process and underlying signaling pathways.

Key Words: Stem cells; Mechanical loading; Tendon repair; Mechanotransduction

Core Tip: Stem cells and mechanical loading are crucial to tendon injuries. In this review, we summarize the sources and roles of endogenous and exogenous stem cells for tendon repair, describe the mechanical response of stem cells, and finally highlight the mechanotransduction process and underlying signaling pathways. The deeper understanding of interactions between stem cells and mechanical loading offers great

Manuscript source: Invited manuscript

Received: April 3, 2020
Peer-review started: April 3, 2020
First decision: April 22, 2020
Revised: May 6, 2020
Accepted: July 19, 2020
Article in press: July 19, 2020
Published online: September 26, 2020

P-Reviewer: Park YS
S-Editor: Ma YJ
L-Editor: Wang TQ
P-Editor: Xing YX

potential for the development of new therapeutic strategies for tendon repair.

Citation: Wang HN, Huang YC, Ni GX. Mechanotransduction of stem cells for tendon repair. *World J Stem Cells* 2020; 12(9): 952-965
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/952.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.952

INTRODUCTION

Tendon is a unique form of connective tissue that links muscle to bone. It is the anatomical structure that transmits muscle-contraction force to the skeleton in order to maintain posture or produce motion. Tendons are composed of triple-helical collagen I molecules assembled into fibrils that, in turn, form fibers, fascicles, and ultimately, tendon[1-3]. This mechanosensitive tissue has specific mechanical properties that enable it to respond and adapt to the loading transmitted by muscles; the collagen fibrils, considered to be the fundamental force-transmitting unit of the tendon, are densely arranged within the extracellular matrix (ECM), oriented parallel to the bone-muscle axis. A multitude of ECM molecules, including collagens, elastin, proteoglycans, and glycoproteins, are involved in tendon-specific collagen I. Tenocytes, the main type of cell located inside the collagen fibers, produce collagen I and ECM molecules[2]. Additionally, tendon stem/progenitor cells (TSPCs), also commonly termed as tendon-derived stem cells (TDSCs) or tendon stem cells (TSCs), located in the fascicular matrix, are responsible for replenishing tendon cells through differentiation and proliferation[4]. Under certain conditions, such as aberrant loading, TSPCs have multidifferentiation potential, namely, TSPCs can differentiate into tenocytes, chondrocytes, osteocytes, and adipocytes[4,5].

Tendon injuries range from chronic to acute, with partial or complete tendon rupture[2,6]. Chronic tendon injury mainly refers to tendinopathy, which is the most common tendon overuse injury and is characterized by pain and impaired function and performance[7]. The pathogenesis of tendinopathy is far from well understood and has been interchangeably defined as a degenerative condition or a failure of the healing process. Moreover, increased expression of inflammatory cytokines, such as COX-2 and interleukin (IL)-6, has been observed in overuse tendinopathy[8]; currently, the production of inflammatory cytokines is not considered to be a classic inflammatory response, but rather, the production expressed by resident tenocytes under overload[9,10]. Histologically, disorganization of collagen, increased noncollagenous ECM, hypercellularity, and neovascularization can be seen[11,12]. The exact relationship between tendinopathy and tendon rupture remains unknown, but it has been reported that tendinopathy increases the risk of tendon rupture[13]. Acute partial or complete tendon rupture interrupts tendon continuity, leading to bleeding, clotting, and the release of PDGF, TGF-β, ATP, and ADP from platelets and of epinephrine and norepinephrine from blood vessels at the wound site[9], resulting in a decrease or even loss of function and, potentially, in the loss of mobility. After injury, tendon undergoes a natural healing process involving successive steps—inflammation and formation of new cells, as well as ECM formation and remodeling[14]. Currently, the treatment for tendon injuries differs from that for other chronic and acute injuries. Conservative treatments include therapeutic exercise[15,16], shockwave[17], and injection therapy[18], which are helpful for reducing pain and improving function in tendinopathy, but pain during treatment, slow recovery, and risk of failure remain. A surgical procedure is usually the first choice for total tendon rupture, but patients may still face postoperative issues, such as proliferation of scar tissue, decreased mobility, and risk of second rupture.

To this end, stem-cell-based therapy has been introduced to clinical practice, and it may have broader future applications with the advantages of offering regeneration and repair. TSPCs, which tend to differentiate into tenocytes and are able to replace the loss of normal tenocytes, have been found to reside in tendon tissue. Thus, they have great potential to become the ideal cell source for stem-cell-based therapy[19,20].

Mechanical loading plays a crucial role in the biology of TSPCs[21]. In particular, proper loading aids in promoting the proliferation and tenogenic differentiation of TSPCs, which is beneficial for tendon repair, whereas aberrant loading might lead to nontenocyte differentiation, which could hinder tendon healing. In order to further

understand the response and mechanism of stem cells to mechanical loading, related to tendon injury and repair, this paper summarizes the sources of stem cells and describes the mechanical response of TSPCs for tendon repair, and it further discusses the underlying signaling pathways of TSPCs responding to mechanical stimuli.

ENDOGENOUS AND EXOGENOUS STEM CELLS/PROGENITORS FOR TENDON REPAIR

Several types of endogenous and exogenous stem cells have proven effective for tendon repair. TSPCs are fibroblast-like cells[22], which have been identified in mice, rabbits, and humans, with typical stem-cell makers[4,22,23]. Nevertheless, the exact source location of TSPCs remains unclear. TSPCs have been isolated and differentiated from tendon[4], peritenon[24], and perivascular sources[25,26]. A recent study reported that a PDGFRA$^+$ cell population expressing tubulin polymerization-promoting protein family member 3 (TPPP3$^+$), which is located in peritenon, has stem-cell characteristics, such that it may generate new tenocytes and self-renew upon injury[27]. TSPCs share some common markers with tenocytes, such as collagen I, collagen III, tenascin C, and tenomodulin (TNMD), but they express still more markers, like Oct-4, SSEA-1/4, and nucleostemin[4,5]. Both TSPCs isolated from the tendon and peritenon regions of mouse Achilles tendons have the Sca1, CD90, and CD44 markers[26], but progenitor cells from the tendon and peritenon regions can be distinguished with genes such as *Scx*, *Mkx*, *Thbs4*, and *Wnt10a*[24]. Moreover, perivascular stem cells isolated and cultured from human supraspinatus tendon biopsies express both tendon-like and stem/precursor-cell-like markers, including musashi-1, nestin, prominin-1/CD133, CD29, CD44, Scx, and Smad8[25]. TSPCs from the tendon proper lack CD133 markers, however, which may help distinguish TSPCs from tendon proper and perivascular sources. TSPCs have shown a high capacity for proliferation and multipotential differentiation into tenocytes, osteoblasts, chondrocytes, and adipocytes[4,28]. Although TSPCs show multipotential differentiation, they also show spontaneous tenogenic differentiation, which can be beneficial for tendon repair[20]. In a tendon-window-wound study, TSPCs participated in tendon repair by proliferation and activation of tenogenesis[29]. The therapeutic effect of TSPCs has also been confirmed by using animal models. It has been reported that TSPCs promote tendon repair by improving cell and collagen-fiber alignment, collagen birefringence, and Young's modulus typical of tendon, as well as by increasing ultimate stress capacity[30]. Similarly, ultimate failure load and the expression of collagen I and collagen III in the ruptured Achilles tendon have been much improved by TSPC transplantation[31]. Nevertheless, the abnormal differentiation of TSPCs into nontendon cells has a negative effect on tendon development, homeostasis, and repair. For instance, tendon progenitor cells of injured tendon have strong chondrogenic potential, which may cause endochondral ossification as a result of ectopic mineralization[32]. To date, very scant clinical research has been performed using TSPCs for tendon-related diseases.

As shown in Table 1, stem cells/progenitors derived from other tissues, such as bone marrow-derived mesenchymal stem cell (BMSCs) and adipose-derived stem cells (ASCs), are much easier to acquire than TSPCs[33,34], and they have been proven efficient for tendon repair[14,19]. BMSCs are spindle-shaped[35] and have the potential of tenogenic differentiation[36,37] and high proliferation[4,38]. Several mechanisms may contribute to tendon repair with exogenic BMSCs. First, BMSCs can differentiate into certain new cells (tenocytes) to replace lost normal cells[19,39]; second, BMSCs can secrete various cytokines and growth factors to promote the proliferation of cells in injured tissue[40]; and third, BMSCs can increase the deposition of collagenous proteins[41]. BMSC-based therapy has been found to improve histological and biomechanical properties and to increase the expression of collagen in animal injury[42,43]. But the application of BMSCs may also carry the risk of nontendon differentiation and of forming ectopic bone during tendon repair[44]. The clinical application of BMSCs was started very early, and four clinical trials (NCT03688308, NCT01788683, NCT02484950, and NCT01687777) using BMSCs for rotator-cuff repair are at the stage of recruiting, and the results have not yet been released. ASCs are spindle-shaped[45] with stem-cell marks[23,35,46]; these cells commonly being isolated from subcutaneous adipose tissue[34] and liposuction aspirates[47], have shown the multipotential ability of differentiation including tenogenic cells[48-50] and high proliferation[23,38,51]. ASC transplantation could enhance the secretion of collagen I and tenascin-C during healing and improve the mechanical strength of tendon[52,53], as well as improve the pathological changes of tendinopathy and the normalization of collagen ratios within the affected tendon[54].

Table 1 Difference among tendon stem/progenitor cells, bone marrow-derived mesenchymal stem cells, and adipose-derived stem cells for tendon repair

	TSPCs	BM-MSCs	ASCs
Morphology	Fibroblast-like shape[22]	Spindle-shaped[35]	Spindle-shaped[45]
Phenotypes	Positive: CD13, C29, CD44, CD54, CD73, CD90, CD105, CD146 and CD166 Negative: CD2, CD3, CD11b, CD14, CD15, CD16, CD18, CD19, CD31, CD34, CD45, CD56, CD71, CD106, CD117, CD123, and CD235a[4,22,23]	Positive: CD13, CD29, CD44, CD73, CD90, and CD105 Negative: CD14, CD19, CD34, CD45[35,37]	Positive: CD13, C29, CD44, CD49d, CD54, CD73, CD90, CD105, and CD166 Negative: CD14, CD19, CD31, CD34, CD45 and CD71[23,35,46]
Proliferation	TSPCs = BM-MSCs[4]; TSPCs ≤ ASCs[23]	BM-MSCs = TSPCs[4]; BM-MSCs < ASCs[38]	ASCs > BM-MSCs[38]; ASCs ≥ TSPCs[23]
Tenogenic differentiation	Spontaneous differentiation[20], or promoted by growth factors[120] and mechanical loading[75]	Induced by growth factors[36] and mechanical loading[39]	Induced by growth factor supplements[48,49] and extracorporeal shockwave[50]
Evidence for tendon repair in vitro	Tenogenic differentiation[20,75,120] and high proliferation potential[4,28]	Tenogenic differentiation and high proliferation potential[4]; enhanced secretion of bioactive factors[40] and the deposition of ECM[41]	Tenogenic differentiation and high proliferation rate[51]
Evidence for tendon repair *in vivo*	High proliferation and activation of tenogenesis[29]; improved collagen alignment and biomechanical properties[30,31]	Improved histological and biomechanical properties; increased expression of collagen[42,43]	Modulation of microenvironment[55]; enhancing the secretion of collagen and mechanical strength of tendon[52,53]
Evidence for tendon repair in clinics	None	Four registered trials, but the results are not available	Reduction of pain, tendon defect areas post intervention[56]
Advantages	Spontaneous tenogenic differentiation[20]; higher proliferation and therapeutic effectiveness[31]	Easier acquirement[33]; enhanced secretion of bioactive factors[40]; increased the deposition of collagenous proteins[41]	Easier acquirement[34]; inhibition of osteogenic differentiation[55]; confirmed clinical outcome[56]
Limitations	Limited number obtained from isolation[63]	High potential of osteogenic differentiation[44]; lower therapeutic effectiveness than TSPCs[31]	Risk of fibrotic tissue formation, scarring[57], and forming adipocytes[58]

TSPCs: Tendon stem/progenitor cells; BMSCs: Bone marrow-derived mesenchymal stem cells; ASCs: Adipose-derived stem cells; ECM: Extracellular matrix.

Recently, a study indicated that ASCs improved tendon repair in tendinopathy by inhibiting inflammation and inducing neovascularization at the early stage of tendon healing, and ASCs are also effective for the inhibition of ectopic ossification *in vivo*[55]. Additionally, the clinical safety and efficacy of ASCs therapy have been reported. After allogeneic ASC treatment, patients with lateral elbow epicondylosis self-reported outcomes with reduced pain and improved function, without safety issues, as well as demonstrated decreased tendon defect areas in ultrasound images at 52 wk post-injection[56]. However, the application of ASCs may give rise to fibrotic tissue formation and scarring[57] as well as forming adipocytes[58] during tendon repair. In addition, induced pluripotent stem cells (iPSCs) can be reprogrammed from adult somatic cells. It has been found that human iPSC-derived neural crest stem cells (iPSC-NCSCs) can differentiate into mesenchymal-lineage tenocytes, which accelerate the process of tendon repair[59]. In a rat patellar-tendon window-defect trial, iPSC-NCSCs promoted healing by improving matrix synthesis and mechanical properties and by increasing fetal tendon-related matrix proteins, stem-cell recruitment factors, and the tenogenic

differentiation factor[60].

Compared with exogenic stem cells/progenitors, TSPCs possess higher regenerative potential for tendon repair. For instance, during treatment of rat Achilles tendon injury, TSPCs have a greater positive effect on morphological and histological alteration and biomechanical strength when compared to BMSC transplantation[31]. This distinction may be because TSPCs proliferate more rapidly and have a greater capacity for colony formation[41,61,62]; additionally, TSPCs undergo spontaneous tenogenic differentiation, whereas BMSCs do not[20]. It has been demonstrated that mouse TSPCs express higher levels of tenogenic markers, such as Scx, Comp, Sox9, and Runx2, than mouse BMSCs; similarly, human TSPCs express more TNMD than BMSCs[4]. Thus, TSPCs more rapidly differentiate to functional tenocytes. Moreover, the expression of collagen I and collagen III is higher in TSPCs, which results in greater biomechanical strength at the early stage of repair[31]. However, the limited number of resident TSPCs hinders the large-scale clinical application[63]. Hence, both endogenous and exogenous stem cells have therapeutic potential. According to current evidence, TSPCs possess some advantages for tendon repair, but the efficacy of endogenic and exogenic stem cells requires further investigation.

MECHANICAL RESPONSE OF STEM CELLS/PROGENITORS FOR TENDON REPAIR

A number of factors influence the homeostasis of tendon, in which mechanical loading plays a critical role[64]. Under normal or physiological loading, the magnitude of loading is much less than the ultimate tensile strength (UTS). Typically, tendon could return to its original length when the strain is less than 4% of elongation; but tendon will have macroscopic tearing and eventually rapture when the strain is beyond 8%-10% of elongation[65]. Researchers usually use 4% cyclic uniaxial stretching to mimic this loading condition in *vitro*, and to moderate treadmill running model of rats (13 m/min, 15 min/d, and 5 d/wk in the first week; 13 m/min, 50 min/d, and 5 d/wk for another 3 wk) *in vivo*[21]. In normal or physiological loading, tendon can maintain homeostasis and respond to loading through cellular anabolic adaptation[3,21]. By contrast, abnormal loading may be different from normal mechanical loading in magnitude, frequency, duration, and/or direction; typically, abnormal loading of tendon can be unload, overload, or high repetitive low load[66]. Compared with explants tensioned with constant 4% strain, nontensioned rabbit patellar tendon decreased linear stiffness, elongation to failure, and maximum failure force after 20 h[67]; undergoing cyclic loading at approximately 35% of the UTS led to tendon rapture in 15 min[68]; also, cyclic loading under 5% of UTS (around 1% strain) resulted in rupture within 15 h[69]. *In vitro*, researchers usually use 8% cyclic uniaxial stretching to mimic the overloading condition, as well as intensive treadmill running (13 m/min, 15 min/d, and 5 d/wk in the first week; 13 m/min, 3 h/d, 4 h/d, and 5 h/d in the second, third, and fourth weeks for 5 d)[21]. Abnormal loading can lead to failed repair or pathological changes by causing anabolic changes in tendon[14,21,70]. After high-intensity repetitive-exercise-induced injury, the expression level of IL-1β increases in mouse tendon[71]. Moreover, a greater production of inflammatory mediators induced by IL-1β, including COX-2, MMP-1, and PGE-2, has been reported for human tendon fibroblasts with excessive stretch loading than with moderate stretching or without stretch loading *in vitro*[72]. Similarly, the level of PGE-2 significantly increases in mouse patellar and Achilles tendons after rigorous treadmill running compared to caged control groups *in vivo*[73], indicating that overloading tendon may lead to a higher production of PGE-2. These inflammatory mediators may, in turn, promote the degradation of tendon, such as through neutrophil infiltration and decreased collagen production, thereby negatively impacting the repair of injured tendon.

TSPCs undergo similar mechanical loading as tenocytes. Mechanical loading, no matter what level, can increase TSC proliferation, which is indeed necessary for healing injured tendon[21]. Patellar and Achilles TSCs isolated from mice after moderate treadmill running have nearly double proliferation rates compared to the TSCs isolated from less active mice *in vitro*[21]; also, compared to inactive mice, cellular production of collagen increases by 70% and 200% for patellar and Achilles TSCs, respectively, for mice completing moderate treadmill running *in vivo*[74]. Currently, few studies have found that the magnitude of stretching could lead to different cell fate. In particular, a higher magnitude of stretching may cause aberrant differentiation compared to a lower magnitude of stretching *in vitro*. It was reported that 4% stretching promoted the differentiation of TSCs into tenocytes with increased gene

expression of collagen I; 8% stretching, however, promoted the differentiation of TSCs into nontenocytes, including adipocytes, chondrocytes, and osteocytes, aside from differentiation into tenocytes, as evidenced by higher expression levels of genes such as *PPARγ*, *collagen II*, *Sox-9*, and *Runx2 in vitro*[75]. Similarly, increased differentiation into adipocytes, chondrocytes, osteocytes, and tenocytes with high gene expression of *LPL*, *Sox-9*, *Runx2*, *Osterix*, *collagen I*, and *TNMD* was found in mice after intensive treadmill running *in vivo*[76]. Also, mechanical loading can influence both TSC proliferation and differentiation due to an inflammatory mediator. In response to rigorous treadmill running, mouse patellar and Achilles tendons increase the production of PGE-2, which can decrease cell proliferation and induce both adipogenesis and osteogenesis of TSCs, as well as promote the production of fatty and calcified tissues within tendon[73]. These findings are consistent with the clinical understanding that complete rest without loading will decrease tendon strength and induce pathologic change in tendon, such that total rest is relatively contraindicated for tendinopathy[77,78]. In short, mechanical loading is necessary for TSC proliferation and collagen production, but excessive loading may cause abnormal differentiation of TSCs into nontenocytes, leading to tendon injury or failed tendon repair.

TSPCs would not be able to respond to mechanical loading without mechanotransduction, which converts mechanical signals from the environment into biochemical signals[79] (Figure 1). As a mechanical signal is transmitted to the microenvironment, it causes the physical perturbation of cells and deformation of the extracellular matrix[80,81]. Both TSPCs and tenocytes reside in the pericellular matrix, such that TSPCs experience a force similar to tenocytes. In tendon, tissue probably undergoes various types of force, including tensile loading, shear, and even compression force[81,82]. As tendon has the function of transmitting force from muscle to bone, tendon is exposed to high-tensile force, whereas the tendon-bone junction area commonly experiences compression force[83]. Further, the midportion of a tendon can potentially be exposed to both shear and compression forces due to the different forces to which the posterior and anterior areas of the tendon are exposed[84]. It has been reported that cyclic tensile loading on tendon may cause interstitial fluid flow, leading to shear force and perhaps hydrostatic force.

Moreover, effector-cell mechanosensors detect mechanical signals and induce an intercellular response *via* various downstream signaling pathways to regulate stem-cell differentiation and proliferation[85,86]. Typically, effector cells can sense mechanical signaling from groups of transmembrane mechanosensitive proteins, namely, mechanosensors such as ion channels, integrins, G-protein coupled receptors (GPCRs), growth factor receptor (GFR), and primary cilium[85]. Ion channels play an essential role in cellular mechanotransduction. Cystic fibrosis transmembrane conductance regulator (CFTR) is a stretch-medicated activation channel that aids in passing chloride and water in and out of cells[87,88]. Recently, a study reported that CFTR regulates tenogenic differentiation through inhibiting the Wnt/β-catenin/ERK1/2 signaling pathway in TSPCs[89]. Also, Ca^{2+} is a greatly powerful second messenger regulating cell migration and influencing the differentiation of stem cells[90,91]. Several studies have shown that transient receptor potential melastatin 7 (TRPM7) works as a calcium channel conducting calcium influx[90,92], which, in turn, activates transcription factor NFATc1, which then induces osteogenesis in mesenchymal stem cells (MSCs)[93,94]. Interestingly, integrins become involved in the intracellular concentration of calcium by interaction with ion channels[95]. In addition to interaction with ion channels, accumulating evidence indicates that integrin systems can detect mechanical signals and then transduce them to nuclear signaling[96,97]. Once integrin receptors bind their ligands in the ECM, they move laterally in the plane of the membrane to form focal adhesions, which play a central role in cell motility and cytoskeletal dynamics, as well as in regulating cell proliferation, differentiation, and gene expression[98]. GPCRs have the ability to sense mechanical loading and regulate molecular mechanisms downstream *via* binding protein ligand. For instance, Wnt binds frizzled receptors, which span the plasma membrane and constitute a distinct family of GPCRs that regulate osteogenic differentiation in MSCs[99]. Studies have found that, when a stretching force is applied to TSPCs, Wnt5a activates a co-receptor, receptor tyrosine kinase-like orphan receptor 2 (ROR2), and regulates Wnt5a/JNK and Wnt5a/RhoA signaling pathways downstream for osteogenic differentiation in TSPCs[100,101]. Additionally, growth factors bind their GFRs and regulate signaling pathways downstream—in TSPCs, GFRs mediate bone morphogenetic protein (BMP) signaling pathways involved in biomechanical loading-induced differentiation[102,103]. Further, primary cilium, an extraordinary organelle that exists on nearly all somatic cells, also plays a role in the detection of mechanical signaling. Currently, research specifically focused on the primary cilium of TSPCs is rare, but it can be hypothesized that the

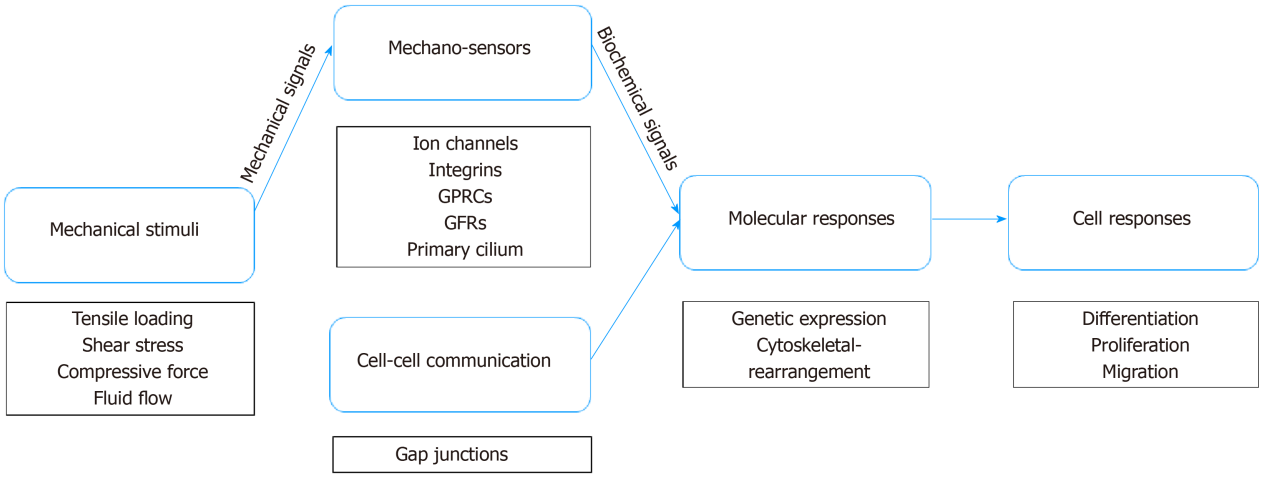


Figure 1 Process of mechanotransduction in stem cells for tendon repair. This figure describes various ways by which mechanical loading stimulates mechanosensors to emit mechanical signals that convert into biochemical signals, which, in turn, activate the rearrangement of cytoskeleton and activate genetic expression *via* various signaling pathways. Cell-cell communication transduces signals from one cell to another cell *via* gap junctions. GPRC: G protein-coupled receptor; GFR: Growth factor receptor.

principle might be similar to tenocytes and MSCs. In tenocytes, the length of primary cilium immediately and significantly increases in a stress-deprived environment and can be reversed by cyclic tensile loading[104]. In MSCs, frizzled receptors are thought to localize cilium membranes, such that primary cilium is involved in controlling differentiation by tuning Wnt signaling pathways in MSCs[105,106].

In addition, even if a cell is not to receive mechanical stimulation, the cell can still respond *via* a process known as cell-cell communication, whereby it can communicate with a distant cell receiving mechanical stimulation[107,108]. To date, limited research has investigated the mechanism of cell-cell communication in TSPCs. Because TSPCs are stem cells/progenitors of tendon, however, they may have similar communication characteristics as tenocytes and MSCs. Connexins form gap junctions between the cytoplasm of adjacent cells allow for the direct intercellular exchange of ions and molecules[109]. Gap junctions are immunohistochemically detected among tenocytes, and connexins 32 and 43 form a three-dimensional network to respond to mechanical stimulation together[110]—connexin 32 has a stimulatory function, whereas connexin 43 is inhibitory[109]. Additionally, cell-cell communication can be altered to some degree by stretching; specifically, communication increases under low-level stretching (4%) and decreases under high-level stretching (8%)[111]. Moreover, a recent study has shown that connexin 43 plays an impactful role in protecting MSCs from premature senescence, which results in the failure to properly differentiate *in vitro*[112]. Connexin 43 may contribute to early tenogenesis in MSCs, but the mechanism in mechanotransduction is still not clear[113].

UNDERLYING SIGNALING PATHWAYS OF STEM CELLS/PROGENITORS RESPONDING TO MECHANICAL STIMULI

As the important role of mechanotransduction in TSPCs responding to mechanical loading has been realized, it has been demonstrated that various underlying signaling pathways transmit biomechanical signals to nuclei (Figure 2). With the noncanonical Wnts signaling pathway, Wnts binds co-receptor ROR2 and regulates signaling pathways downstream, in turn influencing osteogenic differentiation in TSPCs[114]. Mechanical tension promotes osteogenic differentiation of rat TSPCs *via* the Wnt5a/Wnt5b/JNK signaling pathway. Under 8% elongation uniaxial mechanical tension (UMT) stimulation, TSPCs exhibit increased protein levels of Wnt5a, Wnt5b, and P-JNK, as well as increased cytoskeletal rearrangement[100]. The mRNA expression of osteogenic genes, such as *Runx2*, *Dlx5*, *Alpl*, and *collagen Ia1*, also increases[100]. Additionally, UMT induces the appearance of osteogenic differentiation in rat TSCs through the Wnt5a/RhoA signaling pathway. RhoA and its effector protein, ROCK, play an important role in osteogenetic differentiation of MSCs[115], as they show increased mRNA expression of the osteogenic genes *Runx2*, *Alpl*, and *collagen Ia1*,

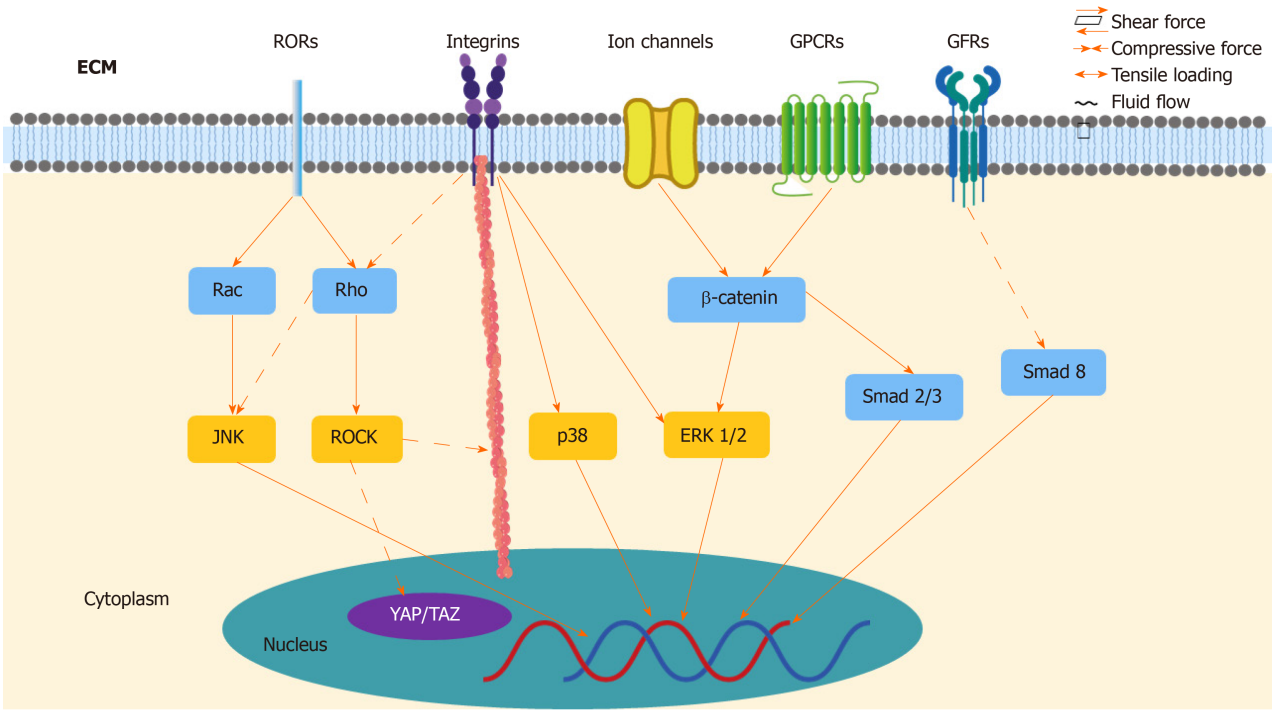


Figure 2 Signaling pathways responding to mechanical stimuli applied to a cell. This figure describes how mechanosensors, such as G-protein coupled receptors, integrins, ion channels, and growth factor receptor, sense mechanical loading and activate a series of signaling pathways downstream, in turn promoting gene expression and/or cytoskeleton rearrangement in tendon repair. ECM: Extracellular matrix; ROR: Receptor tyrosine kinase-like orphan receptor; GPCRs: G-protein coupled receptors; GFRs: Growth factor receptor; JNK: c-Jun N-terminal kinase; ROCK: Rho-associated protein kinase; ERK: Extracellular signal-regulated kinase; YAP: Yes-associated protein; TAZ: PDZ-binding motif.

along with ALP activity, as well as ALP cytochemical staining and Runx2 protein expression after 2% elongation mechanical tension[101]. Only the expression of Wnt5a increased under UMT, not the other noncanonical Wnts, such as Wnt5b, Wnt7a, and Wnt11, but it can be inferred that the difference in Wnt protein levels might be due to varying magnitude of loading[101]. To date, the interaction between Wnt5a/JNK and Wnt5a/RhoA remains to be further investigated, insofar as whether Wnt5a/JNK regulates cells independent of or depending on the RhoA pathway[116].

In addition to noncanonical Wnts signaling pathways, the Wnt/β-catenin signaling pathway also contributes to differentiation in TSPCs. Wnt commonly binds frizzled receptors and downregulates β-catenin in the canonical Wnt/β-catenin signaling pathway[114]. In a rat tendinopathy model and a human tendon with tendinopathy, increased expression of Wnt3 and β-catenin has been observed. Wnt3a can increase ALP activity, calcium nodule formation, and the expression of osteogenic markers in TSPCs[117]. Similarly, strain loading promotes osteogenetic differentiation and inhibits adipogenesis *via* β-catenin[99]. A recent study reported that CFTR, a stretch-mediated activation channel, can regulate TSPCs during tenogenic differentiation under mechanical stretching. Mice with dysfunctional CFTR showed reduced levels of tendon markers, including Scx, TNMD, collagen Ia1 chain, and decorin, as well as abnormally active Wnt/β-catenin signaling, which, in turn, further activated the ERK1/2 signaling pathway[89]. Inhibiting ERK1/2 signaling can promote tenogenic differentiation in TSPCs, both *in vitro* and *in vivo*, however, and increase matrix formation and mechanical properties, which is helpful in the tendon healing process[89]. Moreover, activation of Wnt/β-catenin signaling suppressed the expression of tenogenic genes *Scx*, *Mkx*, and *Tnmd* in TSPCs by reducing the amount of Smad2 and Smad3, which are intracellular mediators for TGF-β signaling[118].

In rat TSPCs, both 4% and 8% stretching can increase the amount of BMP-2, and 4% stretching upregulates BMP-2 genetic expression, when compared to unstretching, which does not obviously promote the expression of BMP-2[119]. Studies have proven that BMP-2 causes aberrant proliferation and differentiation in TSPCs, in other words, the addition of BMP-2 to human TSPC cultures reduces the proliferation of cells and promotes osteogenic differentiation *in vitro*[120]. BMP-2 promotes osteogenic differentiation through ALP cytochemical staining, ALP activity, and calcium nodule formation. Additionally, BMP-2 inhibits tenogenic marker expression, but promotes osteogenic, adipogenic, and chondrogenic differentiation in TSPCs[103]. After BMP-2

stimulation, TSPCs show increased glycosaminoglycan (GAG) production and mRNA expression of aggrecan (*Acan*), along with decreased mRNA expression of decorin (*Dcn*), biglycan (*Bgn*), and fibromodulin (*Fmod*)[103]. To date, it remains to be determined whether the BMP-2 downstream molecular signaling pathways induce differentiation of TSPCs. Smad8 might play a role, as activated Smad8 promotes MSC tenogenic differentiation *via* inhibiting the BMP-2 induced osteogenic pathway[121].

ECM deformation initiates integrin signaling at focal adhesion sites where the ECM binds integrin, which activates downstream proteins, such as ERK1/2, p38, and JNK[98]. Higher matrix stiffness increases TDSC proliferation and forms more stress fibers, as well as inhibits the differentiation of TDSCs into tenogenic, chondrogenic, and osteogenic lineages *via* focal adhesion kinase (FAK) or ERK1/2 signaling pathways[122]. Similarly, another study found that ERK/MAPK signaling pathways increase the tenogenic expression level in mouse MSCs[123]. In addition to ERK, p38 kinases also affect integrin-induced signaling pathways in TSPCs, as it was reported that 8% mechanical stretching caused an upregulated response in ERK1/2 and p38 kinases, as well as altered expression of matrix proteins, integrins, and matrix metalloproteinases[124]. Interestingly, mechanical loading might precisely regulate ERK signaling, as the level of ERK1/2 phosphorylation induced by cyclic uniaxial mechanical stretching is related to stretching time *in vitro*[125]. Furthermore, RhoA/ROCK and the cytoskeleton may also contribute to integrin signaling. This demonstrates that FAK has the ability to regulate mechanical stretch-induced tenogenic differentiation by mediating RhoA/ROCK downstream and interacting with the cytoskeleton in human MSCs[126].

Recent studies have identified two important transcriptional coactivators—Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) in the Hippo signaling pathway[127]. Mechanical loading has the ability to influence YAP/TAZ activities through stretching, geometry, and substrate rigidity, which, in turn, regulates stem-cell fate and behavior[128]. In human MSCs, YAP/TAZ knockdown promotes adipogenic differentiation on rigid substrates, which commonly happens on soft substrates[127]. Also, shear stress can induce human MSC osteogenic and fibrochondrogenic differentiation and promote TAZ nuclear translocation *via* the RhoA/ROCK signaling pathway and YAP/TAZ[129,130]. In addition, another study suggests that YAP/TAZ may be a downstream effector of the noncanonical Wnts signaling pathway, which plays a crucial role in TSPC differentiation. Thus, YAP/TAZ might mediate gene expression, osteogenesis, and cell migration of TSPCs[131]. To date, limited research has been conducted into the deep mechanism of Hippo and YAP/TAZ signaling pathways in tenocytes and TSPCs, but the potential value of this area is certain. In short, several signaling pathways have been demonstrated to participate in the mechanotransduction of TSPCs, such as noncanonical Wnts, Wnt/β-catenin, BMP-2, and integrin, as well as YAP/TAZ.

CONCLUSION

This review summarizes the sources and roles of the endogenous and exogenous stem cells that can be used for tendon repair, describes the mechanical response of stem cells in tendon repair, and finally, highlights the mechanotransduction process and its underlying signaling pathways. Mechanical loading plays a crucial role in both tendon injury and repair. When mechanical loading is applied, the stimulation is detected by mechanosensors, such as ion channels, integrin, GPCRs, GFR, and primary cilium, which then transmute the mechanical signal into a biological signal, and in turn, regulate various downstream signaling pathways. Suitable mechanical loading is helpful for promoting both the proliferation and differentiation of TSPCs, which are crucial for tendon repair. These findings promise a bright future with new therapeutic strategies for tendon repair. Further studies are necessary to identify mechanosensors and deeply understand the signaling pathways of stem cells.

REFERENCES

1 **Kannus P**. Structure of the tendon connective tissue. *Scand J Med Sci Sports* 2000; **10**: 312-320 [PMID: 11085557 DOI: 10.1034/j.1600-0838.2000.010006312.x]
2 **Nourissat G**, Berenbaum F, Duprez D. Tendon injury: from biology to tendon repair. *Nat Rev Rheumatol* 2015; **11**: 223-233 [PMID: 25734975 DOI: 10.1038/nrrheum.2015.26]
3 **Galloway MT**, Lalley AL, Shearn JT. The role of mechanical loading in tendon development, maintenance,

injury, and repair. *J Bone Joint Surg Am* 2013; **95**: 1620-1628 [PMID: 24005204 DOI: 10.2106/JBJS.L.01004]
4 **Bi Y**, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, Li L, Leet AI, Seo BM, Zhang L, Shi S, Young MF. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. *Nat Med* 2007; **13**: 1219-1227 [PMID: 17828274 DOI: 10.1038/nm1630]
5 **Zhang J**, Wang JH. Characterization of differential properties of rabbit tendon stem cells and tenocytes. *BMC Musculoskelet Disord* 2010; **11**: 10 [PMID: 20082706 DOI: 10.1186/1471-2474-11-10]
6 **Flint JH**, Wade AM, Giuliani J, Rue JP. Defining the terms acute and chronic in orthopaedic sports injuries: a systematic review. *Am J Sports Med* 2014; **42**: 235-241 [PMID: 23749341 DOI: 10.1177/0363546513490656]
7 **Scott A**, Squier K, Alfredson H, Bahr R, Cook JL, Coombes B, de Vos RJ, Fu SN, Grimaldi A, Lewis JS, Maffulli N, Magnusson SP, Malliaras P, Mc Auliffe S, Oei EHG, Purdam CR, Rees JD, Rio EK, Gravare Silbernagel K, Speed C, Weir A, Wolf JM, Akker-Scheek IVD, Vicenzino BT, Zwerver J. ICON 2019: International Scientific Tendinopathy Symposium Consensus: Clinical Terminology. *Br J Sports Med* 2020; **54**: 260-262 [PMID: 31399426 DOI: 10.1136/bjsports-2019-100885]
8 **Legerlotz K**, Jones ER, Screen HR, Riley GP. Increased expression of IL-6 family members in tendon pathology. *Rheumatology (Oxford)* 2012; **51**: 1161-1165 [PMID: 22337942 DOI: 10.1093/rheumatology/kes002]
9 **Scott A**, Khan KM, Cook JL, Duronio V. What is "inflammation"? Are we ready to move beyond Celsus? *Br J Sports Med* 2004; **38**: 248-249 [PMID: 15155418 DOI: 10.1136/bjsm.2003.011221]
10 **Riley G**. The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology (Oxford)* 2004; **43**: 131-142 [PMID: 12867575 DOI: 10.1093/rheumatology/keg448]
11 **Sharma P**, Maffulli N. Tendon injury and tendinopathy: healing and repair. *J Bone Joint Surg Am* 2005; **87**: 187-202 [PMID: 15634833 DOI: 10.2106/jbjs.D.01850]
12 **Lipman K**, Wang C, Ting K, Soo C, Zheng Z. Tendinopathy: injury, repair, and current exploration. *Drug Des Devel Ther* 2018; **12**: 591-603 [PMID: 29593382 DOI: 10.2147/DDDT.S154660]
13 **Yasui Y**, Tonogai I, Rosenbaum AJ, Shimozono Y, Kawano H, Kennedy JG. The Risk of Achilles Tendon Rupture in the Patients with Achilles Tendinopathy: Healthcare Database Analysis in the United States. *Biomed Res Int* 2017; **2017**: 7021862 [PMID: 28540301 DOI: 10.1155/2017/7021862]
14 **Docheva D**, Müller SA, Majewski M, Evans CH. Biologics for tendon repair. *Adv Drug Deliv Rev* 2015; **84**: 222-239 [PMID: 25446135 DOI: 10.1016/j.addr.2014.11.015]
15 **Malliaras P**, Barton CJ, Reeves ND, Langberg H. Achilles and patellar tendinopathy loading programmes : a systematic review comparing clinical outcomes and identifying potential mechanisms for effectiveness. *Sports Med* 2013; **43**: 267-286 [PMID: 23494258 DOI: 10.1007/s40279-013-0019-z]
16 **Beyer R**, Kongsgaard M, Hougs Kjær B, Øhlenschlæger T, Kjær M, Magnusson SP. Heavy Slow Resistance Versus Eccentric Training as Treatment for Achilles Tendinopathy: A Randomized Controlled Trial. *Am J Sports Med* 2015; **43**: 1704-1711 [PMID: 26018970 DOI: 10.1177/0363546515584760]
17 **Korakakis V**, Whiteley R, Tzavara A, Malliaropoulos N. The effectiveness of extracorporeal shockwave therapy in common lower limb conditions: a systematic review including quantification of patient-rated pain reduction. *Br J Sports Med* 2018; **52**: 387-407 [PMID: 28954794 DOI: 10.1136/bjsports-2016-097347]
18 **Lin MT**, Chiang CF, Wu CH, Huang YT, Tu YK, Wang TG. Comparative Effectiveness of Injection Therapies in Rotator Cuff Tendinopathy: A Systematic Review, Pairwise and Network Meta-analysis of Randomized Controlled Trials. *Arch Phys Med Rehabil* 2019; **100**: 336-349.e15 [PMID: 30076801 DOI: 10.1016/j.apmr.2018.06.028]
19 **Liu L**, Hindieh J, Leong DJ, Sun HB. Advances of stem cell based-therapeutic approaches for tendon repair. *J Orthop Translat* 2017; **9**: 69-75 [PMID: 29662801 DOI: 10.1016/j.jot.2017.03.007]
20 **Guo J**, Chan KM, Zhang JF, Li G. Tendon-derived stem cells undergo spontaneous tenogenic differentiation. *Exp Cell Res* 2016; **341**: 1-7 [PMID: 26794903 DOI: 10.1016/j.yexcr.2016.01.007]
21 **Zhang J**, Wang JH. The effects of mechanical loading on tendons--an in vivo and in vitro model study. *PLoS One* 2013; **8**: e71740 [PMID: 23977130 DOI: 10.1371/journal.pone.0071740]
22 **Randelli P**, Conforti E, Piccoli M, Ragone V, Creo P, Cirillo F, Masuzzo P, Tringali C, Cabitza P, Tettamanti G, Gagliano N, Anastasia L. Isolation and characterization of 2 new human rotator cuff and long head of biceps tendon cells possessing stem cell-like self-renewal and multipotential differentiation capacity. *Am J Sports Med* 2013; **41**: 1653-1664 [PMID: 23393078 DOI: 10.1177/0363546512473572]
23 **Stanco D**, Viganò M, Perucca Orfei C, Di Giancamillo A, Peretti GM, Lanfranchi L, de Girolamo L. Multidifferentiation potential of human mesenchymal stem cells from adipose tissue and hamstring tendons for musculoskeletal cell-based therapy. *Regen Med* 2015; **10**: 729-743 [PMID: 25565145 DOI: 10.2217/rme.14.92]
24 **Mienaltowski MJ**, Cánovas A, Fates VA, Hampton AR, Pechanec MY, Islas-Trejo A, Medrano JF. Transcriptome profiles of isolated murine Achilles tendon proper- and peritenon-derived progenitor cells. *J Orthop Res* 2019; **37**: 1409-1418 [PMID: 29926971 DOI: 10.1002/jor.24076]
25 **Tempfer H**, Wagner A, Gehwolf R, Lehner C, Tauber M, Resch H, Bauer HC. Perivascular cells of the supraspinatus tendon express both tendon- and stem cell-related markers. *Histochem Cell Biol* 2009; **131**: 733-741 [PMID: 19280209 DOI: 10.1007/s00418-009-0581-5]
26 **Mienaltowski MJ**, Adams SM, Birk DE. Regional differences in stem cell/progenitor cell populations from the mouse achilles tendon. *Tissue Eng Part A* 2013; **19**: 199-210 [PMID: 22871316 DOI: 10.1089/ten.TEA.2012.0182]
27 **Harvey T**, Flamenco S, Fan CM. A $Tppp3^{+}Pdgfra^{+}$ tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis. *Nat Cell Biol* 2019; **21**: 1490-1503 [PMID: 31768046 DOI: 10.1038/s41556-019-0417-z]
28 **Rui YF**, Lui PP, Li G, Fu SC, Lee YW, Chan KM. Isolation and characterization of multipotent rat tendon-derived stem cells. *Tissue Eng Part A* 2010; **16**: 1549-1558 [PMID: 20001227 DOI: 10.1089/ten.TEA.2009.0529]
29 **Tan Q**, Lui PP, Lee YW. In vivo identity of tendon stem cells and the roles of stem cells in tendon healing. *Stem Cells Dev* 2013; **22**: 3128-3140 [PMID: 23815595 DOI: 10.1089/scd.2013.0073]

30 **Ni M**, Lui PP, Rui YF, Lee YW, Lee YW, Tan Q, Wong YM, Kong SK, Lau PM, Li G, Chan KM. Tendon-derived stem cells (TDSCs) promote tendon repair in a rat patellar tendon window defect model. *J Orthop Res* 2012; **30**: 613-619 [PMID: 21928428 DOI: 10.1002/jor.21559]

31 **Al-Ani MKh**, Xu K, Sun Y, Pan L, Xu Z, Yang L. Study of Bone Marrow Mesenchymal and Tendon-Derived Stem Cells Transplantation on the Regenerating Effect of Achilles Tendon Ruptures in Rats. *Stem Cells Int* 2015; **2015**: 984146 [PMID: 26339252 DOI: 10.1155/2015/984146]

32 **Asai S**, Otsuru S, Candela ME, Cantley L, Uchibe K, Hofmann TJ, Zhang K, Wapner KL, Soslowsky LJ, Horwitz EM, Enomoto-Iwamoto M. Tendon progenitor cells in injured tendons have strong chondrogenic potential: the CD105-negative subpopulation induces chondrogenic degeneration. *Stem Cells* 2014; **32**: 3266-3277 [PMID: 25220576 DOI: 10.1002/stem.1847]

33 **Zhang B**, Luo Q, Halim A, Ju Y, Morita Y, Song G. Directed Differentiation and Paracrine Mechanisms of Mesenchymal Stem Cells: Potential Implications for Tendon Repair and Regeneration. *Curr Stem Cell Res Ther* 2017; **12**: 447-454 [PMID: 28464787 DOI: 10.2174/1574888X12666170502102423]

34 **Bunnell BA**, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: isolation, expansion and differentiation. *Methods* 2008; **45**: 115-120 [PMID: 18593609 DOI: 10.1016/j.ymeth.2008.03.006]

35 **Li CY**, Wu XY, Tong JB, Yang XX, Zhao JL, Zheng QF, Zhao GB, Ma ZJ. Comparative analysis of human mesenchymal stem cells from bone marrow and adipose tissue under xeno-free conditions for cell therapy. *Stem Cell Res Ther* 2015; **6**: 55 [PMID: 25884704 DOI: 10.1186/s13287-015-0066-5]

36 **Perucca Orfei C**, Viganò M, Pearson JR, Colombini A, De Luca P, Ragni E, Santos-Ruiz L, de Girolamo L. In Vitro Induction of Tendon-Specific Markers in Tendon Cells, Adipose- and Bone Marrow-Derived Stem Cells is Dependent on TGFβ3, BMP-12 and Ascorbic Acid Stimulation. *Int J Mol Sci* 2019; **20** [PMID: 30609804 DOI: 10.3390/ijms20010149]

37 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

38 **Heo JS**, Choi Y, Kim HS, Kim HO. Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. *Int J Mol Med* 2016; **37**: 115-125 [PMID: 26719857 DOI: 10.3892/ijmm.2015.2413]

39 **Kuo CK**, Tuan RS. Mechanoactive tenogenic differentiation of human mesenchymal stem cells. *Tissue Eng Part A* 2008; **14**: 1615-1627 [PMID: 18759661 DOI: 10.1089/ten.tea.2006.0415]

40 **Baberg F**, Geyh S, Waldera-Lupa D, Stefanski A, Zilkens C, Haas R, Schroeder T, Stühler K. Secretome analysis of human bone marrow derived mesenchymal stromal cells. *Biochim Biophys Acta Proteins Proteom* 2019; **1867**: 434-441 [PMID: 30716505 DOI: 10.1016/j.bbapap.2019.01.013]

41 **Wu T**, Liu Y, Wang B, Sun Y, Xu J, Yuk-Wai LW, Xu L, Zhang J, Li G. The Use of Cocultured Mesenchymal Stem Cells with Tendon-Derived Stem Cells as a Better Cell Source for Tendon Repair. *Tissue Eng Part A* 2016; **22**: 1229-1240 [PMID: 27609185 DOI: 10.1089/ten.TEA.2016.0248]

42 **Chong AK**, Ang AD, Goh JC, Hui JH, Lim AY, Lee EH, Lim BH. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit achilles tendon model. *J Bone Joint Surg Am* 2007; **89**: 74-81 [PMID: 17200313 DOI: 10.2106/jbjs.E.01396]

43 **He M**, Gan AW, Lim AY, Goh JC, Hui JH, Chong AK. Bone Marrow Derived Mesenchymal Stem Cell Augmentation of Rabbit Flexor Tendon Healing. *Hand Surg* 2015; **20**: 421-429 [PMID: 26388004 DOI: 10.1142/S0218810415500343]

44 **Harris MT**, Butler DL, Boivin GP, Florer JB, Schantz EJ, Wenstrup RJ. Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. *J Orthop Res* 2004; **22**: 998-1003 [PMID: 15304271 DOI: 10.1016/j.orthres.2004.02.012]

45 **Arnhold S**, Elashry MI, Klymiuk MC, Geburek F. Investigation of stemness and multipotency of equine adipose-derived mesenchymal stem cells (ASCs) from different fat sources in comparison with lipoma. *Stem Cell Res Ther* 2019; **10**: 309 [PMID: 31640774 DOI: 10.1186/s13287-019-1429-0]

46 **Mohamed-Ahmed S**, Fristad I, Lie SA, Suliman S, Mustafa K, Vindenes H, Idris SB. Adipose-derived and bone marrow mesenchymal stem cells: a donor-matched comparison. *Stem Cell Res Ther* 2018; **9**: 168 [PMID: 29921311 DOI: 10.1186/s13287-018-0914-1]

47 **De Francesco F**, Ricci G, D'Andrea F, Nicoletti GF, Ferraro GA. Human Adipose Stem Cells: From Bench to Bedside. *Tissue Eng Part B Rev* 2015; **21**: 572-584 [PMID: 25953464 DOI: 10.1089/ten.TEB.2014.0608]

48 **Li X**, Pongkitwitoon S, Lu H, Lee C, Gelberman R, Thomopoulos S. CTGF induces tenogenic differentiation and proliferation of adipose-derived stromal cells. *J Orthop Res* 2019; **37**: 574-582 [PMID: 30756417 DOI: 10.1002/jor.24248]

49 **Dai L**, Hu X, Zhang X, Zhu J, Zhang J, Fu X, Duan X, Ao Y, Zhou C. Different tenogenic differentiation capacities of different mesenchymal stem cells in the presence of BMP-12. *J Transl Med* 2015; **13**: 200 [PMID: 26104414 DOI: 10.1186/s12967-015-0560-7]

50 **Rinella L**, Marano F, Paletto L, Fraccalvieri M, Annaratone L, Castellano I, Fortunati N, Bargoni A, Berta L, Frairia R, Catalano MG. Extracorporeal shock waves trigger tenogenic differentiation of human adipose-derived stem cells. *Connect Tissue Res* 2018; **59**: 561-573 [PMID: 29316809 DOI: 10.1080/03008207.2018.1424147]

51 **Cheng X**, Tsao C, Sylvia VL, Cornet D, Nicolella DP, Bredbenner TL, Christy RJ. Platelet-derived growth-factor-releasing aligned collagen-nanoparticle fibers promote the proliferation and tenogenic differentiation of adipose-derived stem cells. *Acta Biomater* 2014; **10**: 1360-1369 [PMID: 24291329 DOI: 10.1016/j.actbio.2013.11.017]

52 **Lee SY**, Kwon B, Lee K, Son YH, Chung SG. Therapeutic Mechanisms of Human Adipose-Derived Mesenchymal Stem Cells in a Rat Tendon Injury Model. *Am J Sports Med* 2017; **45**: 1429-1439 [PMID: 28291954 DOI: 10.1177/0363546517689874]

53 **Norelli JB**, Plaza DP, Stal DN, Varghese AM, Liang H, Grande DA. Tenogenically differentiated adipose-derived stem cells are effective in Achilles tendon repair in vivo. *J Tissue Eng* 2018; **9**: 2041731418811183 [PMID: 30542597 DOI: 10.1177/2041731418811183]

54 **Oshita T**, Tobita M, Tajima S, Mizuno H. Adipose-Derived Stem Cells Improve Collagenase-Induced Tendinopathy in a Rat Model. *Am J Sports Med* 2016; **44**: 1983-1989 [PMID: 27159294 DOI: 10.1177/0363546516640750]

55 **Kokubu S**, Inaki R, Hoshi K, Hikita A. Adipose-derived stem cells improve tendon repair and prevent ectopic ossification in tendinopathy by inhibiting inflammation and inducing neovascularization in the early stage of tendon healing. *Regen Ther* 2020; **14**: 103-110 [PMID: 31989000 DOI: 10.1016/j.reth.2019.12.003]

56 **Lee SY**, Kim W, Lim C, Chung SG. Treatment of Lateral Epicondylosis by Using Allogeneic Adipose-Derived Mesenchymal Stem Cells: A Pilot Study. *Stem Cells* 2015; **33**: 2995-3005 [PMID: 26202898 DOI: 10.1002/stem.2110]

57 **Costa-Almeida R**, Calejo I, Reis RL, Gomes ME. Crosstalk between adipose stem cells and tendon cells reveals a temporal regulation of tenogenesis by matrix deposition and remodeling. *J Cell Physiol* 2018; **233**: 5383-5395 [PMID: 29215729 DOI: 10.1002/jcp.26363]

58 **Uysal AC**, Mizuno H. Tendon regeneration and repair with adipose derived stem cells. *Curr Stem Cell Res Ther* 2010; **5**: 161-167 [PMID: 19941450 DOI: 10.2174/157488810791268609]

59 **Bavin EP**, Smith O, Baird AE, Smith LC, Guest DJ. Equine Induced Pluripotent Stem Cells have a Reduced Tendon Differentiation Capacity Compared to Embryonic Stem Cells. *Front Vet Sci* 2015; **2**: 55 [PMID: 26664982 DOI: 10.3389/fvets.2015.00055]

60 **Xu W**, Wang Y, Liu E, Sun Y, Luo Z, Xu Z, Liu W, Zhong L, Lv Y, Wang A, Tang Z, Li S, Yang L. Human iPSC-derived neural crest stem cells promote tendon repair in a rat patellar tendon window defect model. *Tissue Eng Part A* 2013; **19**: 2439-2451 [PMID: 23815150 DOI: 10.1089/ten.TEA.2012.0453]

61 **Lui PP**, Chan KM. Tendon-derived stem cells (TDSCs): from basic science to potential roles in tendon pathology and tissue engineering applications. *Stem Cell Rev Rep* 2011; **7**: 883-897 [PMID: 21611803 DOI: 10.1007/s12015-011-9276-0]

62 **Thaker H**, Sharma AK. Engaging stem cells for customized tendon regeneration. *Stem Cells Int* 2012; **2012**: 309187 [PMID: 22685473 DOI: 10.1155/2012/309187]

63 **Costa-Almeida R**, Calejo I, Gomes ME. Mesenchymal Stem Cells Empowering Tendon Regenerative Therapies. *Int J Mol Sci* 2019; **20** [PMID: 31248196 DOI: 10.3390/ijms20123002]

64 **Gracey E**, Burssens A, Cambré I, Schett G, Lories R, McInnes IB, Asahara H, Elewaut D. Tendon and ligament mechanical loading in the pathogenesis of inflammatory arthritis. *Nat Rev Rheumatol* 2020; **16**: 193-207 [PMID: 32080619 DOI: 10.1038/s41584-019-0364-x]

65 **Wang JH**, Guo Q, Li B. Tendon biomechanics and mechanobiology--a minireview of basic concepts and recent advancements. *J Hand Ther* 2012; **25**: 133-40; quiz 141 [PMID: 21925835 DOI: 10.1016/j.jht.2011.07.004]

66 **Thornton GM**, Hart DA. The interface of mechanical loading and biological variables as they pertain to the development of tendinosis. *J Musculoskelet Neuronal Interact* 2011; **11**: 94-105 [PMID: 21625046 DOI: 10.1142/S0219635211002725]

67 **Nabeshima Y**, Grood ES, Sakurai A, Herman JH. Uniaxial tension inhibits tendon collagen degradation by collagenase in vitro. *J Orthop Res* 1996; **14**: 123-130 [PMID: 8618154 DOI: 10.1002/jor.1100140120]

68 **Fung DT**, Wang VM, Laudier DM, Shine JH, Basta-Pljakic J, Jepsen KJ, Schaffler MB, Flatow EL. Subrupture tendon fatigue damage. *J Orthop Res* 2009; **27**: 264-273 [PMID: 18683881 DOI: 10.1002/jor.20722]

69 **Parent G**, Huppé N, Langelier E. Low stress tendon fatigue is a relatively rapid process in the context of overuse injuries. *Ann Biomed Eng* 2011; **39**: 1535-1545 [PMID: 21287276 DOI: 10.1007/s10439-011-0254-0]

70 **Snedeker JG**, Foolen J. Tendon injury and repair - A perspective on the basic mechanisms of tendon disease and future clinical therapy. *Acta Biomater* 2017; **63**: 18-36 [PMID: 28867648 DOI: 10.1016/j.actbio.2017.08.032]

71 **Fedorczyk JM**, Barr AE, Rani S, Gao HG, Amin M, Amin S, Litvin J, Barbe MF. Exposure-dependent increases in IL-1beta, substance P, CTGF, and tendinosis in flexor digitorum tendons with upper extremity repetitive strain injury. *J Orthop Res* 2010; **28**: 298-307 [PMID: 19743505 DOI: 10.1002/jor.20984]

72 **Yang G**, Im HJ, Wang JH. Repetitive mechanical stretching modulates IL-1beta induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene* 2005; **363**: 166-172 [PMID: 16226404 DOI: 10.1016/j.gene.2005.08.006]

73 **Zhang J**, Wang JH. Production of PGE(2) increases in tendons subjected to repetitive mechanical loading and induces differentiation of tendon stem cells into non-tenocytes. *J Orthop Res* 2010; **28**: 198-203 [PMID: 19688869 DOI: 10.1002/jor.20962]

74 **Zhang J**, Pan T, Liu Y, Wang JH. Mouse treadmill running enhances tendons by expanding the pool of tendon stem cells (TSCs) and TSC-related cellular production of collagen. *J Orthop Res* 2010; **28**: 1178-1183 [PMID: 20225313 DOI: 10.1002/jor.21123]

75 **Zhang J**, Wang JH. Mechanobiological response of tendon stem cells: implications of tendon homeostasis and pathogenesis of tendinopathy. *J Orthop Res* 2010; **28**: 639-643 [PMID: 19918904 DOI: 10.1002/jor.21046]

76 **Zhang J**, Li B, Wang JH. The role of engineered tendon matrix in the stemness of tendon stem cells in vitro and the promotion of tendon-like tissue formation in vivo. *Biomaterials* 2011; **32**: 6972-6981 [PMID: 21703682 DOI: 10.1016/j.biomaterials.2011.05.088]

77 **Kubo K**, Akima H, Ushiyama J, Tabata I, Fukuoka H, Kanehisa H, Fukunaga T. Effects of 20 days of bed rest on the viscoelastic properties of tendon structures in lower limb muscles. *Br J Sports Med* 2004; **38**: 324-330 [PMID: 15155437 DOI: 10.1136/bjsm.2003.005595]

78 **Uchida H**, Tohyama H, Nagashima K, Ohba Y, Matsumoto H, Toyama Y, Yasuda K. Stress deprivation simultaneously induces over-expression of interleukin-1beta, tumor necrosis factor-alpha, and transforming growth factor-beta in fibroblasts and mechanical deterioration of the tissue in the patellar tendon. *J Biomech* 2005; **38**: 791-798 [PMID: 15713300 DOI: 10.1016/j.jbiomech.2004.05.009]

79 **Elosegui-Artola A**, Trepat X, Roca-Cusachs P. Control of Mechanotransduction by Molecular Clutch Dynamics. *Trends Cell Biol* 2018; **28**: 356-367 [PMID: 29496292 DOI: 10.1016/j.tcb.2018.01.008]

80 **Warden SJ**, Thompson WR. Become one with the force: optimising mechanotherapy through an understanding of mechanobiology. *Br J Sports Med* 2017; **51**: 989-990 [PMID: 28646107 DOI: 10.1136/bjsports-2017-097634]
81 **Khan KM**, Scott A. Mechanotherapy: how physical therapists' prescription of exercise promotes tissue repair. *Br J Sports Med* 2009; **43**: 247-252 [PMID: 19244270 DOI: 10.1136/bjsm.2008.054239]
82 **Docking S**, Samiric T, Scase E, Purdam C, Cook J. Relationship between compressive loading and ECM changes in tendons. *Muscles Ligaments Tendons J* 2013; **3**: 7-11 [PMID: 23885340 DOI: 10.11138/mltj/2013.3.1.007]
83 **Cook JL**, Purdam C. Is compressive load a factor in the development of tendinopathy? *Br J Sports Med* 2012; **46**: 163-168 [PMID: 22113234 DOI: 10.1136/bjsports-2011-090414]
84 **Almekinders LC**, Weinhold PS, Maffulli N. Compression etiology in tendinopathy. *Clin Sports Med* 2003; **22**: 703-710 [PMID: 14560542 DOI: 10.1016/s0278-5919(03)00067-x]
85 **Wall M**, Butler D, El Haj A, Bodle JC, Loboa EG, Banes AJ. Key developments that impacted the field of mechanobiology and mechanotransduction. *J Orthop Res* 2018; **36**: 605-619 [PMID: 28817244 DOI: 10.1002/jor.23707]
86 **Sun Z**, Guo SS, Fässler R. Integrin-mediated mechanotransduction. *J Cell Biol* 2016; **215**: 445-456 [PMID: 27872252 DOI: 10.1083/jcb.201609037]
87 **Cutting GR**. Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat Rev Genet* 2015; **16**: 45-56 [PMID: 25404111 DOI: 10.1038/nrg3849]
88 **Zhang WK**, Wang D, Duan Y, Loy MM, Chan HC, Huang P. Mechanosensitive gating of CFTR. *Nat Cell Biol* 2010; **12**: 507-512 [PMID: 20400957 DOI: 10.1038/ncb2053]
89 **Liu Y**, Xu J, Xu L, Wu T, Sun Y, Lee YW, Wang B, Chan HC, Jiang X, Zhang J, Li G. Cystic fibrosis transmembrane conductance regulator mediates tenogenic differentiation of tendon-derived stem cells and tendon repair: accelerating tendon injury healing by intervening in its downstream signaling. *FASEB J* 2017; **31**: 3800-3815 [PMID: 28495756 DOI: 10.1096/fj.201601181R]
90 **Xiao E**, Yang HQ, Gan YH, Duan DH, He LH, Guo Y, Wang SQ, Zhang Y. Brief reports: TRPM7 Senses mechanical stimulation inducing osteogenesis in human bone marrow mesenchymal stem cells. *Stem Cells* 2015; **33**: 615-621 [PMID: 25263397 DOI: 10.1002/stem.1858]
91 **Tonelli FM**, Santos AK, Gomes DA, da Silva SL, Gomes KN, Ladeira LO, Resende RR. Stem cells and calcium signaling. *Adv Exp Med Biol* 2012; **740**: 891-916 [PMID: 22453975 DOI: 10.1007/978-94-007-2888-2_40]
92 **Liu YS**, Liu YA, Huang CJ, Yen MH, Tseng CT, Chien S, Lee OK. Mechanosensitive TRPM7 mediates shear stress and modulates osteogenic differentiation of mesenchymal stromal cells through Osterix pathway. *Sci Rep* 2015; **5**: 16522 [PMID: 26558702 DOI: 10.1038/srep16522]
93 **Winslow MM**, Pan M, Starbuck M, Gallo EM, Deng L, Karsenty G, Crabtree GR. Calcineurin/NFAT signaling in osteoblasts regulates bone mass. *Dev Cell* 2006; **10**: 771-782 [PMID: 16740479 DOI: 10.1016/j.devcel.2006.04.006]
94 **Fromigué O**, Haÿ E, Barbara A, Marie PJ. Essential role of nuclear factor of activated T cells (NFAT)-mediated Wnt signaling in osteoblast differentiation induced by strontium ranelate. *J Biol Chem* 2010; **285**: 25251-25258 [PMID: 20554534 DOI: 10.1074/jbc.M110.110502]
95 **Xiao E**, Chen C, Zhang Y. The mechanosensor of mesenchymal stem cells: mechanosensitive channel or cytoskeleton? *Stem Cell Res Ther* 2016; **7**: 140 [PMID: 27651019 DOI: 10.1186/s13287-016-0397-x]
96 **Ingber DE**, Wang N, Stamenovic D. Tensegrity, cellular biophysics, and the mechanics of living systems. *Rep Prog Phys* 2014; **77**: 046603 [PMID: 24695087 DOI: 10.1088/0034-4885/77/4/046603]
97 **Lavagnino M**, Wall ME, Little D, Banes AJ, Guilak F, Arnoczky SP. Tendon mechanobiology: Current knowledge and future research opportunities. *J Orthop Res* 2015; **33**: 813-822 [PMID: 25763779 DOI: 10.1002/jor.22871]
98 **Docheva D**, Popov C, Mutschler W, Schieker M. Human mesenchymal stem cells in contact with their environment: surface characteristics and the integrin system. *J Cell Mol Med* 2007; **11**: 21-38 [PMID: 17367499 DOI: 10.1111/j.1582-4934.2007.00001.x]
99 **Sen B**, Xie Z, Case N, Ma M, Rubin C, Rubin J. Mechanical strain inhibits adipogenesis in mesenchymal stem cells by stimulating a durable beta-catenin signal. *Endocrinology* 2008; **149**: 6065-6075 [PMID: 18687779 DOI: 10.1210/en.2008-0687]
100 **Liu X**, Chen W, Zhou Y, Tang K, Zhang J. Mechanical Tension Promotes the Osteogenic Differentiation of Rat Tendon-derived Stem Cells Through the Wnt5a/Wnt5b/JNK Signaling Pathway. *Cell Physiol Biochem* 2015; **36**: 517-530 [PMID: 25966835 DOI: 10.1159/000430117]
101 **Shi Y**, Fu Y, Tong W, Geng Y, Lui PP, Tang T, Zhang X, Dai K. Uniaxial mechanical tension promoted osteogenic differentiation of rat tendon-derived stem cells (rTDSCs) via the Wnt5a-RhoA pathway. *J Cell Biochem* 2012; **113**: 3133-3142 [PMID: 22615126 DOI: 10.1002/jcb.24190]
102 **Brown JP**, Finley VG, Kuo CK. Embryonic mechanical and soluble cues regulate tendon progenitor cell gene expression as a function of developmental stage and anatomical origin. *J Biomech* 2014; **47**: 214-222 [PMID: 24231248 DOI: 10.1016/j.jbiomech.2013.09.018]
103 **Rui YF**, Lui PP, Wong YM, Tan Q, Chan KM. BMP-2 stimulated non-tenogenic differentiation and promoted proteoglycan deposition of tendon-derived stem cells (TDSCs) in vitro. *J Orthop Res* 2013; **31**: 746-753 [PMID: 23238867 DOI: 10.1002/jor.22290]
104 **Gardner K**, Arnoczky SP, Lavagnino M. Effect of in vitro stress-deprivation and cyclic loading on the length of tendon cell cilia in situ. *J Orthop Res* 2011; **29**: 582-587 [PMID: 20957738 DOI: 10.1002/jor.21271]
105 **Satir P**, Pedersen LB, Christensen ST. The primary cilium at a glance. *J Cell Sci* 2010; **123**: 499-503 [PMID: 20144997 DOI: 10.1242/jcs.050377]
106 **Bodle JC**, Loboa EG. Concise Review: Primary Cilia: Control Centers for Stem Cell Lineage Specification and Potential Targets for Cell-Based Therapies. *Stem Cells* 2016; **34**: 1445-1454 [PMID: 26866419 DOI: 10.1002/stem.2341]
107 **Banes AJ**, Weinhold P, Yang X, Tsuzaki M, Bynum D, Bottlang M, Brown T. Gap junctions regulate responses of tendon cells ex vivo to mechanical loading. *Clin Orthop Relat Res* 1999; S356-S370 [PMID:

10546659 DOI: 10.1097/00003086-199910001-00034]
108 **Wall ME**, Banes AJ. Early responses to mechanical load in tendon: role for calcium signaling, gap junctions and intercellular communication. *J Musculoskelet Neuronal Interact* 2005; **5**: 70-84 [PMID: 15788873]
109 **Waggett AD**, Benjamin M, Ralphs JR. Connexin 32 and 43 gap junctions differentially modulate tenocyte response to cyclic mechanical load. *Eur J Cell Biol* 2006; **85**: 1145-1154 [PMID: 16859807 DOI: 10.1016/j.ejcb.2006.06.002]
110 **McNeilly CM**, Banes AJ, Benjamin M, Ralphs JR. Tendon cells in vivo form a three dimensional network of cell processes linked by gap junctions. *J Anat* 1996; **189**: 593-600 [PMID: 8982835 DOI: 10.1076/ejom.34.5.385.13055]
111 **Maeda E**, Ohashi T. Mechano-regulation of gap junction communications between tendon cells is dependent on the magnitude of tensile strain. *Biochem Biophys Res Commun* 2015; **465**: 281-286 [PMID: 26260322 DOI: 10.1016/j.bbrc.2015.08.021]
112 **Shao Q**, Esseltine JL, Huang T, Novielli-Kuntz N, Ching JE, Sampson J, Laird DW. Connexin43 is Dispensable for Early Stage Human Mesenchymal Stem Cell Adipogenic Differentiation But is Protective against Cell Senescence. *Biomolecules* 2019; **9** [PMID: 31514306 DOI: 10.3390/biom9090474]
113 **Theodossiou SK**, Tokle J, Schiele NR. TGFβ2-induced tenogenesis impacts cadherin and connexin cell-cell junction proteins in mesenchymal stem cells. *Biochem Biophys Res Commun* 2019; **508**: 889-893 [PMID: 30538046 DOI: 10.1016/j.bbrc.2018.12.023]
114 **van Amerongen R**, Mikels A, Nusse R. Alternative wnt signaling is initiated by distinct receptors. *Sci Signal* 2008; **1**: re9 [PMID: 18765832 DOI: 10.1126/scisignal.135re9]
115 **Arnsdorf EJ**, Tummala P, Kwon RY, Jacobs CR. Mechanically induced osteogenic differentiation--the role of RhoA, ROCKII and cytoskeletal dynamics. *J Cell Sci* 2009; **122**: 546-553 [PMID: 19174467 DOI: 10.1242/jcs.036293]
116 **Wang C**, Zhao Y, Su Y, Li R, Lin Y, Zhou X, Ye L. C-Jun N-terminal kinase (JNK) mediates Wnt5a-induced cell motility dependent or independent of RhoA pathway in human dental papilla cells. *PLoS One* 2013; **8**: e69440 [PMID: 23844260 DOI: 10.1371/journal.pone.0069440]
117 **Lui PP**, Lee YW, Wong YM, Zhang X, Dai K, Rolf CG. Expression of Wnt pathway mediators in metaplasic tissue in animal model and clinical samples of tendinopathy. *Rheumatology (Oxford)* 2013; **52**: 1609-1618 [PMID: 23776285 DOI: 10.1093/rheumatology/ket214]
118 **Kishimoto Y**, Ohkawara B, Sakai T, Ito M, Masuda A, Ishiguro N, Shukunami C, Docheva D, Ohno K. Wnt/β-catenin signaling suppresses expressions of Scx, Mkx, and Tnmd in tendon-derived cells. *PLoS One* 2017; **12**: e0182051 [PMID: 28750046 DOI: 10.1371/journal.pone.0182051]
119 **Rui YF**, Lui PP, Ni M, Chan LS, Lee YW, Chan KM. Mechanical loading increased BMP-2 expression which promoted osteogenic differentiation of tendon-derived stem cells. *J Orthop Res* 2011; **29**: 390-396 [PMID: 20882582 DOI: 10.1002/jor.21218]
120 **Zhang J**, Wang JH. BMP-2 mediates PGE(2) -induced reduction of proliferation and osteogenic differentiation of human tendon stem cells. *J Orthop Res* 2012; **30**: 47-52 [PMID: 21688312 DOI: 10.1002/jor.21485]
121 **Hoffmann A**, Pelled G, Turgeman G, Eberle P, Zilberman Y, Shinar H, Keinan-Adamsky K, Winkel A, Shahab S, Navon G, Gross G, Gazit D. Neotendon formation induced by manipulation of the Smad8 signalling pathway in mesenchymal stem cells. *J Clin Invest* 2006; **116**: 940-952 [PMID: 16585960 DOI: 10.1172/JCI22689]
122 **Liu C**, Luo JW, Liang T, Lin LX, Luo ZP, Zhuang YQ, Sun YL. Matrix stiffness regulates the differentiation of tendon-derived stem cells through FAK-ERK1/2 activation. *Exp Cell Res* 2018; **373**: 62-70 [PMID: 30138615 DOI: 10.1016/j.yexcr.2018.08.023]
123 **Havis E**, Bonnin MA, Olivera-Martinez I, Nazaret N, Ruggiu M, Weibel J, Durand C, Guerquin MJ, Bonod-Bidaud C, Ruggiero F, Schweitzer R, Duprez D. Transcriptomic analysis of mouse limb tendon cells during development. *Development* 2014; **141**: 3683-3696 [PMID: 25249460 DOI: 10.1242/dev.108654]
124 **Popov C**, Burggraf M, Kreja L, Ignatius A, Schieker M, Docheva D. Mechanical stimulation of human tendon stem/progenitor cells results in upregulation of matrix proteins, integrins and MMPs, and activation of p38 and ERK1/2 kinases. *BMC Mol Biol* 2015; **16**: 6 [PMID: 25880261 DOI: 10.1186/s12867-015-0036-6]
125 **Paxton JZ**, Hagerty P, Andrick JJ, Baar K. Optimizing an intermittent stretch paradigm using ERK1/2 phosphorylation results in increased collagen synthesis in engineered ligaments. *Tissue Eng Part A* 2012; **18**: 277-284 [PMID: 21902469 DOI: 10.1089/ten.TEA.2011.0336]
126 **Xu B**, Song G, Ju Y, Li X, Song Y, Watanabe S. RhoA/ROCK, cytoskeletal dynamics, and focal adhesion kinase are required for mechanical stretch-induced tenogenic differentiation of human mesenchymal stem cells. *J Cell Physiol* 2012; **227**: 2722-2729 [PMID: 21898412 DOI: 10.1002/jcp.23016]
127 **Dupont S**, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S. Role of YAP/TAZ in mechanotransduction. *Nature* 2011; **474**: 179-183 [PMID: 21654799 DOI: 10.1038/nature10137]
128 **Low BC**, Pan CQ, Shivashankar GV, Bershadsky A, Sudol M, Sheetz M. YAP/TAZ as mechanosensors and mechanotransducers in regulating organ size and tumor growth. *FEBS Lett* 2014; **588**: 2663-2670 [PMID: 24747426 DOI: 10.1016/j.febslet.2014.04.012]
129 **Kim KM**, Choi YJ, Hwang JH, Kim AR, Cho HJ, Hwang ES, Park JY, Lee SH, Hong JH. Shear stress induced by an interstitial level of slow flow increases the osteogenic differentiation of mesenchymal stem cells through TAZ activation. *PLoS One* 2014; **9**: e92427 [PMID: 24658423 DOI: 10.1371/journal.pone.0092427]
130 **Zhong W**, Zhang W, Wang S, Qin J. Regulation of fibrochondrogenesis of mesenchymal stem cells in an integrated microfluidic platform embedded with biomimetic nanofibrous scaffolds. *PLoS One* 2013; **8**: e61283 [PMID: 23637803 DOI: 10.1371/journal.pone.0061283]
131 **Park HW**, Kim YC, Yu B, Moroishi T, Mo JS, Plouffe SW, Meng Z, Lin KC, Yu FX, Alexander CM, Wang CY, Guan KL. Alternative Wnt Signaling Activates YAP/TAZ. *Cell* 2015; **162**: 780-794 [PMID: 26276632 DOI: 10.1016/j.cell.2015.07.013]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 966-985

DOI: 10.4252/wjsc.v12.i9.966

ISSN 1948-0210 (online)

REVIEW

Senescent mesenchymal stem/stromal cells and restoring their cellular functions

Qing-Shu Meng, Jing Liu, Lu Wei, Hui-Min Fan, Xiao-Hui Zhou, Xiao-Ting Liang

ORCID number: Qing-Shu Meng 0000-0002-9879-3138; Jing Liu 0000-0003-2126-0146; Lu Wei 0000-0002-7976-5203; Hui-Min Fan 0000-0002-9776-3180; Xiao-Hui Zhou 0000-0002-2965-6914; Xiao-Ting Liang 0000-0003-3262-1858.

Author contributions: Meng QS and Liu J contributed equally to this work; Liang XT and Meng QS defined the study topic; Liu J, Wei L, and Fan HM collected the references; Meng QS wrote the paper; Liu J made the tables; Liang XT and Zhou XH revised the manuscript and resolved all disagreements in discussion with Meng QS and Liu J.

Supported by the National Natural Science Foundation of China, Nos. 81500207, 81670458, and 81470393; Shanghai Municipal Health and Family Planning Commission, No. ZY(2018-2020)-FWTX-2007; Shanghai Key Medical Discipline for Critical Care Medicine, No. 2017zz02017; the National Key Research and Development Program of China, No. 2017YFA0105600; Major Program of Development Fund for Shanghai Zhangjiang National Innovtaion Demonstration Zone < Stem Cell Strategic Biobank and Stem Cell Clinical Technology Transformation Platform>, No. ZJ2018-ZD-004; the Science and

Qing-Shu Meng, Jing Liu, Lu Wei, Hui-Min Fan, Xiao-Hui Zhou, Xiao-Ting Liang, Shanghai Heart Failure Research Center, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, China

Qing-Shu Meng, Jing Liu, Lu Wei, Hui-Min Fan, Xiao-Hui Zhou, Xiao-Ting Liang, Research Center for Translational Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, China

Qing-Shu Meng, Jing Liu, Lu Wei, Hui-Min Fan, Xiao-Hui Zhou, Xiao-Ting Liang, Institute of Integrated Traditional Chinese and Western Medicine for Cardiovascular Chronic Diseases, Tongji University School of Medicine, Shanghai 200120, China

Hui-Min Fan, Department of Heart Failure, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, China

Xiao-Ting Liang, Institute for Regenerative Medicine, Shanghai East Hospital, School of Life Sciences and Technology, Tongji University, Shanghai 200120, China

Corresponding author: Xiao-Ting Liang, MD, PhD, Research Fellow, Institute for Regenerative Medicine, Shanghai East Hospital, School of Life Sciences and Technology, Tongji University, No. 150, Jimo Road, Shanghai 200120, China. liangxt@tongji.edu.cn

Abstract

Mesenchymal stem/stromal cells (MSCs) have various properties that make them promising candidates for stem cell-based therapies in clinical settings. These include self-renewal, multilineage differentiation, and immunoregulation. However, recent studies have confirmed that aging is a vital factor that limits their function and therapeutic properties as standardized clinical products. Understanding the features of senescence and exploration of cell rejuvenation methods are necessary to develop effective strategies that can overcome the shortage and instability of MSCs. This review will summarize the current knowledge on characteristics and functional changes of aged MSCs. Additionally, it will highlight cell rejuvenation strategies such as molecular regulation, non-coding RNA modifications, and microenvironment controls that may enhance the therapeutic potential of MSCs in clinical settings.

Key Words: Mesenchymal stem cells; Senescence; Features; Function; Rejuvenation strategy

Technology Commission of Shanghai Municipality, No. 17431906600; and the Top-level Clinical Discipline Project of Shanghai Pudong, No. PWYgf2018-05.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest related to this work.

Manuscript source: Invited manuscript

Received: May 27, 2020
Peer-review started: May 27, 2020
First decision: June 15, 2020
Revised: June 23, 2020
Accepted: July 19, 2020
Article in press: July 19, 2020
Published online: September 26, 2020

P-Reviewer: Ciccocioppo R
S-Editor: Yan JP
L-Editor: Wang TQ
P-Editor: Xing YX

Core Tip: Mesenchymal stem cell (MSC) administration is a promising therapeutic strategy for various human diseases. However, cell aging limits MSC function and therapeutic properties *via* reducing their activities. We review the morphological changes, molecular expression alterations, and functional degeneration of aged MSCs, and the effects of aged MSCs on immune cells and other target cells. Additionally, we summarize the strategies to rejuvenate aged MSCs to enhance their clinical potential.

Citation: Meng QS, Liu J, Wei L, Fan HM, Zhou XH, Liang XT. Senescent mesenchymal stem/stromal cells and restoring their cellular functions. *World J Stem Cells* 2020; 12(9): 966-985
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/966.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.966

INTRODUCTION

Mesenchymal stem/stromal cells (MSCs) are multipotent progenitor cells that can retain postnatal capacity for both self-renewal and multilineage differentiation. The minimal criteria for MSCs as defined by the International Society for Cellular Therapy in 2006 are adherence to plastic under culture conditions; positivity for cell surface markers CD44, CD90, CD105, and CD73; negativity for hematopoietic markers CD45, CD34, CD14, CD11b, CD79α, CD19, and human leukocyte antigen-DR; and multi-differentiation potential of osteogenesis, chondrogenesis, and adipogenesis[1]. They are a heterogeneous population of cells isolated from a variety of mesodermal tissues. These cells are involved in a wide range of physiological and pathological processes, such as bone development, adipogenesis, fibrosis, and inflammatory regulation[2]. Over the past few decades, the amount of MSC-focused research has grown exponentially. These studies include both preclinical and clinical trials of either autologous or allogeneic MSCs. Infusion of MSCs has been performed to evaluate their safety and therapeutic efficacy in diseases of the immune[3], hematological[4], cardiovascular[5,6], nervous[7,8], respiratory[9], digestive[10], skeletal[11], endocrine[12], and reproductive[13] systems[14]. To date, more than 1000 MSC-based clinical trials have been registered in the United States National Institute of Health database[15,16]. It is well recognized that MSC administration is a safe and effective strategy in the treatment of a variety of diseases.

Emerging evidence has demonstrated that multiple factors, including cell species, tissue source, isolation method, culture conditions, and cellular status, may explain the inconsistency in the features and characteristics of MSCs in some preclinical and clinical trials. A recent study showed that aging is an important factor affecting MSC properties and functions[17]. Age-dependent decline in MSC number and function was found in old individuals[18]. Additionally, MSCs from young donors may also become senescent because of excessive cell passage, oxidative stress, and other injuries[19,20]. The senescent cells manifest a sequence of progressive changes in cellular morphology, biological function, and molecular expression[21,22], as well as weakened efficacy in cell-based therapies[23]. Therefore, appropriate quality controls or cellular rejuvenation processes are required to obtain clinical-grade MSCs. In this review, we will focus on investigations that have assessed the molecular features and functional changes of aged MSCs and highlight rejuvenation strategies that will enable more effective clinical translation.

CHARACTERISTICS AND FUNCTIONAL CHANGES OF AGED MSCS

Aging MSCs exhibit morphological changes and undergo a progressive decline in homeostasis, which contributes to the age-dependent deterioration of MSC function[24]. These changes in senescent MSCs include a general decrease in their regenerative capacity, a switch in differentiation potency, and weakened regulatory functions (such as immunosuppressive effects)[25]. A full understanding of these characteristics is fundamental for the development of strategies to delay or even prevent MSC

senescence. In view of this, the phenotypes and functional characteristics of senescent MSCs will be summarized in this section.

Morphological changes in aged MSCs

The most noticeable changes in aged MSCs are morphological. *In vitro* imaging analysis demonstrated that MSCs from early passages (P1-P3) were remarkably uniform in size[24]. At P5, they exhibited a flattened and enlarged morphology compared with those at P1. Additionally, gradual telomere shortening is a typical characteristic of aging in MSCs[26]. Moreover, these changes in morphology represented the heterogeneous response to the cellular microenvironment *in vitro* and *in vivo*.

Alterations of activity

In aged MSCs, the balance of homeostasis is disrupted, the proliferative ability declines, and mitochondrial dysfunction increases. In addition, both the DNA repair and retrogression of anti-oxidative capacity are reduced[18,27]. Senescent MSCs display delayed self-cloning as well as restricted differentiation potency[28]. Additionally, they exhibit a shift in differentiation potency from osteoblasts to adipocytes[29-33]. Genetic stability, another biological index affecting MSCs activity, is involved in biosafety issues and therapeutic efficacy of these cells[34]. Mounting evidence suggested that long-term cultured MSCs acquired genetic alterations, with the promotion of cell senescence and potential increased risks of transformation. However, the relevance of increased genomic instability with culture passages is still being debated[35-38]. Roselli *et al*[39] reported that MSCs were genetically stable in long-term cultures at least up to passage 10, and abnormal MSC clones showed neither growth advantage nor senescence resistance. Some authors suggested that senescent cells are unlikely to undergo malignant transformation, even if the presence of few tumorigenic cells can not be excluded[40]. The inconsistencies may be caused by tissue sources, culture conditions, culture times, and cuture passages[41]. Nevertheless, it is of critical importance to evaluate MSC genetic stability before clinical application.

Biomarkers for aged MSCs

Several methods may be used to identify MSC senescence. The most widely used measures include increased senescence-associated (SA) beta-galactosidase activity (SA-beta-gal), cell cycle arrest, and persistent DNA damage response signaling[22]. Furthermore, specific markers for senescent MSCs have been discovered. Analysis of the MSC compartment revealed that MSC subpopulations differ between developmental and aged stages. CD271(-)CD146(+) cells only appeared in fetal bone marrow (BM)-containing colony-forming-unit-fibroblasts. The dominant MSC subset in pediatric and fetal samples was the CD271(bright)CD146(+) population, whereas the main cell type in adult samples was CD271(bright)CD146(-)[42]. The proportion of CD11b+, CD3+, Gr-1+, or F4/80+ cells is upregulated in BM from aged mice, while the percentage of B220+ cells was significantly decreased compared with those from young mice BM[43].

Recently, novel specific biomarkers were found to demonstrate the senescent state of MSCs[21]. MSC-derived microvesicles (MVs) are one such biomarker[44], and senescent late passage MSCs displayed a smaller MSC-MV size compared with early passage MSCs. Additionally, when comparing late and early passage MSC-MVs, there was a lower ratio of CD105+ cells and decreased osteogenesis in late passage MSC-MVs[45]. When the percentage of CD264+ cells was greater than 35%, CD264 expression was inversely correlated with the regenerative potential of MSCs. Above the 75% threshold, MSCs were enlarged and showed a decreased proliferation and differentiation potency.

CyBC9, a senescence-specific fluorescent probe, is a promising tool used to rapidly identify both early and late senescent phenotypes in clinically relevant MSCs[46], and it can be applied to live cells as a nontoxic probe. The mitochondrial *Cox1* gene containing the differentially methylated CpG island 4 was upregulated in MSCs from human fetal heart tissues. This demonstrated that CpG hypo-methylation in mitochondria might serve as a biomarker for senescence of human fetal heart MSCs induced by chronic oxidative stress[20].

Portraits of expression profiles

Recent studies have demonstrated significant changes in the expression profiles (including transcriptomic, proteomic, epigenetic, and non-coding RNAs) of senescent MSCs. Transcriptome drift even preceded replicative exhaustion and other aging metrics[47]. Utilizing a microarray assay, transcriptome analyses were performed using

various types of aged MSCs (Table 1).

Transcriptomics: Assays for gene expression profiles have been performed on multiple types of aging MSCs. These MSCs were obtained from various species and tissues, and subjected to different treatments. Benisch *et al*[48] investigated the transcriptional profiles of human BM-MSCs from five elderly patients (79-94 years old) who had osteoporosis (hMSC-OP), the age-matched control group (hMSC-old; donor age 79-89 years), middle-aged donors (hMSC-C; donor age 42-67 years), and healthy middle-aged donors (42-64 years old) until they entered senescence (hMSC-senescent). By using hMSC-C as control cells, they found a small overlap of gene expression in the hMSC-old, hMSC-senescent, and hMSC-OP groups. By comparing the gene expression profiles of hMSC-OP, hMSC-old, and hMSC-senescent with hMSC-C, special transcriptomic features in each group were obtained. The differentially expressed genes in the three groups are mainly involved in proliferation, differentiation, osteoclastogenesis, and DNA repair[48]. Wu *et al*[49] identified six hub genes and eleven transcription factors related to adherens junctions, DNA damage induced by oxidative stress, attribution of telomeres, differentiation, and epigenetic modulation by comparing the gene alterations between hMSC-C and hMSC-old. Yoo *et al*[50] revealed that 19 genes were downregulated and 43 upregulated in senescent human BM-MSCs relative to young MSCs. And these genes were mainly involved in metabolic functions and cell adhesion. Additionally, 394, 1073, and 2077 genes were significantly up-regulated in BM-MSCs from pesticide exposed, P14 MSCs, and MSCs from aged donor, compared with control MSCs (P3), respectively[51]. And 218, 1077, and 1571 genes were down-regulated in BM-MSCs from pesticide exposed, P14 MSCs, and MSCs from aged donor, compared with P3 MSCs, respectively. Insulin-like growth factor-1 (*Igf-1*), *prolactin*, *leptin*, and *Cox-2* were identified as key genes of the predicted protein–protein interactions[51]. In murine BM-MSCs that were freshly sorted by fluorescence-activated cell sorting, 927 differentially expressed genes were obtained in aged BM-MSCs. These genes contained cytokine receptors, chemokines, markers of cell senescence, and other groups, which were seen in the gene expression omnibus[52].

Human umbilical cord (hUC)-derived MSCs, cultured in chemically defined xeno- and serum-free medium, displayed comparable growth trajectories up to passage (P) 9 and variably approached senescence after P10. However, significant changes in the transcription profiles occurred earlier. Microarray analysis of 14500 human genes in aged hUC-MSCs revealed that a nonlinear evolution of aging MSCs appeared after P5 and accumulated rapidly after P9[47]. As for hUC vein-MSCs, young (P9) and senescent (P18) cells were used for transcriptome analysis assays. This study identified 73 differentially expressed genes in senescent cells, compared with young MSCs[19]. Among these, 18 upregulated genes were screened out as characteristic molecular signatures of senescence when comparing senescent and young hMSCs derived from donors with normal or constitutional chromosome inversion karyotype. Among them, 11 novel candidate markers for senescence were identified.

In response to IL-2 priming, human adipose-derived MSCs (ADSCs) showed increased expression of genes encoding potent growth factors, cytokines, angiogenic, and anti-apoptotic promoting factors, and they were defined as novel transcriptional signatures closely associated with senescence[53]. In CD45-CD31-CD34+ ADSCs from murine inguinal fat pads, aging has been shown to affect cellular signaling and function as well[54]. Single-cell transcriptional profiles of ADSCs isolated from both young and aged mice were analyzed by utilizing a microfluidic-based single-cell gene expression platform. About 70 gene targets related to stemness, vasculogenesis, and tissue remodeling were evaluated and used to define ADSC clusters in each group. Ingenuity Pathway Analysis of a subset of this heterogeneous cell collection was performed. This analysis suggested that deficiency of a putatively vasculogenic subpopulation of ADSCs was a potential risk for age-related impairments in ADSCs function (particularly with regard to wound healing)[54].

Proteomics: Proteomics is an efficient and accessible tool used to determine protein expression profiles. An SA secretome, also known as SA secretory phenotype (SASP), usually contains the expression of growth factors, cytokines, and extracellular proteases that modulate the microenvironmental phenotypes caused by senescent cells[55]. The SASP is useful for the development of biological markers and rejuvenation strategies in aged MSCs[22]. Secretome analyses for secretory protein profiles in senescent MSCs are summarized in Table 2. For example, elderly MSCs exhibited increased levels of pro□inflammatory factors, including interleukin-6 (IL-6), IL-8 (IL-8/CXCL8), and monocyte chemoattractant protein-1 (MCP-1/CCL2). Neutralization of these factors improved their immunomodulatory function[56]. In the conditioned

Table 1 Summary of various transcriptomics analyses studies of senescent mesenchymal stem cells

Ref.	Species	Tissue sources	Classification	Cells	Groups	Database	Differentially expressed genes (DEGs)	Identification of targets
Benisch *et al*[48], 2012	Human	Bone marrow of femoral heads	Affymetrix Gene Chip	Cultured in DMEM/Ham's F-12 (1:1) medium supplemented with 10% fetal calf serum (FCS), 1 U/mL penicillin, 100 μg/mL streptomycin, and 50 μg/mL L-ascorbic acid 2-phosphate. Used after 1 to 2 passages	hMSC-C: Middle-aged donors (42-67 yr old); hMSC-old: The age-matched control group (79-89 yr old); hMSC-OP: Patients (79-94 yr old) who had primary osteoporosis; hMSC-senescent: Healthy donors of middle-age (42-64 yr old) until they entered senescence	GEO accession numbers: GSE35955; GSE35956; GSE35957; GSE35958; GSE35959	One gene was upregulated and seven downregulated in all three groups, compared with the hMSC-C group; 38 genes with enhanced and 36 genes with reduced expression in hMSC-OP and hMSC-old groups, compared with hMSC-C; 2477 genes with higher and 1222 genes with lower expression in hMSC-OP, in comparison with hMSC-old	The reliable or promising candidates for osteoporosis, including susceptibility genes: *Lrp5*, *Spp1* (Osteopontin), *Col1a1*, *Sost*, and *Mab21l1*
Yoo *et al*[50], 2013	Human	Bone marrow-derived MSCs	SSH analysis	Purchased from Cambrex Bio Science	Young human MSCs (Y-hMSCs): Approximately 10 population doubling levels (PDL); senescent MSCs (S-hMSCs): Until approximately 30 PDL, at least 80% of the cells were positive for SA-β-Gal staining	NA	Nineteen genes were down-regulated and 43 upregulated in S-hMSCs	Gradually downregulated mRNA in S-hMSCs: *Pdia3*, *Wdr1*, *Fstl1*, *Copg1*, *Lman1*, and *Pdia6*; significantly upregulated genes: *Hsp90b1*, *Eid1*, *Atp2b4*, *Ddah1*, *Prnp*, *Rab1a*, *Psg5*, *Tm4sf1*, and *Ssr3*
Bustos *et al*[52], 2014	Mouse	Bone marrow	Affymetrix Gene Chip	Sorted by fluorescence-activated cell sorting (FACS)	BM-MSCs from young (3-mo-old) and aged (24-mo-old) mice; young donor BM-MSCs *vs* aged ones	GEO accession number: GSE44403	927 genes were differentially expressed	Confirmed by qPCR: Cytokine receptors (15 genes), chemokines (*Ccr7*, *Cxc3cr1*, *Cxcr5*), markers of cell senescence (*CDK*, *p21*, *p27*, and *p53*), *Marcks*, *Mmp9*, and *Timp2*
Duscher *et al*[54], 2014	Mouse	Inguinal fat pads	Microfluidic-based single-cell gene expression platform	CD45-/CD31-/CD34+ cells were sorted	Adipose-derived mesenchymal stem cells (ADSCs) from young (3 mo) and aged (21 mo) mice	NA	Differences in transcriptional profiles of genes related to cell stemness, remodeling, and vasculogenesis: *Adam10*, *Angpt1*, *Angpt2*, *Hif1a*, *Mef2c*, and *Sod2*	Age-related depletion of a subpopulation of MSCs characterized by a pro-vascular transcriptional profile, such as *Angpt1*, *Vegfa*, and *Sod3*
Medeiros *et al*[19], 2017	Human	Umbilical cord veins	The GeneChip Human Genome U133 Plus 2.0 array	The surface markers including CD105, CD73, CD90, CD14, CD34, and CD45 were analyzed by flow cytometry; differentiation capacity toward three lineages was assessed	hMSCs in the 9th (Y-hMSCs) and 18th passages (S-hMSCs) were used for assays, hMSCs/n from the donor with normal karyotype, and hMSCs/inv from the donor with a constitutional inversion of chromosome 3; the comparisons were as follows: (1) Y-hMSCs/n & S-hMSC/n; (2) Y-hMSCs/inv & S-hMSCs/inv; (3) Y-hMSCs/n & Y-hMSCs/inv; and (4) S-hMSCs/n & S-hMSCs/inv	GEO accession number: GSE56530	73 DEGs in S-hMSCs/n compared with Y-hMSCs/n and 279 DEGs in S-hMSCs/inv compared with Y-hMSCs/inv; 93 DEGs in Y-hMSCs/inv compared with Y-hMSCs/n; 425 DEGs in S-hMSCs/inv compared with S-hMSCs/n. The candidates for senescent markers: Dio2, Foxe1, Galnt5, Has3, Krt19, Krt34, Krtap1-55, Oc730755, Mrvi1, Plcb4, and Scube3	Confirmed by qPCR: *Ankrd1* and *Mmp1* in S-hMSC/n *vs* Y-hMSC/n; *Sfrp1*, *Ankrd1*, *G0s2*, and *Ndn* in S-hMSC/inv *vs* Y-hMSC/inv; *Adora2b*, *Sfrp1*, *Kynu*, *G0s2*, *Aldh1a1*, and *Mab21l1* in Y-hMSC/inv *vs* Y-hMSC/inv; and *Adora2b*, *Ccl7*, *Sfrp1*, *Kynu*, *Ankrd1*, *Mmp1*, *Lamc2*, *G0s2*, *M ab21l1*, and *Ndn* in S-hMSC/inv *vs* S-hMSC/n
Wu *et al*[49], 2019	Human	Bone marrow of femoral heads	Affymetrix Gene Chip	Cultured in DMEM/Ham's F-12 (1:1) medium supplemented with 10% FCS, 1 U/mL penicillin, 100 μg/mL streptomycin, and 50 μg/mL L-ascorbic acid 2-phosphate. used after 1 to 2	Middle-aged group *vs* elderly group	GEO accession number: GSE35955	156 up-regulated and 343 down-regulated differentially expressed genes (DEGs)	Six hub genes identified by PPI network analysis: *Ctnnb1*, *Ppp2r1a*, *Fyn*, *Mapk1*, *Pik3c2a* and *Ep300*. 11 TFs identified by TFs screening: Creb1, Cux1, Egr1, Ep300, Foxc1, Hsf2, Mef2a, Plau, Sp1, Stat1 and

				passages				Usf1
Wiese *et al*[47], 2019	Human	The perivascular region of Wharton's jelly from umbilical cords	Affymetrix GeneChip U133A 2.0 arrays	Provided by Tissue Regeneration Therapeutics, Inc. Positive for the cell surface markers CD73, CD90, CD105, CD10, CD140b, CD146 (40%-60%), CD166, and MHC-I; negative for the cell surface markers CD45, CD31, CD34, and HLA-DR. Exhibit trilineage potential in directed differentiation assays	Human umbilical cord perivascular cells (HUCPVCs) from early passages (P2–P5), mid-passages (P6–P9), and pre-senescent passages (P10–P12)	GEO accession number: GSE119987	The transcriptome of HUCPVCs was stable through P5. A single significantly DE gene was identified at P6 and P7 compared with P2, whereas 5 DE genes were detected at P8 and 27 at P9. The number of significantly DE probe sets increased from 27 (P9) to 301 (P10), then to 1094 (P12)	Significant transcriptome drift occurred only after P5
Leveque *et al*[51], 2019	Human	Bone marrow aspirates from the iliac crest of healthy donors (21 to 26 years old)	RNAseq Analysis	The surface markers including CD34, CD45, CD73, CD90, and CD105 were analyzed by flow cytometry; differentiation capacity toward three lineages was assessed	Four groups: Control MSCs (P3); 21 d pesticide mixture exposed MSCs (P4); long-term cultivated MSCs (P14); and MSCs from aging donor (72 yr old)	The SRA database under accession number PRJNA510912	394, 1073, and 2077 EST were significantly increased from pesticide exposed, P14 MSCs, and MSC from aged donor; 218, 1077, and1571 ESTs were down-regulated	Confirmed by QPCR: *Igf-1*, *Prolactin*, *Leptin*, and *Cox-2*

GEO: Gene expression omnibus; DEGs: Differentially expressed genes; hMSC: Human mesenchymal stem cells; MSC: Mesenchymal stem cells; HUCPVCs: Human umbilical cord perivascular cells; FCS: Fetal calf serum; ADSCs: Adipose-derived mesenchymal stem cells; PDL: Population doubling levels; DMEM: Dulbecco's Modified Eagle's medium; FACS: Fluorescence-activated cell sorting; BM: Bone marrow.

medium (CM) from senescent MSCs induced by the HIV protease inhibitor tipranavir, the soluble proteins were evaluated to find dysregulated secreted factors using antibody arrays and liquid chromatography-mass spectrometry (LC-MS)[57]. Semi-quantitative antibody arrays and LC-MS analysis identified altered secretion of 86 proteins related to the extracellular matrix, cell adhesion, angiogenesis, and wound healing. Among the identified secreted factors in the proteomic analysis, a series of TGF-β targets were significantly upregulated. Further investigation revealed that insulin-like growth factor-binding protein 7 (IGFBP7), one of the targets of TGF-β, is independent of any additional factors that induce osteogenesis in hMSCs. IGFBP7 is also essential for the viability of hMSCs during osteogenesis[57].

In the ADSCs from one-year-old male C57BL/6 mice, the anti-senescent protein, telomerase reverse transcriptase (TERT), and the anti-apoptotic transcription factor myocardin were overexpressed to restore their functions. The secretomes in CM and exosome-enriched fractions from the transgenic cells were analyzed using a proteomic approach. This approach involved combining two-dimensional gel electrophoresis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry[58]. The comparative targeted proteomic analysis revealed that both matrix metalloproteinase-2 (MMP-2) and its inhibitor metalloproteinase inhibitor 2 (TIMP2) levels are increased by two-fold in the CM compared with those in mock-transduced cells.

Epigenetics: Epigenetic profiles of aged human BM-MSCs have been reported, and briefly reviewed by Cakouros and Gronthos[59]. Using the human methylation bead

Table 2 Summary of secretome alteration analysis studies of senescent mesenchymal stem cells

Ref.	Species	Tissue sources	Classification	Cells	Groups	Differentially expressed proteins	Identification of targets
Kizilay *et al*[56], 2017	Human	Subcutaneous and pericardial adipose tissue	R&D Systems Human Cytokine Array; multispot electrochemiluminescence immunoassay V-Plex Pro inflammatory Panel	CD44, CD73, CD105, and CD90 expression was more than 95%; CD45, CD34, CD19, CD14, and HLA-DR expression was less than 5%; differentiation capacity toward three lineages was assessed	E-MSCs: MSCs from elderly ATH patients (> 65 yr old); A-MSCs: MSCs from adult ATH patients (< 65 yr old)	The expression of IL-6, IL-8/CXCL8, MCP-1/CCL2, MIF, IFN-g, IL12p70, IL-13, IL-2, and IL-4 was elevated in E-MSCs relative to A-MSCs	Neutralization of IL-6, IL-8/CXCL8, and MCP-1/CCL2 significantly improved the E-MSCs' immunomodulatory function
Infante *et al*[57], 2018	Human	Bone marrow	Semi-quantitative antibody arrays; liquid chromatography-mass spectrometry (LC-MS): Version 4.0.4265.42984, Nonlinear Dynamics	Obtained from Lonza commercially; passages 3-4	Ctrl-hMSCs: Incubated with dimethyl sulfoxide alone; PreA-hMSCs: Treated with the HIV protease inhibitor (tipranavir) every other day until passage 11	A dysregulation in the secretion levels of 42 proteins was detected by antibody arrays; 44 were detected by LS-MS in preA-hMSCs; most of them were overexpressed in preA-hMSCs, in comparison with ctrl-hMSCs	IGFBP7 is essential for hMSCs viability during early osteogenic diferentiation
Madonna *et al*[58], 2019	Mouse	Peri-epididymal visceral adipose tissue from 1-yr-old male C57BL/6 mice	Two-dimensional gel electrophoresis (2DE); matrix-assisted laser desorption/ionization time-of-fight mass spectrometry	The expression of CD45, CD34, CD133, ASMA, Desmin, CD105, CD73, CD90, CD79, and CD160 was analyzed by flow cytometry	Mock AT-MSCs: Mock-transduced AT-MSCs; rTMAT-MSCs: Rejuvenated by TERT and the anti-apoptotic transcription factor myocardin overexpression	113 protein spots were picked up and identified from the whole CM and exosome-enriched fraction in rTMAT-MSCs	Two novel candidates supporting angiogenesis in the whole CM of rTMAT-MSCs: MMP2 and its inhibitor TIMP2

hMSC: Human mesenchymal stem cells; MSC: Mesenchymal stem cells; TERT: Telomerase reverse transcriptase; MMP2: Matrix metalloproteinase-2; CM: Conditioned medium; LC-MS: Liquid chromatography-mass spectrometry.

ChIP array, researchers identified a series of hypomethylated, hypermethylated, and hydroxymethylated CpG sites in MSCs from aged subjects[60-62]. However, differentially methylated CpG sites are a robust age-related DNA methylation signature, illustrating similar DNA changes independently of disease state, sex, tissue, and cell type[59,63]. Additionally, aged human BM-MSCs from long-term culture exhibited consistent epigenetic changes *in vitro* when the methylation profile of human BM-MSCs at early and late passages was assessed[64]. Following the application of enhanced reduced representation bisulfite sequencing, the global DNA methylation profiling demonstrated a greater breadth than previously reported. The genome-wide analyses using whole-genome bisulfite sequencing provided a better understanding of how the epigenetic modifications alter gene expression and regulate the biological characteristics[65].

Non-coding RNAs: It has been previously reported that some non-coding RNAs are associated with cellular senescence in different cell types[66-70]. MicroRNAs (miRNAs) are small non-coding RNAs that have highly conserved sequences and regulate target genes in cellular functions of metabolism, proliferation, apoptosis, and senescence[71,72]. In senescent MSCs, 43 miRNAs were identified and characterized using a miScript miRNA assay[73]. Among them, the expression of 24 miRNAs was closed related to

cellular senescence as referred to previous studies. As for the rest, fourteen miRNAs (miR-10, miR-27b, miR-30b, miR-30d, miR-103a, miR-103a-2, miR-136, miR-140-5p, miR-323-3p, miR-330-5p, miR-361-5p, miR-409-3p, miR-424, and miR-455-3p) were upregulated, and five miRNAs (miR-16-2, miR-29b, miR-199b-5p, miR-454, and miR-618) were downregulated in response to cellular aging. Additionally, miR-29b and miR-199b-5p modulated cellular senescence *via* LAMC networks[73].

In MSCs cultured under hypoxic conditions, miRNA expression profiles of MSCs from young (≤ 30 years old) and aged (≥ 60 years old) donors were analyzed using an Agilent Technologies Bioanalyzer high sensitivity DNA chip[74]. Principal component analysis demonstrated differentially expressed miRNAs in normal and hypoxic groups. There was > 2-fold upregulation of nine miRNAs in young MSCs and two miRNAs in aged MSCs after culturing under hypoxic conditions. Also, the hypoxia induced downregulation of four miRNAs in young MSCs and thirty-one miRNAs in aged MSCs, respectively. MiR-543 and miR-590-3p were identified as regulators of cellular aging in hMSCs through direct binding to the aminoacyl tRNA synthetase-interacting multifunctional protein-3/p18 transcripts and decreasing the protein expression levels[75].

Long non-coding RNAs (lncRNAs) are non-coding transcripts, longer than 200 nucleotides, that play critical roles in the regulation of MSCs senescence. They are not only involved in age-related lineage fate switching but also in the reprogramming of old cells[76,77]. LncRNA microarray analysis of young and aged Sca-1+CD29+CD45-CD11b- murine BM-MSCs has demonstrated that 92 lncRNAs showed altered expression[76]. Among them, 83 lncRNAs were downregulated, and 9 were upregulated in cells isolated from aged mice. Further investigation demonstrated that the candidate BM stem cell-related lncRNA (BMNCR) was highly expressed in the BM-MSCs of young mice, and significantly decreased during aging. Moreover, the BMNCR levels in human BM-MSCs were negatively correlated with age. The effects of BMNCR were evidenced by Bmncr-KO and Bmncr-Tg mice simultaneously[76].

Functional degeneration of aged MSCs

The senescent MSCs exhibit significant impairments in paracrine functions[78], resistance to oxidative stress, hypoxia, or serum deprivation-induced apoptosis[79-81]. The age-dependent decrease in cytokines, chemokines, and growth factors released by MSCs will impact cellular functions such as apoptosis, migration, osteogenesis, angiogenesis, cell adhesion, and immunomodulation[52,54,82]. Finally, the aged MSCs delay wound healing and exacerbate tissue injuries [54]. In summary, the functional regression of senescent MSCs limits their application in tissue engineering and regenerative medicine.

Age-related effects of MSCs on target cells

Bidirectional signaling exists between MSCs and their target cells[83,84]. The interaction between MSCs and target cells has been shown to occasionally follow a time-dependent model of regulation and feedback[85]. MSC senescence decreases the functions of a large variety of immune cells, hematopoietic stem and progenitor cells (HSPCs), oligodendrocytes, senescent chondrocytes, and other target cells through either direct or indirect cross-talk (Figure 1)[78,84,86].

Immune cells: Previous studies have demonstrated that MSC senescence retards immunosuppression in various types of immune cells. Replicative senescence of MSCs derived from BM or adipose tissue showed decreased ability to suppress T-cell, but not natural killer and B-cell proliferation[87,88]. Long-term expansion of MSCs reduced the capacity to inhibit CD4+ and CD8+ T cell proliferation. This phenomenon was observed by co-culturing MSCs with alphaCD3CD28-activated peripheral blood mononuclear cells. The inhibitory effect on T-cell proliferation significantly decreased along with increased passage number of hBM-MSCs, and the effect in hUC-MSCs was even more substantial[88]. ADSCs derived from elderly subjects also displayed a diminished capacity to suppress the proliferation of activated T cells. Similar results were observed in MSCs isolated in parallel from Lewis and Brown Norway rats of young (less than 4 wk old) and aged (older than 15 mo) groups[89]. Aside from proliferation, the senescent MSCs impair the suppressive effects of the activation-antigen expression and cytokine production in phytohemagglutinin-stimulated T cells[79]. Soluble factors and direct cell-cell contact partially mediate the decreased suppressive effect of aged MSCs on T cells[56].

In addition to lymphocytes, MSC senescence affects the phenotypes and functions of macrophages and dendritic cells[29,90]. When co-cultured with BM-MSCs from young

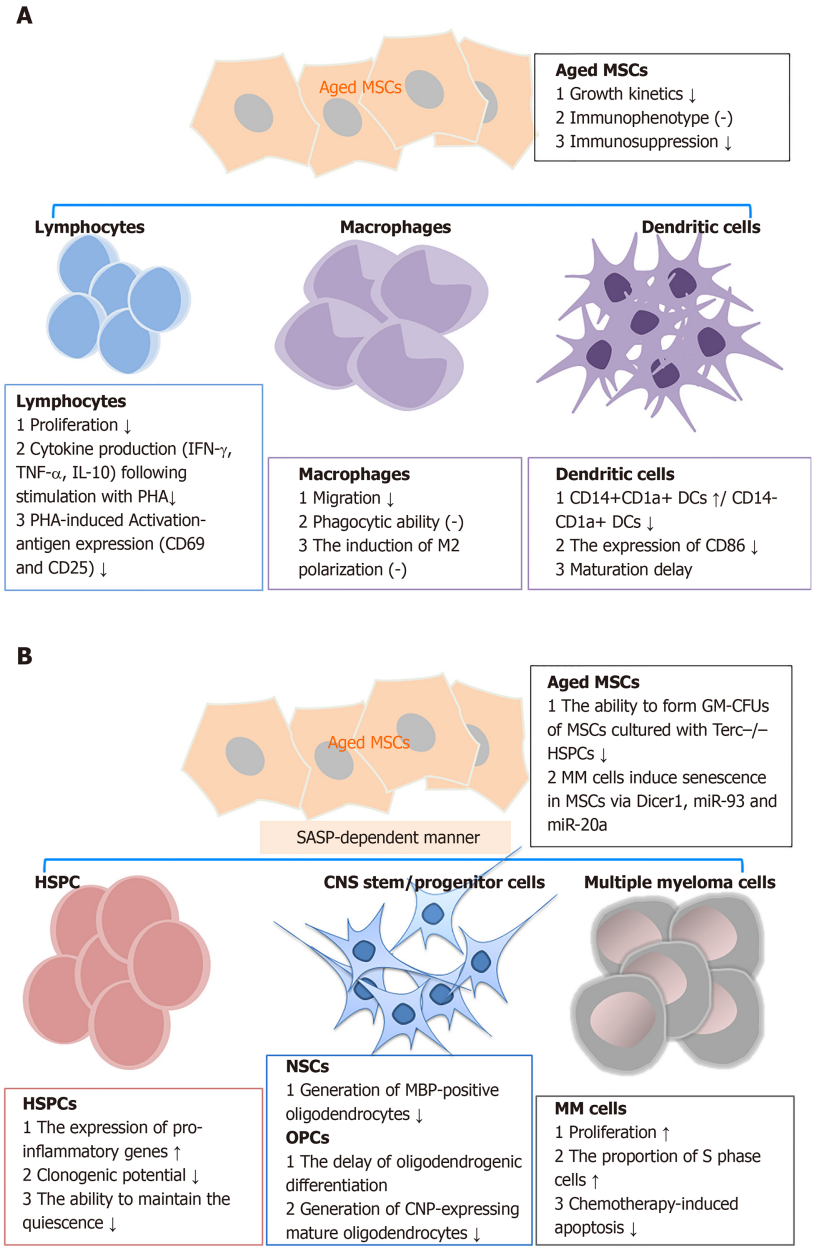


Figure 1 Effects of senescent mesenchymal stem cells on target cells. A: Effects of aged mesenchymal stem cells (MSCs) on immune cells; B: Effects of aged MSCs on other target cells, including hematopoietic stem and progenitor cells, neural stem cells, and multiple myeloma cells. DCs: Dendritic cells; HSPCs: Hematopoietic stem and progenitor cells; NSCs: Neural stem cells; OPCs: Oligodendrocyte progenitor cells; MM cells: Multiple myeloma cells; MSCs: Mesenchymal stem cells; PHA: Phytohemagglutinin; GM-CFUs: Granulocyte macrophage-clone formation units; MBP: Myelin basic protein; CNP: 2',3'-cyclic-nucleotide 3'-phosphodiesterase.

mice, a macrophage cell line (RAW264.7 cells) exhibited higher migration rates, although they displayed similar phagocytic ability and induction of macrophage M2 polarization[29]. In dendritic cells, cellular maturation was inhibited when cultured with expanded marrow stromal cells relative to the parental MSCs[91].

Other target cells: Apart from immune cells, senescent MSCs show impaired activity against multiple target cells. Senescent MSCs enhanced the expression of pro-inflammatory SASP factors in HSPCs and inhibited their clonogenic potentials[92]. These cells also have a reduced capacity to maintain CD34+CD38- HSPCs quiescence, as a result of increased IL-6 secretion[93]. In a study on telomere dysfunction in MSCs, using Terc-/- mice, Ju *et al*[94] found that aged Terc-/- BM-MSCs depressed the functions of HSPCs and early hematopoietic progenitors. Aging MSCs had a reduced ability to induce oligodendrogenic differentiation in neural stem cells. Additionally, the production of 2′,3′-cyclic-nucleotide 3′-phosphodiesterase-positive oligodendrocytes in oligodendrocyte progenitor cells was reduced. The impaired differentiation suppressed the generation of myelin-like sheaths during central

nervous system remyelination[95]. Also, in the peri-infarct cortex of rats subjected to transient middle cerebral artery occlusion, aged MSC administration resulted in more microglia and reduced pericyte infiltration compared with that for young MSCs. The changes in cellular components probably correlated with reduced expression of brain-derived neurotrophic factor and MCP-1[96]. In a myocardial infarction (MI) model, the infusion of old MSCs resulted in a switch of the cellular profile in the infarct region. This was characterized by fewer endothelial cells, vascular smooth muscle cells, and macrophages, and more fibroblasts compared with young MSCs[97]. Similarly, senescent MSCs facilitated the growth of multiple myeloma (MM) cells, which aggravated disease progression[98]. In turn, MM cells (such as NCI-H929, OPM-2, KMS-12-BM, and primary CD138+ tumors cells) induced senescence in MSCs derived from healthy controls with decreased expression of Dicer1, miR-93, and miR-20a.

CELLULAR REJUVENATION STRATEGIES

Simple isolation, standardized culture methods, and potential autologous application make MSCs a superior cell source for the treatment of various diseases and injuries[13]. Therefore, optimizing the viability and function of MSCs for infusion is of great significance. With an increased understanding of the characteristics of MSC senescence, further investigations are ongoing to resolve challenges linked to the clinical application of cellular therapies. To date, the rejuvenation strategies have demonstrated therapeutic potential including molecular regulation, non-coding RNA modification, and control of the microenvironment.

Molecular regulation

Multiple molecules have been confirmed to restore the proliferative ability and biological function of MSCs *via* gene modification, or the administration of recombinant proteins, agonists, or inhibitors.

Sirtuins: The sirtuins (SIRTs), which include seven sirtuin homologs, are wellknown for their ability to delay cellular senescence and extend the lifespan of organisms ranging from yeast to humans[99]. SIRT1 overexpression in aged MSCs ameliorated the senescence phenotype, recapitulated angiogenesis, and protected cells from oxidative stress. Infusion of SIRT1-modified aged MSCs promoted the expression of pro-angiogenic factors, such as angiopoietin 1, and basic fibroblast growth factor (bFGF). Additionally, SIRT1-modified aged MSCs increased Bcl-2/Bax ratio at the protein level, promoted cellular survival, inhibited fibrosis, upregulated vascular density, and improved heart function in an MI model, compared with vector-aged MSCs[100]. SIRT1 pathway activators, including nicotinamide mononucleotide[101], nicotinamide phosphoribosyl transferase[102], cell-deposited decellularized extracellular matrix[103], and SRT1720[104] have been applied in aged human MSCs. They improve cell viability and osteogenesis while inhibiting apoptosis and adipogenesis in aged MSCs. Pretreatment of aged MSCs with SRT1720 enhanced therapeutic efficacy by promoting angiogenesis and repressing fibrosis following rat MI[104].

Likewise, the overexpression of SIRT3 improves the antioxidant capacity and promotes the survival of old human MSCs through activating forkhead box O3a in the nucleus, manganese-superoxide dismutase, and catalase. In an MI model, the application of old human MSCs overexpressing SIRT3 enhanced cardiac function and decreased infarct size[105]. SIRT6 maintains hMSC homeostasis by co-activating the antioxidant nuclear factor erythroid 2-related factor 2 pathway, RNA polymerase II, and heme oxygenase 1[106].

Growth factors: Growth factors are a superfamily of proteins that promote cell survival, expansion, migration, and differentiation, as well as prevent disruption of homeostasis *in vitro* and *in vivo*[107]. Through stimulation of the FGFR1/2 pathway, LY294002 (a PI3K inhibitor) rescued MSCs from senescence[108]. Acadesine activates adenosine 5′-monophosphate-activated protein kinase (AMPK), a downstream signal of FGF21, and abrogates the depletion of FGF21-induced senescence by inhibiting mitochondrial fusion[109]. Silencing mitofusin-2 has been found to inhibit MSC senescence induced by the abrogation of FGF21 as well. Knockdown of insulin-like growth factor binding protein 4 restored the osteogenic potency of aged MSCs *via* the activation of Erk and Smad signals[110]. Pretreatment of aged MSCs with macrophage migration inhibitory factor (MIF) enhanced the secretion of vascular endothelial growth factor (VEGF), bFGF, hepatocyte growth factor, and insulin-like growth factor,

which promoted their growth, paracrine function, and survival[80]. MIF-rejuvenated MSCs release growth factors through interactions with CD74 and subsequent activation of AMPK-FOXO3a signaling, which protects cells from doxorubicin-induced senescence by modulating the PI3K-Akt signaling pathway[111].

Additional potential regulators: The AKT pathway plays an important role in the rejuvenation of features and functions of aged MSCs. This pathway can act *via* ERBB4/PI3K/AKT[112], lactoferrin/AKT[113], Vc/AKT/mTOR[114], and fucoidan/FAK-Akt-TWIST axes[115]. Administration of rapamycin, an inhibitor of the mTOR signaling pathway, raised the expression level of NANOG, postponed replicative arrest, and enhanced the lifespan increment of BM-MSCs[116]. NANOG, an embryonic transcription factor, is a pluripotency marker that facilitates myogenic differentiation and restores the contractile function of senescent MSCs[117]. Additionally, a high number of potential regulators involved in senescent MSC rejuvenation have been screened and investigated *in vitro*. For example, both L-carnitine[118] and curcumin[119] affect the methylation status of the *TERT* promoter, increase the telomerase activity, and consequently alleviate the aging-related features of ADSCs. In human BM-MSCs, the addition of L-carnitine during expansion also elevates cell production[120].

Many *in vivo* experiments using various disease models have been conducted to confirm the efficacy of the rescue strategies to rejuvenate aged MSCs. It is reported that melatonin can protect MSCs from senescence *via* prion protein (PrPc)-dependent enhancement of mitochondrial function[121]. Implantation of genetically-modified old human MSCs with tissue inhibitor of matrix metalloproteinase-3 or VEGF promotes angiogenesis, prevents adverse remodeling, and preserves cardiac function to a similar extent compared with young hMSCs[122]. Stem cell antigen 1 (Sca-1)+ MSCs resident in the heart increase angiogenesis, and activate cell proliferation in the infarcted heart, which improves cardiac function after MI[123,124]. Overexpression of neuron-derived neurotrophic factor rejuvenates human ADSCs and BM-MSCs from the elderly, reduces the ischemic area, and repairs cardiac function after MI by improving angiogenesis and decreasing apoptosis[125,126]. Ethyl pyruvate, a HMGB1 inhibitor, restores the senescent phenotype of BM-MSCs, alleviates clinical signs of lupus nephritis, and prolongs survival of MRL/Mp-lpr/lpr mice *via* TLR4-NF-kappaB signaling[127]. These candidates (both *in vitro* and *in vivo*) may be valuable for the identification of suitable targets with utility in the production of clinical-grade MSCs.

Non-coding RNA modification

Non-coding RNAs are novel genetic regulators involved in regenerative medicine. With respect to aging, transfection of the miR-195 inhibitor restores the expression of anti-aging factors, including TERT and SIRT1, as well as phosphorylation of AKT and FOXO1. The miR-195 inhibitor reduced the expression of SA-beta-gal, which significantly induced telomere relengthening and restored the proliferative abilities of old MSCs. Administration of aged MSCs with miR-195 knockout alleviated infarction size and improved left ventricular function[128]. Additionally, miR-10a, miR-29c-3p, and miR-130b have been reported to rejuvenate MSC senescence by targeting different downstream pathways[128-131]. The overexpression of miR-10a in aged hBM-MSCs stimulates angiogenesis by inducing the expression of angiogenic factors *via* activated Akt. These cells then enhance implanted stem cell survival and improve cardiac function after MI[129]. The miR-29b-3p derived from BM-MSCs regulates aging-associated insulin resistance[132]. In multiple myeloma-MSCs, Dicer1 overexpression and upregulation of miR-93/miR-20a could reverse the effects on differentiation and reduce cellular senescence[98]. The lncRNA Bmncr regulates the age-related lineage switch between osteogenic and adipogenic differentiation in BM-MSCs. Overexpression of Bmncr (Bmncr-Tg) reduced bone loss and fat accumulation by maintaining extracellular matrix protein fibromodulin and activating the bone morphogenetic protein 2 pathway[76].

Microenvironment modulation

A conducive microenvironment is essential for maintaining MSC activity[133]. When BM-MSCs were treated with BM supernatant from systemic lupus erythernatosus (SLE) patients, they demonstrated characteristics of senescence. An inflammatory microenvironment is considered to play a primary role in the senescence of SLE BM-MSCs[127]. In unbalanced microenvironments caused by aging, the survival, proliferation, colony formation, migration, and appropriate differentiation of grafted BM-MSCs were significantly suppressed[43,84]. In addition, the BM pCO_2 and HCO_3 levels displayed a close correlation with MSC differentiation and proliferation[134].

Therefore, microenvironment regulation is a promising strategy to reverse the decline of aged MSCs and promote the clinical efficacy[135,136].

Rejuvenating the senescent MSCs is more effective in hypoxic conditions compared with that in normal conditions. The neuroprotective effects of CM from aged human BM-MSCs against cerebral ischemia were enhanced by hypoxia conditioning *in vitro* [137,138]. Stem cell-deposited decellularized extracellular matrix can rescue hUC-MSCs from oxidative stress-induced premature senescence and facilitate their clinical application in regenerative medicine[103]. The co-culture system is a convenient means of modulating the cellular microenvironment. BM-MSCs co-cultured with young (P3) human umbilical vein endothelial cells demonstrated a higher proliferative ability and decreased pro-inflammatory (cytokines and miRNA) phenotype, compared with the old cells (P13)[139]. In conclusion, the enhancement of the microenvironment has a significant effect on the prevention of MSC senescence.

Other factors influencing rejuvenation

Besides restoring the activities of aged autologous MSCs, some researchers have attempted to find an accessible source for the replenishment of autologous MSCs. In the past few decades, the differentiation of pluripotent stem cells into MSC-like cells has been explored to address the problems of viability and scarcity of autologous MSCs derived from old individuals[140-142]. Induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), which acquire a rejuvenation gene signature, are the alternative sources of MSCs[141,143]. However, the production protocols used to derive MSCs from iPSCs and ESCs require optimization. The introduction of new technologies, such as 3D culture and gene engineering, might make them more valuable for further clinical application[142].

Biomaterials and various cellular components are potential carriers used for the modification of aged MSCs. MVs carrying mRNAs, miRNAs, non-coding RNAs, proteins, and DNA are a novel and promising tool used to reverse aging in cells by mediating intercellular communication[44,108]. For instance, exosomes containing miR-17 and miR-34a from young MSCs rejuvenate aged murine hematopoietic stem cells *via* AKT/autophagy-related mRNAs[144]. Extracellular vesicles from human iPSCs can reduce cellular reactive oxygen species levels and alleviate aged phenotypes of senescent MSCs by partially delivering intracellular peroxiredoxin antioxidant enzymes[145]. Media supplied with human platelet lysate from younger donors were able to facilitate MSC expansion and osteogenic differentiation[146]. Additionally, many bioactive hydrogels[147], biomimetic scaffolds[148], and other biomaterials[149,150] have been tested to assess whether they can modify aged MSCs. Removal of senescent cells in a high-throughput manner is another strategy that can be used to address the challenge of senescence[151]; this strategy, which has been explored in clinical trials, involves the isolation and enumeration of senescent MSCs from undiluted human whole blood.

Some chemical compounds and foods rejuvenate senescent MSCs. Zinc sulfate significantly reduced the doubling time and increased *TERT* gene expression of rat ADSCs under extremely low-frequency-electromagnetic field[152]. It also enhanced telomere length extension in human ADSCs by regulating telomerase and methylation of the *TERT* gene promoter CpG island[153]. Besides zinc sulfate, resveratrol mimics the effects of dietary restriction, improves osteogenic function, and promotes mitochondrial activities of senescent MSCs through the regulation of mitofilin[154]. NT-020, a dietary supplement containing blueberry, green tea, vitamin D3, and carnosine, rescued the reduced proliferation of MSCs in serum from aged rats[155]. Additionally, *Undaria pinnatifida* and its ethanol extracts improve replication ability and ameliorate functional decline in senescent hBM-MSCs (P17)[156].

The rejuvenation methods mentioned above have potentials to optimize the functional status of aged MSCs. However, most of them were *in vitro* or rodent model studies. Further research is needed to evaluate their long-term safety and efficacy before it can be clinically useful.

CURRENT CHALLENGES AND FUTURE PERSPECTIVES

Senescence is an inevitable biological process for MSCs obtained from old individuals or long-term cultures. Although recent studies have revealed the characteristics and mechanisms of MSC senescence and attempted to rejuvenate aged MSCs, many issues remain unresolved. First, in studies of age-correlated phenotypic alterations, the expression of CD90 and CD73 in intervertebral disc cells was reduced in older individuals, while CD146 expression was increased[157]. However, the expression of

these factors (MSC markers) is rarely compared between young and aged MSCs. The comparison of these two types of cells provides a better understanding of senescent MSCs. Second, the effects of cellular rejuvenation for aged MSCs need to be determined *in vivo*, especially in the context of the multidirectional functions of regulators[158]. For example, hypoxia not only promotes the expansion of MSCs[159,160], but also influences the activity of MSCs during osteogenic differentiation[161]. Future work *in vivo* can provide more information about clinical efficacy. Additionally, MSCs isolated from specific tissues usually maintain lineage differentiation towards a specific cell type, and this plays a crucial role in regenerative therapy[161]. Therefore, the directional differentiation capacities in aged MSCs must be clarified following the increase in available tissue sources. Finally, many newly developing technologies, such as MVs, three-dimensional spheroid culture, and nanobiotechnology, will aid in improving aged MSC function in clinical therapies. Additionally, the functional discrepancies in various rejuvenation factors reported in different studies should be evaluated. For example, although a decline of osteogenesis capacitiy in aged MSCs was reported, other studies suggested that bone formation capacity was not affected in aged MSCs[162]. The function of pigment epithelium-derived factor (PEDF) responding to the senescence is unanticipated to demonstrate the different results in different research teams[97,163]. Liang *et al*[97] showed that increased PEDF secretion resulted in the impaired therapeutic ability of aged MSCs. However, Cao *et al*[163] showed that PEDF delayed cellular senescence and allowed a greater expansion of MSCs by suppressing oxidative stress and preserving differentiation potentials, compared with that in the control group. The different PEDF functions are possibly attributable to MSC heterogeneity, varying research objectives, and the specific experimental models used.

CONCLUSION

The rejuvenation of aged MSCs holds great promise for the accelerated translation of cell-based approaches (especially autologous cell administration) into clinically relevant therapies.

REFERENCES

1 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

2 **Naji A**, Eitoku M, Favier B, Deschaseaux F, Rouas-Freiss N, Suganuma N. Biological functions of mesenchymal stem cells and clinical implications. *Cell Mol Life Sci* 2019; **76**: 3323-3348 [PMID: 31055643 DOI: 10.1007/s00018-019-03125-1]

3 **Jiang W**, Xu J. Immune modulation by mesenchymal stem cells. *Cell Prolif* 2020; **53**: e12712 [PMID: 31730279 DOI: 10.1111/cpr.12712]

4 **Kuci Z**, Jordan C, Wehner S, Sörensen J, Jarisch A, Salzmann-Manrique E, Pfeffermann LM, Klingebiel T, Bader P, Kuçi S. The Phenotype and Functional Activity of Mesenchymal Stromal Cells in Pediatric Patients with Non-Malignant Hematological Diseases. *Cells* 2020; **9**: 431 [PMID: 32059574 DOI: 10.3390/cells9020431]

5 **Mathiasen AB**, Qayyum AA, Jørgensen E, Helqvist S, Kofoed KF, Haack-Sørensen M, Ekblond A, Kastrup J. Bone marrow-derived mesenchymal stromal cell treatment in patients with ischaemic heart failure: final 4-year follow-up of the MSC-HF trial. *Eur J Heart Fail* 2020; **22**: 884-892 [PMID: 31863561 DOI: 10.1002/ejhf.1700]

6 **Hong Y**, He H, Jiang G, Zhang H, Tao W, Ding Y, Yuan D, Liu J, Fan H, Lin F, Liang X, Li X, Zhang Y. miR-155-5p inhibition rejuvenates aged mesenchymal stem cells and enhances cardioprotection following infarction. *Aging Cell* 2020; **19**: e13128 [PMID: 32196916 DOI: 10.1111/acel.13128]

7 **Staff NP**, Jones DT, Singer W. Mesenchymal Stromal Cell Therapies for Neurodegenerative Diseases. *Mayo Clin Proc* 2019; **94**: 892-905 [PMID: 31054608 DOI: 10.1016/j.mayocp.2019.01.001]

8 **Berry JD**, Cudkowicz ME, Windebank AJ, Staff NP, Owegi M, Nicholson K, McKenna-Yasek D, Levy YS, Abramov N, Kaspi H, Mehra M, Aricha R, Gothelf Y, Brown RH. NurOwn, phase 2, randomized, clinical trial in patients with ALS: Safety, clinical, and biomarker results. *Neurology* 2019; **93**: e2294-e2305 [PMID: 31740545 DOI: 10.1212/WNL.0000000000008620]

9 **Matthay MA**, Calfee CS, Zhuo H, Thompson BT, Wilson JG, Levitt JE, Rogers AJ, Gotts JE, Wiener-Kronish JP, Bajwa EK, Donahoe MP, McVerry BJ, Ortiz LA, Exline M, Christman JW, Abbott J, Delucchi KL, Caballero L, McMillan M, McKenna DH, Liu KD. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. *Lancet Respir Med* 2019; **7**: 154-162 [PMID: 30455077 DOI: 10.1016/S2213-2600(18)30418-1]

10 **Carvello M**, Lightner A, Yamamoto T, Kotze PG, Spinelli A. Mesenchymal Stem Cells for Perianal Crohn's Disease. *Cells* 2019; **8**: 764 [PMID: 31340546 DOI: 10.3390/cells8070764]

11 **Colombini A**, Perucca Orfei C, Kouroupis D, Ragni E, De Luca P, ViganÒ M, Correa D, de Girolamo L. Mesenchymal stem cells in the treatment of articular cartilage degeneration: New biological insights for an old-timer cell. *Cytotherapy* 2019; **21**: 1179-1197 [PMID: 31784241 DOI: 10.1016/j.jcyt.2019.10.004]

12 **Yin Y**, Hao H, Cheng Y, Zang L, Liu J, Gao J, Xue J, Xie Z, Zhang Q, Han W, Mu Y. Human umbilical cord-derived mesenchymal stem cells direct macrophage polarization to alleviate pancreatic islets dysfunction in type 2 diabetic mice. *Cell Death Dis* 2018; **9**: 760 [PMID: 29988034 DOI: 10.1038/s41419-018-0801-9]

13 **Fazeli Z**, Abedindo A, Omrani MD, Ghaderian SMH. Mesenchymal Stem Cells (MSCs) Therapy for Recovery of Fertility: a Systematic Review. *Stem Cell Rev Rep* 2018; **14**: 1-12 [PMID: 28884412 DOI: 10.1007/s12015-017-9765-x]

14 **Galipeau J**, Sensébé L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell Stem Cell* 2018; **22**: 824-833 [PMID: 29859173 DOI: 10.1016/j.stem.2018.05.004]

15 **Nie WB**, Zhang D, Wang LS. Growth Factor Gene-Modified Mesenchymal Stem Cells in Tissue Regeneration. *Drug Des Devel Ther* 2020; **14**: 1241-1256 [PMID: 32273686 DOI: 10.2147/DDDT.S243944]

16 **Andrzejewska A**, Lukomska B, Janowski M. Concise Review: Mesenchymal Stem Cells: From Roots to Boost. *Stem Cells* 2019; **37**: 855-864 [PMID: 30977255 DOI: 10.1002/stem.3016]

17 **Khademi-Shirvan M**, Ghorbaninejad M, Hosseini S, Baghaban Eslaminejad M. The Importance of Stem Cell Senescence in Regenerative Medicine. *Adv Exp Med Biol* 2020 [PMID: 32026416 DOI: 10.1007/5584_2020_489]

18 **Yu J**, Shi J, Zhang Y, Zhang Y, Huang Y, Chen Z, Yang J. The replicative senescent mesenchymal stem / stromal cells defect in DNA damage response and anti-oxidative capacity. *Int J Med Sci* 2018; **15**: 771-781 [PMID: 30008586 DOI: 10.7150/ijms.24635]

19 **Medeiros Tavares Marques JC**, Cornélio DA, Nogueira Silbiger V, Ducati Luchessi A, de Souza S, Batistuzzo de Medeiros SR. Identification of new genes associated to senescent and tumorigenic phenotypes in mesenchymal stem cells. *Sci Rep* 2017; **7**: 17837 [PMID: 29259202 DOI: 10.1038/s41598-017-16224-5]

20 **Yu D**, Du Z, Pian L, Li T, Wen X, Li W, Kim SJ, Xiao J, Cohen P, Cui J, Hoffman AR, Hu JF. Mitochondrial DNA Hypomethylation Is a Biomarker Associated with Induced Senescence in Human Fetal Heart Mesenchymal Stem Cells. *Stem Cells Int* 2017; **2017**: 1764549 [PMID: 28484495 DOI: 10.1155/2017/1764549]

21 **Zhai W**, Yong D, El-Jawhari JJ, Cuthbert R, McGonagle D, Win Naing M, Jones E. Identification of senescent cells in multipotent mesenchymal stromal cell cultures: Current methods and future directions. *Cytotherapy* 2019; **21**: 803-819 [PMID: 31138507 DOI: 10.1016/j.jcyt.2019.05.001]

22 **Nadeau S**, Cheng A, Colmegna I, Rodier F. Quantifying Senescence-Associated Phenotypes in Primary Multipotent Mesenchymal Stromal Cell Cultures. *Methods Mol Biol* 2019; **2045**: 93-105 [PMID: 31020633 DOI: 10.1007/7651_2019_217]

23 **Turinetto V**, Vitale E, Giachino C. Senescence in Human Mesenchymal Stem Cells: Functional Changes and Implications in Stem Cell-Based Therapy. *Int J Mol Sci* 2016; **17**: 1164 [PMID: 27447618 DOI: 10.3390/ijms17071164]

24 **Oja S**, Komulainen P, Penttilä A, Nystedt J, Korhonen M. Automated image analysis detects aging in clinical-grade mesenchymal stromal cell cultures. *Stem Cell Res Ther* 2018; **9**: 6 [PMID: 29321040 DOI: 10.1186/s13287-017-0740-x]

25 **Jung JS**, Volk C, Marga C, Navarrete Santos A, Jung M, Rujescu D, Navarrete Santos A. Adipose-Derived Stem/Stromal Cells Recapitulate Aging Biomarkers and Show Reduced Stem Cell Plasticity Affecting Their Adipogenic Differentiation Capacity. *Cell Reprogram* 2019; **21**: 187-199 [PMID: 31298565 DOI: 10.1089/cell.2019.0010]

26 **Ganguly P**, El-Jawhari JJ, Giannoudis PV, Burska AN, Ponchel F, Jones EA. Age-related Changes in Bone Marrow Mesenchymal Stromal Cells: A Potential Impact on Osteoporosis and Osteoarthritis Development. *Cell Transplant* 2017; **26**: 1520-1529 [PMID: 29113463 DOI: 10.1177/0963689717721201]

27 **Stab BR 2nd**, Martinez L, Grismaldo A, Lerma A, Gutiérrez ML, Barrera LA, Sutachan JJ, Albarracín SL. Mitochondrial Functional Changes Characterization in Young and Senescent Human Adipose Derived MSCs. *Front Aging Neurosci* 2016; **8**: 299 [PMID: 28018212 DOI: 10.3389/fnagi.2016.00299]

28 **Li JJ**, Ma FX, Wang YW, Chen F, Lu SH, Chi Y, Du WJ, Song BQ, Hu LD, Chen H, Han ZC. Knockdown of IL-8 Provoked Premature Senescence of Placenta-Derived Mesenchymal Stem Cells. *Stem Cells Dev* 2017; **26**: 912-931 [PMID: 28418782 DOI: 10.1089/scd.2016.0324]

29 **Yin Y**, Wu RX, He XT, Xu XY, Wang J, Chen FM. Influences of age-related changes in mesenchymal stem cells on macrophages during in-vitro culture. *Stem Cell Res Ther* 2017; **8**: 153 [PMID: 28646912 DOI: 10.1186/s13287-017-0608-0]

30 **Chen Q**, Shou P, Zheng C, Jiang M, Cao G, Yang Q, Cao J, Xie N, Velletri T, Zhang X, Xu C, Zhang L, Yang H, Hou J, Wang Y, Shi Y. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? *Cell Death Differ* 2016; **23**: 1128-1139 [PMID: 26868907 DOI: 10.1038/cdd.2015.168]

31 **D'Ippolito G**, Schiller PC, Ricordi C, Roos BA, Howard GA. Age-related osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. *J Bone Miner Res* 1999; **14**: 1115-1122 [PMID: 10404011 DOI: 10.1359/jbmr.1999.14.7.1115]

32 **Li CJ**, Cheng P, Liang MK, Chen YS, Lu Q, Wang JY, Xia ZY, Zhou HD, Cao X, Xie H, Liao EY, Luo XH. MicroRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation. *J Clin Invest* 2015; **125**: 1509-1522 [PMID: 25751060 DOI: 10.1172/JCI77716]

33 **Li H**, Liu P, Xu S, Li Y, Dekker JD, Li B, Fan Y, Zhang Z, Hong Y, Yang G, Tang T, Ren Y, Tucker HO, Yao Z, Guo X. FOXP1 controls mesenchymal stem cell commitment and senescence during skeletal aging. *J Clin Invest* 2017; **127**: 1241-1253 [PMID: 28240601 DOI: 10.1172/JCI89511]

34 **Neri S**. Genetic Stability of Mesenchymal Stromal Cells for Regenerative Medicine Applications: A Fundamental Biosafety Aspect. *Int J Mol Sci* 2019; **20**: 2406 [PMID: 31096604 DOI: 10.3390/ijms20102406]

35 **Hladik D**, Höfig I, Oestreicher U, Beckers J, Matjanovski M, Bao X, Scherthan H, Atkinson MJ, Rosemann M. Long-term culture of mesenchymal stem cells impairs ATM-dependent recognition of DNA breaks and

increases genetic instability. *Stem Cell Res Ther* 2019; **10**: 218 [PMID: 31358047 DOI: 10.1186/s13287-019-1334-6]

36 **Wang Y**, Wu H, Yang Z, Chi Y, Meng L, Mao A, Yan S, Hu S, Zhang J, Zhang Y, Yu W, Ma Y, Li T, Cheng Y, Wang Y, Wang S, Liu J, Han J, Li C, Liu L, Xu J, Han ZB, Han ZC. Human mesenchymal stem cells possess different biological characteristics but do not change their therapeutic potential when cultured in serum free medium. *Stem Cell Res Ther* 2014; **5**: 132 [PMID: 25476802 DOI: 10.1186/scrt522]

37 **Kundrotas G**, Gasperskaja E, Slapsyte G, Gudleviciene Z, Krasko J, Stumbryte A, Liudkeviciene R. Identity, proliferation capacity, genomic stability and novel senescence markers of mesenchymal stem cells isolated from low volume of human bone marrow. *Oncotarget* 2016; **7**: 10788-10802 [PMID: 26910916 DOI: 10.18632/oncotarget.7456]

38 **Sharma S**, Bhonde R. Mesenchymal stromal cells are genetically stable under a hostile in vivo-like scenario as revealed by in vitro micronucleus test. *Cytotherapy* 2015; **17**: 1384-1395 [PMID: 26264182 DOI: 10.1016/j.jcyt.2015.07.004]

39 **Roselli EA**, Lazzati S, Iseppon F, Manganini M, Marcato L, Gariboldi MB, Maggi F, Grati FR, Simoni G. Fetal mesenchymal stromal cells from cryopreserved human chorionic villi: cytogenetic and molecular analysis of genome stability in long-term cultures. *Cytotherapy* 2013; **15**: 1340-1351 [PMID: 24094486 DOI: 10.1016/j.jcyt.2013.06.019]

40 **Prockop DJ**, Keating A. Relearning the lessons of genomic stability of human cells during expansion in culture: implications for clinical research. *Stem Cells* 2012; **30**: 1051-1052 [PMID: 22495826 DOI: 10.1002/stem.1103]

41 **Rebuzzini P**, Zuccotti M, Redi CA, Garagna S. Chromosomal Abnormalities in Embryonic and Somatic Stem Cells. *Cytogenet Genome Res* 2015; **147**: 1-9 [PMID: 26583376 DOI: 10.1159/000441645]

42 **Maijenburg MW**, Kleijer M, Vermeul K, Mul EP, van Alphen FP, van der Schoot CE, Voermans C. The composition of the mesenchymal stromal cell compartment in human bone marrow changes during development and aging. *Haematologica* 2012; **97**: 179-183 [PMID: 21993672 DOI: 10.3324/haematol.2011.047753]

43 **Yang YM**, Li P, Cui DC, Dang RJ, Zhang L, Wen N, Jiang XX. Effect of aged bone marrow microenvironment on mesenchymal stem cell migration. *Age (Dordr)* 2015; **37**: 16 [PMID: 25693923 DOI: 10.1007/s11357-014-9743-z]

44 **Lei Q**, Liu T, Gao F, Xie H, Sun L, Zhao A, Ren W, Guo H, Zhang L, Wang H, Chen Z, Guo AY, Li Q. Microvesicles as Potential Biomarkers for the Identification of Senescence in Human Mesenchymal Stem Cells. *Theranostics* 2017; **7**: 2673-2689 [PMID: 28819455 DOI: 10.7150/thno.18915]

45 **Madsen SD**, Russell KC, Tucker HA, Glowacki J, Bunnell BA, O'Connor KC. Decoy TRAIL receptor CD264: a cell surface marker of cellular aging for human bone marrow-derived mesenchymal stem cells. *Stem Cell Res Ther* 2017; **8**: 201 [PMID: 28962588 DOI: 10.1186/s13287-017-0649-4]

46 **Ang J**, Lee YA, Raghothaman D, Jayaraman P, Teo KL, Khan FJ, Reuveny S, Chang YT, Kang NY, Oh S. Rapid Detection of Senescent Mesenchymal Stromal Cells by a Fluorescent Probe. *Biotechnol J* 2019; **14**: e1800691 [PMID: 31218816 DOI: 10.1002/biot.201800691]

47 **Wiese DM**, Ruttan CC, Wood CA, Ford BN, Braid LR. Accumulating Transcriptome Drift Precedes Cell Aging in Human Umbilical Cord-Derived Mesenchymal Stromal Cells Serially Cultured to Replicative Senescence. *Stem Cells Transl Med* 2019; **8**: 945-958 [PMID: 30924318 DOI: 10.1002/sctm.18-0246]

48 **Benisch P**, Schilling T, Klein-Hitpass L, Frey SP, Seefried L, Raaijmakers N, Krug M, Regensburger M, Zeck S, Schinke T, Amling M, Ebert R, Jakob F. The transcriptional profile of mesenchymal stem cell populations in primary osteoporosis is distinct and shows overexpression of osteogenic inhibitors. *PLoS One* 2012; **7**: e45142 [PMID: 23028809 DOI: 10.1371/journal.pone.0045142]

49 **Wu Y**, Yang J, Ai Z, Yu M, Li J, Li S. Identification of key genes and transcription factors in aging mesenchymal stem cells by DNA microarray data. *Gene* 2019; **692**: 79-87 [PMID: 30641220 DOI: 10.1016/j.gene.2018.12.063]

50 **Yoo JK**, Choi SJ, Kim JK. Expression profiles of subtracted mRNAs during cellular senescence in human mesenchymal stem cells derived from bone marrow. *Exp Gerontol* 2013; **48**: 464-471 [PMID: 23466301 DOI: 10.1016/j.exger.2013.02.022]

51 **Leveque X**, Hochane M, Geraldo F, Dumont S, Gratas C, Oliver L, Gaignier C, Trichet V, Layrolle P, Heymann D, Herault O, Vallette FM, Olivier C. Low-Dose Pesticide Mixture Induces Accelerated Mesenchymal Stem Cell Aging In Vitro. *Stem Cells* 2019; **37**: 1083-1094 [PMID: 30977188 DOI: 10.1002/stem.3014]

52 **Bustos ML**, Huleihel L, Kapetanaki MG, Lino-Cardenas CL, Mroz L, Ellis BM, McVerry BJ, Richards TJ, Kaminski N, Cerdenes N, Mora AL, Rojas M. Aging mesenchymal stem cells fail to protect because of impaired migration and antiinflammatory response. *Am J Respir Crit Care Med* 2014; **189**: 787-798 [PMID: 24559482 DOI: 10.1164/rccm.201306-1043OC]

53 **Niu P**, Smagul A, Wang L, Sadvakas A, Sha Y, Pérez LM, Nussupbekova A, Amirbekov A, Akanov AA, Gálvez BG, Jordan IK, Lunyak VV. Transcriptional profiling of interleukin-2-primed human adipose derived mesenchymal stem cells revealed dramatic changes in stem cells response imposed by replicative senescence. *Oncotarget* 2015; **6**: 17938-17957 [PMID: 26255627 DOI: 10.18632/oncotarget.4852]

54 **Duscher D**, Rennert RC, Januszyk M, Anghel E, Maan ZN, Whittam AJ, Perez MG, Kosaraju R, Hu MS, Walmsley GG, Atashroo D, Khong S, Butte AJ, Gurtner GC. Aging disrupts cell subpopulation dynamics and diminishes the function of mesenchymal stem cells. *Sci Rep* 2014; **4**: 7144 [PMID: 25413454 DOI: 10.1038/srep07144]

55 **Malaquin N**, Martinez A, Rodier F. Keeping the senescence secretome under control: Molecular reins on the senescence-associated secretory phenotype. *Exp Gerontol* 2016; **82**: 39-49 [PMID: 27235851 DOI: 10.1016/j.exger.2016.05.010]

56 **Kizilay Mancini Ö**, Lora M, Shum-Tim D, Nadeau S, Rodier F, Colmegna I. A Proinflammatory Secretome Mediates the Impaired Immunopotency of Human Mesenchymal Stromal Cells in Elderly Patients with Atherosclerosis. *Stem Cells Transl Med* 2017; **6**: 1132-1140 [PMID: 28194905 DOI: 10.1002/sctm.16-0221]

57 **Infante A**, Rodríguez CI. Secretome analysis of in vitro aged human mesenchymal stem cells reveals

IGFBP7 as a putative factor for promoting osteogenesis. *Sci Rep* 2018; **8**: 4632 [PMID: 29545581 DOI: 10.1038/s41598-018-22855-z]

58 **Madonna R**, Angelucci S, Di Giuseppe F, Doria V, Giricz Z, Görbe A, Ferdinandy P, De Caterina R. Proteomic analysis of the secretome of adipose tissue-derived murine mesenchymal cells overexpressing telomerase and myocardin. *J Mol Cell Cardiol* 2019; **131**: 171-186 [PMID: 31055035 DOI: 10.1016/j.yjmcc.2019.04.019]

59 **Cakouros D**, Gronthos S. Epigenetic Regulation of Bone Marrow Stem Cell Aging: Revealing Epigenetic Signatures associated with Hematopoietic and Mesenchymal Stem Cell Aging. *Aging Dis* 2019; **10**: 174-189 [PMID: 30705777 DOI: 10.14336/AD.2017.1213]

60 **Choi MR**, In YH, Park J, Park T, Jung KH, Chai JC, Chung MK, Lee YS, Chai YG. Genome-scale DNA methylation pattern profiling of human bone marrow mesenchymal stem cells in long-term culture. *Exp Mol Med* 2012; **44**: 503-512 [PMID: 22684242 DOI: 10.3858/emm.2012.44.8.057]

61 **Fernández AF**, Bayón GF, Urdinguio RG, Toraño EG, García MG, Carella A, Petrus-Reurer S, Ferrero C, Martinez-Camblor P, Cubillo I, García-Castro J, Delgado-Calle J, Pérez-Campo FM, Riancho JA, Bueno C, Menéndez P, Mentink A, Mareschi K, Claire F, Fagnani C, Medda E, Toccaceli V, Brescianini S, Moran S, Esteller M, Stolzing A, de Boer J, Nisticò L, Stazi MA, Fraga MF. H3K4me1 marks DNA regions hypomethylated during aging in human stem and differentiated cells. *Genome Res* 2015; **25**: 27-40 [PMID: 25271306 DOI: 10.1101/gr.169011.113]

62 **Toraño EG**, Bayón GF, Del Real Á, Sierra MI, García MG, Carella A, Belmonte T, Urdinguio RG, Cubillo I, García-Castro J, Delgado-Calle J, Pérez-Campo FM, Riancho JA, Fraga MF, Fernández AF. Age-associated hydroxymethylation in human bone-marrow mesenchymal stem cells. *J Transl Med* 2016; **14**: 207 [PMID: 27393146 DOI: 10.1186/s12967-016-0966-x]

63 **Bork S**, Pfister S, Witt H, Horn P, Korn B, Ho AD, Wagner W. DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. *Aging Cell* 2010; **9**: 54-63 [PMID: 19895632 DOI: 10.1111/j.1474-9726.2009.00535.x]

64 **Redaelli S**, Bentivegna A, Foudah D, Miloso M, Redondo J, Riva G, Baronchelli S, Dalprà L, Tredici G. From cytogenomic to epigenomic profiles: monitoring the biologic behavior of in vitro cultured human bone marrow mesenchymal stem cells. *Stem Cell Res Ther* 2012; **3**: 47 [PMID: 23168092 DOI: 10.1186/scrt138]

65 **Pasumarthy KK**, Doni Jayavelu N, Kilpinen L, Andrus C, Battle SL, Korhonen M, Lehenkari P, Lund R, Laitinen S, Hawkins RD. Methylome Analysis of Human Bone Marrow MSCs Reveals Extensive Age- and Culture-Induced Changes at Distal Regulatory Elements. *Stem Cell Reports* 2017; **9**: 999-1015 [PMID: 28844656 DOI: 10.1016/j.stemcr.2017.07.018]

66 **Dellago H**, Preschitz-Kammerhofer B, Terlecki-Zaniewicz L, Schreiner C, Fortschegger K, Chang MW, Hackl M, Monteforte R, Kühnel H, Schosserer M, Gruber F, Tschachler E, Scheideler M, Grillari-Voglauer R, Grillari J, Wieser M. High levels of oncomiR-21 contribute to the senescence-induced growth arrest in normal human cells and its knock-down increases the replicative lifespan. *Aging Cell* 2013; **12**: 446-458 [PMID: 23496142 DOI: 10.1111/acel.12069]

67 **Bonifacio LN**, Jarstfer MB. MiRNA profile associated with replicative senescence, extended cell culture, and ectopic telomerase expression in human foreskin fibroblasts. *PLoS One* 2010; **5**: e12519 [PMID: 20824140 DOI: 10.1371/journal.pone.0012519]

68 **Tzatsos A**, Paskaleva P, Lymperi S, Contino G, Stoykova S, Chen Z, Wong KK, Bardeesy N. Lysine-specific demethylase 2B (KDM2B)-let-7-enhancer of zester homolog 2 (EZH2) pathway regulates cell cycle progression and senescence in primary cells. *J Biol Chem* 2011; **286**: 33061-33069 [PMID: 21757686 DOI: 10.1074/jbc.M111.257667]

69 **Qiu W**, Kassem M. miR-141-3p inhibits human stromal (mesenchymal) stem cell proliferation and differentiation. *Biochim Biophys Acta* 2014; **1843**: 2114-2121 [PMID: 24937190 DOI: 10.1016/j.bbamcr.2014.06.004]

70 **Ukai T**, Sato M, Akutsu H, Umezawa A, Mochida J. MicroRNA-199a-3p, microRNA-193b, and microRNA-320c are correlated to aging and regulate human cartilage metabolism. *J Orthop Res* 2012; **30**: 1915-1922 [PMID: 22674437 DOI: 10.1002/jor.22157]

71 **Lee Y**, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004; **23**: 4051-4060 [PMID: 15372072 DOI: 10.1038/sj.emboj.7600385]

72 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438 DOI: 10.1016/s0092-8674(04)00045-5]

73 **Yoo JK**, Kim CH, Jung HY, Lee DR, Kim JK. Discovery and characterization of miRNA during cellular senescence in bone marrow-derived human mesenchymal stem cells. *Exp Gerontol* 2014; **58**: 139-145 [PMID: 25087724 DOI: 10.1016/j.exger.2014.07.020]

74 **Mohd Ali N**, Boo L, Yeap SK, Ky H, Satharasinghe DA, Liew WC, Ong HK, Cheong SK, Kamarul T. Probable impact of age and hypoxia on proliferation and microRNA expression profile of bone marrow-derived human mesenchymal stem cells. *PeerJ* 2016; **4**: e1536 [PMID: 26788424 DOI: 10.7717/peerj.1536]

75 **Lee S**, Yu KR, Ryu YS, Oh YS, Hong IS, Kim HS, Lee JY, Kim S, Seo KW, Kang KS. miR-543 and miR-590-3p regulate human mesenchymal stem cell aging via direct targeting of AIMP3/p18. *Age (Dordr)* 2014; **36**: 9724 [PMID: 25465621 DOI: 10.1007/s11357-014-9724-2]

76 **Li CJ**, Xiao Y, Yang M, Su T, Sun X, Guo Q, Huang Y, Luo XH. Long noncoding RNA Bmncr regulates mesenchymal stem cell fate during skeletal aging. *J Clin Invest* 2018; **128**: 5251-5266 [PMID: 30352426 DOI: 10.1172/JCI99044]

77 **Bernardes de Jesus B**, Marinho SP, Barros S, Sousa-Franco A, Alves-Vale C, Carvalho T, Carmo-Fonseca M. Silencing of the lncRNA Zeb2-NAT facilitates reprogramming of aged fibroblasts and safeguards stem cell pluripotency. *Nat Commun* 2018; **9**: 94 [PMID: 29311544 DOI: 10.1038/s41467-017-01921-6]

78 **Cárdenes N**, Álvarez D, Sellarés J, Peng Y, Corey C, Wecht S, Nouraie SM, Shanker S, Sembrat J, Bueno M, Shiva S, Mora AL, Rojas M. Senescence of bone marrow-derived mesenchymal stem cells from patients with idiopathic pulmonary fibrosis. *Stem Cell Res Ther* 2018; **9**: 257 [PMID: 30257725 DOI: 10.1186/s13287-018-0970-6]

79 **Li XY**, Ding J, Zheng ZH, Li XY, Wu ZB, Zhu P. Long-term culture in vitro impairs the

immunosuppressive activity of mesenchymal stem cells on T cells. *Mol Med Rep* 2012; **6**: 1183-1189 [PMID: 22923041 DOI: 10.3892/mmr.2012.1039]

80 **Xia W**, Zhang F, Xie C, Jiang M, Hou M. Macrophage migration inhibitory factor confers resistance to senescence through CD74-dependent AMPK-FOXO3a signaling in mesenchymal stem cells. *Stem Cell Res Ther* 2015; **6**: 82 [PMID: 25896286 DOI: 10.1186/s13287-015-0076-3]

81 **Malaise O**, Tachikart Y, Constantinides M, Mumme M, Ferreira-Lopez R, Noack S, Krettek C, Noël D, Wang J, Jorgensen C, Brondello JM. Mesenchymal stem cell senescence alleviates their intrinsic and seno-suppressive paracrine properties contributing to osteoarthritis development. *Aging (Albany NY)* 2019; **11**: 9128-9146 [PMID: 31644429 DOI: 10.18632/aging.102379]

82 **Abolhasani M**, Rezaee MA, Mohammadi M, Ghadimi T, Mohammadi M, Rahmani MR. Immunomodulatory properties of umbilical cord vein mesenchymal stromal cells influenced by gestational age and in vitro expansion. *Immunol Lett* 2018; **194**: 62-68 [PMID: 29175314 DOI: 10.1016/j.imlet.2017.11.008]

83 **Ding W**, Nowakowski GS, Knox TR, Boysen JC, Maas ML, Schwager SM, Wu W, Wellik LE, Dietz AB, Ghosh AK, Secreto CR, Medina KL, Shanafelt TD, Zent CS, Call TG, Kay NE. Bi-directional activation between mesenchymal stem cells and CLL B-cells: implication for CLL disease progression. *Br J Haematol* 2009; **147**: 471-483 [PMID: 19751240 DOI: 10.1111/j.1365-2141.2009.07868.x]

84 **Cao X**, Luo P, Huang J, Liang C, He J, Wang Z, Shan D, Peng C, Wu S. Intraarticular senescent chondrocytes impair the cartilage regeneration capacity of mesenchymal stem cells. *Stem Cell Res Ther* 2019; **10**: 86 [PMID: 30867061 DOI: 10.1186/s13287-019-1193-1]

85 **Petri RM**, Hackel A, Hahnel K, Dumitru CA, Bruderek K, Flohe SB, Paschen A, Lang S, Brandau S. Activated Tissue-Resident Mesenchymal Stromal Cells Regulate Natural Killer Cell Immune and Tissue-Regenerative Function. *Stem Cell Reports* 2017; **9**: 985-998 [PMID: 28781075 DOI: 10.1016/j.stemcr.2017.06.020]

86 **Lepperdinger G**. Inflammation and mesenchymal stem cell aging. *Curr Opin Immunol* 2011; **23**: 518-524 [PMID: 21703839 DOI: 10.1016/j.coi.2011.05.007]

87 **Loisel S**, Dulong J, Ménard C, Renoud ML, Meziere N, Isabelle B, Latour M, Bescher N, Pedeux R, Bertheuil N, Flecher E, Sensebé L, Tarte K. Brief Report: Proteasomal Indoleamine 2,3-Dioxygenase Degradation Reduces the Immunosuppressive Potential of Clinical Grade-Mesenchymal Stromal Cells Undergoing Replicative Senescence. *Stem Cells* 2017; **35**: 1431-1436 [PMID: 28142215 DOI: 10.1002/stem.2580]

88 **de Witte SFH**, Lambert EE, Merino A, Strini T, Douben HJCW, O'Flynn L, Elliman SJ, de Klein AJEMM, Newsome PN, Baan CC, Hoogduijn MJ. Aging of bone marrow- and umbilical cord-derived mesenchymal stromal cells during expansion. *Cytotherapy* 2017; **19**: 798-807 [PMID: 28462821 DOI: 10.1016/j.jcyt.2017.03.071]

89 **Wu LW**, Wang YL, Christensen JM, Khalifian S, Schneeberger S, Raimondi G, Cooney DS, Lee WP, Brandacher G. Donor age negatively affects the immunoregulatory properties of both adipose and bone marrow derived mesenchymal stem cells. *Transpl Immunol* 2014; **30**: 122-127 [PMID: 24632513 DOI: 10.1016/j.trim.2014.03.001]

90 **Xu LL**, Fu HX, Zhang JM, Feng FE, Wang QM, Zhu XL, Xue J, Wang CC, Chen Q, Liu X, Wang YZ, Qin YZ, Kong Y, Chang YJ, Xu LP, Liu KY, Huang XJ, Zhang XH. Impaired Function of Bone Marrow Mesenchymal Stem Cells from Immune Thrombocytopenia Patients in Inducing Regulatory Dendritic Cell Differentiation Through the Notch-1/Jagged-1 Signaling Pathway. *Stem Cells Dev* 2017; **26**: 1648-1661 [PMID: 28946811 DOI: 10.1089/scd.2017.0078]

91 **Dao MA**, Tate CC, Aizman I, McGrogan M, Case CC. Comparing the immunosuppressive potency of naïve marrow stromal cells and Notch-transfected marrow stromal cells. *J Neuroinflammation* 2011; **8**: 133 [PMID: 21982515 DOI: 10.1186/1742-2094-8-133]

92 **Gnani D**, Crippa S, Della Volpe L, Rossella V, Conti A, Lettera E, Rivis S, Ometti M, Fraschini G, Bernardo ME, Di Micco R. An early-senescence state in aged mesenchymal stromal cells contributes to hematopoietic stem and progenitor cell clonogenic impairment through the activation of a pro-inflammatory program. *Aging Cell* 2019; **18**: e12933 [PMID: 30828977 DOI: 10.1111/acel.12933]

93 **O'Hagan-Wong K**, Nadeau S, Carrier-Leclerc A, Apablaza F, Hamdy R, Shum-Tim D, Rodier F, Colmegna I. Increased IL-6 secretion by aged human mesenchymal stromal cells disrupts hematopoietic stem and progenitor cells' homeostasis. *Oncotarget* 2016; **7**: 13285-13296 [PMID: 26934440 DOI: 10.18632/oncotarget.7690]

94 **Ju Z**, Jiang H, Jaworski M, Rathinam C, Gompf A, Klein C, Trumpp A, Rudolph KL. Telomere dysfunction induces environmental alterations limiting hematopoietic stem cell function and engraftment. *Nat Med* 2007; **13**: 742-747 [PMID: 17486088 DOI: 10.1038/nm1578]

95 **Rivera FJ**, de la Fuente AG, Zhao C, Silva ME, Gonzalez GA, Wodnar R, Feichtner M, Lange S, Errea O, Priglinger E, O'Sullivan A, Romanelli P, Jadasz JJ, Brachtl G, Greil R, Tempfer H, Traweger A, Bátiz LF, Küry P, Couillard-Despres S, Franklin RJM, Aigner L. Aging restricts the ability of mesenchymal stem cells to promote the generation of oligodendrocytes during remyelination. *Glia* 2019; **67**: 1510-1525 [PMID: 31038798 DOI: 10.1002/glia.23624]

96 **Yamaguchi S**, Horie N, Satoh K, Ishikawa T, Mori T, Maeda H, Fukuda Y, Ishizaka S, Hiu T, Morofuji Y, Izumo T, Nishida N, Matsuo T. Age of donor of human mesenchymal stem cells affects structural and functional recovery after cell therapy following ischaemic stroke. *J Cereb Blood Flow Metab* 2018; **38**: 1199-1212 [PMID: 28914133 DOI: 10.1177/0271678X17731964]

97 **Liang H**, Hou H, Yi W, Yang G, Gu C, Lau WB, Gao E, Ma X, Lu Z, Wei X, Pei J, Yi D. Increased expression of pigment epithelium-derived factor in aged mesenchymal stem cells impairs their therapeutic efficacy for attenuating myocardial infarction injury. *Eur Heart J* 2013; **34**: 1681-1690 [PMID: 21606086 DOI: 10.1093/eurheartj/ehr131]

98 **Guo J**, Zhao Y, Fei C, Zhao S, Zheng Q, Su J, Wu D, Li X, Chang C. Dicer1 downregulation by multiple myeloma cells promotes the senescence and tumor-supporting capacity and decreases the differentiation potential of mesenchymal stem cells. *Cell Death Dis* 2018; **9**: 512 [PMID: 29724992 DOI: 10.1038/s41419-018-0545-6]

99 **Lee SH**, Lee JH, Lee HY, Min KJ. Sirtuin signaling in cellular senescence and aging. *BMB Rep* 2019; **52**: 24-34 [PMID: 30526767 DOI: 10.5483/BMBRep.2019.52.1.290]

100 **Liu X**, Chen H, Zhu W, Chen H, Hu X, Jiang Z, Xu Y, Zhou Y, Wang K, Wang L, Chen P, Hu H, Wang C, Zhang N, Ma Q, Huang M, Hu D, Zhang L, Wu R, Wang Y, Xu Q, Yu H, Wang J. Transplantation of SIRT1-engineered aged mesenchymal stem cells improves cardiac function in a rat myocardial infarction model. *J Heart Lung Transplant* 2014; **33**: 1083-1092 [PMID: 25034794 DOI: 10.1016/j.healun.2014.05.008]

101 **Song J**, Li J, Yang F, Ning G, Zhen L, Wu L, Zheng Y, Zhang Q, Lin D, Xie C, Peng L. Nicotinamide mononucleotide promotes osteogenesis and reduces adipogenesis by regulating mesenchymal stromal cells via the SIRT1 pathway in aged bone marrow. *Cell Death Dis* 2019; **10**: 336 [PMID: 31000692 DOI: 10.1038/s41419-019-1569-2]

102 **Ma C**, Pi C, Yang Y, Lin L, Shi Y, Li Y, Li Y, He X. Nampt Expression Decreases Age-Related Senescence in Rat Bone Marrow Mesenchymal Stem Cells by Targeting Sirt1. *PLoS One* 2017; **12**: e0170930 [PMID: 28125705 DOI: 10.1371/journal.pone.0170930]

103 **Zhou L**, Chen X, Liu T, Zhu C, Si M, Jargstorf J, Li M, Pan G, Gong Y, Luo ZP, Yang H, Pei M, He F. SIRT1-dependent anti-senescence effects of cell-deposited matrix on human umbilical cord mesenchymal stem cells. *J Tissue Eng Regen Med* 2018; **12**: e1008-e1021 [PMID: 28107614 DOI: 10.1002/term.2422]

104 **Liu X**, Hu D, Zeng Z, Zhu W, Zhang N, Yu H, Chen H, Wang K, Wang Y, Wang L, Zhao J, Zhang L, Wu R, Hu X, Wang J. SRT1720 promotes survival of aged human mesenchymal stem cells via FAIM: a pharmacological strategy to improve stem cell-based therapy for rat myocardial infarction. *Cell Death Dis* 2017; **8**: e2731 [PMID: 28383554 DOI: 10.1038/cddis.2017.107]

105 **Zhang DY**, Zhang CF, Fu BC, Sun L, Wang XQ, Chen W, Liu W, Liu KY, Du GQ, Ma CY, Jiang SL, Li RK, Tian H. Sirtuin3 protects aged human mesenchymal stem cells against oxidative stress and enhances efficacy of cell therapy for ischaemic heart diseases. *J Cell Mol Med* 2018; **22**: 5504-5517 [PMID: 30091830 DOI: 10.1111/jcmm.13821]

106 **Pan H**, Guan D, Liu X, Li J, Wang L, Wu J, Zhou J, Zhang W, Ren R, Zhang W, Li Y, Yang J, Hao Y, Yuan T, Yuan G, Wang H, Ju Z, Mao Z, Li J, Qu J, Tang F, Liu GH. SIRT6 safeguards human mesenchymal stem cells from oxidative stress by coactivating NRF2. *Cell Res* 2016; **26**: 190-205 [PMID: 26768768 DOI: 10.1038/cr.2016.4]

107 **Mitchell AC**, Briquez PS, Hubbell JA, Cochran JR. Engineering growth factors for regenerative medicine applications. *Acta Biomater* 2016; **30**: 1-12 [PMID: 26555377 DOI: 10.1016/j.actbio.2015.11.007]

108 **Kulkarni RS**, Bajaj MS, Kale VP. Induction and Detection of Autophagy in Aged Hematopoietic Stem Cells by Exposing Them to Microvesicles Secreted by HSC-Supportive Mesenchymal Stromal Cells. *Methods Mol Biol* 2019; **1854**: 21-34 [PMID: 29951740 DOI: 10.1007/7651_2018_166]

109 **Li X**, Hong Y, He H, Jiang G, You W, Liang X, Fu Q, Han S, Lian Q, Zhang Y. FGF21 Mediates Mesenchymal Stem Cell Senescence via Regulation of Mitochondrial Dynamics. *Oxid Med Cell Longev* 2019; **2019**: 4915149 [PMID: 31178962 DOI: 10.1155/2019/4915149]

110 **Wu J**, Wang C, Miao X, Wu Y, Yuan J, Ding M, Li J, Shi Z. Age-Related Insulin-Like Growth Factor Binding Protein-4 Overexpression Inhibits Osteogenic Differentiation of Rat Mesenchymal Stem Cells. *Cell Physiol Biochem* 2017; **42**: 640-650 [PMID: 28595186 DOI: 10.1159/000477873]

111 **Xia W**, Hou M. Macrophage migration inhibitory factor rescues mesenchymal stem cells from doxorubicin-induced senescence though the PI3K-Akt signaling pathway. *Int J Mol Med* 2018; **41**: 1127-1137 [PMID: 29207187 DOI: 10.3892/ijmm.2017.3282]

112 **Liang X**, Ding Y, Lin F, Zhang Y, Zhou X, Meng Q, Lu X, Jiang G, Zhu H, Chen Y, Lian Q, Fan H, Liu Z. Overexpression of ERBB4 rejuvenates aged mesenchymal stem cells and enhances angiogenesis via PI3K/AKT and MAPK/ERK pathways. *FASEB J* 2019; **33**: 4559-4570 [PMID: 30566395 DOI: 10.1096/fj.201801690R]

113 **Park SY**, Jeong AJ, Kim GY, Jo A, Lee JE, Leem SH, Yoon JH, Ye SK, Chung JW. Lactoferrin Protects Human Mesenchymal Stem Cells from Oxidative Stress-Induced Senescence and Apoptosis. *J Microbiol Biotechnol* 2017; **27**: 1877-1884 [PMID: 28870012 DOI: 10.4014/jmb.1707.07040]

114 **Yang M**, Teng S, Ma C, Yu Y, Wang P, Yi C. Ascorbic acid inhibits senescence in mesenchymal stem cells through ROS and AKT/mTOR signaling. *Cytotechnology* 2018; **70**: 1301-1313 [PMID: 29777434 DOI: 10.1007/s10616-018-0220-x]

115 **Lee JH**, Yun CW, Hur J, Lee SH. Fucoidan Rescues p-Cresol-Induced Cellular Senescence in Mesenchymal Stem Cells via FAK-Akt-TWIST Axis. *Mar Drugs* 2018; **16**: 121 [PMID: 29642406 DOI: 10.3390/md16040121]

116 **Antonioli E**, Torres N, Ferretti M, Piccinato CA, Sertie AL. Individual response to mTOR inhibition in delaying replicative senescence of mesenchymal stromal cells. *PLoS One* 2019; **14**: e0204784 [PMID: 30703123 DOI: 10.1371/journal.pone.0204784]

117 **Shahini A**, Mistriotis P, Asmani M, Zhao R, Andreadis ST. NANOG Restores Contractility of Mesenchymal Stem Cell-Based Senescent Microtissues. *Tissue Eng Part A* 2017; **23**: 535-545 [PMID: 28125933 DOI: 10.1089/ten.TEA.2016.0494]

118 **Farahzadi R**, Fathi E, Mesbah-Namin SA, Zarghami N. Anti-aging protective effect of L-carnitine as clinical agent in regenerative medicine through increasing telomerase activity and change in the hTERT promoter CpG island methylation status of adipose tissue-derived mesenchymal stem cells. *Tissue Cell* 2018; **54**: 105-113 [PMID: 30309499 DOI: 10.1016/j.tice.2018.08.012]

119 **Pirmoradi S**, Fathi E, Farahzadi R, Pilehvar-Soltanahmadi Y, Zarghami N. Curcumin Affects Adipose Tissue-Derived Mesenchymal Stem Cell Aging Through TERT Gene Expression. *Drug Res (Stuttg)* 2018; **68**: 213-221 [PMID: 29017189 DOI: 10.1055/s-0043-119635]

120 **Fujisawa K**, Takami T, Fukui Y, Quintanilha LF, Matsumoto T, Yamamoto N, Sakaida I. Evaluating effects of L-carnitine on human bone-marrow-derived mesenchymal stem cells. *Cell Tissue Res* 2017; **368**: 301-310 [PMID: 28197778 DOI: 10.1007/s00441-017-2569-0]

121 **Han YS**, Kim SM, Lee JH, Jung SK, Noh H, Lee SH. Melatonin protects chronic kidney disease mesenchymal stem cells against senescence via PrP^{C} -dependent enhancement of the mitochondrial function. *J Pineal Res* 2019; **66**: e12535 [PMID: 30372554 DOI: 10.1111/jpi.12535]

122 **Yao J**, Jiang SL, Liu W, Liu C, Chen W, Sun L, Liu KY, Jia ZB, Li RK, Tian H. Tissue inhibitor of matrix metalloproteinase-3 or vascular endothelial growth factor transfection of aged human mesenchymal stem cells enhances cell therapy after myocardial infarction. *Rejuvenation Res* 2012; **15**: 495-506 [PMID: 22950427 DOI: 10.1089/rej.2012.1325]

123 **Li SH**, Sun L, Yang L, Li J, Shao Z, Du GQ, Wu J, Weisel RD, Li RK. Young Bone-Marrow Sca-1$^+$ Stem Cells Rejuvenate the Aged Heart and Improve Function after Injury through PDGFRβ-Akt pathway. *Sci Rep* 2017; **7**: 41756 [PMID: 28139736 DOI: 10.1038/srep41756]

124 **Li J**, Li SH, Dong J, Alibhai FJ, Zhang C, Shao ZB, Song HF, He S, Yin WJ, Wu J, Weisel RD, Liu SM, Li RK. Long-term repopulation of aged bone marrow stem cells using young Sca-1 cells promotes aged heart rejuvenation. *Aging Cell* 2019; **18**: e13026 [PMID: 31385396 DOI: 10.1111/acel.13026]

125 **Yang K**, Song HF, He S, Yin WJ, Fan XM, Ru F, Gong H, Zhai XY, Zhang J, Peng ZX, Xi GX, Xie J, Li RK. Effect of neuron-derived neurotrophic factor on rejuvenation of human adipose-derived stem cells for cardiac repair after myocardial infarction. *J Cell Mol Med* 2019; **23**: 5981-5993 [PMID: 31287219 DOI: 10.1111/jcmm.14456]

126 **Song HF**, He S, Li SH, Yin WJ, Wu J, Guo J, Shao ZB, Zhai XY, Gong H, Lu L, Wei F, Weisel RD, Xie J, Li RK. Aged Human Multipotent Mesenchymal Stromal Cells Can Be Rejuvenated by Neuron-Derived Neurotrophic Factor and Improve Heart Function After Injury. *JACC Basic Transl Sci* 2017; **2**: 702-716 [PMID: 30062183 DOI: 10.1016/j.jacbts.2017.07.014]

127 **Ji J**, Fu T, Dong C, Zhu W, Yang J, Kong X, Zhang Z, Bao Y, Zhao R, Ge X, Sha X, Lu Z, Li J, Gu Z. Targeting HMGB1 by ethyl pyruvate ameliorates systemic lupus erythematosus and reverses the senescent phenotype of bone marrow-mesenchymal stem cells. *Aging (Albany NY)* 2019; **11**: 4338-4353 [PMID: 31303606 DOI: 10.18632/aging.102052]

128 **Okada M**, Kim HW, Matsu-ura K, Wang YG, Xu M, Ashraf M. Abrogation of Age-Induced MicroRNA-195 Rejuvenates the Senescent Mesenchymal Stem Cells by Reactivating Telomerase. *Stem Cells* 2016; **34**: 148-159 [PMID: 26390028 DOI: 10.1002/stem.2211]

129 **Dong J**, Zhang Z, Huang H, Mo P, Cheng C, Liu J, Huang W, Tian C, Zhang C, Li J. miR-10a rejuvenates aged human mesenchymal stem cells and improves heart function after myocardial infarction through KLF4. *Stem Cell Res Ther* 2018; **9**: 151 [PMID: 29848383 DOI: 10.1186/s13287-018-0895-0]

130 **Shang J**, Yao Y, Fan X, Shangguan L, Li J, Liu H, Zhou Y. miR-29c-3p promotes senescence of human mesenchymal stem cells by targeting CNOT6 through p53-p21 and p16-pRB pathways. *Biochim Biophys Acta* 2016; **1863**: 520-532 [PMID: 26792405 DOI: 10.1016/j.bbamcr.2016.01.005]

131 **Xu J**, Huang Z, Lin L, Fu M, Song Y, Shen Y, Ren D, Gao Y, Su Y, Zou Y, Chen Y, Zhang D, Hu W, Qian J, Ge J. miRNA-130b is required for the ERK/FOXM1 pathway activation-mediated protective effects of isosorbide dinitrate against mesenchymal stem cell senescence induced by high glucose. *Int J Mol Med* 2015; **35**: 59-71 [PMID: 25355277 DOI: 10.3892/ijmm.2014.1985]

132 **Su T**, Xiao Y, Xiao Y, Guo Q, Li C, Huang Y, Deng Q, Wen J, Zhou F, Luo XH. Bone Marrow Mesenchymal Stem Cells-Derived Exosomal MiR-29b-3p Regulates Aging-Associated Insulin Resistance. *ACS Nano* 2019; **13**: 2450-2462 [PMID: 30715852 DOI: 10.1021/acsnano.8b09375]

133 **Redondo J**, Sarkar P, Kemp K, Virgo PF, Pawade J, Norton A, Emery DC, Guttridge MG, Marks DI, Wilkins A, Scolding NJ, Rice CM. Reduced cellularity of bone marrow in multiple sclerosis with decreased MSC expansion potential and premature ageing in vitro. *Mult Scler* 2018; **24**: 919-931 [PMID: 28548004 DOI: 10.1177/1352458517711276]

134 **Miettinen JA**, Salonen RJ, Ylitalo K, Niemelä M, Kervinen K, Säily M, Koistinen P, Savolainen ER, Mäkikallio TH, Huikuri HV, Lehenkari P. The effect of bone marrow microenvironment on the functional properties of the therapeutic bone marrow-derived cells in patients with acute myocardial infarction. *J Transl Med* 2012; **10**: 66 [PMID: 22462635 DOI: 10.1186/1479-5876-10-66]

135 **Oses C**, Olivares B, Ezquer M, Acosta C, Bosch P, Donoso M, Léniz P, Ezquer F. Preconditioning of adipose tissue-derived mesenchymal stem cells with deferoxamine increases the production of pro-angiogenic, neuroprotective and anti-inflammatory factors: Potential application in the treatment of diabetic neuropathy. *PLoS One* 2017; **12**: e0178011 [PMID: 28542352 DOI: 10.1371/journal.pone.0178011]

136 **Di Maggio N**, Mehrkens A, Papadimitropoulos A, Schaeren S, Heberer M, Banfi A, Martin I. Fibroblast growth factor-2 maintains a niche-dependent population of self-renewing highly potent non-adherent mesenchymal progenitors through FGFR2c. *Stem Cells* 2012; **30**: 1455-1464 [PMID: 22495904 DOI: 10.1002/stem.1106]

137 **Zhang Y**, Ma L, Su Y, Su L, Lan X, Wu D, Han S, Li J, Kvederis L, Corey S, Borlongan CV, Ji X. Hypoxia conditioning enhances neuroprotective effects of aged human bone marrow mesenchymal stem cell-derived conditioned medium against cerebral ischemia in vitro. *Brain Res* 2019; **1725**: 146432 [PMID: 31491422 DOI: 10.1016/j.brainres.2019.146432]

138 **Ratushnyy AY**, Rudimova YV, Buravkova LB. Alteration of Hypoxia-Associated Gene Expression in Replicatively Senescent Mesenchymal Stromal Cells under Physiological Oxygen Level. *Biochemistry (Mosc)* 2019; **84**: 263-271 [PMID: 31221064 DOI: 10.1134/S0006297919030088]

139 **Lazzarini R**, Caffarini M, Tang H, Cerqueni G, Pellegrino P, Monsurrò V, Di Primio R, Orciani M. The senescent status of endothelial cells affects proliferation, inflammatory profile and SOX2 expression in bone marrow-derived mesenchymal stem cells. *Exp Gerontol* 2019; **120**: 21-27 [PMID: 30822486 DOI: 10.1016/j.exger.2019.02.014]

140 **Umezaki Y**, Hashimoto Y, Nishishita N, Kawamata S, Baba S. Human Gingival Integration-Free iPSCs; a Source for MSC-Like Cells. *Int J Mol Sci* 2015; **16**: 13633-13648 [PMID: 26084043 DOI: 10.3390/ijms160613633]

141 **Spitzhorn LS**, Megges M, Wruck W, Rahman MS, Otte J, Degistirici Ö, Meisel R, Sorg RV, Oreffo ROC, Adjaye J. Human iPSC-derived MSCs (iMSCs) from aged individuals acquire a rejuvenation signature. *Stem Cell Res Ther* 2019; **10**: 100 [PMID: 30885246 DOI: 10.1186/s13287-019-1209-x]

142 **Zhao C**, Ikeya M. Generation and Applications of Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells. *Stem Cells Int* 2018; **2018**: 9601623 [PMID: 30154868 DOI: 10.1155/2018/9601623]

143 **Wang LT**, Jiang SS, Ting CH, Hsu PJ, Chang CC, Sytwu HK, Liu KJ, Yen BL. Differentiation of

Mesenchymal Stem Cells from Human Induced Pluripotent Stem Cells Results in Downregulation of c-Myc and DNA Replication Pathways with Immunomodulation Toward CD4 and CD8 Cells. *Stem Cells* 2018; **36**: 903-914 [PMID: 29396902 DOI: 10.1002/stem.2795]

144 **Kulkarni R**, Bajaj M, Ghode S, Jalnapurkar S, Limaye L, Kale VP. Intercellular Transfer of Microvesicles from Young Mesenchymal Stromal Cells Rejuvenates Aged Murine Hematopoietic Stem Cells. *Stem Cells* 2018; **36**: 420-433 [PMID: 29230885 DOI: 10.1002/stem.2756]

145 **Liu S**, Mahairaki V, Bai H, Ding Z, Li J, Witwer KW, Cheng L. Highly Purified Human Extracellular Vesicles Produced by Stem Cells Alleviate Aging Cellular Phenotypes of Senescent Human Cells. *Stem Cells* 2019; **37**: 779-790 [PMID: 30811771 DOI: 10.1002/stem.2996]

146 **Lohmann M**, Walenda G, Hemeda H, Joussen S, Drescher W, Jockenhoevel S, Hutschenreuter G, Zenke M, Wagner W. Donor age of human platelet lysate affects proliferation and differentiation of mesenchymal stem cells. *PLoS One* 2012; **7**: e37839 [PMID: 22662236 DOI: 10.1371/journal.pone.0037839]

147 **Pescador D**, Ibáñez-Fonseca A, Sánchez-Guijo F, Briñón JG, Arias FJ, Muntión S, Hernández C, Girotti A, Alonso M, Del Cañizo MC, Rodríguez-Cabello JC, Blanco JF. Regeneration of hyaline cartilage promoted by xenogeneic mesenchymal stromal cells embedded within elastin-like recombinamer-based bioactive hydrogels. *J Mater Sci Mater Med* 2017; **28**: 115 [PMID: 28647792 DOI: 10.1007/s10856-017-5928-1]

148 **Manferdini C**, Guarino V, Zini N, Raucci MG, Ferrari A, Grassi F, Gabusi E, Squarzoni S, Facchini A, Ambrosio L, Lisignoli G. Mineralization behavior with mesenchymal stromal cells in a biomimetic hyaluronic acid-based scaffold. *Biomaterials* 2010; **31**: 3986-3996 [PMID: 20172605 DOI: 10.1016/j.biomaterials.2010.01.148]

149 **Šponer P**, Kučera T, Brtková J, Urban K, Kočí Z, Měřička P, Bezrouk A, Konrádová Š, Filipová A, Filip S. Comparative Study on the Application of Mesenchymal Stromal Cells Combined with Tricalcium Phosphate Scaffold into Femoral Bone Defects. *Cell Transplant* 2018; **27**: 1459-1468 [PMID: 30203687 DOI: 10.1177/0963689718794918]

150 **Li WJ**, Tuli R, Okafor C, Derfoul A, Danielson KG, Hall DJ, Tuan RS. A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. *Biomaterials* 2005; **26**: 599-609 [PMID: 15282138 DOI: 10.1016/j.biomaterials.2004.03.005]

151 **Chen Y**, Mao P, Snijders AM, Wang D. Senescence chips for ultrahigh-throughput isolation and removal of senescent cells. *Aging Cell* 2018; **17**: e12722 [PMID: 29336105 DOI: 10.1111/acel.12722]

152 **Fathi E**, Farahzadi R. Zinc Sulphate Mediates the Stimulation of Cell Proliferation of Rat Adipose Tissue-Derived Mesenchymal Stem Cells Under High Intensity of EMF Exposure. *Biol Trace Elem Res* 2018; **184**: 529-535 [PMID: 29189996 DOI: 10.1007/s12011-017-1199-4]

153 **Farahzadi R**, Fathi E, Mesbah-Namin SA, Zarghami N. Zinc sulfate contributes to promote telomere length extension via increasing telomerase gene expression, telomerase activity and change in the TERT gene promoter CpG island methylation status of human adipose-derived mesenchymal stem cells. *PLoS One* 2017; **12**: e0188052 [PMID: 29145503 DOI: 10.1371/journal.pone.0188052]

154 **Lv YJ**, Yang Y, Sui BD, Hu CH, Zhao P, Liao L, Chen J, Zhang LQ, Yang TT, Zhang SF, Jin Y. Resveratrol counteracts bone loss via mitofilin-mediated osteogenic improvement of mesenchymal stem cells in senescence-accelerated mice. *Theranostics* 2018; **8**: 2387-2406 [PMID: 29721087 DOI: 10.7150/thno.23620]

155 **Bickford PC**, Kaneko Y, Grimmig B, Pappas C, Small B, Sanberg CD, Sanberg PR, Tan J, Douglas Shytle R. Nutraceutical intervention reverses the negative effects of blood from aged rats on stem cells. *Age (Dordr)* 2015; **37**: 103 [PMID: 26410618 DOI: 10.1007/s11357-015-9840-7]

156 **Jeong SG**, Oh YS, Joe IS, Jeong SY, Cho HM, Lee JS, Oh WK, Cho TO, Cho GW. Functional restoration of replicative senescent mesenchymal stem cells by the brown alga *Undaria pinnatifida*. *Anim Cells Syst (Seoul)* 2017; **21**: 108-114 [PMID: 30460058 DOI: 10.1080/19768354.2017.1292951]

157 **Molinos M**, Cunha C, Almeida CR, Gonçalves RM, Pereira P, Silva PS, Vaz R, Barbosa MA. Age-Correlated Phenotypic Alterations in Cells Isolated From Human Degenerated Intervertebral Discs With Contained Hernias. *Spine (Phila Pa 1976)* 2018; **43**: E274-E284 [PMID: 28678109 DOI: 10.1097/BRS.0000000000002311]

158 **Čamernik K**, Zupan J. Complete Assessment of Multilineage Differentiation Potential of Human Skeletal Muscle-Derived Mesenchymal Stem/Stromal Cells. *Methods Mol Biol* 2019; **2045**: 131-144 [PMID: 30499024 DOI: 10.1007/7651_2018_200]

159 **Gao S**, Xiang C, Qin K, Sun C. Mathematical Modeling Reveals the Role of Hypoxia in the Promotion of Human Mesenchymal Stem Cell Long-Term Expansion. *Stem Cells Int* 2018; **2018**: 9283432 [PMID: 29861746 DOI: 10.1155/2018/9283432]

160 **Dos Santos F**, Andrade PZ, Boura JS, Abecasis MM, da Silva CL, Cabral JM. Ex vivo expansion of human mesenchymal stem cells: a more effective cell proliferation kinetics and metabolism under hypoxia. *J Cell Physiol* 2010; **223**: 27-35 [PMID: 20020504 DOI: 10.1002/jcp.21987]

161 **Xu N**, Liu H, Qu F, Fan J, Mao K, Yin Y, Liu J, Geng Z, Wang Y. Hypoxia inhibits the differentiation of mesenchymal stem cells into osteoblasts by activation of Notch signaling. *Exp Mol Pathol* 2013; **94**: 33-39 [PMID: 22964414 DOI: 10.1016/j.yexmp.2012.08.003]

162 **Carbonneau CL**, Despars G, Beaudry GM, Benabdallah B, Bouhanik S, Dépôt J, Moreau A, Beauséjour CM. Heterotopic bone formation derived from multipotent stromal cells is not inhibited in aged mice. *Cytotherapy* 2014; **16**: 1073-1079 [PMID: 24934305 DOI: 10.1016/j.jcyt.2014.03.004]

163 **Cao Y**, Yang T, Gu C, Yi D. Pigment epithelium-derived factor delays cellular senescence of human mesenchymal stem cells in vitro by reducing oxidative stress. *Cell Biol Int* 2013; **37**: 305-313 [PMID: 23359450 DOI: 10.1002/cbin.10041]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 986-1000

DOI: 10.4252/wjsc.v12.i9.986

ISSN 1948-0210 (online)

REVIEW

Mechanisms of action of neuropeptide Y on stem cells and its potential applications in orthopaedic disorders

Jian-Qun Wu, Nan Jiang, Bin Yu

ORCID number: Jian-Qun Wu 0000-0002-6805-7746; Nan Jiang 0000-0003-2416-1653; Bin Yu 0000-0002-3109-2062.

Author contributions: Wu JQ and Jiang N contributed equally to this study; Jiang N and Yu B contributed to the conception and design; Wu JQ contributed to the literature search; Wu JQ and Jiang N wrote the paper; all authors contributed to the critical revision and approval of the final version.

Supported by the National Natural Science Foundation of China, No. 81830079; Guangzhou Health Science and Technology Project, No. 20191A011116; and Science and Technology Project of Guangzhou Huadu District, No. 18-HDWS-003.

Conflict-of-interest statement: The authors certify that they, or any members of their immediate families, have no funding or commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article.

Jian-Qun Wu, Department of Orthopedics and Traumatology, Huadu District People's Hospital, Guangzhou 510800, Guangdong Province, China

Nan Jiang, Bin Yu, Division of Orthopaedics and Traumatology, Department of Orthopedics, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China

Nan Jiang, Bin Yu, Guangdong Provincial Key Laboratory of Bone and Cartilage Regenerative Medicine, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China

Corresponding author: Bin Yu, MD, PhD, Professor, Surgeon, Department of Orthopedics, Nanfang Hospital, Southern Medical University, No. 1838 North Guangzhou Avenue, Baiyun District, Guangzhou 510515, Guangdong Province, China. yubin@smu.edu.cn

Abstract

Musculoskeletal disorders are the leading causes of disability and result in reduced quality of life. The neuro-osteogenic network is one of the most promising fields in orthopaedic research. Neuropeptide Y (NPY) system has been reported to be involved in the regulations of bone metabolism and homeostasis, which also provide feedback to the central NPY system *via* NPY receptors. Currently, potential roles of peripheral NPY in bone metabolism remain unclear. Growing evidence suggests that NPY can regulate biological actions of bone marrow mesenchymal stem cells, hematopoietic stem cells, endothelial cells, and chondrocytes *via* a local autocrine or paracrine manner by different NPY receptors. The regulative activities of NPY may be achieved through the plasticity of NPY receptors, and interactions among the targeted cells as well. In general, NPY can influence proliferation, apoptosis, differentiation, migration, mobilization, and cytokine secretion of different types of cells, and play crucial roles in the development of bone delayed/non-union, osteoporosis, and osteoarthritis. Further basic research should clarify detailed mechanisms of action of NPY on stem cells, and clinical investigations are also necessary to comprehensively evaluate potential applications of NPY and its receptor-targeted drugs in management of musculoskeletal disorders.

Key Words: Neuropeptide Y; Bone marrow mesenchymal stem cells; Hematopoietic stem cells; Fracture; Osteoporosis; Osteoarthritis

Manuscript source: Invited manuscript

Received: March 25, 2020
Peer-review started: March 25, 2020
First decision: April 29, 2020
Revised: May 25, 2020
Accepted: June 2, 2020
Article in press: June 2, 2020
Published online: September 26, 2020

P-Reviewer: Emara KM
S-Editor: Yan JP
L-Editor: Wang TQ
P-Editor: Xing YX

Core tip: Neuropeptide Y (NPY) system is crucial for bone metabolism and homeostasis. NPY can regulate biological effects of different types of cells through central and peripheral nervous systems. Here, we summarize recent findings regarding the roles of NPY and its receptors in bone metabolism and homeostasis, and discuss the biological actions and underlying mechanisms of NPY on bone marrow mesenchymal stem cells, hematopoietic stem cells, endothelial cells, and chondrocytes. We also review the potential applications and efficacy of NPY and NPY receptor-targeted drugs in the treatment of fracture healing, osteoporosis, and osteoarthritis.

Citation: Wu JQ, Jiang N, Yu B. Mechanisms of action of neuropeptide Y on stem cells and its potential applications in orthopaedic disorders. *World J Stem Cells* 2020; 12(9): 986-1000
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/986.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.986

INTRODUCTION

As a substantial portion of the whole body, skeletal muscles are integral for locomotion and metabolic health[1]. Musculoskeletal disorders, such as fracture, delayed/non-union, osteoporosis, and osteoarthritis, are the primary causes of disability and lead to reduced life quality. Skeletal metabolism and homeostasis are accurately regulated by neural signal networks between nervous systems and bone cells.

Recently, neuropeptide Y (NPY) has emerged as one of the major regulators of bone metabolism and homeostasis. NPY, a 36-amino-acid peptide first isolated from the porcine brain by Tatemoto[2] in 1982, is widely distributed in the central nervous system (CNS) and peripheral nervous system[3]. It is produced by nerve endings, and acts as a critical molecule in the interactions between nerves and the osseous system through central- and peripheral-mediated pathways[4,5]. NPY can maintain the homeostases of bone, blood vessels, and immune system *via* different NPY receptors[6,7]. Previous studies have indicated that NPY can stimulate proliferation, promote osteoblastic differentiation, and prevent apoptosis of bone marrow mesenchymal stem cells (BMSCs) though Y1R[8]. In addition, NPY is also able to facilitate neuroprotection, restore bone marrow dysfunction, and thus mediate bone marrow microenvironment[9]. Aside from BMSCs, treatment with NPY caused a decreased number of osteoclasts by promoting mobilization of hematopoietic stem cells (HSCs) *via* Y2R and Y5R. Furthermore, NPY can accelerate endothelial cell (EC) proliferation and capillary tube formation though Y1R, Y2R, and Y5R[9,10]. As NPY possesses capacities of regulating different types of cells, it determines the relative rates of bone formation and resorption process, which is critical for prevention against bone structure damage and bone metabolic disorders[11].

In recent years, stem cell therapy is a burgeoning field in regeneration and restoration of the impaired musculoskeletal tissues[12]. Enrichment and differentiation of stem cells have been confirmed for the benefits in bone regeneration and maintenance of bone homeostasis. As both experiments and clinical investigations have revealed definite efficacy of stem cells in treatment of fractures, osteoporosis, cartilage, and ligament injuries[13-15], it has become an attractive avenue of research for therapeutic applications in musculoskeletal disorders.

This article reviews the current knowledge of NPY, potential roles of NPY receptors, and interactions among NPY, NPY receptors, and stem cells, primarily BMSCs and HSCs. In addition, the potential influences of NPY on biological functions of ECs and chondrocytes, which are also involved in the development of many orthopaedic disorders, are also discussed. Furthermore, the potential applications and efficacy of NPY and its receptor-targeted drugs in the treatment of fracture, osteoporosis, and osteoarthritis are also summarized.

NPY AND RECEPTORS

Recent studies have shown that NPY participates in many physiological and psychological processes, such as inhibition of vascular smooth muscle contraction and alleviation of anxiety and depression[16,17]. Till now, five primary NPY receptors (Y1R, Y2R, Y4R, Y5R, and y6R) have been found in mammals, and all of them belong to the superfamily of G protein-coupled receptors[18]. Different receptors are distributed differently in the human body, play different biological functions, and have different affinities for NPY (Y2R > Y1R > Y5R, Y4R = y6R)[19]. Y1R and Y2R are reported to be involved in bone homeostasis. Y4R is distributed in both brain and peripheral tissues[20]. Y5R, predominant in the CNS, consistently co-localizes with Y1R[21]. y6R, also observed in rat genome, seems to be non-functional in humans[22].

Current evidence indicates that hypothalamic secreted NPY inhibits bone formation. Previous *in vivo* studies found that an increase of NPY in the hypothalamus led to a decreased volume of cancellous bone and inhibition of the osteoblasts activity[23,24]. These results are similar to the finding of another study that overexpression of hypothalamus-specific NPY in the wild-type (WT) mice displayed anti-osteogenic effects[25]. Likewise, both deficiency of NPY [NPY (-/-)] in mice and inhibition of central NPY signaling pathway can induce bone gain[5,26]. As targeted deletion of the *NPY* gene in the hypothalamus has not been realized yet, current research of the potential effects of hypothalamus-specific NPY on peripheral stem cells may be conducted *via* the approach of isolating BMSCs or other stem cells from mice deficient in hypothalamus-specific NPY.

It is known that both Y1R and Y2R are distributed abundantly in the CNS. As an auto-receptor, Y2R is primarily located presynaptically, and inhibits NPY expression and release[27,28]. Both hypothalamus and germline deletions of Y2R produce an identically promotive bone anabolic phenotype, implying that hypothalamic Y2R may modulate bone formation *via* CNS mechanism, and also protect against central NPY-induced bone loss[26,29]. A previous study showed that the osteoblastic activity of the mice with double knockout of *Y4R* and *Y2R* in the hypothalamus was more obvious than those with only knockout of *Y2R*, demonstrating the probably synergistic role of Y4R in hypothalamic control of the bone mass[30]. Nonetheless, targeted deletion of *Y1R* in the hypothalamus failed to recapitulate this increased bone mass phenotype, which had been observed in systematic deficiency of Y1R [Y1R (-/-)] mice. This implies that Y1R may participate in the regulation of bone regulation by non-hypothalamic pathways[31]. Interestingly, double deletions of global *Y1R* and *Y2R* in mice did not result in any additive effects on bone phenotype, suggesting that Y1R and Y2R may share a common pathway in regulation of bone homeostasis[31]. As for y6R, its mRNA is only expressed in the hypothalamus but not in bone components, such as BMSCs, osteoblasts, or osteoclasts. Compared to the WT controls, significantly decreased bone density and volumes of cortical and cancellous bone were found in the mice with y6R(-/-) in the hypothalamus, which is primarily attributed to the increasing number of osteoclast precursors in y6R(-/-) mice[22].

Peripheral NPY acts directly in a paracrine fashion to maintain the bone homeostasis. Growing evidence shows that NPY is expressed in non-neuronal cells in the bone marrow microenvironment, such as osteoblasts[32], osteocytes[33], BMSCs[8,34], and ECs[35]. *In vitro* studies have indicated that NPY has direct effects on osteoblast lineage by inhibiting differentiation of mesenchymal progenitors and mineralization of mature osteoblasts[33,36]. Overexpression of osteoblasts-specific NPY (Col2.3NPY) resulted in decreases in bone trabecular number, thickness, and volume[37], whereas changes of serological NPY levels did not reveal such effects[37]. These results imply that overexpression of local NPY may cause changes of bone phenotypes. Outcomes of clinical studies have revealed that patients with craniocerebral injury had accelerated fracture healing and higher serological NPY levels, the latter of which showed a positive correlation with the severity of craniocerebral injury[38]. Gu *et al*[38] inferred that serum NPY may originate from cerebrospinal fluid, as the NPY level in the serum was almost equal to that in the cerebrospinal fluid among the patients with craniocerebral injury, and severe craniocerebral injury may cause leakage of cerebrospinal fluid[38,39].

It is definite that Y1R is expressed in osseous tissue, including adipocytes, osteoblasts, and bone marrow cells[8,33,40-42]; however, whether Y2R is expressed in the bone or not remains in debate. Some studies reported that Y2R can be found in MC3T3 and BMSCs[41,42], but not in osteoblasts[43]. It is speculated that such differences may be explained by two reasons. The first reason is that Y2R exists in the bone, but may be expressed under specific conditions, and thus, it cannot always be detected. Second, potential limitations of the current techniques may also affect the detection rate. As for Y1R, both *in vitro* and *in vivo* studies have shown that the mice with knockout of

osteoblast-specific *Y1R* recapitulate the bone phenotype of germline Y1R deficient mice[5,36]. These findings confirm the role of Y1R in inhibiting bone formation, as well as in enlarging the negative effects of NPY on osteocytes. Although the selective deletion of Y1R from osteoblasts did not hinder the process of fracture healing, lack of systemic Y1R caused delays of endochondral fracture repair[44]. Considering that Y1R plays important roles in many physiological functions, aside from the above-mentioned issues, other underlying mechanisms may also account for the effects of global *Y1R* deletion on bone healing process (Figure 1).

MECHANISMS OF ACTIONS OF NPY ON BMSCs AND HSCs

NPY and BMSCs

BMSCs, isolated from bone marrow, are termed as a "fibroblast-like" osteogenic cell population. With plastic-adherent culture characteristics, BMSCs express specific surface antigens CD105 and CD90 (> 95%) but not CD45 or CD34 (< 2%)[45], and are capable of differentiating into osteoblasts, chondrocytes, and adipocytes[19,46]. As BMSCs play a vital role in repairing musculoskeletal tissues, fracture healing and spinal injury regeneration can be accelerated following application of BMSCs[47]. Local injection of BMSCs has also yielded promising results in the treatment of bone nonunion and bone defect[48].

Currently, conflicting results still exist regarding the potential role of NPY in proliferation of BMSCs[8,36,42]. Our previous study revealed that NPY can enhance proliferation and prevent apoptosis of BMSCs in a concentration-dependent manner by activating the Wnt/β-catenin signaling pathway. Such NPY-induced activities were partially blocked by PD160170, a Y1R antagonist[8], implying that NPY-induced proliferative and anti-apoptotic effects of BMSCs may be partially achieved through Y1R. Another study also found that NPY is able to stimulate proliferation of BMSCs derived from rats of different ages, and this capacity was blocked by Y5R antagonist, demonstrating an inhibitory role of Y5R in the proliferation of rat BMSCs. In addition, Igura *et al*[42] found that NPY increased the proliferation of BMSCs of transgenic overexpression of Y5R in the elder rats by activating extracellular signal-regulated kinase 1/2 (Erk1/2) pathways. Aside from BMSCs, NPY can also promote the proliferation of human embryonic stem cells, which was achieved *via* NPY/Y1R/Y5R by activation of ERK1/2 pathways[49]. However, one study failed to find a promotive role of NPY on BMSC proliferation. Lee *et al*[36] reported that BMSCs isolated from Y1R (-/-) mice formed a greater number and larger size of colonies than the WT controls, implying that NPY may inhibit BMSC proliferation.

Apart from its potential influences on BMSC proliferation, NPY can also facilitate the migration of BMSCs by upregulated expression of CXC chemokin receptor 4[50], which is in accordance with the finding of our study that NPY therapy significantly increased the total migration distance and speed of BMSCs[51].

In addition to the controversy regarding the effect of NPY on the proliferation of BMSCs, the potential activities of NPY on osteogenic differentiation of BMSCs and the underlying mechanisms are also in debate. Some studies found that NPY stimulated the differentiation of BMSCs into osteoblasts, which was supported by upregulating the expression of alkaline phosphatase (ALP), collagen type I (COL-I), osteocalcin (OCN), and runt-related transcription factor 2 (Runx2) through the Wnt signaling pathway[51,52]. Other studies reported that NPY inhibited osteogenic differentiation of BMSCs, evidenced by the findings of decreased ALP and OCN expression, and reduced mineralization of BMSCs as well[41]. Besides, another study also found the inhibitory effect of NPY on isoprenaline-induced differentiation into osteoblasts from BMSCs[40]. Interestingly, BMSCs isolated from NPY (-/-) mice display an increased ability in osteogenic differentiation, which was confirmed by increases in ALP activity, *OCN* gene expression, and mineralization[52]. These diverse outcomes can be explained by the fact that NPY-induced osteogenic differentiation of BMSCs may be achieved *via* auto-regulation mechanisms.

Growing evidence has revealed that auto-regulation mechanisms of NPY may correlate to the plasticity of its receptors. It has been noted that NPY led to upregulated expression of Y1R throughout BMSC osteogenic differentiation[36,38,41]. In a recent study, Wee *et al*[52] found that during osteogenic differentiation of BMSCs in the WT mice, the expression of Y1R was increased while NPY was decreased. However, as in NPY (-/-) mice, Y1R expression level did not alter during differentiation, demonstrating that increased Y1R expression during BMSC differentiation may be assisted or induced by NPY. Outcomes of NPY or its receptor gene knockout animal

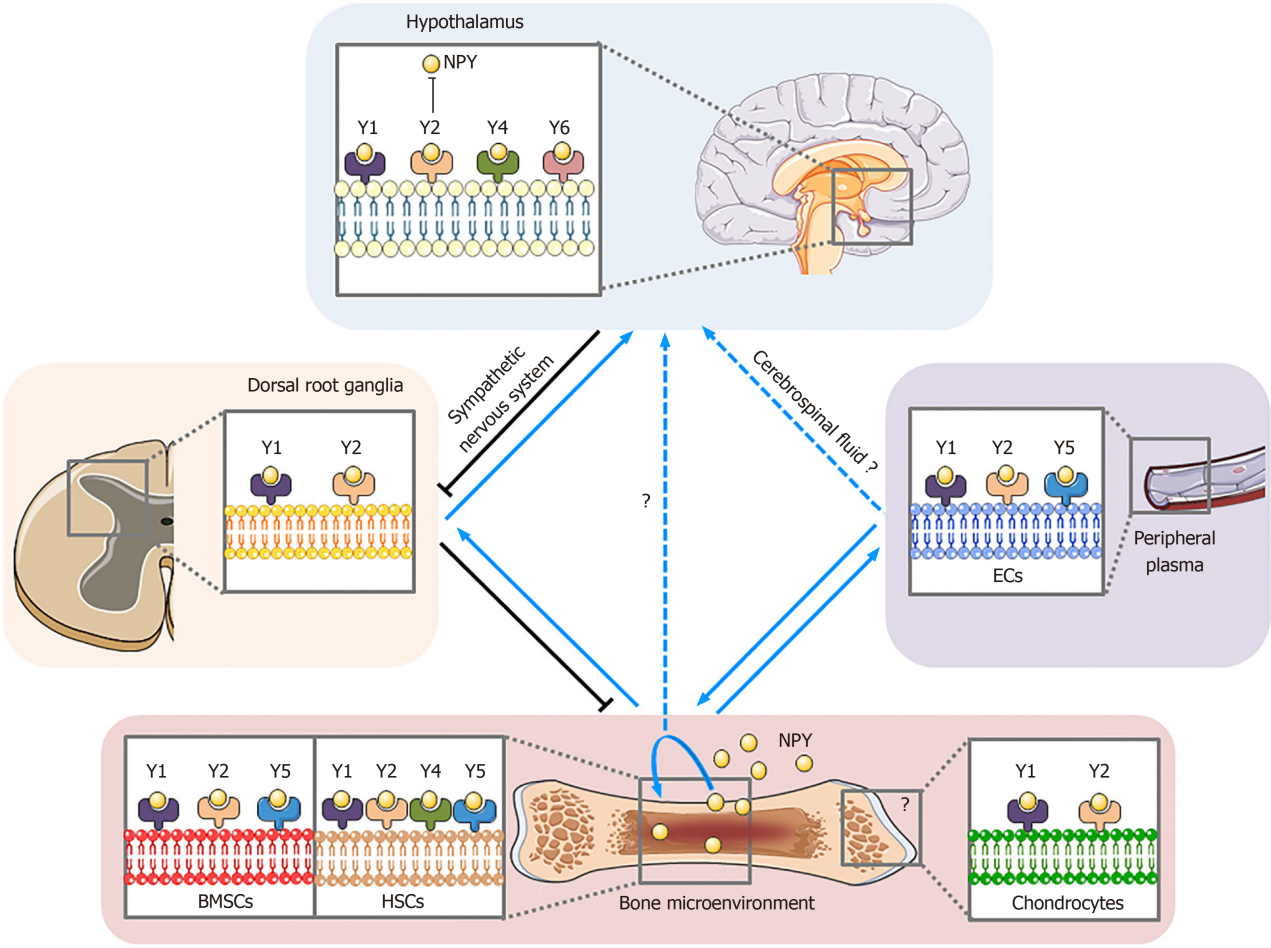


Figure 1 Neuropeptide Y is involved in the regulation of bone microenvironment through central- and peripheral-mediated pathways *via* different neuropeptide Y receptors. NPY: Neuropeptide Y; BMSCs: Bone marrow mesenchymal stem cells; HSCs: Hematopoietic stem cells; ECs: Endothelial cells.

models revealed that the presence of functional Y1R directly hindered the osteogenesis of BMSCs or bone cells, as evidenced by the finding that BMSCs isolated from Y1R (-/-) mice displayed an increased capacity of osteogenic differentiation[36]. An *in vitro* experiment showed that blockade of Y1R by PD160170 facilitated osteogenic differentiation of BMSCs[53]. On the contrary, Dong *et al*[34] reported that melatonin can upregulate the expression of NPY and Y1R, and promote MSC osteoblastic differentiation. Apart from BMSCs, lack of Y1R also promoted differentiation of mesenchymal progenitor cells and activated mature osteoblasts[36]. Yahara *et al*[54] also noticed that inhibition of Y1R increased the ALP activity and mineralization in mouse pre-osteoblast MC3T3-E1 cells.

Aside Y1R, Y2R may also participate in osteogenic differentiation of BMSCs, which is supported by the finding that BMSCs, treated with either NPY1–36 (universal Y2R agonist) or PYY3-36 (Y2R preferring agonist), revealed significantly elevated levels of ALP activity and OCN expression. Such effects of NPY1-36 were blocked by a Y2R antagonist, BII0246, and a marked decrease of Y1R protein level was also found following treatment with exogenous PYY3–36 or NPY1–36[41].

During osteogenic differentiation of BMSCs, potential interactions may exist between Y1R and Y2R[42,43]. By detecting the NPY ligand-receptor system in BMSCs derived from rats of different ages, Igura *et al*[42] found that, although NPY expression increased with age, Y1R protein was upregulated whereas Y2R protein was downregulated with an increase in age. In addition, Lundberg *et al*[43] found that the lack of Y2R signaling mediated downregulation of Y1R in BMSCs, which was proved by the finding of decreased expression of Y1R in BMSCs from Y2R (-/-) mice. They inferred that downregulation of Y1R is possibly due to the lack of feedback inhibition of NPY release and resulted in elevated levels of NPY, which in turn caused subsequent overstimulation of Y1R, following desensitization and downregulation of the Y1R population[43]. Moreover, alternation of Y1R expression may have different

effects on NPY. The precursor osteogenic cells (*e.g.*, BMSCs and osteoblast precursor cells), which are able to inhibit osteogenic differentiation through Y1R, have low expression levels of Y1R. However, in osteogenic conditions (*e.g.*, osteogenic induction medium), Y1R expression in these cells is increased, with Y2R expression decreased.

In addition to Y1R and Y2R, y6R may also be involved in the regulation of BMSC differentiation into osteoblasts. BMSCs isolated from y6R (-/-) mice showed significant reductions of ALP, osterix, and mineralizing surface, implying possibly opposite roles of Y6R to those of Y1R and Y2R in BMSC differentiation and activities[22].

Currently, some studies also reported potential mechanisms of NPY in mediation of BMSC osteogenic differentiation from another perspectives. Gu *et al*[38] indicated that NPY can directly promote the osteogenic differentiation of MSCs by upregulating Runx2. Ma *et al*[55] reported that the anabolic activity of osteoblasts treated with NPY may also enhance the gap junction intercellular communication (GJIC). Tang *et al*[56] noticed that the levels of NPY and p-ERK1/2 in fracture model rats were significantly higher than those in the controls. They also found that use of BIBP3226, a Y1R antagonist, inhibited the fracture healing process by downregulating the p-ERK expression in the fracture site, indicating that the ERK pathway may participate in the NPY-induced effects on fracture healing. Additionally, Sharma *et al*[57] also observed that activation of ERK could facilitate osteogenic differentiation of human MSCs.

In summary, considering the still existing controversies regarding the role of NPY in BMSCs and still not-well-understood mechanisms, future more studies are warranted to provide more definitive evidence regarding the links between NPY/NPY receptors and BMSCs.

NPY and HSCs

HSCs, or hematopoietic stem/progenitor cells, responsible for regeneration and repopulation of all blood cell lineages, contact osteoblasts in endosteal microenvironments or sinusoidal endothelium[58]. NPY receptors Y1, Y2, Y4, and Y5 were found to be highly expressed in HSCs, and NPY plays a crucial role in the proliferation and mobilization of HSCs[59,60].

Growing evidence has suggested that NPY acts directly or indirectly in the regulation of HSC proliferation. NPY can directly inhibit the proliferation of HSCs in cell cultures, as confirmed by an increased number of HSCs in G0 phase, with decreased numbers of cells in S and G2/M phases[60]. Besides, the number of HSCs in NPY (-/-) mice is decreased, suggesting that NPY may protect HSCs in the bone marrow microenvironment[9,61].

In addition to potential influences on HSC proliferation, NPY and its receptors are also involved in regulating the survival and mobilization of HSCs. It was reported that NPY/Y1R can improve the survival and apoptosis of HSCs, which maintains the survival of nestin+ cells, and thus be linked to retention of HSCs[9]. NPY-induced biological effects on HSCs, osteoblasts, and osteoclasts play an important role in the process of fracture healing. Nonetheless, detailed mechanisms of such effects remain largely unclear. Several studies explored the underlying mechanisms from different perspectives. Park *et al*[61] indicated that the effect of NPY on HSC mobilization might be achieved by increasing the expression levels of matrix metalloproteinase 9 (MMP-9) in osteoblasts *via* Y1R, which was strengthened by the finding that NPY failed to display a positive activity on HSC mobilization in mice with Y1R (-/-) in osteoblasts. Singh *et al*[59] reported that NPY3-36, a agonist of Y2R and Y5R, facilitated the mobilization of HSCs to the peripheral blood, whereas selective Y2R and Y5R antagonists hindered such activity. It has been noted that NPY can be also secreted by HSCs, indicating that HSCs may exert regulatory feedback on itself by releasing NPY[9]. In ovariectomized mice, NPY therapy could help reduce the bone loss due to HSC mobilization, and result in an increased number of osteoblasts and a decreased number of osteoclasts[61]. Using an ovariectomy-induced osteoporosis mouse model, Park *et al*[62] also found that NPY-based recombinant peptides could relieve ovariectomy-induced bone loss and may be used for osteoporosis treatment.

In summary, current evidence suggests that NPY is able to induce the rapid mobilization of HSCs into the peripheral blood, and increase the number of osteoblasts. Therefore, it is reasonable to believe that a comprehensive understanding of the role of NPY in HSCs may pave the way for its future clinical applications[63].

MECHANISMS OF ACTIONS OF NPY ON ECs AND CHONDROCYTES

NPY and ECs

NPY-positive fibers predominantly localize alongside blood vessels in bone tissue and bone marrow, and associate with EC migration, capillary tube formation, and self-renewal[10,64,65]. ECs express Y1R and Y2R and can produce, store, and respond to NPY, suggesting an autocrine regulatory mechanism of NPY in the endothelium (Figure 2)[10,35,66].

In vitro studies have revealed that NPY can promote the migration and capillary tube formation of human ECs *via* Y1R, Y2R, and Y5R[10,65]. Previous studies also indicated that NPY-induced angiogenic effect on ECs was achieved primarily through Y2R[65,67]. Furthermore, NPY can regulate the angiogenic process by influencing the proliferation of ECs, which was achieved mainly *via* Y5R[10]. NPY3-36, an agonist of Y2R and Y5R, was found to be able to reduce EC contact and increase vascular permeability, and selective Y2R and Y5R antagonists restored the vascular integrity[59]. Interactions may exist among BMSCs, HSCs, and ECs *via* NPY. It has been observed that NPY originating from platelet lysate caused an decreased angiogenic activity of human adipose stromal cells, which may be linked to reduced expression of vascular endothelial growth factor (VEGF) and a lower intracellular calcium level[68]. As a ubiquitous and potent peptide, VEGF is involved in many angiogenic cascades. Increased levels of VEGF facilitate angiogenesis and osteoblastic differentiation of BMSCs[69], and the level of VEGF secreted by BMSCs is elevated following treatment with NPY[51]. Wang *et al*[50] found that NPY promoted endothelial differentiation and tube formation of BMSCs, the latter of which is involved in all the stages of EC angiogenesis, including survival and proliferation, migration, tube formation, and maturation of blood vessels[70].

NPY and chondrocytes

Although chondroblasts and osteoblasts share the same progenitor reservoir (BMSCs), specific effects of NPY on chondrocytes have not been clarified. However, anatomical studies have indicated that NPY-positive sympathetic nerve fibers are located in cartilage, and chondrocytes can also secret NPY[32].

NPY can promote the proliferation of both chondrocytes and articular cartilage, and the effect of NPY on chondrogenesis may be achieved through autocrine mechanisms *via* Y1R[32]. A new study found that intra-articular injection of NPY caused more severe osteoarthritis phenotypes, which was evidenced by more severe cartilage degradation and fibrillation, whereas a combination of NPY with BIIE0246 (Y2R antagonist) but not BIBO3304 (Y1R antagonist) significantly alleviated the above negative effects, indicating that Y2R may play an important role in NPY-induced chondrocyte hypertrophy and cartilage matrix degradation[71]. In addition, NPY-induced chondrocyte hypertrophy was also marked by increased levels of col10a1 (a biomarker indicating hypertrophy of chondrocytes), MMP-13, and ADAMTS-5 through activation of mTORC1 in a Y2R-dependent manner[71]. Chen *et al*[72] found that NPY markedly augmented the expression of Col2a1 (a biomarker indicating proliferation of chondrocytes), Col10a1, and OCN in the murine chondrogenic cell line ATDC5. And inhibition of Y1R partly hindered the capability of NPY, demonstrating that Y1R may participate in NPY-induced activities (proliferation, chondrogenesis, and mineralization) in ATDC5 cells[72]. It is known that Runx2 and Osterix are biomarkers for chondrocyte differentiation and cartilage mineralization. In the Y1R (-/-) mice, the expression levels of Runx2 and Osterix increased in the long bones[36], both of which facilitated the formation of cartilage callus. Based on current findings, potential strategies to solve NPY-related hypertrophy of chondrocytes and cartilage degradation may be a research hotspot in the future.

APPLICATIONS OF NPY IN ORTHOPAEDIC DISORDERS

Fracture

Fracture healing process can be divided into inflammatory, reparative, and remodeling stages. During the initial phase, BMSCs are recruited to the fracture site and differentiate into chondrocytes simultaneously, and the level of NYP in the peripheral blood is increased[56]. NPY- and Y1R-positive BMSCs and osteoblasts can be found in the new osseous tissue[64]. Innervation of NPY occurs in a spatio-temporal dependent manner in the inflammatory and remodeling stages[73], demonstrating

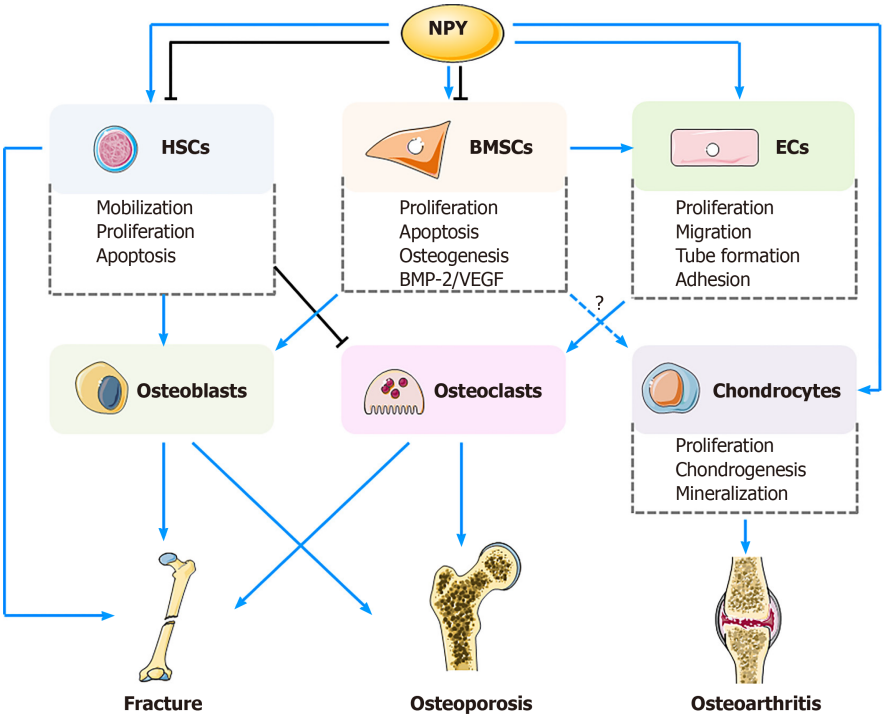


Figure 2 Neuropeptide Y exerts potential regulatory effects on biological functions of bone marrow mesenchymal stem cells, hematopoietic stem cells, endothelial cells, and chondrocytes, and their potential applications in management of fracture healing, osteoporosis, and osteoarthritis. NPY: Neuropeptide Y; BMSCs: Bone marrow mesenchymal stem cells; HSCs: Hematopoietic stem cells; ECs: Endothelial cells; BMP-2; Bone morphogenetic protein 2; VEGF: Vascular endothelial growth factor.

different functions of NPY among different fracture stages. Previous studies found that the volumes of cortical and cancellous bones in NPY-deficient mice were significantly increased, which may result from elevated osteoblast activities and upregulated expression of Runx2 and Osterix[23,24]. Lundberg *et al*[43] reported that, compared with the WT controls, Y2R (-/-) mice presented with greater bone mass, which may correlate with the reinforced abilities of BMSCs, thus being able to produce more mineralized extracellular matrix as well as increased expression of ALP and OCN. As mentioned previously, NPY can directly increase the number and viability of BMSCs by promoting their proliferation and inhibiting apoptosis[8], together with increased osteogenic differentiation and BMP-2 expression[51]. Aside from *in vitro* and *in vivo* experiments, clinical observations also confirmed the role of NPY in fracture healing process. Gu *et al*[38] reported that, compared with those with a single fracture, patients with a fracture and accompanying traumatic brain injury had higher serological levels of NPY and accelerated fracture healing process. This finding can be explained by the fact that NPY can directly promote osteogenic differentiation of MSCs[38]. Besides, Y1R antagonist play a direct role in regulation of BMSCs. Liu *et al*[53] noted that treatment with PD160170, a Y1R antagonist, promoted osteogenic differentiation of BMSCs, with higher expression of COL-I, OCN, and Runx2. The *in vivo* outcomes also showed increases in bone volume/total volume (BV/TV), bone mineral density, and bone trabeculae number after PD160170 intervention[53]. However, considering the still limited evidence from clinical investigations, more studies are necessary. In short, NPY can induce proliferation and angiogenic and osteogenic differentiation of BMSCs, facilitating bone regeneration.

VEGF is one of the most important components in angiogenesis, which is regarded as an essential factor that directly influences the fracture healing process[74]. BMSCs can promote vessel sprouting and vascularization, which is considered to be important for bone formation, whilst ECs can accelerate bone repair by facilitating the recruitment of osteoclast precursors and stimulating the osteoclastogenic process[75]. NPY can also facilitate vessel sprouting, adhesion, migration, proliferation, and capillary tube formation in ECs[10,59,65]. Also, during the process of angiogenesis, NPY receptors are indispensable. For example, Lee *et al*[67] observed that NPY-induced aortic sprouting and *in vivo* matrigel capillary formation were decreased by 50% after deleting *Y2R* gene or using its antagonists, implying the vital role of Y2R in angiogenesis. During the angiogenesis process, continuous nutrients and cytokines are transported to the

fracture site, providing synergistic effects in fracture healing.

Osteoporosis

Osteoporosis, a disorder characterized by progressive bone loss and thus an increased risk of fracture, often results from menopausal loss of estrogen in women[76]. Currently, the primary methods to investigate the effects of NPY system on osteoporosis include examining bone phenotypes in gene knockout models and using NPY receptor antagonists.

Increasing number of studies indicated that Y2R is essential in medicating bone gain and loss. For example, mice lacking Y2R globally or specifically in the hypothalamus are characterized by greater bone loss compared to the WT controls, indicating that Y2R is critical in protection against bone loss[77,78]. In addition, ovariectomized mice injected with JNJ-31020028, a Y2R antagonist, showed increases in the whole-body bone mineral density, vertebral trabecular bone volume, and trabecular thickness[79] (Table 1). NPY3-36, an agonist of Y2R and Y5R, strongly lowered the expression level of receptor activator of Nfkb ligand (RANKL) and upregulated the basal levels of osteoprotegerin (OPG) in BMSCs, suggesting that Y2R may induce bone formation by affecting the expression of RANKL and OPG in BMSCs[40,41]. These results also hint that selective pharmacological manipulation of Y2R may be a potential strategy for anabolic treatment of osteoporosis.

Aside from Y2R, Y1R may be also involved in regulation of bone homeostasis. Sousa *et al*[80] found that in mice, oral administration of BIBO3304, a Y1R antagonist, led to increased rates of mineral deposition in both cortical and cancellous bones, which may result from an increased number of osteoblasts following direct actions of NPY/Y1R on osteoclasts[80] (Table 1). Although there is still a lack of enough evidence to support the belief that NPY can improve the status of osteoporosis *via* direct regulations of BMSCs, based on the potential role of Y1R in osteogenic differentiation of BMSCs, it can be speculated that inhibition of Y1R may be a potential anabolic strategy for prevention of bone loss.

Nonetheless, other studies displayed different outcomes. Tang *et al*[56] reported that, compared with the controls, the rats treated with the Y1R antagonist BIBP3226 showed remarkable reductions in volumes of callus bone and tissue, and BV/TV as well. This demonstrates a potential negative effect of Y1R antagonist on bone healing (Table 1). Similarly, Sousa *et al*[44] also observed a lower rate of bone healing in Y1R (-/-) mice, with a decreased volume of bone callus and a weaker bone strength. Osteoclasts of hematopoietic origin also express high levels of NPY and Y1R[33]. Since osteoclasts are differentiated from HSCs, NPY can effectively alleviate ovariectomy-induced bone loss by decreasing the number of osteoclasts in bone marrow microenvironment through HSC mobilization, and increasing the number of osteoblasts as well[61]. These findings imply that NPY may act as a potential indicator of HSC mobilization, and supplementation of NPY may provide a therapeutic effect for bone loss.

Osteoarthritis

Stem cells can provide preventive and regenerative effects in early stages of osteoarthritis[81]. Neurotransmitters, such as substance P (SP) and calcitonin gene-related peptide (CGRP), modulate osteo-chondrogenic differentiation of mesenchymal progenitor cells during endochondral ossification in limb development[82]. Guo *et al*[83] found that fracture- related pain in a rat model was partially relieved by an NK1 (substance P receptor) antagonist LY303870. In a rat knee model evaluating SP coupled with self-assembled peptide hydrogels, Kim *et al*[84] observed that cartilage regeneration and regenerative properties were markedly improved by recruiting MSCs. However, to date, few studies have addressed the role of NPY and its receptors in chondrocytes and their potential applications in osteoarthritis therapy.

One of the most frequent symptoms of osteoarthritis is pain of the affected joint, which associates with irritation of sensory nerve endings following pathological changes in the subchondral bone and synovial lining, with neuropeptide-containing nerves in the joint tissue as an important regulatory element[85,86]. NPY is able to bind to free nerve endings in accessory ligaments, synovium, subchondral bone, menisci, articular cartilage, and periosteum[87], and changes afferent sensitivity[88]. The visual analog scale (VAS) score, used to rate pain, was negatively correlated with the mean optical density values for NPY by immunohistochemical analysis in all patients with osteoarthritis, implying that NPY may be involved in the generation of pain[89]. Similarly, the mean level of CGRP in the synovial tissue of patients with severe osteoarthritis-related pain (VAS > 6) was significantly higher than that of patients with moderate to lower pain (VAS < 6)[90]. In another study, higher levels of CGRP were detected in the infrapatellar fat pad in patients with knee osteoarthritis, which is

Table 1 Effects of neuropeptide Y receptor-targeted drugs on bone metabolism

Antagonist	Bone phenotype	Administration and subjects
Y1R antagonist BIBP3226[56]	Callus tissue volume ↓, callus bone volume and callus ↓, BV/TV↓	Intraperitoneal injection Sprague-Dawley rats
Y2R antagonist JNJ-31020028[79]	Vertebral trabecular bone volume ↑, bone mineral density ↑, trabecular thickness ↑; P1NP and CTX biomarkers ↓	Intraperitoneal injection Ovariectomized Mice
Y1R antagonist BIBO3304[80]	Osteoblast activity ↑, cortical and cancellous bone volumes ↑; no adverse effects	Oral administration Mice

P1NP: Procollagen type 1 N-terminal pro-peptide; CTX: Collagen type I C-telopeptide; BV/TV: Bone volume/total volume.

implicated as a possible cause of osteoarthritis development and related pain[91].

Traditionally, the rationale application of MSCs for management of osteoarthritis stems from their ability to differentiate into chondrogenic lineage rather than osteoblasts, which can help improve degeneration of the cartilage, and alleviate joint damage and related pain[92]. A decrease in the subchondral bone lesions may help reduce joint pain, which may be a future treatment target in relief of osteoarthritis-related pain[93]. Besides, NPY can increase the VEGF expression and promote osteoblastic differentiation and angiogenesis of BMSCs[38,51,55]. As cartilage does not contain blood vessels, it may be considered a hostile environment for spreading of vascular channels[82]. VEGF, a crucial regulator of angiogenesis, can induce tube formation activity into cartilage channels, likely resulting in calcification and stiffness of subchondral bone, accompanied by increased innervation and subsequent increases in nociceptors[94]. Therefore, blocking NPY could be of interest in pain treatment and osteoarthritis prevention.

Inflammation plays a crucial role in the pathogenesis of osteoarthritis. Prostaglandins are the key mediators of inflammation and pain in osteoarthritis[87]. NPY is considered to have pro-inflammatory effects. In a clinical study, Hernanz *et al*[95] found that the level of NPY in the synovial fluid of the knee joint in patients with rheumatoid arthritis was higher than the healthy controls, and NPY stimulated the expression of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α). In a murine chondrogenic cell line, NPY was found to be able to promote proliferation and increase viable cell count of ATDC5 cells. Besides, the chondrogenic differentiation of ATDC5 can be enhanced by NPY *via* upregulating Runx2 and Col10a1. In the tissue engineering field, stem cell-scaffold-NPY combinations may be used to repair cartilage and tissues.

NPY receptor-targeted drugs

As mentioned previously, Y1R and Y2R antagonists have been applied to minimize the bone loss caused by different musculoskeletal disorders. Y1R and Y2R have been identified as novel therapeutic targets for the treatment of obesity and cancer[96,97]. Recently, a group of Y1R antagonists have been commercially available. Among these, BIBO3304, a Y1R antagonist, is the most potent[98]. However, the effect and efficacy of BIBO3304 have been only evaluated in animals, and clinical trials are required to confirm its role[56,79,80](Table 1).

CONCLUSION

This review introduces NPY and NPY receptors, and the potential mechanisms of actions of NPY on BMSCs, HSCs, ECs, and chondrocytes. NPY exerts different biological effects in different types of cells, including proliferation, apoptosis, differentiation, migration, mobilization, and cytokine secretion. NPY and its receptors play important roles in promoting bone union and anti-osteoporosis by regulating relative cell functions. Currently, limited studies have investigated the role of NPY in chondrocytes and its efficacy in controlling pain and inflammation in osteoarthritis. Improved clinical investigations addressing the role of NPY and its receptors in orthopaedic disorders may provide new insights into the stem cell research and

therapy.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Song Y, from Nanfang Hospital, Southern Medical University for her help in figure preparation.

REFERENCES

1 **Reid KF**, Fielding RA. Skeletal muscle power: a critical determinant of physical functioning in older adults. *Exerc Sport Sci Rev* 2012; **40**: 4-12 [PMID: 22016147 DOI: 10.1097/JES.0b013e31823b5f13]
2 **Tatemoto K**. Neuropeptide Y: complete amino acid sequence of the brain peptide. *Proc Natl Acad Sci USA* 1982; **79**: 5485-5489 [PMID: 6957876 DOI: 10.1073/pnas.79.18.5485]
3 **Lee NJ**, Herzog H. NPY regulation of bone remodelling. *Neuropeptides* 2009; **43**: 457-463 [PMID: 19748118 DOI: 10.1016/j.npep.2009.08.006]
4 **Apel PJ**, Crane D, Northam CN, Callahan M, Smith TL, Teasdall RD. Effect of selective sensory denervation on fracture-healing: an experimental study of rats. *J Bone Joint Surg Am* 2009; **91**: 2886-2895 [PMID: 19952252 DOI: 10.2106/JBJS.H.01878]
5 **Lee NJ**, Nguyen AD, Enriquez RF, Doyle KL, Sainsbury A, Baldock PA, Herzog H. Osteoblast specific Y1 receptor deletion enhances bone mass. *Bone* 2011; **48**: 461-467 [PMID: 21040809 DOI: 10.1016/j.bone.2010.10.174]
6 **Peng S**, Zhou YL, Song ZY, Lin S. Effects of Neuropeptide Y on Stem Cells and Their Potential Applications in Disease Therapy. *Stem Cells Int* 2017; **2017**: 6823917 [PMID: 29109742 DOI: 10.1155/2017/6823917]
7 **Aerts-Kaya F**, Ulum B, Mammadova A, Köse S, Aydin G, Korkusuz P, Uçkan-Çetinkaya D. Neurological Regulation of the Bone Marrow Niche. *Adv Exp Med Biol* 2020; **1212**: 127-153 [PMID: 31342461 DOI: 10.1007/5584_2019_398]
8 **Wu J**, Liu S, Meng H, Qu T, Fu S, Wang Z, Yang J, Jin D, Yu B. Neuropeptide Y enhances proliferation and prevents apoptosis in rat bone marrow stromal cells in association with activation of the Wnt/β-catenin pathway in vitro. *Stem Cell Res* 2017; **21**: 74-84 [PMID: 28411439 DOI: 10.1016/j.scr.2017.04.001]
9 **Park MH**, Jin HK, Min WK, Lee WW, Lee JE, Akiyama H, Herzog H, Enikolopov GN, Schuchman EH, Bae JS. Neuropeptide Y regulates the hematopoietic stem cell microenvironment and prevents nerve injury in the bone marrow. *EMBO J* 2015; **34**: 1648-1660 [PMID: 25916827 DOI: 10.15252/embj.201490174]
10 **Movafagh S**, Hobson JP, Spiegel S, Kleinman HK, Zukowska Z. Neuropeptide Y induces migration, proliferation, and tube formation of endothelial cells bimodally via Y1, Y2, and Y5 receptors. *FASEB J* 2006; **20**: 1924-1926 [PMID: 16891622 DOI: 10.1096/fj.05-4770fje]
11 **Im GI**. Clinical use of stem cells in orthopaedics. *Eur Cell Mater* 2017; **33**: 183-196 [PMID: 28266690 DOI: 10.22203/eCM.v033a14]
12 **Luby AO**, Ranganathan K, Lynn JV, Nelson NS, Donneys A, Buchman SR. Stem Cells for Bone Regeneration: Current State and Future Directions. *J Craniofac Surg* 2019; **30**: 730-735 [PMID: 30817525 DOI: 10.1097/SCS.0000000000005250]
13 **Eagan MJ**, Zuk PA, Zhao KW, Bluth BE, Brinkmann EJ, Wu BM, McAllister DR. The suitability of human adipose-derived stem cells for the engineering of ligament tissue. *J Tissue Eng Regen Med* 2012; **6**: 702-709 [PMID: 21953999 DOI: 10.1002/term.474]
14 **Lee SY**, Kwon B, Lee K, Son YH, Chung SG. Therapeutic Mechanisms of Human Adipose-Derived Mesenchymal Stem Cells in a Rat Tendon Injury Model. *Am J Sports Med* 2017; **45**: 1429-1439 [PMID: 28291954 DOI: 10.1177/0363546517689874]
15 **Sanghani-Kerai A**, McCreary D, Lancashire H, Osagie L, Coathup M, Blunn G. Stem Cell Interventions for Bone Healing: Fractures and Osteoporosis. *Curr Stem Cell Res Ther* 2018; **13**: 369-377 [PMID: 29637866 DOI: 10.2174/1574888X13666180410160511]
16 **Saraf R**, Mahmood F, Amir R, Matyal R. Neuropeptide Y is an angiogenic factor in cardiovascular regeneration. *Eur J Pharmacol* 2016; **776**: 64-70 [PMID: 26875634 DOI: 10.1016/j.ejphar.2016.02.033]
17 **Reichmann F**, Holzer P. Neuropeptide Y: A stressful review. *Neuropeptides* 2016; **55**: 99-109 [PMID: 26441327 DOI: 10.1016/j.npep.2015.09.008]
18 **Mörl K**, Beck-Sickinger AG. Intracellular Trafficking of Neuropeptide Y Receptors. *Prog Mol Biol Transl Sci* 2015; **132**: 73-96 [PMID: 26055055 DOI: 10.1016/bs.pmbts.2015.02.011]
19 **Horsnell H**, Baldock PA. Osteoblastic Actions of the Neuropeptide Y System to Regulate Bone and Energy Homeostasis. *Curr Osteoporos Rep* 2016; **14**: 26-31 [PMID: 26872458 DOI: 10.1007/s11914-016-0300-9]
20 **Blomqvist AG**, Herzog H. Y-receptor subtypes--how many more? *Trends Neurosci* 1997; **20**: 294-298 [PMID: 9223221 DOI: 10.1016/s0166-2236(96)01057-0]
21 **Gerald C**, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL. A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature* 1996; **382**: 168-171 [PMID: 8700207 DOI: 10.1038/382168a0]
22 **Khor EC**, Yulyaningsih E, Driessler F, Kovačić N, Wee NKY, Kulkarni RN, Lee NJ, Enriquez RF, Xu J, Zhang L, Herzog H, Baldock PA. The y6 receptor suppresses bone resorption and stimulates bone formation in mice via a suprachiasmatic nucleus relay. *Bone* 2016; **84**: 139-147 [PMID: 26721736 DOI: 10.1016/j.bone.2015.12.011]
23 **Baldock PA**, Lee NJ, Driessler F, Lin S, Allison S, Stehrer B, Lin EJ, Zhang L, Enriquez RF, Wong IP, McDonald MM, During M, Pierroz DD, Slack K, Shi YC, Yulyaningsih E, Aljanova A, Little DG, Ferrari

SL, Sainsbury A, Eisman JA, Herzog H. Neuropeptide Y knockout mice reveal a central role of NPY in the coordination of bone mass to body weight. *PLoS One* 2009; **4**: e8415 [PMID: 20027231 DOI: 10.1371/journal.pone.0008415]

24 **Baldock PA**, Sainsbury A, Allison S, Lin EJ, Couzens M, Boey D, Enriquez R, During M, Herzog H, Gardiner EM. Hypothalamic control of bone formation: distinct actions of leptin and y2 receptor pathways. *J Bone Miner Res* 2005; **20**: 1851-1857 [PMID: 16160743 DOI: 10.1359/jbmr.050523]

25 **Allison SJ**, Baldock PA, Enriquez RF, Lin E, During M, Gardiner EM, Eisman JA, Sainsbury A, Herzog H. Critical interplay between neuropeptide Y and sex steroid pathways in bone and adipose tissue homeostasis. *J Bone Miner Res* 2009; **24**: 294-304 [PMID: 18847327 DOI: 10.1359/jbmr.081013]

26 **Baldock PA**, Sainsbury A, Couzens M, Enriquez RF, Thomas GP, Gardiner EM, Herzog H. Hypothalamic Y2 receptors regulate bone formation. *J Clin Invest* 2002; **109**: 915-921 [PMID: 11927618 DOI: 10.1172/jci14588]

27 **Chen X**, DiMaggio DA, Han SP, Westfall TC. Autoreceptor-induced inhibition of neuropeptide Y release from PC-12 cells is mediated by Y2 receptors. *Am J Physiol* 1997; **273**: H1737-H1744 [PMID: 9362238 DOI: 10.1152/ajpheart.1997.273.4.H1737]

28 **Broberger C**, Landry M, Wong H, Walsh JN, Hökfelt T. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. *Neuroendocrinology* 1997; **66**: 393-408 [PMID: 9430445 DOI: 10.1159/000127265]

29 **Allison SJ**, Baldock P, Sainsbury A, Enriquez R, Lee NJ, Lin EJ, Klugmann M, During M, Eisman JA, Li M, Pan LC, Herzog H, Gardiner EM. Conditional deletion of hypothalamic Y2 receptors reverts gonadectomy-induced bone loss in adult mice. *J Biol Chem* 2006; **281**: 23436-23444 [PMID: 16785231 DOI: 10.1074/jbc.M604839200]

30 **Sainsbury A**, Baldock PA, Schwarzer C, Ueno N, Enriquez RF, Couzens M, Inui A, Herzog H, Gardiner EM. Synergistic effects of Y2 and Y4 receptors on adiposity and bone mass revealed in double knockout mice. *Mol Cell Biol* 2003; **23**: 5225-5233 [PMID: 12861009 DOI: 10.1128/mcb.23.15.5225-5233.2003]

31 **Baldock PA**, Allison SJ, Lundberg P, Lee NJ, Slack K, Lin EJ, Enriquez RF, McDonald MM, Zhang L, During MJ, Little DG, Eisman JA, Gardiner EM, Yulyaningsih E, Lin S, Sainsbury A, Herzog H. Novel role of Y1 receptors in the coordinated regulation of bone and energy homeostasis. *J Biol Chem* 2007; **282**: 19092-19102 [PMID: 17491016 DOI: 10.1074/jbc.M700644200]

32 **Nunes AF**, Liz MA, Franquinho F, Teixeira L, Sousa V, Chenu C, Lamghari M, Sousa MM. Neuropeptide Y expression and function during osteoblast differentiation--insights from transthyretin knockout mice. *FEBS J* 2010; **277**: 263-275 [PMID: 19954489 DOI: 10.1111/j.1742-4658.2009.07482.x]

33 **Igwe JC**, Jiang X, Paic F, Ma L, Adams DJ, Baldock PA, Pilbeam CC, Kalajzic I. Neuropeptide Y is expressed by osteocytes and can inhibit osteoblastic activity. *J Cell Biochem* 2009; **108**: 621-630 [PMID: 19670271 DOI: 10.1002/jcb.22294]

34 **Dong P**, Gu X, Zhu G, Li M, Ma B, Zi Y. Melatonin Induces Osteoblastic Differentiation of Mesenchymal Stem Cells and Promotes Fracture Healing in a Rat Model of Femoral Fracture via Neuropeptide Y/Neuropeptide Y Receptor Y1 Signaling. *Pharmacology* 2018; **102**: 272-280 [PMID: 30227410 DOI: 10.1159/000492576]

35 **Silva AP**, Kaufmann JE, Vivancos C, Fakan S, Cavadas C, Shaw P, Brunner HR, Vischer U, Grouzmann E. Neuropeptide Y expression, localization and cellular transducing effects in HUVEC. *Biol Cell* 2005; **97**: 457-467 [PMID: 15850450 DOI: 10.1042/bc20040102]

36 **Lee NJ**, Doyle KL, Sainsbury A, Enriquez RF, Hort YJ, Riepler SJ, Baldock PA, Herzog H. Critical role for Y1 receptors in mesenchymal progenitor cell differentiation and osteoblast activity. *J Bone Miner Res* 2010; **25**: 1736-1747 [PMID: 20200977 DOI: 10.1002/jbmr.61]

37 **Matic I**, Matthews BG, Kizivat T, Igwe JC, Marijanovic I, Ruohonen ST, Savontaus E, Adams DJ, Kalajzic I. Bone-specific overexpression of NPY modulates osteogenesis. *J Musculoskelet Neuronal Interact* 2012; **12**: 209-218 [PMID: 23196263 DOI: 10.1055/s-0032-1305278]

38 **Gu XC**, Zhang XB, Hu B, Zi Y, Li M. Neuropeptide Y accelerates post-fracture bone healing by promoting osteogenesis of mesenchymal stem cells. *Neuropeptides* 2016; **60**: 61-66 [PMID: 27720230 DOI: 10.1016/j.npep.2016.09.005]

39 **Liao KH**, Wang JY, Lin HW, Lui TN, Chen KY, Yen DH, Jeng MJ. Risk of death in patients with post-traumatic cerebrospinal fluid leakage--analysis of 1773 cases. *J Chin Med Assoc* 2016; **79**: 58-64 [PMID: 26617240 DOI: 10.1016/j.jcma.2015.10.002]

40 **Amano S**, Arai M, Goto S, Togari A. Inhibitory effect of NPY on isoprenaline-induced osteoclastogenesis in mouse bone marrow cells. *Biochim Biophys Acta* 2007; **1770**: 966-973 [PMID: 17383824 DOI: 10.1016/j.bbagen.2007.02.009]

41 **Teixeira L**, Sousa DM, Nunes AF, Sousa MM, Herzog H, Lamghari M. NPY revealed as a critical modulator of osteoblast function in vitro: new insights into the role of Y1 and Y2 receptors. *J Cell Biochem* 2009; **107**: 908-916 [PMID: 19459152 DOI: 10.1002/jcb.22194]

42 **Igura K**, Haider HKh, Ahmed RP, Sheriff S, Ashraf M. Neuropeptide y and neuropeptide y y5 receptor interaction restores impaired growth potential of aging bone marrow stromal cells. *Rejuvenation Res* 2011; **14**: 393-403 [PMID: 21595512 DOI: 10.1089/rej.2010.1129]

43 **Lundberg P**, Allison SJ, Lee NJ, Baldock PA, Brouard N, Rost S, Enriquez RF, Sainsbury A, Lamghari M, Simmons P, Eisman JA, Gardiner EM, Herzog H. Greater bone formation of Y2 knockout mice is associated with increased osteoprogenitor numbers and altered Y1 receptor expression. *J Biol Chem* 2007; **282**: 19082-19091 [PMID: 17491022 DOI: 10.1074/jbc.M609629200]

44 **Sousa DM**, McDonald MM, Mikulec K, Peacock L, Herzog H, Lamghari M, Little DG, Baldock PA. Neuropeptide Y modulates fracture healing through Y1 receptor signaling. *J Orthop Res* 2013; **31**: 1570-1578 [PMID: 23733357 DOI: 10.1002/jor.22400]

45 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

46 **L PK**, Kandoi S, Misra R, S V, K R, Verma RS. The mesenchymal stem cell secretome: A new paradigm towards cell-free therapeutic mode in regenerative medicine. *Cytokine Growth Factor Rev* 2019; **46**: 1-9 [PMID: 30954374 DOI: 10.1016/j.cytogfr.2019.04.002]

47 **Sheyn D**, Shapiro G, Tawackoli W, Jun DS, Koh Y, Kang KB, Su S, Da X, Ben-David S, Bez M, Yalon E, Antebi B, Avalos P, Stern T, Zelzer E, Schwarz EM, Gazit Z, Pelled G, Bae HM, Gazit D. PTH Induces Systemically Administered Mesenchymal Stem Cells to Migrate to and Regenerate Spine Injuries. *Mol Ther* 2016; **24**: 318-330 [PMID: 26585691 DOI: 10.1038/mt.2015.211]

48 **Maiti SK**, Shivakumar MU, Mohan D, Kumar N, Singh KP. Mesenchymal Stem Cells of Different Origin-Seeded Bioceramic Construct in Regeneration of Bone Defect in Rabbit. *Tissue Eng Regen Med* 2018; **15**: 477-492 [PMID: 30603571 DOI: 10.1007/s13770-018-0129-7]

49 **Son MY**, Kim MJ, Yu K, Koo DB, Cho YS. Involvement of neuropeptide Y and its Y1 and Y5 receptors in maintaining self-renewal and proliferation of human embryonic stem cells. *J Cell Mol Med* 2011; **15**: 152-165 [PMID: 19874423 DOI: 10.1111/j.1582-4934.2009.00956.x]

50 **Wang Y**, Zhang D, Ashraf M, Zhao T, Huang W, Ashraf A, Balasubramaniam A. Combining neuropeptide Y and mesenchymal stem cells reverses remodeling after myocardial infarction. *Am J Physiol Heart Circ Physiol* 2010; **298**: H275-H286 [PMID: 19897711 DOI: 10.1152/ajpheart.00765.2009]

51 **Liu S**, Jin D, Wu JQ, Xu ZY, Fu S, Mei G, Zou ZL, Ma SH. Neuropeptide Y stimulates osteoblastic differentiation and VEGF expression of bone marrow mesenchymal stem cells related to canonical Wnt signaling activating in vitro. *Neuropeptides* 2016; **56**: 105-113 [PMID: 26707636 DOI: 10.1016/j.npep.2015.12.008]

52 **Wee NKY**, Sinder BP, Novak S, Wang X, Stoddard C, Matthews BG, Kalajzic I. Skeletal phenotype of the neuropeptide Y knockout mouse. *Neuropeptides* 2019; **73**: 78-88 [PMID: 30522780 DOI: 10.1016/j.npep.2018.11.009]

53 **Liu S**, Wu JQ, Hu JJ, Wang L, Wang Z, Meng H, Zhuo LJ, Zheng JX. [Neuropeptide Y Y1 receptor antagonist PD160170 promotes osteogenic differentiation of rat bone marrow mesenchymal stem cells in vitro and femoral defect repair in rats]. *Nan Fang Yi Ke Da Xue Xue Bao* 2018; **38**: 669-676 [PMID: 29997088]

54 **Yahara M**, Tei K, Tamura M. Inhibition of neuropeptide Y Y1 receptor induces osteoblast differentiation in MC3T3-E1 cells. *Mol Med Rep* 2017; **16**: 2779-2784 [PMID: 28656295 DOI: 10.3892/mmr.2017.6866]

55 **Ma WH**, Liu YJ, Wang W, Zhang YZ. Neuropeptide Y, substance P, and human bone morphogenetic protein 2 stimulate human osteoblast osteogenic activity by enhancing gap junction intercellular communication. *Braz J Med Biol Res* 2015; **48**: 299-307 [PMID: 25714881 DOI: 10.1590/1414-431X20144226]

56 **Tang P**, Duan C, Wang Z, Wang C, Meng G, Lin K, Yang Q, Yuan Z. NPY and CGRP Inhibitor Influence on ERK Pathway and Macrophage Aggregation during Fracture Healing. *Cell Physiol Biochem* 2017; **41**: 1457-1467 [PMID: 28315869 DOI: 10.1159/000468405]

57 **Sharma R**, Wu X, Rhodes SD, Chen S, He Y, Yuan J, Li J, Yang X, Li X, Jiang L, Kim ET, Stevenson DA, Viskochil D, Xu M, Yang FC. Hyperactive Ras/MAPK signaling is critical for tibial nonunion fracture in neurofibromin-deficient mice. *Hum Mol Genet* 2013; **22**: 4818-4828 [PMID: 23863460 DOI: 10.1093/hmg/ddt333]

58 **Park MH**, Min WK, Jin HK, Bae JS. Role of neuropeptide Y in the bone marrow hematopoietic stem cell microenvironment. *BMB Rep* 2015; **48**: 645-646 [PMID: 26538251 DOI: 10.5483/bmbrep.2015.48.12.22]

59 **Singh P**, Hoggatt J, Kamocka MM, Mohammad KS, Saunders MR, Li H, Speth J, Carlesso N, Guise TA, Pelus LM. Neuropeptide Y regulates a vascular gateway for hematopoietic stem and progenitor cells. *J Clin Invest* 2017; **127**: 4527-4540 [PMID: 29130940 DOI: 10.1172/JCI94687]

60 **Ulum B**, Mammadova A, Özyüncü Ö, Uçkan-Çetinkaya D, Yanık T, Aerts-Kaya F. Neuropeptide Y is involved in the regulation of quiescence of hematopoietic stem cells. *Neuropeptides* 2020; **80**: 102029 [PMID: 32127176 DOI: 10.1016/j.npep.2020.102029]

61 **Park MH**, Lee JK, Kim N, Min WK, Lee JE, Kim KT, Akiyama H, Herzog H, Schuchman EH, Jin HK, Bae JS. Neuropeptide Y Induces Hematopoietic Stem/Progenitor Cell Mobilization by Regulating Matrix Metalloproteinase-9 Activity Through Y1 Receptor in Osteoblasts. *Stem Cells* 2016; **34**: 2145-2156 [PMID: 27090492 DOI: 10.1002/stem.2383]

62 **Park MH**, Kim N, Jin HK, Bae JS. Neuropeptide Y-based recombinant peptides ameliorate bone loss in mice by regulating hematopoietic stem/progenitor cell mobilization. *BMB Rep* 2017; **50**: 138-143 [PMID: 27998395 DOI: 10.5483/bmbrep.2017.50.3.191]

63 **Szczesny G**. Molecular aspects of bone healing and remodeling. *Pol J Pathol* 2002; **53**: 145-153 [PMID: 12476617]

64 **Alves CJ**, Alencastre IS, Neto E, Ribas J, Ferreira S, Vasconcelos DM, Sousa DM, Summavielle T, Lamghari M. Bone Injury and Repair Trigger Central and Peripheral NPY Neuronal Pathways. *PLoS One* 2016; **11**: e0165465 [PMID: 27802308 DOI: 10.1371/journal.pone.0165465]

65 **Zukowska-Grojec Z**, Karwatowska-Prokopczuk E, Rose W, Rone J, Movafagh S, Ji H, Yeh Y, Chen WT, Kleinman HK, Grouzmann E, Grant DS. Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. *Circ Res* 1998; **83**: 187-195 [PMID: 9686758 DOI: 10.1161/01.res.83.2.187]

66 **Kuo LE**, Kitlinska JB, Tilan JU, Li L, Baker SB, Johnson MD, Lee EW, Burnett MS, Fricke ST, Kvetnansky R, Herzog H, Zukowska Z. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat Med* 2007; **13**: 803-811 [PMID: 17603492 DOI: 10.1038/nm1611]

67 **Lee EW**, Grant DS, Movafagh S, Zukowska Z. Impaired angiogenesis in neuropeptide Y (NPY)-Y2 receptor knockout mice. *Peptides* 2003; **24**: 99-106 [PMID: 12576090 DOI: 10.1016/s0196-9781(02)00281-4]

68 **Businaro R**, Scaccia E, Bordin A, Pagano F, Corsi M, Siciliano C, Capoano R, Procaccini E, Salvati B, Petrozza V, Totta P, Vietri MT, Frati G, De Falco E. Platelet Lysate-Derived Neuropeptide y Influences Migration and Angiogenesis of Human Adipose Tissue-Derived Stromal Cells. *Sci Rep* 2018; **8**: 14365 [PMID: 30254326 DOI: 10.1038/s41598-018-32623-8]

69 **Zhang LF**, Qi J, Zuo G, Jia P, Shen X, Shao J, Kang H, Yang H, Deng L. Osteoblast-secreted factors promote proliferation and osteogenic differentiation of bone marrow stromal cells via VEGF/heme-

oxygenase-1 pathway. *PLoS One* 2014; **9**: e99946 [PMID: 24940620 DOI: 10.1371/journal.pone.0099946]
70 **Duffy GP**, Ahsan T, O'Brien T, Barry F, Nerem RM. Bone marrow-derived mesenchymal stem cells promote angiogenic processes in a time- and dose-dependent manner in vitro. *Tissue Eng Part A* 2009; **15**: 2459-2470 [PMID: 19327020 DOI: 10.1089/ten.TEA.2008.0341]
71 **Kang X**, Qian Z, Liu J, Feng D, Li H, Zhang Z, Jin X, Ma Z, Xu M, Li F, Zhang Y, Gao X, Sun H, Wu S. Neuropeptide Y Acts Directly on Cartilage Homeostasis and Exacerbates Progression of Osteoarthritis Through NPY2R. *J Bone Miner Res* 2020 [PMID: 32101625 DOI: 10.1002/jbmr.3991]
72 **Chen R**, Hao Z, Chen X, Fu Q, Ma Y. Neuropeptide Y enhances proliferation and chondrogenic differentiation of ATDC5 cells. *Neuropeptides* 2020; **80**: 102022 [PMID: 31987472 DOI: 10.1016/j.npep.2020.102022]
73 **Long H**, Ahmed M, Ackermann P, Stark A, Li J. Neuropeptide Y innervation during fracture healing and remodeling. A study of angulated tibial fractures in the rat. *Acta Orthop* 2010; **81**: 639-646 [PMID: 20860441 DOI: 10.3109/17453674.2010.504609]
74 **Keramaris NC**, Calori GM, Nikolaou VS, Schemitsch EH, Giannoudis PV. Fracture vascularity and bone healing: a systematic review of the role of VEGF. *Injury* 2008; **39** Suppl 2: S45-S57 [PMID: 18804573 DOI: 10.1016/S0020-1383(08)70015-9]
75 **Cui Y**, Fu S, Hou T, Wu X. Endothelial Progenitor Cells Enhance the Migration and Osteoclastic Differentiation of Bone Marrow-Derived Macrophages in vitro and in a Mouse Femur Fracture Model through Talin-1. *Cell Physiol Biochem* 2018; **49**: 555-564 [PMID: 30165361 DOI: 10.1159/000492993]
76 **Hart EC**, Charkoudian N. Sympathetic neural regulation of blood pressure: influences of sex and aging. *Physiology (Bethesda)* 2014; **29**: 8-15 [PMID: 24382867 DOI: 10.1152/physiol.00031.2013]
77 **Baldock PA**, Lin S, Zhang L, Karl T, Shi Y, Driessler F, Zengin A, Hörmer B, Lee NJ, Wong IP, Lin EJ, Enriquez RF, Stehrer B, During MJ, Yulyaningsih E, Zolotukhin S, Ruohonen ST, Savontaus E, Sainsbury A, Herzog H. Neuropeptide y attenuates stress-induced bone loss through suppression of noradrenaline circuits. *J Bone Miner Res* 2014; **29**: 2238-2249 [PMID: 24535841 DOI: 10.1002/jbmr.2205]
78 **Baldock PA**, Allison S, McDonald MM, Sainsbury A, Enriquez RF, Little DG, Eisman JA, Gardiner EM, Herzog H. Hypothalamic regulation of cortical bone mass: opposing activity of Y2 receptor and leptin pathways. *J Bone Miner Res* 2006; **21**: 1600-1607 [PMID: 16995815 DOI: 10.1359/jbmr.060705]
79 **Seldeen KL**, Halley PG, Volmar CH, Rodríguez MA, Hernandez M, Pang M, Carlsson SK, Suva LJ, Wahlestedt C, Troen BR, Brothers SP. Neuropeptide Y Y2 antagonist treated ovariectomized mice exhibit greater bone mineral density. *Neuropeptides* 2018; **67**: 45-55 [PMID: 29129406 DOI: 10.1016/j.npep.2017.11.005]
80 **Sousa DM**, Baldock PA, Enriquez RF, Zhang L, Sainsbury A, Lamghari M, Herzog H. Neuropeptide Y Y1 receptor antagonism increases bone mass in mice. *Bone* 2012; **51**: 8-16 [PMID: 22484690 DOI: 10.1016/j.bone.2012.03.020]
81 **Goldberg A**, Mitchell K, Soans J, Kim L, Zaidi R. The use of mesenchymal stem cells for cartilage repair and regeneration: a systematic review. *J Orthop Surg Res* 2017; **12**: 39 [PMID: 28279182 DOI: 10.1186/s13018-017-0534-y]
82 **Grässel SG**. The role of peripheral nerve fibers and their neurotransmitters in cartilage and bone physiology and pathophysiology. *Arthritis Res Ther* 2014; **16**: 485 [PMID: 25789373 DOI: 10.1186/s13075-014-0485-1]
83 **Guo TZ**, Offley SC, Boyd EA, Jacobs CR, Kingery WS. Substance P signaling contributes to the vascular and nociceptive abnormalities observed in a tibial fracture rat model of complex regional pain syndrome type I. *Pain* 2004; **108**: 95-107 [PMID: 15109512 DOI: 10.1016/j.pain.2003.12.010]
84 **Kim SJ**, Kim JE, Kim SH, Kim SJ, Jeon SJ, Kim SH, Jung Y. Therapeutic effects of neuropeptide substance P coupled with self-assembled peptide nanofibers on the progression of osteoarthritis in a rat model. *Biomaterials* 2016; **74**: 119-130 [PMID: 26454050 DOI: 10.1016/j.biomaterials.2015.09.040]
85 **Dirmeier M**, Capellino S, Schubert T, Angele P, Anders S, Straub RH. Lower density of synovial nerve fibres positive for calcitonin gene-related peptide relative to substance P in rheumatoid arthritis but not in osteoarthritis. *Rheumatology (Oxford)* 2008; **47**: 36-40 [PMID: 18077488 DOI: 10.1093/rheumatology/kem301]
86 **Mapp PI**, Walsh DA. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. *Nat Rev Rheumatol* 2012; **8**: 390-398 [PMID: 22641138 DOI: 10.1038/nrrheum.2012.80]
87 **Witt KL**, Vilensky JA. The anatomy of osteoarthritic joint pain. *Clin Anat* 2014; **27**: 451-454 [PMID: 22730047 DOI: 10.1002/ca.22120]
88 **Grässel S**, Muschter D. Peripheral Nerve Fibers and Their Neurotransmitters in Osteoarthritis Pathology. *Int J Mol Sci* 2017; **18**: 931 [PMID: 28452955 DOI: 10.3390/ijms18050931]
89 **Xiao J**, Yu W, Wang X, Wang B, Chen J, Liu Y, Li Z. Correlation between neuropeptide distribution, cancellous bone microstructure and joint pain in postmenopausal women with osteoarthritis and osteoporosis. *Neuropeptides* 2016; **56**: 97-104 [PMID: 26706183 DOI: 10.1016/j.npep.2015.12.006]
90 **Takano S**, Uchida K, Inoue G, Minatani A, Miyagi M, Aikawa J, Iwase D, Onuma K, Mukai M, Takaso M. Increase and regulation of synovial calcitonin gene-related peptide expression in patients with painful knee osteoarthritis. *J Pain Res* 2017; **10**: 1099-1104 [PMID: 28546767 DOI: 10.2147/JPR.S135939]
91 **Aikawa J**, Uchida K, Takano S, Inoue G, Minatani A, Miyagi M, Iwase D, Sekiguchi H, Mukai M, Takaso M. Expression of calcitonin gene-related peptide in the infrapatellar fat pad in knee osteoarthritis patients. *J Orthop Surg Res* 2017; **12**: 65 [PMID: 28431586 DOI: 10.1186/s13018-017-0568-1]
92 **Jayaram P**, Ikpeama U, Rothenberg JB, Malanga GA. Bone Marrow-Derived and Adipose-Derived Mesenchymal Stem Cell Therapy in Primary Knee Osteoarthritis: A Narrative Review. *PM R* 2019; **11**: 177-191 [PMID: 30010050 DOI: 10.1016/j.pmrj.2018.06.019]
93 **Gatenholm B**, Brittberg M. Neuropeptides: important regulators of joint homeostasis. *Knee Surg Sports Traumatol Arthrosc* 2019; **27**: 942-949 [PMID: 30039292 DOI: 10.1007/s00167-018-5074-4]
94 **Bonnet CS**, Walsh DA. Osteoarthritis, angiogenesis and inflammation. *Rheumatology (Oxford)* 2005; **44**: 7-16 [PMID: 15292527 DOI: 10.1093/rheumatology/keh344]
95 **Hernanz A**, Tato E, De la Fuente M, de Miguel E, Arnalich F. Differential effects of gastrin-releasing peptide, neuropeptide Y, somatostatin and vasoactive intestinal peptide on interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha production by whole blood cells from healthy young and old subjects. *J*

Neuroimmunol 1996; **71**: 25-30 [PMID: 8982099 DOI: 10.1016/s0165-5728(96)00118-x]
96 **Yulyaningsih E**, Zhang L, Herzog H, Sainsbury A. NPY receptors as potential targets for anti-obesity drug development. *Br J Pharmacol* 2011; **163**: 1170-1202 [PMID: 21545413 DOI: 10.1111/j.1476-5381.2011.01363.x]
97 **Ailanen L**, Vähätalo LH, Salomäki-Myftari H, Mäkelä S, Orpana W, Ruohonen ST, Savontaus E. Peripherally Administered Y_2-Receptor Antagonist BIIE0246 Prevents Diet-Induced Obesity in Mice With Excess Neuropeptide Y, but Enhances Obesity in Control Mice. *Front Pharmacol* 2018; **9**: 319 [PMID: 29674968 DOI: 10.3389/fphar.2018.00319]
98 **Dailey MJ**, Bartness TJ. Appetitive and consummatory ingestive behaviors stimulated by PVH and perifornical area NPY injections. *Am J Physiol Regul Integr Comp Physiol* 2009; **296**: R877-R892 [PMID: 19193934 DOI: 10.1152/ajpregu.90568.2008]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 1001-1012

DOI: 10.4252/wjsc.v12.i9.1001

ISSN 1948-0210 (online)

MINIREVIEWS

Stem cell treatments for oropharyngeal dysphagia: Rationale, benefits, and challenges

Eric K Tran, Kevin O Juarez, Jennifer L Long

ORCID number: Eric K Tran 0000-0003-4506-9266; Kevin O Juarez 0000-0002-5104-6047; Jennifer L Long 0000-0002-4185-2328.

Author contributions: All authors contributed to literature review, drafting, and revising manuscript.

Conflict-of-interest statement: No potential conflicts of interest.

Manuscript source: Invited manuscript

Received: February 28, 2020
Peer-review started: February 28, 2020
First decision: July 5, 2020
Revised: July 28, 2020
Accepted: September 1, 2020

Eric K Tran, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA 90095, United States

Kevin O Juarez, Department of Otolaryngology, Yale School of Medicine, New Haven, CT 06511, United States

Jennifer L Long, Department of Head and Neck Surgery, David Geffen School of Medicine, University of California-Los Angeles, Los Angeles, CA 90095, United States

Jennifer L Long, Greater Los Angeles VA Healthcare System, Los Angeles, CA 90073, United States

Corresponding author: Jennifer L Long, MD, PhD, Associate Professor, Department of Head and Neck Surgery, David Geffen School of Medicine, University of California-Los Angeles, 650 Charles Young Dr, CHS Room 62-150, Los Angeles, CA 90095, United States. jlong@mednet.ucla.edu

Abstract

Dysphagia, defined as difficulty swallowing, is a common symptom negatively impacting millions of adults annually. Estimated prevalence ranges from 14 to 33 percent in those over age 65 to over 70 percent in a nursing home setting. The elderly, those with neurodegenerative diseases, head and neck cancer patients, and those with autoimmune conditions such as Sjögren's syndrome are disproportionately affected. Oropharyngeal dysphagia refers specifically to difficulty in initiating a swallow due to dysfunction at or above the upper esophageal sphincter, and represents a large proportion of dysphagia cases. Current treatments are limited and are often ineffective. Stem cell therapy is a new and novel advancement that may fill a much-needed role in our treatment regimen. Here, we review the current literature regarding stem cell treatments for oropharyngeal dysphagia. Topics discussed include tissue regeneration advancements as a whole and translation of these principles into research surrounding tongue dysfunction, xerostomia, cricopharyngeal dysfunction, and finally an overview of the challenges and future directions for investigation. Although this field of study remains in its early stages, initial promising results show potential for the use of stem cell-based therapies to treat oropharyngeal dysphagia and warrant further research.

Key Words: Dysphagia; Xerostomia; Hemiglossectomy; Stem cells; Myoblasts;

Article in press: September 1, 2020
Published online: September 26, 2020

P-Reviewer: Lo Furno D, Yang RL
S-Editor: Huang P
L-Editor: A
P-Editor: Xing YX

Mesenchymal stem cells

Core Tip: Oropharyngeal dysphagia, despite its widespread prevalence, is a difficult condition to treat, particularly in those who have undergone irradiation or resection in the head and neck. This challenge stems from the lack of native functional tissue upon which current therapies such as physical rehabilitation rely. There have been several studies examining the use of stem cell therapy as a potential new treatment option for these patients. Our objective is to review and consolidate the current literature regarding this topic and discuss the recent advancements, challenges, and future directions for research in this field.

Citation: Tran EK, Juarez KO, Long JL. Stem cell treatments for oropharyngeal dysphagia: Rationale, benefits, and challenges. *World J Stem Cells* 2020; 12(9): 1001-1012
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/1001.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.1001

INTRODUCTION

Dysphagia is an extremely common yet morbid condition affecting over 9 million or over 1 in 25 adults in the United States annually[1]. Of those reporting swallowing problems, 32% report their condition to be moderate while 25% perceive it as a large problem greatly impacting their quality of life. Stroke is the most common etiology followed by other neurologic diseases such as Parkinson's disease, amyotrophic lateral sclerosis, and muscular dystrophy[2]. Head and neck cancer and its associated treatments, including resection and irradiation, often cause oropharyngeal dysphagia as well.

Given the oropharyngeal structures' location and vital function in promoting a patent airway, speech articulation, and deglutition, damage to these structures and resulting dysphagia leads to significantly increased risk for malnutrition, aspiration pneumonia, and death. Despite the significant morbidity associated with this condition however, there exists few reliable treatments that have proven to dramatically improve outcomes. Current therapies are limited and often heavily rely on physical rehabilitation of oral cavity and oropharynx swallowing function. These include isometric and resistance lingual strengthening exercises in cases of impaired tongue functioning or muscles intended to strengthen oral cavity and pharyngeal muscles[3,4]. A study conducted by Robbins *et al*[5] showed that among ten stroke patients who underwent isometric lingual exercise program, all subjects showed significantly increased isometric and swallowing pressures, reduction in airway invasion, and even increased lingual volume. Still, although improvement has been found in young and older healthy adults and those suffering from stroke, this success has not reliably translated toward head and neck cancer patients, who may have had extensive resection or irradiation in tumor removal. In these patients, the lack of normal oropharyngeal tissue impedes the efficacy and feasibility of swallowing rehabilitation exercises. Thus, these patients may benefit from newer, more innovative therapies.

Stem cell therapy has, in recent years, generated much interest in regards to its therapeutic potential to replace damaged tissue, guide wound healing, and restore function. Current research on this topic is extremely limited and primarily in investigational stages of study, although a few have advanced to clinical trials[6]. We aim to describe the current literature regarding the promising results of stem cell administration to restore swallowing function.

PHYSIOLOGY OF SWALLOWING

To understand dysphagia, we must first appreciate the mechanisms and anatomy underlying normal physiologic swallowing (Figure 1). Typically, swallowing consists of three stages: An oral stage, pharyngeal stage, and esophageal stage. The oral stage is

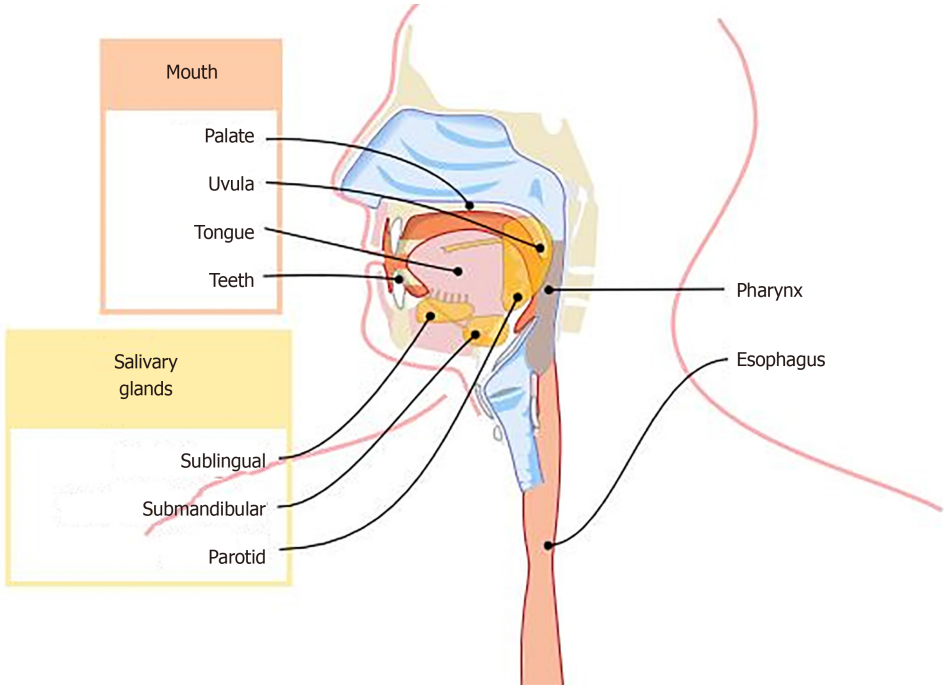


Figure 1 Anatomy of upper gastrointestinal tract. Depiction of upper gastrointestinal tract which clearly delineates the anatomically correlated oral, pharyngeal, and esophageal stages of swallowing. Complex interactions between numerous components facilitates this process and dysfunction in any may lead to dysphagia. This depiction also illustrates potential target areas for stem cell intervention.

further subdivided into an oral preparatory stage where a bolus of food or liquid is taken into the mouth and held in the anterior portion of the oral cavity, and an oral propulsive stage where this bolus is propelled posteriorly by the tongue into the oropharynx. Variations exist between swallowing liquids and solids. Solid food swallowing requires mastication and allows food to reside in the posterior oral cavity during bolus preparation. Liquids transit much more rapidly and are more difficult to control. Following the oral phase, the pharyngeal phase consists of two vital functions: Propulsion of the food downwards and through the upper esophageal sphincter (UES), and protection of the airway to prevent aspiration. First, the soft palate elevates and forms a seal with the posterior pharynx, preventing food from traveling upwards into the nasopharynx. The pharyngeal constrictor muscles then contract rhythmically, squeezing the bolus downwards towards the UES. Opening of the UES to allow passage into the esophagus is dependent on relaxation of the cricopharyngeus muscle, pressure from the bolus to expand the sphincter, and mechanical forces exerted by the thyrohyoid and suprahyoid muscles which lift the larynx and pull open the UES. Concurrently, the vocal folds close to seal the glottis and as the entire larynx is lifted by the thyrohyoid and suprahyoid muscles, the epiglottis is pressed against the laryngeal inlet and the oblique interarytenoid muscles contract to complete the seal. This serves to protect the airway. Finally in the esophageal stage, the bolus is propelled downwards *via* a peristaltic wave. The upper 1/3 of the esophagus is comprised primarily of skeletal muscle while the lower 2/3 is largely smooth muscle. The lower esophageal sphincter opens to allow passage into the stomach[7,8]. The entire process requires precise timing to transfer from one phase to the next. Given the enormous complexity and coordination required in swallowing, it is unsurprising that dysfunction of involved structures along any of these steps can result in dysphagia. Stem cell-based replacement therapy, thus, attempts to restore normal tissue or function in each of these structures.

STEM CELL THERAPIES FOR LARYNGEAL MUSCLE

Within the field of skeletal muscle regeneration, laryngeal muscles are of particular interest with regards to dysphagia given the function of the larynx in protecting the airway during deglutition. Vocal fold adduction is an important mechanism in preventing aspiration and facilitating coughing should particles enter the airway, as may often occur among patients suffering from dysphagia[9]. Denervation models of

the laryngeal muscles mimic iatrogenic injuries to the recurrent laryngeal nerves or neurodegenerative diseases that commonly cause problems with swallowing. Additionally, findings regarding regeneration of bulbar muscle (innervated by cranial nerves) may differ from somatic skeletal muscles, and thus be more directly applicable to the bulbar muscles of swallowing.

Halum *et al*[10] described a denervation model of vocal fold paralysis with subsequent autologous muscle-derived stem cell (MDSC) injection. 16 male rats underwent unilateral denervation of the recurrent laryngeal nerve (RLN). Myoblasts were isolated from sternocleidomastoid muscle biopsy and cultured at subconfluency to avoid myotube formation. One month post injury, autologous MDSCs labeled with a fluorescent marker were injected into the laryngeal thyroarytenoid (TA) muscle of half the animals while the remaining half received saline alone. MDSC persistence was found at both two weeks and two months post-treatment, with evidence of fusion with native muscle fibers at two months. TA muscle volume was increased in the myoblast injection group at both time points, and muscle fiber diameter increased within the myoblast injection group by two months. Upon laryngoscopy visualization, two animals achieved weak adductor motion of the vocal folds at two months, while no adduction was observed among the control group. Researchers did not find significant differences in reinnervation between the groups indicating that the improvements were due to muscle enhancements rather than neurologic signals[10]. As a follow up, the same group studied the use of trophic factors to enhance MDSC survival following injection. They found co-administration with insulin-like growth factor (IGF-1) and ciliary neurotrophic factor (CNTF) significantly increased survival, and cell persistence correlated with decreased myofiber atrophy[11]. Furthermore, CNTF was found to enhance neuron outgrowth and branching within vagus nerve motor neuron cultures *in vitro*[12]. When these findings were translated to a larger model utilizing canines, increased laryngeal adductor force was observed in a small group of MDSC-injected larynges. Three dogs underwent RLN injury and were separated into non-injection control, MDSC injection, and MDSC-enhanced injection as defined by use of acetylcholine chloride to increase motor end plate conduction. Compared to pre-injury, the non-injected control demonstrated adduction force measurements 60% of baseline, while the MDSC-injected group and MDSC-enhanced injection group demonstrated 98% and 128% adductor force measurements respectively[13]. While swallowing function was not directly measured in any of these studies, clinical experience links laryngeal adductor function with prevention of aspiration during swallowing in a subset of patients.

STEM CELL THERAPIES FOR TONGUE DYSFUNCTION

Several groups have attempted stem cell therapies in the tongue intended to improve swallowing following treatment for head and neck cancer. Current treatments have not reliably improved swallow function in patients who have undergone partial and total glossectomy, which remain mainstay for treatment of cancer of the tongue. Post-surgical outcomes have revealed higher incidence of inadequate tongue control, inadequate chewing, delayed oral transit time, aspiration or penetration, residue within the vallecula and piriform recesses, and inadequate laryngeal elevation after surgery[14], leading to varying degrees of dysphagia. The base of tongue is particularly critical for swallow function, with resection of only a quarter of the tongue base resulting in impaired ability to trigger the pharyngeal swallow[15,16]. Current treatment consists of reconstructive options including myocutaneous, pedicled, and free flaps to restore bulk to the tissue, albeit typically without innervation. The field lacks clear guidelines and research into optimal reconstructive strategies, and long-term functional outcomes are poor. In a multicenter retrospective study comparing long-term functional outcomes of total glossectomy patients with or without laryngectomy, Lin *et al*[17] report 55% gastrotomy tube dependence at last follow-up, similar to the 50% dependence found by Dziegielewski *et al*[18] and 71% dependence by Rihani *et al*[19] Clearly, newer approaches are needed which can more closely imitate native, normal tissue.

Among the first to investigate the use of muscle stem cell tissue engineering for tongue defects was Kim *et al*[20] in 2003, who described the use of a collagen-rich hydrogel populated with neonatal rat myoblasts for a partial glossectomy defect. Thirty rats underwent unilateral mucosal-sparing glossectomy, producing pockets which were then filled with either saline, collagen-rich hydrogel alone, or collagen-rich hydrogel with myoblasts. After six weeks, the group receiving the cell-containing

hydrogel demonstrated a statistically significant increase in tongue weight of the operated side compared with the control side. In contrast, the saline and hydrogel groups demonstrated loss of tongue weight. Histologically, this corresponded to islands of desmin-stain positive cells signaling presence and persistence of skeletal muscle with evidence of neovascularization and preliminary neurotization. Comparatively, the control group which received only isotonic sodium chloride demonstrated diffuse fibrosis and disorganized myofibril architecture, while the hydrogel group showed no evidence of new tissue formation.

Bunaprasert *et al*[21] in the same laboratory explored this hemiglossectomy-pocket approach further and reported similar findings. Sixty rats were separated into five groups: Hemiglossectomy alone, collagen gel implant alone, collagen gel implant with suspended myoblasts, myoblasts cultured for 7 d in a pre-molded collagen gel, and myoblasts cultured for 7 d in a collagen gel with 2% horse serum to promote muscle differentiation. All groups gained weight appropriately without statistically significant difference between them, up to 16 wk. However, those groups containing gel alone or scar alone resulted in significant scar formation and lack of new muscle formation, whereas myoblast-containing gel contained interdigitating new muscle fibers, as confirmed by desmin, and neuroma-like fibers as well. A semi-quantitative histology score was applied to rate scar tissue and muscle formation, with best results occurring for both the undifferentiated myoblast groups irrespective of collagen culture method. Differentiated myoblasts scored better than controls but worse than their undifferentiated counterparts, suggesting either a benefit to multipotency or a detriment of the differentiation medium containing horse serum.

The same laboratory then assessed the cellular persistence in the collagen-filled pockets by implanting myoblasts that were pre-labeled with membrane dyes[22]. At six weeks, myoblasts were identified in all 12 rats studied. Cells primarily remained within their treatment pocket, with limited migration into normal tissue. Treatment group tongues showed significantly less scar contracture, increased muscle diameter, and similar architecture to normal tongue compared to control. Markedly increased desmin positivity was also observed within the treatment group as opposed to control, and microscopy revealed myoblast proliferation and fusion into viable myotubes. Note that cells alone were not tested in this series of reports.

More recently, human cells have been investigated in immune-deficient rodents, taking a step closer to human translation. Kuhn *et al*[23] demonstrated persistence of human MDSCs injections in immune deficient mice. MDSC survival was assessed using *in vivo* imaging to visualize luciferase-transduced MDSCs. At 12 wk, the human MDSC group exhibited greater bioluminescence than immediately following injection, and increased weight gain compared to mice receiving saline injections only. Separately, Vahabzadeh *et al*[24] performed partial glossectomy in 18 athymic rats. After two weeks for scar formation to mimic a tumor resection scenario, animals were treated with either low- or high-dose mesenchymal stem cell (MSC) or saline control. Three weeks post-injection, researchers found significantly reduced cross sectional scar burden and pathologic score for inflammation and fibrosis in the high-dose MSC group (250000 cells injected) relative to controls. Low-dose MSC (70000 cells injected) did not differ from controls. These recent studies support human stem cell persistence and efficacy in xenograft cell injection models within immunosuppressed animals, supporting the concept of autologous human stem cell injection to avoid immune rejection.

In addition to studies looking at structural improvement using stem cells, one study evaluated functional improvement in one animal. Plowman *et al*[25] utilized a partially denervated ovine tongue model to study tongue force following injury and treatment. Two animals were denervated and injected, but only one could undergo eventual tongue force testing. A very high dose of 500 million GFP-labelled autologous MDSCs were injected into the tongues one month after bilateral hypoglossal nerve crush; histology and tongue force recordings were collected 2 mo post injection. Researchers found GFP+ muscle fibers in both tongues, indicating MDSC survival at 2 mo. They also noted increased muscle fiber diameter in GFP+ fibers suggesting reduced atrophy and even increased diameter compared to pre-injury, suggesting new muscle growth. Regarding functional improvement, the authors reported a 27% increase in maximal tongue force and a 54% increase in base of tongue pressure compared to pre-injection in the one animal tested[25]. Clearly, such a small sample size warrants further investigation to obtain more robust results. Nevertheless, this novel denervation model and functional analysis indicating not only anatomical but also improvement in force generation shows promise for further research. Whether reduced tongue atrophy and increased forced production translates into improved swallowing function, remains to be seen.

STEM CELL THERAPIES FOR XEROSTOMIA

Dysfunctional salivary glands prove to be another major etiology of dysphagia. Saliva plays an important role in swallowing, serving to solubilize food substance, form a solid bolus by mixing with food particles, add moisture, and facilitate lubrication of the mucosa during bolus propulsion, among its other vital functions[26]. Patients with dry mouth, also known as xerostomia, due to insufficient saliva production, often report extreme difficulty in swallowing. Causes of dysfunctional salivary glands are numerous and include but are not limited to: Medications, radiation therapy for head and neck cancers, resection of salivary glands, Sjögren's syndrome, and other autoimmune disease. Regardless of cause, the symptom impacts many Americans, with a prevalence ranging from 5.5% to 46%[27]. Current therapies consist of sialogogues such as pilocarpine or cevimeline which stimulate secretion of saliva *via* binding of muscarinic receptors, although these carry their respective side effect profiles and more importantly, depend on intact functional glandular tissue to be effective. Other remedies include intraoral electrostimulation and acupuncture. However, there has not been sufficient research to confirm these as viable treatment options[27].

A number of groups have explored the use of stem cells for xerostomia, although these are still in early stages of investigation. Lombaert *et al*[28] described an approach towards the restoration of salivary gland function by using salivary gland stem cells cultured *in vitro* then transplanted into irradiated glands. Cells isolated from murine submandibular glands and cultured *in vitro* were injected in low numbers into irradiated glands. Researchers found close to 20% more surface area of acinar cells per gland compared to the untreated irradiated group. Functionally, they observed a 42% increase in saliva production in the cell injection group compared to untreated irradiated glands[28].

Likewise, Nanduri *et al*[29] found improved saliva production (54.59% *vs* 21.5%) in intra-glandularly injected cells *vs* irradiated control in addition to improved tissue healing, as indicated by persistence of ductal stem cell markers and revascularization. These novel studies demonstrated much promise into the therapeutic potential of regenerative medicine for restoration of salivary gland function, but knowledge regarding salivary gland stem cell behavior and characteristics was still lacking. Notably, only general stem cell markers such Sca-1, c-Kit, and Musashi-1 were used to identify the cells of interest, but a specific salivary gland stem cell marker was unable to be identified, making it difficult to track activity and persistence over time[30,31]. Furthermore, these studies were unable to maintain cells in prolonged culture, showing limited self-renewal capacity *in vitro* that serves as an additional barrier for further research.

To this end, Ikeura *et al*[32] successfully cultured cells derived from wild type murine submandibular glands for over 80 passages while maintaining their proliferative ability and morphology. Cells were isolated and cultured in low calcium, serum-free growth media with the addition of cholera toxin and epithelial growth factor to inhibit fibroblast growth and stimulate an epithelial phenotype. Notably, at passages 20, 50 and even 80, their cells did not form aggregates and salivary spheres, implying lack of cellular transformation and maintenance of a basal ductal cell phenotype which expressed keratin 14, 18 and p63, markers for basal, ductal and basal duct cells respectively[32]. This opens up exciting new avenues for research into stem cell regeneration of damaged salivary gland tissue, as researchers are now more capable of closely mimicking *in vivo* salivary gland cellular phenotypes for further study rather than relying on immortalized tumor-derived cell lines which may not behave similarly to normal cells.

Dai *et al*[33] described a different approach, whereby they demonstrated the potential for adipose tissue-derived stem cells (ADSCs) to differentiate into acinar-like epithelium capable of secreting α-amylase and expressing AQP-5. ADSCs are multipotent and may provide the advantage of differentiation into different components of salivary gland epithelium, both secretory and ductal, in addition to providing local trophic effects to improve tissue healing response following injury[34]. Platelet-rich fibrin was co-administered to promote transdifferentiating into an acinar-like phenotype and was found to increase levels of both α-amylase and AQP-5. Still, a unique and reliable marker for salivary gland stem cells remains to be identified and continues to be an active area of research.

Clinically, these promising initial results have been translated into a landmark randomized, placebo-controlled trial in Denmark (MESRIX trial). Grønhøj *et al*[35] randomized 30 patients to receive either ultrasound-guided injection of autologous ADSCs or placebo in submandibular glands after radiation therapy for HPV-positive

squamous cell carcinoma of the oropharynx. Cell doses administered were not clearly noted, although a planned dose of 2.8 million cells per volumetric milliliter of the gland was stated; total cell numbers harvested were between 13 and 82 million cells. No adverse events were reported. At both one and four months, patients within the ADSC arm displayed significantly increased unstimulated salivary flow rates compared to placebo, with 33% improvement over baseline at one month and 50% improvement at four months. In the placebo arm, salivary flow rates decreased remained largely unchanged. Patient-centered questionnaires revealed markedly decreased symptom reports from the ADSC group compared to placebo, reporting less difficulty within the domains of thirst and eating solid foods. Biopsies revealed increases in serous gland tissue and decrease in fibrosis and fatty deposits within the ADSC arm compared to placebo[35]. While long-term follow up is required and ongoing[36], the findings of this initial trial are exciting. Meanwhile, a clinical trial of allogeneic mesenchymal stem cell transplantation, MESRIX-SAFE[37], is currently recruiting participants.

STEM CELL TREATMENT FOR CRICOPHARYNGEAL DYSFUNCTION

Further along the alimentary canal, food must travel past the UES to enter the esophagus. As mentioned before, relaxation of the UES to allow food passage largely depends upon cricopharyngeus muscle (CP) relaxation. Several disorders impair this function, leading to oropharyngeal dysphagia and food trapped upstream. The pressure buildup may eventually lead to complications such as outpouching of the mucosa, known as Zenker's diverticulum. One common etiology of CP dysfunction includes stroke, one of the leading causes of dysphagia. A study by Yang *et al*[38] utilizing videofluoroscopic imaging to assess swallowing function in patients suffering from dysphagia due to stroke found an incidence of CP dysfunction to be 5.7% among first-time ischemic stroke patients. This incidence increased to 14.3% among those with infratentorial lesions and decreased to 2.8% among those with supratentorial lesions, suggesting focal lesions, primarily to the dorsal motor nucleus of the vagus, to be the primary culprit[38]. These lesions are typical of lateral medullary syndrome. Other causes include radiation for head and neck cancer, iatrogenic nerve injury, and oculopharyngeal muscular dystrophy (OPMD); many patients have idiopathic dysfunction. Current treatment consists of swallowing therapy, balloon dilatation, botulinum toxin injection, and surgical CP myotomy[39,40]. However, although effective, these are often temporary measures and have high rates of recurrence.

Current literature surrounding the use of muscle stem cells to repair cricopharyngeal dysfunction is sparse. A study published in 2014 by Périé *et al*[41] described a clinical pilot study, whereby 12 patients with OPMD and indication for CP myotomy were injected with a median of 178 million myoblasts at various sites of pharyngeal constrictors following CP myotomy. Pharyngeal propulsion, as measured by videoendoscopy and videofluoroscopy remained largely unchanged at both 12 mo and 24 mo. UES function, defined by quality of UES opening and closure and pooling within the hypopharynx, improved in 6/12 patients at 24 mo using videoendoscopy but only in 2/12 patients at 24 mo using the more sensitive videofluoroscopy. Of note, investigators did not elaborate on the discrepancy between endoscopy *vs* fluoroscopy results and specify scoring mechanisms for assessing function *via* these two visualization modalities. Swallowing function as assessed using the Salassa score and McHorney score improved at both time points. Finally, average time to swallow 80 mL of water decreased from 23.7 s to 10.2 s[41]. It is important to highlight the lack of a control group within the study, since all 12 patients received myoblast injection following CP myotomy, making it difficult to compare to improvement had patients received CP myotomy alone. Furthermore, small scarifications were induced with a scalpel during surgery to induce muscle regeneration. The effects of these small injuries may or may not have affected the end outcomes of pharyngeal function. Lastly, the discrepancy between videoendoscopy and videofluoroscopy scores weaken the overall body of evidence. Further study with larger sample size would improve statistical power and warrants investigation following this proof of principle research.

STEM CELL THERAPIES FOR DYSPHAGIA IN THE SETTING OF STROKE

Post-stroke dysphagia is a very common morbidity affecting up to 50% of cerebrovascular accident patients at 6 mo after the initial insult[42]. Etiology is multi-

factorial including deficits in mastication, oropharyngeal bolus propulsion due to muscle atrophy[43], cricopharyngeal dysfunction, and perhaps most importantly, neurologic coordination of the precisely-timed swallow cascade. With the global neurologic function in mind, SC treatment for ischemic brain injury could have significant benefits on post-stroke swallowing. This is an active area of research and has primarily focused on neuron regeneration and structural improvement within the cortex. Multiple human clinical trials of SC transplantation have been conducted that provide evidence of safety, however the evidence of efficacy in these trials remains lacking[44-48]. Stem cell transplantation methods include direct injection into infarcted brain parenchyma, infusion through intravenous and intra-arterial vasculature, and infusion into cerebrospinal fluid through lumbar puncture. Implanted neural stem cells migrate to ischemic cortex and impart benefits including stimulating endogenous neurogenesis[49,50], differentiating into functional neurons and astrocytes, inhibiting inflammation and glial scar formation[51,52], and becoming neuroprotective if injected during acute ischemic injury[53]. Functional improvement has been limited to memory tests and improvement in skeletal muscle coordination within animals[54] and NIH Stroke Scale in humans, neither of which directly test dysphagia outcomes following neural stem cell transplantation[44-47]. While improvement of coordination is encouraging, extrapolation of gross movements of the extremities cannot be reliably transferred to improvements of fine deglutition muscle coordination and functional improvement.

STEM CELL TREATMENT FOR ESOPHAGEAL REPAIR

Esophageal disorders represent a large proportion of cases underlying dysphagia. Etiologies are numerous, ranging from obstructive lesions such as strictures, webs, and tumors, to gastroesophageal reflux disease (GERD), eosinophilic esophagitis, achalasia, autoimmune causes such as Sjögren's syndrome, systemic lupus erythematous, and systemic sclerosis as part of the CREST syndrome, medications, and neurological diseases[55,56]. As such, management is extremely varied based upon underlying pathophysiology and is beyond the scope of this review. It is worth noting the use of stem cells to manage esophageal dysphagia has not been studied extensively, perhaps due to difficulty in isolating targeted points of intervention given the broad range of conditions which can lead to esophageal dysfunction. In cases of autoimmune or neurologic etiologies for example, management of the primary disease likely represents a more effective strategy than stem cell-based therapies. For more primary causes of esophageal dysphagia, however, such as inherent dysmotility, achalasia, or even treatment refractory GERD, stem cell therapy may fill a niche yet to be occupied. Given the lack of literature surrounding this topic and the substantial decrease in quality of life for patients suffering from esophageal motility disorders, this may represent a highly beneficial avenue of study in the future.

CHALLENGES

It is important to realize that despite many of these recent advancements in stem cell therapy for treatment of dysphagia (Table 1), the overwhelming majority of research remains in the basic and translational stage. The behavior of multipotent cells following injection into native tissue continues to be a point of discussion for researchers studying regenerative medicine. Although many of the above studies correlate persistence of cells as evidence of successful engraftment, studies in other organ systems such as cardiac tissue have found benefit despite cell elimination. Davani *et al*[57] and Iso *et al*[58] both demonstrated *in vivo* improvements in cardiac function following MSC injection, even without long-term engraftment and differentiation. When compared to injection with pure cardiac progenitor cells (CPCs), combination CPCs with MSCs actually improved cardiac ejection fraction more than CPC injection alone[59]. Particularly for the case of multipotent mesenchymal stem cells, it is thought apoptosis of these cells actually plays an important immunomodulatory role during tissue repair, exhibiting paracrine immunomodulation to promote tissue regeneration[60]. Our lack of understanding regarding the direct mechanisms whereby stem cells promote tissue healing represents a significant roadblock towards widespread stem cell therapy in the near future.

Moreover, it is difficult to translate structural outcome measurements such as increased tongue mass and reinnervation into actual functional improvement in

Table 1 Recent advances in targeted stem cell therapy for oropharyngeal dysphagia

Ref.	Sample, *n*	Main findings
Tongue dysfunction		
Kim *et al*[20] (2003)	30 rats	Myoblast-containing collagen hydrogel group demonstrated statistically significant increase in tongue weight and desmin-positive cells at the surgery site
Bunaprasert *et al*[21] (2003)	60 rats	Myoblast-containing gel group showed interdigitating new muscle and neuroma-like fibers, with less scar tissue formation
Luxameechanporn *et al*[22] (2006)	12 rats	Six weeks post surgery, myoblasts persisted in all 12 rats, had limited migration, showed less scar contracture, and had increased muscle diameter
Kuhn *et al*[23] (2017)	16 mice	Human MDSCs injected into a hemiglossectomy model survived to the endpoint demonstrating 132% bioluminescence compared to 15% in control groups, and exhibited the greatest weight gain among all groups
Vahabzadeh *et al*[24] (2018)	18 athymic rats	Three weeks post MSC injection following partial glossectomy, rats which received MSC treatment were found to have reduced x-sectional scar burden and pathologic scores for inflammation and fibrosis
Plowman *et al*[25] (2014)	1 ovine	MDSCs survived 2 mo following bilateral hypoglossal nerve crush, and demonstrated increased muscle fiber diameter, 27% increase in maximal tongue force, and 54% increase in base of tongue pressure compared to pre cell injection
Xerostomia		
Lombaert *et al*[28] (2008)	13 mice	Salivary glands cultured *in vitro* then injected into irradiated glands demonstrated increased surface area of acinar cells and 42% increase in saliva production compared to untreated irradiated group
Nanduri *et al*[29] (2013)	15 mice	54.59% increase in salivary gland function following intraglandular injection of salisphere-derived c-kit positive cells in irradiated mice, compared to 21.5% in untreated mice
Ikeura *et al*[32] (2016)		Salivary gland basal and ductal cells isolated form submandibular glands were successfully cultured for over 80 passages while maintaining their proliferative ability and morphology
Dai *et al*[33] (2019)		ADSCs demonstrated the ability to differentiate into acinar-like epithelium capable of secreting α-amylase and expressing AQP-5
Grønhøj *et al*[35] (2018)	30 patients	Patients who received ADSC injection in submandibular glands demonstrated 50% improved unstimulated salivary flow rates at four months, markedly decreased symptom reports, increase in serous glands, and decreased fibrosis and fatty deposit on biopsies
Cricopharyngeal dysfunction		
Périé *et al*[41] (2014)	12 patients	6/12 patients by videoendoscopy but only 2/12 patients by videofluoroscopy demonstrated improved UES function following myoblast injection and CP myotomy. Swallowing function improved in all patients subjectively and mean swallowing time decreased in all patients from 23.7 s to 10.2 s

MDSC: Muscle-derived stem cell; MSC: Mesenchymal stem cell; ADSC: Adipose tissue-derived stem cells; UES: Upper esophageal sphincter; CP: Cricopharyngeus muscle.

swallowing. The few studies that do attempt to assess functional measurements, such as videofluoroscopy analysis, are limited by small sample sizes. A large portion of this challenge stems from the incredible complexity and coordination needed for swallowing. The interplay between different components of a swallow may mean recovery of one structure does not translate into recovery of swallowing function as a whole. It is reasonable to infer, particularly in cases of denervation injury, that physical therapy will remain an essential component of therapy to relearn swallowing and achieve coordination between a regenerated tissue and native tissue.

CONCLUSION

Dysphagia is a highly prevalent yet challenging disorder to treat, particularly among patients who lack native, functional tissue upon which many conventional treatments exert their effects. In these patients, more novel therapies in the form of stem cell implantation may represent a new avenue to pursue to improve swallowing function. Initial research targeting different domains of swallowing have shown promise in restoring both structure and function. Large strides have been made in animal studies involving tongue dysfunction and xerostomia, with comparatively fewer studies about pharyngeal, esophageal, and neuromuscular etiologies of dysphagia. It is important to realize this field of research is still relatively young and far from becoming a part of standard clinical practice, with very few studies progressing into human clinical trials. Nevertheless, it represents an exciting new area of study with promising initial results

and is worth pursuing for its large potential to improve dysphagia in difficult to treat patients.

REFERENCES

1 **Bhattacharyya N**. The prevalence of dysphagia among adults in the United States. *Otolaryngol Head Neck Surg* 2014; **151**: 765-769 [PMID: 25193514 DOI: 10.1177/0194599814549156]
2 **Steele CM**, Greenwood C, Ens I, Robertson C, Seidman-Carlson R. Mealtime difficulties in a home for the aged: not just dysphagia. *Dysphagia* 1997; **12**: 43-50; discussion 51 [PMID: 8997832 DOI: 10.1007/pl00009517]
3 **Langmore SE**. Efficacy of behavioral treatment for oropharyngeal dysphagia. *Dysphagia* 1995; **10**: 259-262 [PMID: 7493507 DOI: 10.1007/bf00431419]
4 **Logemann JA**. Oropharyngeal dysphagia and nutritional management. *Curr Opin Clin Nutr Metab Care* 2007; **10**: 611-614 [PMID: 17693745 DOI: 10.1097/MCO.0b013e328285d872]
5 **Robbins J**, Kays SA, Gangnon RE, Hind JA, Hewitt AL, Gentry LR, Taylor AJ. The effects of lingual exercise in stroke patients with dysphagia. *Arch Phys Med Rehabil* 2007; **88**: 150-158 [PMID: 17270511 DOI: 10.1016/j.apmr.2006.11.002]
6 **Chhetri D**, Dewan K. Dysphagia Evaluation and Management in Otolaryngology. Elsevier; 2019 [DOI: 10.1016/C2017-0-01118-7]
7 **Matsuo K**, Palmer JB. Anatomy and physiology of feeding and swallowing: normal and abnormal. *Phys Med Rehabil Clin N Am* 2008; **19**: 691-707, vii [PMID: 18940636 DOI: 10.1016/j.pmr.2008.06.001]
8 **Speyer R**, Baijens L, Heijnen M, Zwijnenberg I. Effects of therapy in oropharyngeal dysphagia by speech and language therapists: a systematic review. *Dysphagia* 2010; **25**: 40-65 [PMID: 19760458 DOI: 10.1007/s00455-009-9239-7]
9 **Pitts T**. Airway protective mechanisms. *Lung* 2014; **192**: 27-31 [PMID: 24297325 DOI: 10.1007/s00408-013-9540-y]
10 **Halum SL**, Naidu M, Delo DM, Atala A, Hingtgen CM. Injection of autologous muscle stem cells (myoblasts) for the treatment of vocal fold paralysis: a pilot study. *Laryngoscope* 2007; **117**: 917-922 [PMID: 17473696 DOI: 10.1097/MLG.0b013e31803e8c8d]
11 **Halum SL**, Hiatt KK, Naidu M, Sufyan AS, Clapp DW. Optimization of autologous muscle stem cell survival in the denervated hemilarynx. *Laryngoscope* 2008; **118**: 1308-1312 [PMID: 18401272 DOI: 10.1097/MLG.0b013e31816c438e]
12 **Halum SL**, McRae B, Bijangi-Vishehsaraei K, Hiatt K. Neurotrophic factor-secreting autologous muscle stem cell therapy for the treatment of laryngeal denervation injury. *Laryngoscope* 2012; **122**: 2482-2496 [PMID: 22965802 DOI: 10.1002/lary.23519]
13 **Paniello RC**, Brookes S, Bhatt NK, Bijangi-Vishehsaraei K, Zhang H, Halum S. Improved adductor function after canine recurrent laryngeal nerve injury and repair using muscle progenitor cells. *Laryngoscope* 2018; **128**: E241-E246 [PMID: 29219186 DOI: 10.1002/lary.26992]
14 **Son YR**, Choi KH, Kim TG. Dysphagia in tongue cancer patients. *Ann Rehabil Med* 2015; **39**: 210-217 [PMID: 25932417 DOI: 10.5535/arm.2015.39.2.210]
15 **Pauloski BR**, Logemann JA, Rademaker AW, McConnel FM, Heiser MA, Cardinale S, Shedd D, Lewin J, Baker SR, Graner D. Speech and swallowing function after anterior tongue and floor of mouth resection with distal flap reconstruction. *J Speech Hear Res* 1993; **36**: 267-276 [PMID: 8487519 DOI: 10.1044/jshr.3602.267]
16 **Zuydam AC**, Lowe D, Brown JS, Vaughan ED, Rogers SN. Predictors of speech and swallowing function following primary surgery for oral and oropharyngeal cancer. *Clin Otolaryngol* 2005; **30**: 428-437 [PMID: 16232247 DOI: 10.1111/j.1365-2273.2005.01061.x]
17 **Lin DT**, Yarlagadda BB, Sethi RK, Feng AL, Shnayder Y, Ledgerwood LG, Diaz JA, Sinha P, Hanasono MM, Yu P, Skoracki RJ, Lian TS, Patel UA, Leibowitz J, Purdy N, Starmer H, Richmon JD. Long-term Functional Outcomes of Total Glossectomy With or Without Total Laryngectomy. *JAMA Otolaryngol Head Neck Surg* 2015; **141**: 797-803 [PMID: 26291031 DOI: 10.1001/jamaoto.2015.1463]
18 **Dziegielewski PT**, Ho ML, Rieger J, Singh P, Langille M, Harris JR, Seikaly H. Total glossectomy with laryngeal preservation and free flap reconstruction: objective functional outcomes and systematic review of the literature. *Laryngoscope* 2013; **123**: 140-145 [PMID: 22952109 DOI: 10.1002/lary.23505]
19 **Rihani J**, Lee MR, Lee T, Ducic Y. Flap selection and functional outcomes in total glossectomy with laryngeal preservation. *Otolaryngol Head Neck Surg* 2013; **149**: 547-553 [PMID: 23884285 DOI: 10.1177/0194599813498063]
20 **Kim J**, Hadlock T, Cheney M, Varvares M, Marler J. Muscle tissue engineering for partial glossectomy defects. *Arch Facial Plast Surg* 2003; **5**: 403-407 [PMID: 12975138 DOI: 10.1001/archfaci.5.5.403]
21 **Bunaprasert T**, Hadlock T, Marler J, Kobler J, Cowan D, Faquin W, Varvares M. Tissue engineered muscle implantation for tongue reconstruction: a preliminary report. *Laryngoscope* 2003; **113**: 1792-1797 [PMID: 14520108 DOI: 10.1097/00005537-200310000-00025]
22 **Luxameechanporn T**, Hadlock T, Shyu J, Cowan D, Faquin W, Varvares M. Successful myoblast transplantation in rat tongue reconstruction. *Head Neck* 2006; **28**: 517-524 [PMID: 16619280 DOI: 10.1002/hed.20325]
23 **Kuhn MA**, Black AB, Siddiqui MT, Nolta JA, Belafsky PC. Novel murine xenograft model for the evaluation of stem cell therapy for profound dysphagia. *Laryngoscope* 2017; **127**: E359-E363 [PMID: 28543406 DOI: 10.1002/lary.26666]
24 **Vahabzadeh-Hagh AM**, Goel AN, Frederick JW, Berke GS, Long JL. Transplanted human multipotent stromal cells reduce acute tongue fibrosis in rats. *Laryngoscope Investig Otolaryngol* 2018; **3**: 450-456 [PMID: 30599029 DOI: 10.1002/lio2.202]
25 **Plowman EK**, Bijangi-Vishehsaraei K, Halum S, Cates D, Hanenberg H, Domer AS, Nolta JA, Belafsky PC.

Autologous myoblasts attenuate atrophy and improve tongue force in a denervated tongue model: a pilot study. *Laryngoscope* 2014; **124**: E20-E26 [PMID: 23929623 DOI: 10.1002/lary.24352]
26 **Pedersen AM**, Bardow A, Jensen SB, Nauntofte B. Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. *Oral Dis* 2002; **8**: 117-129 [PMID: 12108756 DOI: 10.1034/j.1601-0825.2002.02851.x]
27 **Villa A**, Connell CL, Abati S. Diagnosis and management of xerostomia and hyposalivation. *Ther Clin Risk Manag* 2015; **11**: 45-51 [PMID: 25653532 DOI: 10.2147/TCRM.S76282]
28 **Lombaert IM**, Brunsting JF, Wierenga PK, Faber H, Stokman MA, Kok T, Visser WH, Kampinga HH, de Haan G, Coppes RP. Rescue of salivary gland function after stem cell transplantation in irradiated glands. *PLoS One* 2008; **3**: e2063 [PMID: 18446241 DOI: 10.1371/journal.pone.0002063]
29 **Nanduri LS**, Lombaert IM, van der Zwaag M, Faber H, Brunsting JF, van Os RP, Coppes RP. Salisphere derived c-Kit+ cell transplantation restores tissue homeostasis in irradiated salivary gland. *Radiother Oncol* 2013; **108**: 458-463 [PMID: 23769181 DOI: 10.1016/j.radonc.2013.05.020]
30 **Min S**, Song EC, Oyelakin A, Gluck C, Smalley K, Romano RA. Functional characterization and genomic studies of a novel murine submandibular gland epithelial cell line. *PLoS One* 2018; **13**: e0192775 [PMID: 29462154 DOI: 10.1371/journal.pone.0192775]
31 **Coppes RP**, Stokman MA. Stem cells and the repair of radiation-induced salivary gland damage. *Oral Dis* 2011; **17**: 143-153 [PMID: 20796229 DOI: 10.1111/j.1601-0825.2010.01723.x]
32 **Ikeura K**, Kawakita T, Tsunoda K, Nakagawa T, Tsubota K. Characterization of Long-Term Cultured Murine Submandibular Gland Epithelial Cells. *PLoS One* 2016; **11**: e0147407 [PMID: 26800086 DOI: 10.1371/journal.pone.0147407]
33 **Dai TQ**, Zhang LL, An Y, Xu FF, An R, Xu HY, Liu YP, Liu B. In vitro transdifferentiation of adipose tissue-derived stem cells into salivary gland acinar-like cells. *Am J Transl Res* 2019; **11**: 2908-2924 [PMID: 31217863]
34 **Fu Y**, Karbaat L, Wu L, Leijten J, Both SK, Karperien M. Trophic Effects of Mesenchymal Stem Cells in Tissue Regeneration. *Tissue Eng Part B Rev* 2017; **23**: 515-528 [PMID: 28490258 DOI: 10.1089/ten.TEB.2016.0365]
35 **Grønhøj C**, Jensen DH, Vester-Glowinski P, Jensen SB, Bardow A, Oliveri RS, Fog LM, Specht L, Thomsen C, Darkner S, Jensen M, Müller V, Kiss K, Agander T, Andersen E, Fischer-Nielsen A, von Buchwald C. Safety and Efficacy of Mesenchymal Stem Cells for Radiation-Induced Xerostomia: A Randomized, Placebo-Controlled Phase 1/2 Trial (MESRIX). *Int J Radiat Oncol Biol Phys* 2018; **101**: 581-592 [PMID: 29678523 DOI: 10.1016/j.ijrobp.2018.02.034]
36 **Rigshospitalet, Denmark**. Long-term Follow-up Study of Mesenchymal Stem Cells for Radiation-induced Xerostomia (MESRIX). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: https://ClinicalTrials.gov/show/NCT03876197 NLM Identifier: NCT03876197
37 **Rigshospitalet, Denmark**. Allogeneic Mesenchymal Stem Cells for Radiation-induced Hyposalivation and Xerostomia/Dry Mouth. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). Available from: https://ClinicalTrials.gov/show/NCT03874572 NLM Identifier: NCT03874572
38 **Yang H**, Yi Y, Han Y, Kim HJ. Characteristics of Cricopharyngeal Dysphagia After Ischemic Stroke. *Ann Rehabil Med* 2018; **42**: 204-212 [PMID: 29765873 DOI: 10.5535/arm.2018.42.2.204]
39 **Bergeron JL**, Chhetri DK. Indications and outcomes of endoscopic CO2 laser cricopharyngeal myotomy. *Laryngoscope* 2014; **124**: 950-954 [PMID: 24114581 DOI: 10.1002/lary.24415]
40 **Coiffier L**, Périé S, Laforêt P, Eymard B, St Guily JL. Long-term results of cricopharyngeal myotomy in oculopharyngeal muscular dystrophy. *Otolaryngol Head Neck Surg* 2006; **135**: 218-222 [PMID: 16890071 DOI: 10.1016/j.otohns.2006.03.015]
41 **Périé S**, Trollet C, Mouly V, Vanneaux V, Mamchaoui K, Bouazza B, Marolleau JP, Laforêt P, Chapon F, Eymard B, Butler-Browne G, Larghero J, St Guily JL. Autologous myoblast transplantation for oculopharyngeal muscular dystrophy: a phase I/IIa clinical study. *Mol Ther* 2014; **22**: 219-225 [PMID: 23831596 DOI: 10.1038/mt.2013.155]
42 **Mann G**, Hankey GJ, Cameron D. Swallowing function after stroke: prognosis and prognostic factors at 6 months. *Stroke* 1999; **30**: 744-748 [PMID: 10187872 DOI: 10.1161/01.str.30.4.744]
43 **Schimmel M**, Ono T, Lam OL, Müller F. Oro-facial impairment in stroke patients. *J Oral Rehabil* 2017; **44**: 313-326 [PMID: 28128465 DOI: 10.1111/joor.12486]
44 **Steinberg GK**, Kondziolka D, Wechsler LR, Lunsford LD, Coburn ML, Billigen JB, Kim AS, Johnson JN, Bates D, King B, Case C, McGrogan M, Yankee EW, Schwartz NE. Clinical Outcomes of Transplanted Modified Bone Marrow-Derived Mesenchymal Stem Cells in Stroke: A Phase 1/2a Study. *Stroke* 2016; **47**: 1817-1824 [PMID: 27256670 DOI: 10.1161/STROKEAHA.116.012995]
45 **Kalladka D**, Sinden J, Pollock K, Haig C, McLean J, Smith W, McConnachie A, Santosh C, Bath PM, Dunn L, Muir KW. Human neural stem cells in patients with chronic ischaemic stroke (PISCES): a phase 1, first-in-man study. *Lancet* 2016; **388**: 787-796 [PMID: 27497862 DOI: 10.1016/S0140-6736(16)30513-X]
46 **Prasad K**, Sharma A, Garg A, Mohanty S, Bhatnagar S, Johri S, Singh KK, Nair V, Sarkar RS, Gorthi SP, Hassan KM, Prabhakar S, Marwaha N, Khandelwal N, Misra UK, Kalita J, Nityanand S; InveST Study Group. Intravenous autologous bone marrow mononuclear stem cell therapy for ischemic stroke: a multicentric, randomized trial. *Stroke* 2014; **45**: 3618-3624 [PMID: 25378424 DOI: 10.1161/STROKEAHA.114.007028]
47 **Hess DC**, Wechsler LR, Clark WM, Savitz SI, Ford GA, Chiu D, Yavagal DR, Uchino K, Liebeskind DS, Auchus AP, Sen S, Sila CA, Vest JD, Mays RW. Safety and efficacy of multipotent adult progenitor cells in acute ischaemic stroke (MASTERS): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Neurol* 2017; **16**: 360-368 [PMID: 28320635 DOI: 10.1016/S1474-4422(17)30046-7]
48 **Nagpal A**, Choy FC, Howell S, Hillier S, Chan F, Hamilton-Bruce MA, Koblar SA. Safety and effectiveness of stem cell therapies in early-phase clinical trials in stroke: a systematic review and meta-analysis. *Stem Cell Res Ther* 2017; **8**: 191 [PMID: 28854961 DOI: 10.1186/s13287-017-0643-x]
49 **Cheng Y**, Zhang J, Deng L, Johnson NR, Yu X, Zhang N, Lou T, Zhang Y, Wei X, Chen Z, He S, Li X, Xiao J. Intravenously delivered neural stem cells migrate into ischemic brain, differentiate and improve functional recovery after transient ischemic stroke in adult rats. *Int J Clin Exp Pathol* 2015; **8**: 2928-2936

[PMID: 26045801]

50 **Bernstock JD**, Peruzzotti-Jametti L, Ye D, Gessler FA, Maric D, Vicario N, Lee YJ, Pluchino S, Hallenbeck JM. Neural stem cell transplantation in ischemic stroke: A role for preconditioning and cellular engineering. *J Cereb Blood Flow Metab* 2017; **37**: 2314-2319 [PMID: 28303738 DOI: 10.1177/0271678X17700432]

51 **Bacigaluppi M**, Russo GL, Peruzzotti-Jametti L, Rossi S, Sandrone S, Butti E, De Ceglia R, Bergamaschi A, Motta C, Gallizioli M, Studer V, Colombo E, Farina C, Comi G, Politi LS, Muzio L, Villani C, Invernizzi RW, Hermann DM, Centonze D, Martino G. Neural Stem Cell Transplantation Induces Stroke Recovery by Upregulating Glutamate Transporter GLT-1 in Astrocytes. *J Neurosci* 2016; **36**: 10529-10544 [PMID: 27733606 DOI: 10.1523/JNEUROSCI.1643-16.2016]

52 **Hou B**, Ma J, Guo X, Ju F, Gao J, Wang D, Liu J, Li X, Zhang S, Ren H. Exogenous Neural Stem Cells Transplantation as a Potential Therapy for Photothrombotic Ischemia Stroke in Kunming Mice Model. *Mol Neurobiol* 2017; **54**: 1254-1262 [PMID: 26820680 DOI: 10.1007/s12035-016-9740-6]

53 **Watanabe M**, Yavagal DR. Intra-arterial delivery of mesenchymal stem cells. *Brain Circ* 2016; **2**: 114-117 [PMID: 30276284 DOI: 10.4103/2394-8108.192522]

54 **Hosseini SM**, Farahmandnia M, Razi Z, Delavari S, Shakibajahromi B, Sarvestani FS, Kazemi S, Semsar M. Combination cell therapy with mesenchymal stem cells and neural stem cells for brain stroke in rats. *Int J Stem Cells* 2015; **8**: 99-105 [PMID: 26019759 DOI: 10.15283/ijsc.2015.8.1.99]

55 **Koch KL**, Bitar KN, Fortunato JE. Tissue engineering for neuromuscular disorders of the gastrointestinal tract. *World J Gastroenterol* 2012; **18**: 6918-6925 [PMID: 23322989 DOI: 10.3748/wjg.v18.i47.6918]

56 **Philpott H**, Garg M, Tomic D, Balasubramanian S, Sweis R. Dysphagia: Thinking outside the box. *World J Gastroenterol* 2017; **23**: 6942-6951 [PMID: 29097867 DOI: 10.3748/wjg.v23.i38.6942]

57 **Davani S**, Marandin A, Mersin N, Royer B, Kantelip B, Hervé P, Etievent JP, Kantelip JP. Mesenchymal progenitor cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a rat cellular cardiomyoplasty model. *Circulation* 2003; **108** Suppl 1: II253-II258 [PMID: 12970242 DOI: 10.1161/01.cir.0000089186.09692.fa]

58 **Iso Y**, Spees JL, Serrano C, Bakondi B, Pochampally R, Song YH, Sobel BE, Delafontaine P, Prockop DJ. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. *Biochem Biophys Res Commun* 2007; **354**: 700-706 [PMID: 17257581 DOI: 10.1016/j.bbrc.2007.01.045]

59 **Marbán E**, Malliaras K. Boot camp for mesenchymal stem cells. *J Am Coll Cardiol* 2010; **56**: 735-737 [PMID: 20723803 DOI: 10.1016/j.jacc.2010.02.064]

60 **Galleu A**, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, von Bonin M, Barbieri L, Halai K, Ward S, Weng L, Chakraverty R, Lombardi G, Watt FM, Orchard K, Marks DI, Apperley J, Bornhauser M, Walczak H, Bennett C, Dazzi F. Apoptosis in mesenchymal stromal cells induces *in vivo* recipient-mediated immunomodulation. *Sci Transl Med* 2017; 9 [PMID: 29141887 DOI: 10.1126/scitranslmed.aam7828]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 1013-1022

DOI: 10.4252/wjsc.v12.i9.1013

ISSN 1948-0210 (online)

MINIREVIEWS

Perspectives on mesenchymal stem/progenitor cells and their derivates as potential therapies for lung damage caused by COVID-19

Aleksandra Klimczak

ORCID number: Aleksandra Klimczak 0000-0001-6590-4420.

Author contributions: Klimczak A conceived the study, designed and wrote the manuscript.

Conflict-of-interest statement: The author declares that there is no conflict of interests regarding the publication of this paper.

Manuscript source: Invited manuscript

Received: June 12, 2020
Peer-review started: June 12, 2020
First decision: July 30, 2020
Revised: August 24, 2020

Aleksandra Klimczak, Laboratory of Biology of Stem and Neoplastic Cells, Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wrocław 53-114, Poland

Corresponding author: Aleksandra Klimczak, PhD, DSc, Full Professor, Laboratory of Biology of Stem and Neoplastic Cells, Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Rudolfa Weigla 12, Wrocław 53-114, Poland. aleksandra.klimczak@hirszfeld.pl

Abstract

The new coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which emerged in December 2019 in Wuhan, China, has reached worldwide pandemic proportions, causing coronavirus disease 2019 (COVID-19). The clinical manifestations of COVID-19 vary from an asymptomatic disease course to clinical symptoms of acute respiratory distress syndrome and severe pneumonia. The lungs are the primary organ affected by SARS-CoV-2, with a very slow turnover for renewal. SARS-CoV-2 enters the lungs *via* angiotensin-converting enzyme 2 receptors and induces an immune response with the accumulation of immunocompetent cells, causing a cytokine storm, which leads to target organ injury and subsequent dysfunction. To date, there is no effective antiviral therapy for COVID-19 patients, and therapeutic strategies are based on experience treating previously recognized coronaviruses. In search of new treatment modalities of COVID-19, cell-based therapy with mesenchymal stem cells (MSCs) and/or their secretome, such as soluble bioactive factors and extracellular vesicles, is considered supportive therapy for critically ill patients. Multipotent MSCs are able to differentiate into different types of cells of mesenchymal origin, including alveolar epithelial cells, lung epithelial cells, and vascular endothelial cells, which are severely damaged in the course of COVID-19 disease. Moreover, MSCs secrete a variety of bioactive factors that can be applied for respiratory tract regeneration in COVID-19 patients thanks to their trophic, anti-inflammatory, immunomodulatory, anti-apoptotic, pro-regenerative, and proangiogenic properties.

Key Words: Mesenchymal stem cells; Stem/progenitor cells; Lung damage; Mesenchymal stem cell secretome; COVID-19 disease; COVID-19 pneumonia

Accepted: September 1, 2020
Article in press: September 1, 2020
Published online: September 26, 2020

P-Reviewer: Long X
S-Editor: Wang DM
L-Editor: Filipodia
P-Editor: Xing YX

Core Tip: The new severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has reached pandemic proportions, causing coronavirus disease (COVID-19), which leads to severe pneumonia. The lungs are the primary organ affected by SARS-CoV-2, with a very slow turnover for renewal. SARS-CoV-2 enters the lungs and induces immune response with cytokine storm and subsequent organ dysfunction. To date, there is no effective antiviral therapy for COVID-19. Cell-based therapy involving mesenchymal stem cells and/or their secretome is considered a supportive therapy for critically ill COVID-19 patients. Mesenchymal stem cells can regenerate severely injured respiratory tract cells through their trophic, anti-inflammatory, and immunomodulatory properties.

Citation: Klimczak A. Perspectives on mesenchymal stem/progenitor cells and their derivates as potential therapies for lung damage caused by COVID-19. *World J Stem Cells* 2020; 12(9): 1013-1022
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/1013.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.1013

INTRODUCTION

The new virus, which emerged in December 2019 in Wuhan, China, was initially named coronavirus 2019-nCoV, and based on its phylogeny and taxonomy, was later renamed severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). It has reached worldwide pandemic proportions, causing coronavirus disease 2019 (COVID-19)[1,2]. As of 11 June 2020, the World Health Organization (WHO) Situation Report-143 states that COVID-19 has been confirmed globally in 7273958 patients and resulted in 413372 deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports).

The clinical manifestations of COVID-19 vary and include an asymptomatic disease course, acute respiratory disease, and pneumonia with different stages of severity. The asymptomatic disease course, without fever or respiratory or gastrointestinal symptoms, does not protect the patients from SARS-CoV-2 transmission, which is transmitted from person-to-person by direct contact[2,3]. Based on current observations, it is unknown whether asymptomatic patients recover without adverse organ damage, or if complications appear as the late effects of the disease in the future. Patients with clinical signs of acute respiratory disease have revealed fever and cough with no signs of pneumonia, and more than 30% of patients require oxygen therapy but not mechanical ventilation[4]. Uncommon gastrointestinal symptoms such as vomiting, nausea, and diarrhea have also been observed. The most severe symptoms of COVID-19 including fever, cough, headache, dyspnea, and sputum production have been observed in patients who developed pneumonia. Most of them (> 70%) have needed oxygen therapy, and almost 30% require mechanical ventilation[2,4]. Severely affected patients are usually older and have coexisting illnesses, including hypertension, chronic obstructive pulmonary disease, diabetes mellitus, and cardiovascular disease, which often lead to death[4-6]. Most patients with SARS-CoV-2 infection have a good clinical outcome and prognosis; however, in elderly patients (aged > 65 years) with comorbidities, severe complications can occur including acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, coagulation dysfunction, and multiple organ failure, causing an increased risk of death[5-8].

The basis of the pathogenesis of SARS-CoV-2 virus is binding of the S protein, expressed on the surface of the coronavirus, to angiotensin-converting enzyme 2 (ACE2) receptors[9-11], which are widely distributed on the surface of human cells, especially alveolar type II cells, and on the capillary endothelium of the lung[12]. Moreover, cellular transmembrane protease, serine 2 (TMPRSS2), which is abundantly expressed on alveolar cells, is essential for SARS-CoV-2 entry into target cells and spreading[13,14]. In addition, ACE2 receptors are present on the cells in different tissues and organs including the heart, liver, kidney, and gut. The SARS-CoV-2 virus uses the ACE2 receptor for entry and initiates fusion with the host cells, infecting them and inducing the immune response from the host's innate immune system[9]. The virus-induced immune response leads to the accumulation of immunocompetent cells, which produce a large number of proinflammatory cytokines, leading to target organ

damage, and consequently, fatal organ dysfunction. One of the first studies with COVID-19 patients reported that severely affected patients with pneumonia, in the acute phase of the disease, had high levels of proinflammatory cytokines and chemokines including interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1Ra), IL-7, IL-8, IL-9, IL-10, basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon-γ (IFN-γ), IFN-γ-induced protein 10 kDa, macrophage inflammatory protein-1 (MIP-1α), MIP-1β, and tumor necrosis factor-α (TNF-α)[6]. Activity of the innate immune response is necessary to contain and eliminate the virus infection; however, an out-of-control immune response leads to immunopathological changes.

Current therapies for COVID-19

To date, there are no effective therapies against COVID-19. To overcome this problem, global medical, scientific, pharma and funding groups have rapidly initiated more than 500 COVID-19 clinical trials based on currently available anti-viral drugs in various combinations[15]. Current antiviral therapies are based on experience in treatment strategies against the previously recognized SARS-CoV and MERS-CoV[16]. Remdesivir and chloroquine or hydroxychloroquine are commonly used to treat pneumonia in COVID-19 patients[17-20]. Other currently available potent antiviral agents and their combinations repurposed for COVID-19 treatment are also widely used[5,21,22]. New therapies include passive antibody transfer from the sera of convalescent patients[23,24] and blocking the ACE2 receptor by the serine protease TMPRSS2 inhibitor (approved for clinical use)[13].

The ACE2 receptor is not expressed in the bone marrow, lymph nodes, spleen, or on immune cells such as T and B lymphocytes and macrophages[12]. This biological feature of these cells suggests that immunotherapy can be used to treat severely infected patients. In search of new treatment modalities for COVID-19, early studies on cell-based therapy with mesenchymal stem cells (MSCs) have been employed as supportive treatment for critically ill patients[25]. Currently, a total of 28 trials exploring the potential of MSCs and their derivates for the treatment of critically ill COVID-19 patients have been approved and registered at the WHO International Clinical Trials Registry Platform www.clinicaltrials.gov[15], and more than 20 have been registered in the Chinese clinical trial registry (www.chictr.org.cn)[26].

LUNG STEM/PROGENITOR CELLS AND TISSUE HOMEOSTASIS

Most human tissues and organs, including the pulmonary tract, contain stem/progenitor cells responsible for the maintenance of tissue homeostasis[27]. The lung is a conditionally renewing organ, and in normal conditions, the turnover of airway epithelial cells is less than 1% per day, in contrast to other adult organs such as the skin, intestines, or bone marrow. However, severe damage increases the self-renewing capability of different types of endogenous epithelial stem/progenitor cells that reside in the lung and are important for regulation of the regeneration of damaged tissue[28]. The lung contains various types of epithelial cells that reside in several regions along the pulmonary airways.

Endogenous epithelial stem and progenitor cells in the adult lung are organized specifically according to their regional decomposition and functional activity along the proximal-distal axis of the pulmonary tract. The proximal part of the respiratory tract encompasses the cartilaginous trachea, lined by columnar pseudostratified epithelial cells, and different types of stem/progenitor cells with distinct roles in lung regeneration, including basal, secretory, ciliated, and neuroendocrine cells. The regenerative processes in the pulmonary tract involve local stem/progenitor cells, which are characterized by high proliferative activity during the perinatal period and a slow turnover during adulthood. In response to injury, a population of basal cells, which represent the stem/progenitor cells of the bronchiolar epithelium, migrate from the bronchiolar niche into the damaged alveolar epithelium and proliferate in order to repair the lung alveolar cells[28,29]. The distal part of the airways is lined by a columnar epithelium, and includes secretory club cells (also known as Clara cells) and populations of ciliated cells, goblet cells, and pulmonary neuroendocrine cells[28,29]. To maintain epithelial homeostasis, club cells are capable of self-renewal and can generate ciliated cells, whereas ciliated cells do not have self-regeneration capacity[29]. Another population of stem and progenitor cells residing in the distal airway, involved in epithelial homeostasis and regeneration, is a rare population of cells called the bronchioalveolar stem/progenitor cells with self-renewal potential. Their number

increases following bronchiolar damage, and these cells are able to differentiate into bronchiolar and alveolar colonies, thus contributing to tissue repair[28]. The terminal part of the airway tree is composed of alveoli containing specific alveolar progenitor cells, which differentiate into surfactant-producing alveolar epithelial cells type II and squamous gas-exchanging alveolar epithelial cells type I, responsible for the maintenance and restoration of the gas exchange units of the distal part of the pulmonary tract[28,29].

Mesenchymal stromal/stem cells residing in the lung constitute a key component supporting epithelial progenitor niches along the proximal-distal axis of the airway tree. The lung mesenchymal stromal/stem cells secrete a variety of bioactive factors, including FGF10, a critical trophic factor necessary for coordinating differentiation in the developing lung and supporting epithelial regeneration in steady-state conditions and after injury[29]. Moreover, lung mesenchymal stromal/stem cells modulate the local microenvironment *via* a paracrine-mediated anti-inflammatory effect and support the proliferation and differentiation of lung epithelial progenitor cells.

The different populations of endogenous stem and progenitor cells residing in distinct niches of the pulmonary tract contribute to region-specific epithelial cell repair, and the balance between the immune regulation and promotion of tissue regeneration ensures homeostasis of the lung[27].

BIOLOGICAL PROPERTIES OF MSCS IN TISSUE REGENERATION

MSCs are multipotent cells, which are able to differentiate into different types of cells of mesenchymal origin including alveolar epithelial cells, lung epithelial cells, and vascular endothelial cells[30,31]. MSCs are extensively studied for their clinical application in regenerative medicine due to their trophic, anti-inflammatory, and immunomodulatory properties[32,33]. The capability of MSCs to restore tissues is also accomplished through their ability to secrete a variety of bioactive proteins, including growth factors and chemokines, to induce the proliferation of tissue-resident progenitor cells and angiogenesis[33]. In response to inflammatory cytokines, such as IL-1, IL-2, IL-12, TNF-α, and IFN-γ secreted by immunocompetent cells, MSCs secrete a variety of growth factors and anti-inflammatory proteins including prostaglandin 2 (PGE 2), transforming growth factor-1β (TGF-β1), stromal-derived factor-1 (SDF-1), IL-4, IL-6, IL-10, and IL-1Ra[31]. Soluble factors secreted by the MSCs prevent the proliferation and function of many immunocompetent cells including T lymphocytes, B lymphocytes, natural killer cells, monocytes, macrophages, and dendritic cells. The immunomodulatory activity of MSCs involves decreasing the level of IFN-γ and increasing the level of IL-4 and IL-10, thus promoting a shift from T helper type 1 (Th1) to Th2 lymphocytes and a shift in macrophage balance from the M1 (proinflammatory) to M2 (anti-inflammatory) phenotype[31,34,35].

The trophic properties of MSCs are associated with the secretion of growth factors and chemokines, such as TGF-α, TGF-β, hepatocyte growth factor (HGF), epithelial growth factor (EGF), insulin-like growth factor 1, bFGF, vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and other bioactive factors involved in cell proliferation and angiogenesis, as confirmed by many studies[31,36] including research conducted by the author of this article[33,37,38].

The advantage of MSCs as a therapeutic option is the low or moderate expression of human leukocyte antigen (HLA) class I antigens and the lack of expression of HLA class II antigens, which makes MSCs "undetectable" by recipient immunocompetent cells in the allogeneic condition. However, a proinflammatory environment and IFN-γ production may increase the expression of their HLA class II antigens[31]. The immunomodulatory activity of MSCs related to dendritic cells is associated with their capacity to produce anti-inflammatory factors (PGE 2 and TGF-β), which inhibit the activation and maturation of dendritic cells, impairing their function[31].

MSCS AS SUPPORTIVE THERAPY IN COVID-19 PATIENTS

COVID-19 triggers a strong immune response with cytokine storm, especially in the lower airway, leading to lung damage[5,6]. MSCs are the ideal candidate for respiratory tract regeneration because they not only contribute to structural tissue repair but also have immunomodulatory, anti-inflammatory, proangiogenic, and anti-fibrotic properties[39,40]. This biological activity of MSCs may also affect tissue repair through modulation of the local microenvironment. The immunomodulatory properties of

MSCs can diminish the inflammatory response and ameliorate the cytokine storm, as documented in clinical trials conducted among patients with steroid-resistant graft-versus-host disease[41] and among patients with an autoimmune disease[42]. Cell-based therapies with allogeneic MSCs of bone marrow or adipose tissue origin have also been applied to patients with an acute lung injury and ARDS[43-45]. In these studies, the administration of MSCs was safe and feasible; however, the clinical effect suggests that this strategy needs further optimization. ARDS is characterized by substantial damage to the capillary endothelium and alveolar epithelium, which leads to an increase in alveolar-capillary permeability, causing pulmonary edema and the formation and accumulation of inflammatory cells in the interstitial and alveolar space[46]; extensive regeneration of the tissues is required to restore pulmonary function.

A clinical study that used MSCs to treat patients infected with influenza A (H7N9), who displayed symptoms similar to COVID-19 patients including cough, fever, shortness of breath, and dyspnea accompanied by ARDS and subsequent pneumonia, as well as corresponding multi-organ dysfunction, suggested that MSCs can be used as supportive therapy to treat SARS-CoV-2-infected patients[47].

In the case of patients with COVID-19, MSCs may attenuate the cytokine storm by means of paracrine secretion of a variety of anti-inflammatory cytokines including TGF-β1, SDF-1, IL-4, IL-6, IL-10, and IL-1Ra, which decrease the overactivation of immunocompetent cells, thus regulating the inflammatory response (Figure 1). A decreased immune response modifies the microenvironment of the damaged tissue and promotes tissue repair and regeneration. It is well known that MSCs transplanted intravenously are trapped by organs with a large capillary bed including the liver, spleen, and lung. MSCs accumulating in the lung may improve the pulmonary microenvironment, protect alveolar epithelial cells, prevent dysfunction of capillary endothelial cells, and prevent pulmonary fibrosis, thus helping to recover lung function[25,48]. Moreover, systemic delivery of MSCs may ameliorate multi-organ dysfunction associated with SARS-CoV-2 infection including cardiovascular, renal, or hepatic damage[25]. The therapeutic potential of MSCs for the treatment of patients in critical condition caused by COVID-19 pneumonia was proved in a pilot study on intravenous MSC transplantation[25]. The delivery of MSCs significantly improved the functional outcome and pulmonary function of the patients within 2 d following transplantation. This effect was associated with the immunomodulatory properties of MSCs, which caused a shift in the immune response from Th1 towards Th2, resulting in a decreased level of the proinflammatory cytokine TNF-α and an increased level of the anti-inflammatory cytokine IL-10. The MSC therapy also resulted in a high production of the proangiogenic VEGF, which can help restore the function of capillary endothelial cells. A very important biological characteristic of MSCs, assessed by 10x scRNA-seq analysis, showed that MSCs transplanted to patients did not express the ACE2 receptor or TMPRSS2, thus providing resistance to COVID-19 infection[25].

The progression of COVID-19 leads to the development of pulmonary diseases, such as idiopathic pulmonary fibrosis or ARDS, commonly associated with damage to alveolar epithelial cells, which in turn is related to a severe hypoxia of the alveolar cells, leading to a massive apoptosis, therefore, contributing to the pathophysiology of lung fibrosis[49]. Experimental studies have indicated that not only MSCs but also their derivates, such as a conditioned medium containing a variety of bioactive factors or extracellular vesicles (EVs) (microvesicles and exosomes) carrying various cytoplasmic components, including lipids, DNA fragments, and RNA (including mRNA and microRNA), contribute to the recovery of alveolar epithelial cells and endothelial cells and modify the function of inflammatory infiltrates in paracrine and endocrine manners[50,51].

An experimental study on the lung alveolar cells of the rat showed that conditioned medium of human MSC culture had a paracrine anti-apoptotic effects on the hypoxia-induced apoptosis of the alveolar cells. The anti-apoptotic properties of MSCs involve the secretion of keratinocyte growth factor and HGF, which downregulate the proapoptotic signal caused by the hypoxia-inducible factor-1 alpha and reactive oxygen species[49]. A similar study in a rat model using conditioned medium from a culture of MSCs of bone marrow or adipose tissue origin confirmed that MSC-derived bioactive factors protected alveolar epithelial cells from damage in hypoxic conditions by decreasing the secretion of proinflammatory cytokines, augmenting the production of IL-10, and delaying cell apoptosis[52].

MSC-derived EVs, similarly to their parent cells, exhibit proregenerative, anti-inflammatory, anti-apoptotic, anti-oxidative, prometabolomic, and immunoregulatory properties with respect to the damaged tissue microenvironment. The effectiveness of treatment of lung damage using MSC-derived EVs is currently being tested *in vitro*

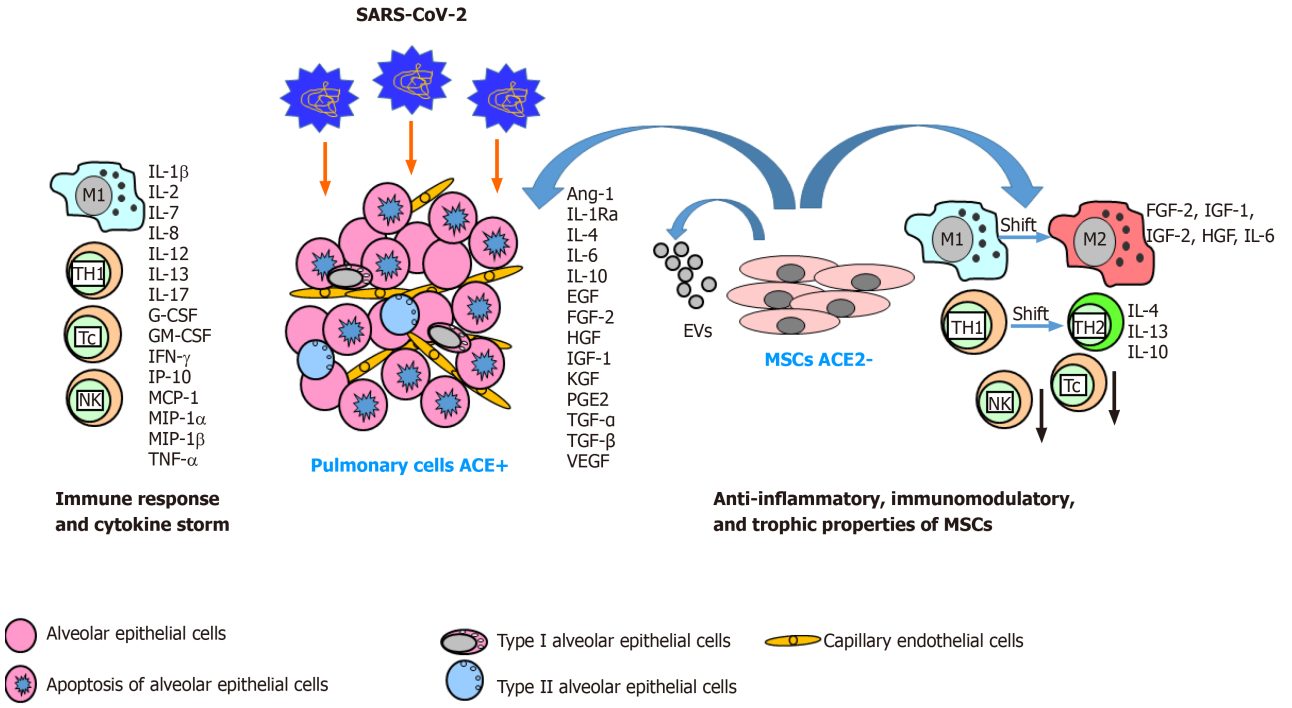


Figure 1 Immune response to severe acute respiratory syndrome coronavirus-2 infection and immunoregulatory activity of mesenchymal stem cells for treatment in patients with coronavirus disease 2019 pneumonia. Upon entry into the alveolar epithelium, SARS-CoV-2 triggers a strong immune response with cytokine storm. Cytokines and trophic factors released by MSCs (ACE2 negative) and their derivate EVs modulate the inflammatory microenvironment within the damaged pulmonary cells and modulate immune response, promoting a shift from T helper type 1 to Th2 lymphocytes, and a shift in macrophage balance from the M1 (proinflammatory) to M2 (anti-inflammatory) phenotype, and decreasing the activity of cytotoxic T lymphocytes (Tc) and natural killer lymphocytes. ACE2: Angiotensin-converting enzyme 2; MSCs: Mesenchymal stem cells; Ang-1: Angiopoietin-1; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; COVID-19: Coronavirus disease 2019; bFGF: Basic fibroblast growth factor; EGF: Epithelial growth factor; EVs: Extracellular vesicles; G-CSF: Granulocyte-colony stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; HGF: Hepatocyte growth factor; IFN-γ: Interferon-γ; IGF-1: Insulin-like growth factor-1; IL-: Interleukin-; KGF: Keratinocyte growth factor; MIP-1α: Macrophage inflammatory protein-1α; MIP-1β: Macrophage inflammatory protein-1β; SDF-1: Stromal-derived factor-1; TGF-α: Transforming growth factor-α; TGF-β: Transforming growth factor-β; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor; MCP-1: Monocyte chemoattractant protein-1; PGE2: Prostaglandin 2.

and in different preclinical experimental models[51]. The beneficial effects of MSC-derived EVs have been demonstrated in influenza-induced acute lung injury in a pig model. Studies have shown that MSC-derived EVs, delivered 12 h after virus infection, result in reduced viral replication and shedding and a decreased level of proinflammatory cytokines[53]. In addition to anti-inflammatory factors, MSC-derived EVs also contain Ang-1 mRNA, an angiogenic trophic factor that is essential in endothelial cell stabilization, and during injury, diminishes the interactions between leukocytes and vessel endothelial cells, and preserves vascular barrier integrity. The therapeutic effects of MSC-derived EVs in an experimental murine model of lipopolysaccharide-induced acute lung damage confirmed that a transfer of Ang-1 mRNA by EVs significantly contributed to the restoration of pulmonary capillary permeability[54]. Moreover, MSC-derived EVs affected the immunomodulatory properties of the murine macrophage cell line by suppressing the expression of TNF-α and inducing the secretion of IL-10, thus attenuating inflammation. Transfer of Ang-1 mRNA to the injured endothelium restores partial protein permeability across the injured human lung microvascular endothelial cells through the internalization of MSC-derived EVs into the injured cells[55]. A study in an experimental mouse model revealed that exosomes, isolated from MSCs derived from human Wharton's jelly and bone marrow, improved lung development, decreased lung fibrosis, and improved pulmonary vascular remodeling in a neonatal hyperoxia model of bronchopulmonary dysplasia. Exosomes originating from MSCs act as a paracrine anti-inflammatory mediator by modifying the pulmonary macrophage phenotype from M1 (proinflammatory) to M2 (anti-inflammatory) and suppressing lung inflammation and the immune response in favor of proper organ development[34].

MSC-derived EVs, widely used in pre-clinical experimental models of pulmonary injury and disease, are a promising alternative to MSC-based therapy[51]. However, many scientific and clinical questions regarding EV production, purification,

characterization, route of delivery (intravenous or inhalation), and bio-distribution need to be explored before clinical application. The first prospective non-randomized single-center clinical study using bone marrow-derived exosomes (ExoFlo™) was performed to address the safety and efficacy for the treatment of severely affected COVID-19 patients[56]. Twenty-four patients with severe and moderate-to-severe symptoms of ARDS enrolled in this study received a single dose of ExoFlo™ administered intravenously, and no adverse effect was observed 72 h after exosome delivery. The survival rate was 83%, and the study demonstrated a profound reversal of hypoxia, downregulation of cytokine storm, and immune reconstitution. However, the biological characteristics of the delivered exosomes (ExoFlo™) were not presented. To determine the therapeutic potential of the applied therapy, future randomized controlled trials with a detailed characterization of the delivered exosomes are needed.

Extensive research on COVID-19 treatment have suggested that the MSC secretome should be employed as a supportive therapy in patients infected with SARS-CoV-2. Experimental pre-clinical studies on the biological activity of the MSC secretome, composed of both soluble bioactive factors (including cytokines, chemokines, and trophic factors) and EVs, suggest that the MSC secretome can be applied for cell-free therapy in severely affected COVID-19 patients[57]. The bioactive proteins and EVs released by MSCs activate endogenous lung stem/progenitor cells, inducing their proliferation and differentiation, inhibit apoptosis, diminish inflammatory response, restore capillary barrier function, and reduce fibrosis and can also be used to treat acute and chronic lung injury, as they act similarly to parental MSCs[50,57]. The advantages of cell-free therapy with the MSC secretome is its formulation as inhalable dosage forms or injectable dosage forms for potential clinical use[58]. Both types of formulation are stored as freeze-dried powder and can be used to treat critically ill patients with COVID-19 pneumonia. The authors of the study introduced two Chinese clinical trials to investigate the inhaled secretome for the treatment of COVID-19 pneumonia (NCT04276987) and assess its tolerance in healthy volunteers (NCT04313647)[57].

The current status of clinical investigations of cell-based therapy for COVID-19 patients has been reviewed very well by Khoury *et al*[59] in the context of cell sources, doses, dosing strategies, and targeted patient populations. Khoury *et al*[59] also highlighted the importance of upholding ethical standards to create a rational evidence-based platform for the potential therapeutic use of cell-based therapies in patients infected with SARS-CoV-2. A very recent editorial article, introduced by worldwide famous experts in the field of infectious diseases and MSCs therapies, discusses the rationale behind the use of MSCs in the treatment trials of patients with severe COVID-19 disease[15]. The authors emphasized that the registered trials differ in design, sources of MSCs, doses and schedules of MSCs administration, and patient selection. All of these aspects indicate the need for standardizing protocols through a worldwide consortium network on cellular therapies for COVID-19 and other infectious diseases.

CONCLUSION

In summary, MSCs and their derivates, such as the MSC secretome, may have a great curative potential for COVID-19 patients thanks to their trophic, paracrine, immunomodulatory, anti-inflammatory, anti-apoptotic, anti-oxidative, and prometabolomic activities. Moreover, the immunosuppressive properties of MSCs may decrease the alloreactivity of host immune cells in allogenic conditions. MSCs are attractive candidates for the supportive therapy of severely affected COVID-19 patients thanks to their high proliferative activity, multipotent ability to regenerate tissues *via* direct differentiation into the desired cells and tissues, and their immunomodulatory activity. MSCs are easily obtainable from different sources, such as bone marrow, adipose tissue, skin, or perinatal tissues, including the umbilical cord, cord blood, Warton's jelly, and amniotic fluid, and can be expanded into clinical grade and stored for potential clinical use. The most important biological characteristic of MSCs is that they do not express the ACE2 receptor or serine protease TMPRSS2, which makes them safe for the treatment of SARS-CoV-2 infection.

REFERENCES

1 **Li Q**, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N Engl J Med* 2020; **382**: 1199-1207 [PMID: 31995857 DOI: 10.1056/NEJMoa2001316]

2 **Lai CC**, Liu YH, Wang CY, Wang YH, Hsueh SC, Yen MY, Ko WC, Hsueh PR. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Facts and myths. *J Microbiol Immunol Infect* 2020; **53**: 404-412 [PMID: 32173241 DOI: 10.1016/j.jmii.2020.02.012]

3 **Rothe C**, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirglmaier K, Zange S, Wölfel R, Hoelscher M. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. *N Engl J Med* 2020; **382**: 970-971 [PMID: 32003551 DOI: 10.1056/NEJMc2001468]

4 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

5 **Guo YR**, Cao QD, Hong ZS, Tan YY, Chen SD, Jin HJ, Tan KS, Wang DY, Yan Y. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res* 2020; **7**: 11 [PMID: 32169119 DOI: 10.1186/s40779-020-00240-0]

6 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

7 **Wang W**, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *J Med Virol* 2020; **92**: 441-447 [PMID: 31994742 DOI: 10.1002/jmv.25689]

8 **Yang X**, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; **8**: 475-481 [PMID: 32105632 DOI: 10.1016/S2213-2600(20)30079-5]

9 **Lu R**, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; **395**: 565-574 [PMID: 32007145 DOI: 10.1016/S0140-6736(20)30251-8]

10 **Kuba K**, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; **11**: 875-879 [PMID: 16007097 DOI: 10.1038/nm1267]

11 **Letko M**, Marzi A, Munster V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol* 2020; **5**: 562-569 [PMID: 32094589 DOI: 10.1038/s41564-020-0688-y]

12 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: 15141377 DOI: 10.1002/path.1570]

13 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280.e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]

14 **Iwata-Yoshikawa N**, Okamura T, Shimizu Y, Hasegawa H, Takeda M, Nagata N. TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection. *J Virol* 2019; **93** [PMID: 30626688 DOI: 10.1128/JVI.01815-18]

15 **Zumla A**, Wang FS, Ippolito G, Petrosillo N, Agrati C, Azhar EI, Chang C, El-Kafrawy SA, Osman M, Zitvogel L, Galle PR, Locatelli F, Gorman E, Cordon-Cardo C, O'Kane C, McAuley D, Maeurer M. Reducing mortality and morbidity in patients with severe COVID-19 disease by advancing ongoing trials of Mesenchymal Stromal (stem) Cell (MSC) therapy - Achieving global consensus and visibility for cellular host-directed therapies. *Int J Infect Dis* 2020; **96**: 431-439 [PMID: 32425638 DOI: 10.1016/j.ijid.2020.05.040]

16 **Zumla A**, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses - drug discovery and therapeutic options. *Nat Rev Drug Discov* 2016; **15**: 327-347 [PMID: 26868298 DOI: 10.1038/nrd.2015.37]

17 **Wang M**, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhong W, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020; **30**: 269-271 [PMID: 32020029 DOI: 10.1038/s41422-020-0282-0]

18 **Gao J**, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends* 2020; **14**: 72-73 [PMID: 32074550 DOI: 10.5582/bst.2020.01047]

19 **Holshue ML**, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A, Diaz G, Cohn A, Fox L, Patel A, Gerber SI, Kim L, Tong S, Lu X, Lindstrom S, Pallansch MA, Weldon WC, Biggs HM, Uyeki TM, Pillai SK; Washington State 2019-nCoV Case Investigation Team. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med* 2020; **382**: 929-936 [PMID: 32004427

DOI: 10.1056/NEJMoa2001191]
20 **Yao X**, Ye F, Zhang M, Cui C, Huang B, Niu P, Liu X, Zhao L, Dong E, Song C, Zhan S, Lu R, Li H, Tan W, Liu D. In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis* 2020; **71**: 732-739 [PMID: 32150618 DOI: 10.1093/cid/ciaa237]
21 **Sheahan TP**, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Montgomery SA, Hogg A, Babusis D, Clarke MO, Spahn JE, Bauer L, Sellers S, Porter D, Feng JY, Cihlar T, Jordan R, Denison MR, Baric RS. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat Commun* 2020; **11**: 222 [PMID: 31924756 DOI: 10.1038/s41467-019-13940-6]
22 **Li CC**, Wang XJ, Wang HR. Repurposing host-based therapeutics to control coronavirus and influenza virus. *Drug Discov Today* 2019; **24**: 726-736 [PMID: 30711575 DOI: 10.1016/j.drudis.2019.01.018]
23 **Ahn JY**, Sohn Y, Lee SH, Cho Y, Hyun JH, Baek YJ, Jeong SJ, Kim JH, Ku NS, Yeom JS, Roh J, Ahn MY, Chin BS, Kim YS, Lee H, Yong D, Kim HO, Kim S, Choi JY. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. *J Korean Med Sci* 2020; **35**: e149 [PMID: 32281317 DOI: 10.3346/jkms.2020.35.e149]
24 **Alzoughool F**, Alanagreh L. Coronavirus drugs: Using plasma from recovered patients as a treatment for COVID-19. *Int J Risk Saf Med* 2020; **31**: 47-51 [PMID: 32310190 DOI: 10.3233/JRS-201017]
25 **Leng Z**, Zhu R, Hou W, Feng Y, Yang Y, Han Q, Shan G, Meng F, Du D, Wang S, Fan J, Wang W, Deng L, Shi H, Li H, Hu Z, Zhang F, Gao J, Liu H, Li X, Zhao Y, Yin K, He X, Gao Z, Wang Y, Yang B, Jin R, Stambler I, Lim LW, Su H, Moskalev A, Cano A, Chakrabarti S, Min KJ, Ellison-Hughes G, Caruso C, Jin K, Zhao RC. Transplantation of ACE2^{-} Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia. *Aging Dis* 2020; **11**: 216-228 [PMID: 32257537 DOI: 10.14336/AD.2020.0228]
26 **Golchin A**, Seyedjafari E, Ardeshirylajimi A. Mesenchymal Stem Cell Therapy for COVID-19: Present or Future. *Stem Cell Rev Rep* 2020; **16**: 427-433 [PMID: 32281052 DOI: 10.1007/s12015-020-09973-w]
27 **Klimczak A**, Kozlowska U. Mesenchymal Stromal Cells and Tissue-Specific Progenitor Cells: Their Role in Tissue Homeostasis. *Stem Cells Int* 2016; **2016**: 4285215 [PMID: 26823669 DOI: 10.1155/2016/4285215]
28 **Bertoncello I**, McQualter JL. Lung stem cells: do they exist? *Respirology* 2013; **18**: 587-595 [PMID: 23433037 DOI: 10.1111/resp.12073]
29 **Leeman KT**, Fillmore CM, Kim CF. Lung stem and progenitor cells in tissue homeostasis and disease. *Curr Top Dev Biol* 2014; **107**: 207-233 [PMID: 24439808 DOI: 10.1016/B978-0-12-416022-4.00008-1]
30 **Fan XL**, Zhang Z, Ma CY, Fu QL. Mesenchymal stem cells for inflammatory airway disorders: promises and challenges. *Biosci Rep* 2019; **39** [PMID: 30610158 DOI: 10.1042/BSR20182160]
31 **Murphy MB**, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. *Exp Mol Med* 2013; **45**: e54 [PMID: 24232253 DOI: 10.1038/emm.2013.94]
32 **Prockop DJ**, Oh JY. Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. *Mol Ther* 2012; **20**: 14-20 [PMID: 22008910 DOI: 10.1038/mt.2011.211]
33 **Kozlowska U**, Krawczenko A, Futoma K, Jurek T, Rorat M, Patrzalek D, Klimczak A. Similarities and differences between mesenchymal stem/progenitor cells derived from various human tissues. *World J Stem Cells* 2019; **11**: 347-374 [PMID: 31293717 DOI: 10.4252/wjsc.v11.i6.347]
34 **Willis GR**, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, Kwong A, Mitsialis SA, Kourembanas S. Mesenchymal Stromal Cell Exosomes Ameliorate Experimental Bronchopulmonary Dysplasia and Restore Lung Function through Macrophage Immunomodulation. *Am J Respir Crit Care Med* 2018; **197**: 104-116 [PMID: 28853608 DOI: 10.1164/rccm.201705-0925OC]
35 **Waterman RS**, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. *PLoS One* 2010; **5**: e10088 [PMID: 20436665 DOI: 10.1371/journal.pone.0010088]
36 **L PK**, Kandoi S, Misra R, S V, K R, Verma RS. The mesenchymal stem cell secretome: A new paradigm towards cell-free therapeutic mode in regenerative medicine. *Cytokine Growth Factor Rev* 2019; **46**: 1-9 [PMID: 30954374 DOI: 10.1016/j.cytogfr.2019.04.002]
37 **Kraskiewicz H**, Paprocka M, Bielawska-Pohl A, Krawczenko A, Panek K, Kaczyńska J, Szyposzyńska A, Psurski M, Kuropka P, Klimczak A. Can supernatant from immortalized adipose tissue MSC replace cell therapy? An *in vitro* study in chronic wounds model. *Stem Cell Res Ther* 2020; **11**: 29 [PMID: 31964417 DOI: 10.1186/s13287-020-1558-5]
38 **Krawczenko A**, Bielawska-Pohl A, Paprocka M, Kraskiewicz H, Szyposzynska A, Wojdat E, Klimczak A. Microvesicles from Human Immortalized Cell Lines of Endothelial Progenitor Cells and Mesenchymal Stem/Stromal Cells of Adipose Tissue Origin as Carriers of Bioactive Factors Facilitating Angiogenesis. *Stem Cells Int* 2020; **2020**: 1289380 [PMID: 32612661 DOI: 10.1155/2020/1289380]
39 **Behnke J**, Kremer S, Shahzad T, Chao CM, Böttcher-Friebertshäuser E, Morty RE, Bellusci S, Ehrhardt H. MSC Based Therapies-New Perspectives for the Injured Lung. *J Clin Med* 2020; **9** [PMID: 32138309 DOI: 10.3390/jcm9030682]
40 **Rogers CJ**, Harman RJ, Bunnell BA, Schreiber MA, Xiang C, Wang FS, Santidrian AF, Minev BR. Rationale for the clinical use of adipose-derived mesenchymal stem cells for COVID-19 patients. *J Transl Med* 2020; **18**: 203 [PMID: 32423449 DOI: 10.1186/s12967-020-02380-2]
41 **Le Blanc K**, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, Dini G, Egeler RM, Bacigalupo A, Fibbe W, Ringdén O; Developmental Committee of the European Group for Blood and Marrow Transplantation. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 2008; **371**: 1579-1586 [PMID: 18468541 DOI: 10.1016/S0140-6736(08)60690-X]
42 **Wang D**, Zhang H, Liang J, Wang H, Hua B, Feng X, Gilkeson GS, Farge D, Shi S, Sun L. A Long-Term Follow-Up Study of Allogeneic Mesenchymal Stem/Stromal Cell Transplantation in Patients with Drug-Resistant Systemic Lupus Erythematosus. *Stem Cell Reports* 2018; **10**: 933-941 [PMID: 29478901 DOI: 10.1016/j.stemcr.2018.01.029]
43 **Matthay MA**, Calfee CS, Zhuo H, Thompson BT, Wilson JG, Levitt JE, Rogers AJ, Gotts JE, Wiener-Kronish JP, Bajwa EK, Donahoe MP, McVerry BJ, Ortiz LA, Exline M, Christman JW, Abbott J, Delucchi

KL, Caballero L, McMillan M, McKenna DH, Liu KD. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. *Lancet Respir Med* 2019; **7**: 154-162 [PMID: 30455077 DOI: 10.1016/S2213-2600(18)30418-1]

44 **Zheng G**, Huang L, Tong H, Shu Q, Hu Y, Ge M, Deng K, Zhang L, Zou B, Cheng B, Xu J. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. *Respir Res* 2014; **15**: 39 [PMID: 24708472 DOI: 10.1186/1465-9921-15-39]

45 **Liu S**, Peng D, Qiu H, Yang K, Fu Z, Zou L. Mesenchymal stem cells as a potential therapy for COVID-19. *Stem Cell Res Ther* 2020; **11**: 169 [PMID: 32366290 DOI: 10.1186/s13287-020-01678-8]

46 **Matthay MA**, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. *J Clin Invest* 2012; **122**: 2731-2740 [PMID: 22850883 DOI: 10.1172/JCI60331]

47 **Chen J**, Hu C, Chen L, Tang L, Zhu Y, Xu X, Chen L, Gao H, Lu X, Yu L, Dai X, Xiang C, Li L. Clinical study of mesenchymal stem cell treating acute respiratory distress syndrome induced by epidemic Influenza A (H7N9) infection, a hint for COVID-19 treatment. *Engineering (Beijing)* 2020 [PMID: 32292627 DOI: 10.1016/j.eng.2020.02.006]

48 **Atluri S**, Manchikanti L, Hirsch JA. Expanded Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) as a Therapeutic Strategy in Managing Critically Ill COVID-19 Patients: The Case for Compassionate Use. *Pain Physician* 2020; **23**: E71-E83 [PMID: 32214286]

49 **Bernard O**, Jeny F, Uzunhan Y, Dondi E, Terfous R, Label R, Sutton A, Larghero J, Vanneaux V, Nunes H, Boncoeur E, Planès C, Dard N. Mesenchymal stem cells reduce hypoxia-induced apoptosis in alveolar epithelial cells by modulating HIF and ROS hypoxic signaling. *Am J Physiol Lung Cell Mol Physiol* 2018; **314**: L360-L371 [PMID: 29167125 DOI: 10.1152/ajplung.00153.2017]

50 **Mohammadipoor A**, Antebi B, Batchinsky AI, Cancio LC. Therapeutic potential of products derived from mesenchymal stem/stromal cells in pulmonary disease. *Respir Res* 2018; **19**: 218 [PMID: 30413158 DOI: 10.1186/s12931-018-0921-x]

51 **Worthington EN**, Hagood JS. Therapeutic Use of Extracellular Vesicles for Acute and Chronic Lung Disease. *Int J Mol Sci* 2020; **21** [PMID: 32230828 DOI: 10.3390/ijms21072318]

52 **Shologu N**, Scully M, Laffey JG, O'Toole D. Human Mesenchymal Stem Cell Secretome from Bone Marrow or Adipose-Derived Tissue Sources for Treatment of Hypoxia-Induced Pulmonary Epithelial Injury. *Int J Mol Sci* 2018; **19** [PMID: 30274394 DOI: 10.3390/ijms19102996]

53 **Khatri M**, Richardson LA, Meulia T. Mesenchymal stem cell-derived extracellular vesicles attenuate influenza virus-induced acute lung injury in a pig model. *Stem Cell Res Ther* 2018; **9**: 17 [PMID: 29378639 DOI: 10.1186/s13287-018-0774-8]

54 **Tang XD**, Shi L, Monsel A, Li XY, Zhu HL, Zhu YG, Qu JM. Mesenchymal Stem Cell Microvesicles Attenuate Acute Lung Injury in Mice Partly Mediated by Ang-1 mRNA. *Stem Cells* 2017; **35**: 1849-1859 [PMID: 28376568 DOI: 10.1002/stem.2619]

55 **Hu S**, Park J, Liu A, Lee J, Zhang X, Hao Q, Lee JW. Mesenchymal Stem Cell Microvesicles Restore Protein Permeability Across Primary Cultures of Injured Human Lung Microvascular Endothelial Cells. *Stem Cells Transl Med* 2018; **7**: 615-624 [PMID: 29737632 DOI: 10.1002/sctm.17-0278]

56 **Sengupta V**, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes Derived from Bone Marrow Mesenchymal Stem Cells as Treatment for Severe COVID-19. *Stem Cells Dev* 2020; **29**: 747-754 [PMID: 32380908 DOI: 10.1089/scd.2020.0080]

57 **Bari E**, Ferrarotti I, Saracino L, Perteghella S, Torre ML, Corsico AG. Mesenchymal Stromal Cell Secretome for Severe COVID-19 Infections: Premises for the Therapeutic Use. *Cells* 2020; **9** [PMID: 32283815 DOI: 10.3390/cells9040924]

58 **Bari E**, Ferrarotti I, Torre ML, Corsico AG, Perteghella S. Mesenchymal stem/stromal cell secretome for lung regeneration: The long way through "pharmaceuticalization" for the best formulation. *J Control Release* 2019; **309**: 11-24 [PMID: 31326462 DOI: 10.1016/j.jconrel.2019.07.022]

59 **Khoury M**, Cuenca J, Cruz FF, Figueroa FE, Rocco PRM, Weiss DJ. Current status of cell-based therapies for respiratory virus infections: applicability to COVID-19. *Eur Respir J* 2020; **55** [PMID: 32265310 DOI: 10.1183/13993003.00858-2020]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 1023-1031

DOI: 10.4252/wjsc.v12.i9.1023

ISSN 1948-0210 (online)

MINIREVIEWS

Mass acquisition of human periodontal ligament stem cells

Hidefumi Maeda

ORCID number: Hidefumi Maeda 0000-0002-1347-995x.

Supported by Japan Society for the Promotion of Science, No. JP17H01598.

Conflict-of-interest statement: There is no conflict of interest.

Manuscript source: Invited manuscript

Received: May 19, 2020
Peer-review started: May 19, 2020
First decision: June 5, 2020
Revised: June 8, 2020
Accepted: August 1, 2020
Article in press: August 1, 2020
Published online: September 26, 2020

P-Reviewer: Fang FC, Yang RL

Hidefumi Maeda, Department of Endodontology and Operative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Fukuoka 8128582, Japan

Corresponding author: Hidefumi Maeda, DDS, PhD, Chairman, Professor, Department of Endodontology and Operative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 8128582, Japan. hide@dent.kyushu-u.ac.jp

Abstract

The periodontal ligament (PDL) is an essential fibrous tissue for tooth retention in the alveolar bone socket. PDL tissue further functions to cushion occlusal force, maintain alveolar bone height, allow orthodontic tooth movement, and connect tooth roots with bone. Severe periodontitis, deep caries, and trauma cause irreversible damage to this tissue, eventually leading to tooth loss through the destruction of tooth retention. Many patients suffer from these diseases worldwide, and its prevalence increases with age. To address this issue, regenerative medicine for damaged PDL tissue as well as the surrounding tissues has been extensively investigated regarding the potential and effectiveness of stem cells, scaffolds, and cytokines as well as their combined applications. In particular, PDL stem cells (PDLSCs) have been well studied. In this review, I discuss comprehensive studies on PDLSCs performed *in vivo* and contemporary reports focusing on the acquisition of large numbers of PDLSCs for therapeutic applications because of the very small number of PDLSCs available *in vivo*.

Key Words: Induced pluripotent stem cells; Mesoderm specific transcript; Periodontal ligament stem cells; Periodontal tissue; Regenerative medicine; Semaphorin 3A

Core tip: For patients with severe periodontitis, deep caries, and trauma, which can lead to tooth loss, the development of highly effective regenerative therapies for severely damaged periodontal tissue is an urgent concern. As one possible method to address this issue, cell-based therapy using periodontal ligament stem cells (PDLSCs) shows great promise. However, the number of PDLSCs present *in vivo* is too small for implementation of this method, and PDLSC isolation requires patients to undergo invasive surgery. In this review, ways to acquire large numbers of PDLSCs and advances in periodontal regenerative therapy during the past two decades are summarized.

S-Editor: Zhang L
L-Editor: Filipodia
P-Editor: Ma YJ

Citation: Maeda H. Mass acquisition of human periodontal ligament stem cells. *World J Stem Cells* 2020; 12(9): 1023-1031
URL: https://www.wjgnet.com/1948-0210/full/v12/i9/1023.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.1023

HOW ARE REGENERATIVE TREATMENTS OF PERIODONTAL DEFECTS PERFORMED USING CELL-RELATED THERAPY?

Stem cell-based therapy (Figure 1)

In humans, autologous transplantation of cultured periosteum sheets[1], periodontal ligament (PDL) progenitor cells[2], PDL cell sheets[3], dental pulp stem cells[4,5], gingiva-derived cells[6], and tissue-engineered bone constituted by bone marrow mesenchymal stem cells (BMMSCs)[7] into patients with periodontal defects have been reported. All of the cited studies clinically verified the potency of stem cells for periodontal regeneration. In contrast, Chen *et al*[8] transplanted PDL stem cell (PDLSC) sheets and found no significant improvement compared with the control group. However, these authors targeted very small periodontal defects and applied scaffolding materials as controls, possibly making it difficult to detect significant differences.

In animal periodontal defect models, Iwasaki *et al*[9] reported no significant advantage of spheroid formation by human PDLSCs in rats, despite increased expression of genes related to angiogenesis and anti-inflammation[9]. Nevertheless, a recent study demonstrated that coculture of human PDLSC spheroids with vascular endothelial cells promoted rat periodontal regeneration[10]. In addition, a previous study revealed that pellets of cultured human PDLSCs showed periodontal regeneration capacity in mice[11]. Meanwhile, application of other immature cells, including adipose-derived stem cells[12], stem cells from human exfoliated deciduous teeth[13], dental pulp stem cells[14], dental follicle cells[15], induced pluripotent stem (iPS) cells[16], and iPS-derived mesenchymal stem cells (MSCs)[17] was shown to induce periodontal regeneration *in vivo*. These reports suggest that the indicated cell sources may have potential for clinical use.

Gene/noncoding RNA modified cell therapy

No clinical studies on gene or noncoding RNA modified cell therapy for the treatment of patients with periodontal disease have been reported because of the associated safety issues. However, there have been some reports involving animal models with experimentally produced periodontal defects. *Osteoprotegerin* gene-transferred rabbit PDLSCs and *platelet-derived growth factor-BB*-transduced human PDLSCs exhibited increased bone formation in periodontal defects[18,19].

The development and characterization of other tissue-derived cells with gene transduction have been reported. Specifically, *bone morphogenetic protein 2*-transfected canine BMMSCs[20], *fibroblast growth factor 2*-transduced canine BMMSCs[21], *hepatocyte growth factor*-transduced human dental pulp cells[22], and leptin-transduced rat BMMSCs[23] were able to restore periodontal defects *in vivo*.

Modification of PDLSCs using noncoding RNA, including microRNA, long noncoding RNA, and circular RNA have been reported to induce their osteogenic differentiation, suggesting the application of these cells to bone defective in periodontitis[24].

Although the above studies indicated the potential of novel therapies for repair of severe periodontal defects, further basic studies are indispensable for future clinical trials.

Cell culture conditioned medium and exosomes

Conditioned medium from cultured cells and extracellular vesicles secreted from stem cells have various effects, including tissue regeneration, cell proliferation, chemotactic and metabolic activities, anti-inflammation, and cell-cell communication[25]. Because these cells and vesicles possess great potential, researchers have examined their roles in periodontal regeneration studies, but related clinical trials have not been reported.

There have been some studies on the effects of conditioned medium or exosomes from PDLSCs as well as other tissue-derived stem cells in animal models. Recently, Nagata *et al*[26] demonstrated periodontal regeneration activity of conditioned medium from cultured human PDLSCs injected into rat periodontal defects. Another study showed the capability of conditioned medium from human gingival stem cells as well

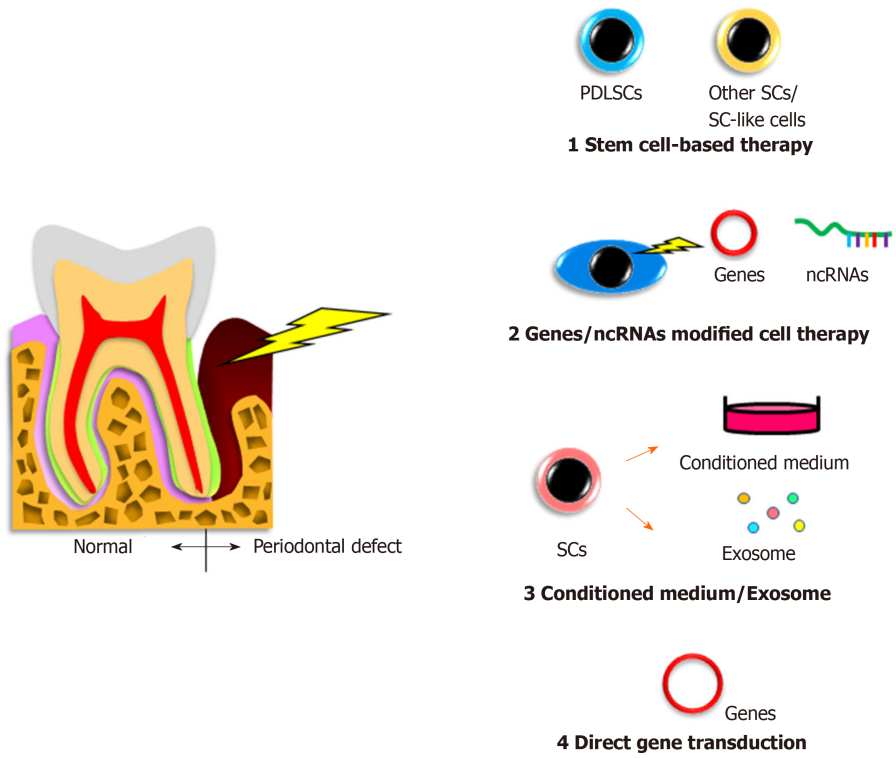


Figure 1 Cell-related therapies for periodontal regeneration. The regenerative treatments of periodontal defects performed using cell-related therapy are illustrated, which include the transplantation of stem cells, gene or noncoding RNA modified cells, application of conditioned medium or exosome from cell culture, and direct gene transfection to the lesion. PDLSC: Periodontal ligament stem cell; SC: Stem cell; ncRNA: noncoding RNA.

as human cultured human PDLSCs for periodontal regeneration[27]. Furthermore, conditioned medium from human BMMSCs was clearly able to repair canine and rat periodontal defects[28,29]. A recent report applying exosomes from human BMMSCs to rat periodontal defects showed induction of newly-formed bone and PDL tissue[30].

Although the above effects do not reflect direct contributions of stem cells to treatment, these indirect effects of stem cells may deserve further consideration as treatment options.

Gene therapy

Direct *in vivo* gene transfer of the *bone morphogenetic protein 2/7*[31] or *platelet-derived growth factor*[32] genes to rat periodontal tissue promoted bone growth or bone regeneration and cementum formation, respectively. Meanwhile, another group directly transferred the *bone morphogenetic protein 4* gene to rat PDL tissue by electroporation but did not detect any obvious bone augmentation[33]. Similarly, embedding of a *platelet-derived growth factor-B* plasmid with collagen gel into alveolar bone defects in rats had no significant effects[34].

The gene therapy method has not been investigated in human patients, and its effectiveness needs to be fully verified before it can be used as a relatively easy therapeutic modality.

WHAT ARE PDLSCs?

Periodontal tissue (periodontium) is a complex tissue mainly composed of two hard tissues (alveolar bone and cementum coating tooth root surfaces) and two soft tissues (PDL tissue and gingival tissue)[35]. In particular, PDL tissue has crucial roles in supporting the tooth and integrating the tissues.

PDLSCs are somatic stem cells localized in PDL tissue[36] and derived from cranial neural crest cells[37,38]. PDLSCs have similar features to BMMSCs and exhibit self-renewal capacity and multipotency[39]. These cells have the potential to undergo triploblastic differentiation with the ability to differentiate into not only osteoblasts, adipocytes, chondrocytes, cementoblasts, and tendon/ligament fibroblasts[40,41] but also myocytes[42], neural cells[43], retinal cells[44], endothelial cells[45], pancreatic islet cells[46],

and hepatic cells[47]. In addition, PDLSCs express cell surface markers such as STRO-1, CD146/MUC18[36], CD44, and CD90 (markers associated with stromal cells), CD105 and CD166 (markers associated with stromal cells and endothelial cells)[48], and CD10, CD26, CD29, CD73, and CD349/FZD9[49] but do not express hematopoietic cell surface markers such as CD31 and CD45, similar to BMMSCs[50].

PDLSCs also express embryonic stem cell-related transcription factors like NANOG and OCT-4 and embryonic stem cell antigens like stage-specific embryonic antigen-1 (SSEA-1)/CD15, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, alkaline phosphatase, and REX1/ZFP42[49,51]. However, PDLSCs from aged people exhibit decreased capacities for proliferation, migration, and multiple differentiation with reduced SSEA-4 expression[52].

Finally, PDLSCs possess immunomodulatory properties[48], among which reactive oxygen species production may be interestingly regulated by dual mechanisms depending on the degree of inflammation[53].

WHERE ARE PDLSCs REQUIRED?

Severe periodontitis, deep caries, and trauma cause irreversible damage to PDL tissue as well as the surrounding tissues such as alveolar bone, gingiva, and cementum, eventually resulting in tooth loss. The 8020 Promotion Foundation survey for causes of tooth loss in Japan (https://www.8020zaidan.or.jp/english/) performed in 2018 reported that periodontitis, caries, and tooth fracture accounted for 84.0% of all causes of tooth loss. The Global Burden of Disease 2015 study suggested that 7.4% of people worldwide suffered from severe periodontitis[54]. Meanwhile, the National Survey of Dental Diseases in Japan performed in 2016 (https://www.mhlw.go.jp/toukei/list/62-28.html) reported that Japanese people with periodontal health (< 4 mm periodontal pocket depth) comprised less than 37.0% of people aged ≥ 50 years.

To date, transplantation of PDLSCs has led to successful periodontal regeneration in experimentally produced periodontal defects in dogs[55,56], rats[57], and pigs[58]. Furthermore, Yan *et al*[59] performed a systematic review and meta-analysis and decisively stated that cell-based therapy is an effective therapy for regeneration of lost periodontal tissue.

PDLSCs have been proposed as the most promising cells for regeneration of severely damaged PDL tissue, among other stem cells such as dental pulp stem cells, stem cells from human exfoliated deciduous, dental follicle stem cells, stem cells from apical papilla[60], BMMSCs, and alveolar periosteal cells[61].

Interestingly, the fate of transplanted PDLSCs was examined in a rat periodontal defect model[62]. The findings revealed that PDLSCs contributed to periodontal repair but did not become markedly engrafted, suggesting a supportive role of PDLSCs for activating the regenerative capability in the damaged periodontium. Considering the critical role of PDLSCs in periodontal regenerative therapy, PDLSCs themselves may be difficult to engraft into defect sites, but the use of 2D or 3D construction methods combined with extracellular matrices may be effective.

WHY ARE LARGE AMOUNTS OF PDLSCs NEEDED?

In human PDL tissue, STRO1$^+$/CD146$^+$ cells, regarded as candidate PDLSCs[36,63], were reported to comprise only about 0.07% of the total cells[64]. Another study described that PDLSCs comprised 2.4% of the total cells[63]. Regardless of the actual numbers, both studies indicated that very few PDLSCs are present in PDL tissue.

While the defect volumes in cases reported in human clinical studies have been very limited as described above, the defect areas in severe cases leading to tooth loss can vary across a wide range. Therefore, clinical application of PDLSCs to regenerative therapy of periodontal defects in humans will require the acquisition of large numbers of PDLSCs. Meanwhile, delivery of autologous PDLSCs to patients will necessitate the patients to undergo surgically invasive procedures. In addition, the subsequent expansion of small numbers of PDLSCs *in vitro* could lead to loss of their stemness. In this regard, it is of concern that large amounts of PDLSCs are needed for regenerative treatment. To address this issue, methods to acquire large numbers of PDLSCs have been explored.

HOW TO SOLVE THE INSUFFICIENCY OF PDLSCs?

Reprogrammed cells (Figure 2)

We have reported unique methods for conversion of PDL cells to PDLSCs by gene transduction[65,66]. In a previous study, semaphorin 3A-transduced human PDL cells were converted into stem-like cells that showed multipotency and expressed both embryonic stem cell and MSC markers[65]. Furthermore, we recently demonstrated that an unexplored gene, *mesoderm-specific transcript*, was expressed in PDLSCs and that human PDL cells transduced with the *mesoderm-specific transcript* gene acquired PDLSC properties similar to semaphorin 3A-transduced cells[66]. In addition, the transduction changed the spindle shape of PDL cells to a stem cell-like round shape. Therefore, although the safety of these cells *in vivo* needs to be confirmed for clinical use, cell transformation with these genes is a potential method for mass acquisition of PDLSCs.

iPS cells

Our group was the first to report the development of PDLSC-like cells from human skin fibroblast-derived iPS cells[67]. Our study indicated that iPS cells themselves were unable to directly differentiate into PDLSCs, whereas neural crest-like cells developed from iPS cells attained PDLSC properties when cultured on extracellular matrix secreted from human primary PDL cells. We believe that this method may have great potential to solve the issue of insufficient numbers of PDLSCs. In addition, a recent study produced human leukocyte antigen homozygous iPS cells by gene modification, which have immune compatibility[68]. This development will enable the clinical use of iPS cell-derived PDLSCs benefiting many patients with severe periodontal defects. However, the issue of cost needs to be solved.

CONCLUSION

Many researchers have attempted to develop innovative and critical methods for periodontal therapy from various angles to support people's health and life and address the aging society. PDLSC-based therapy is one of these methods, and we believe that it has the potential to deliver sustainable oral health to people around the world.

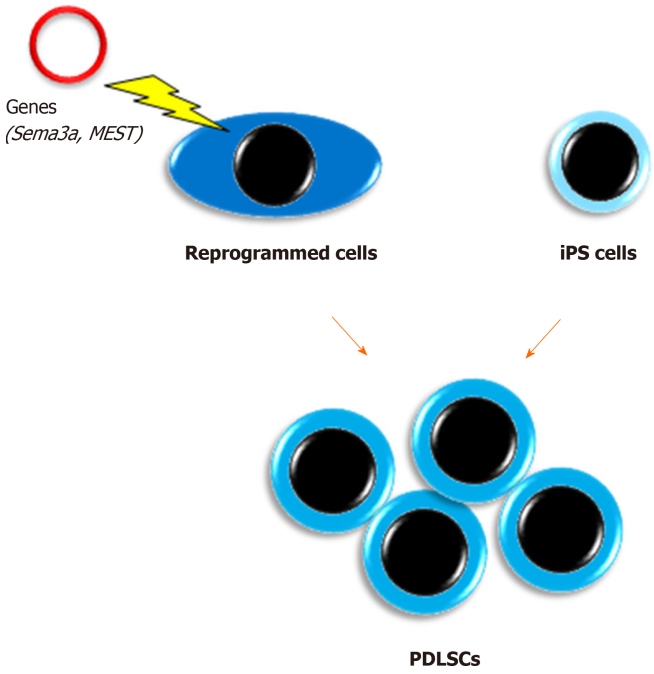


Figure 2 Acquisition of a large number of periodontal ligament stem cells. The illustration shows that the reprogramming of periodontal ligament cells with *semaphorin 3A* or *mesoderm-specific transcript*, or differentiation induction of induced pluripotent stem cells to human periodontal ligament stem cell lineage are promising to acquire a large number of periodontal ligament stem cells. iPS cells: Induced pluripotent stem cells; PDLSCs: Periodontal ligament stem cells. *Sema3a*: *Semaphorin 3A*; *MEST*: *Mesoderm-specific transcript*.

ACKNOWLEDGEMENTS

The author thanks Drs. Tomokiyo, Hamano, Hasegawa, Sugii, Yoshida, and Itoyama for their great support in the preparation of this review.

REFERENCES

1 **Yamamiya K**, Okuda K, Kawase T, Hata K, Wolff LF, Yoshie H. Tissue-engineered cultured periosteum used with platelet-rich plasma and hydroxyapatite in treating human osseous defects. *J Periodontol* 2008; **79**: 811-818 [PMID: 18454659 DOI: 10.1902/jop.2008.070518]
2 **Feng F**, Akiyama K, Liu Y, Yamaza T, Wang TM, Chen JH, Wang BB, Huang GT, Wang S, Shi S. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. *Oral Dis* 2010; **16**: 20-28 [PMID: 20355278 DOI: 10.1111/j.1601-0825.2009.01593.x]
3 **Iwata T**, Yamato M, Washio K, Yoshida T, Tsumanuma Y, Yamada A, Onizuka S, Izumi Y, Ando T, Okano T, Ishikawa I. Periodontal regeneration with autologous periodontal ligament-derived cell sheets - A safety and efficacy study in ten patients. *Regen Ther* 2018; **9**: 38-44 [PMID: 30525074 DOI: 10.1016/j.reth.2018.07.002]
4 **Aimetti M**, Ferrarotti F, Cricenti L, Mariani GM, Romano F. Autologous dental pulp stem cells in periodontal regeneration: a case report. *Int J Periodontics Restorative Dent* 2014; **34** Suppl 3: s27-s33 [PMID: 24956088 DOI: 10.11607/prd.1635]
5 **d'Aquino R**, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur Cell Mater* 2009; **18**: 75-83 [PMID: 19908196 DOI: 10.22203/ecm.v018a07]
6 **Hou LT**, Tsai AY, Liu CM, Feng F. Autologous transplantation of gingival fibroblast-like cells and a hydroxylapatite complex graft in the treatment of periodontal osseous defects: cell cultivation and long-term report of cases. *Cell Transplant* 2003; **12**: 787-797 [PMID: 14653625 DOI: 10.3727/000000003108747262]
7 **Yamada Y**, Nakamura S, Ito K, Umemura E, Hara K, Nagasaka T, Abe A, Baba S, Furuichi Y, Izumi Y, Klein OD, Wakabayashi T. Injectable bone tissue engineering using expanded mesenchymal stem cells. *Stem Cells* 2013; **31**: 572-580 [PMID: 23225744 DOI: 10.1002/stem.1300]
8 **Chen FM**, Gao LN, Tian BM, Zhang XY, Zhang YJ, Dong GY, Lu H, Chu Q, Xu J, Yu Y, Wu RX, Yin Y, Shi S, Jin Y. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial. *Stem Cell Res Ther* 2016; **7**: 33 [PMID: 26895633 DOI: 10.1186/s13287-016-0288-1]
9 **Iwasaki K**, Nagata M, Akazawa K, Watabe T, Morita I. Changes in characteristics of periodontal ligament stem cells in spheroid culture. *J Periodontal Res* 2019; **54**: 364-373 [PMID: 30597545 DOI: 10.1111/jre.12637]
10 **Sano K**, Usui M, Moritani Y, Nakazawa K, Hanatani T, Kondo H, Nakatomi M, Onizuka S, Iwata T, Sato T, Togari A, Ariyoshi W, Nishihara T, Nakashima K. Co-cultured spheroids of human periodontal ligament

mesenchymal stem cells and vascular endothelial cells enhance periodontal tissue regeneration. *Regen Ther* 2020; **14**: 59-71 [PMID: 31988996 DOI: 10.1016/j.reth.2019.12.008]
11 **Yang Z**, Jin F, Zhang X, Ma D, Han C, Huo N, Wang Y, Zhang Y, Lin Z, Jin Y. Tissue engineering of cementum/periodontal-ligament complex using a novel three-dimensional pellet cultivation system for human periodontal ligament stem cells. *Tissue Eng Part C Methods* 2009; **15**: 571-581 [PMID: 19534606 DOI: 10.1089/ten.tec.2008.0561]
12 **Tobita M**, Uysal CA, Guo X, Hyakusoku H, Mizuno H. Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. *Cytotherapy* 2013; **15**: 1517-1526 [PMID: 23849975 DOI: 10.1016/j.jcyt.2013.05.007]
13 **Fu X**, Jin L, Ma P, Fan Z, Wang S. Allogeneic stem cells from deciduous teeth in treatment for periodontitis in miniature swine. *J Periodontol* 2014; **85**: 845-851 [PMID: 24001042 DOI: 10.1902/jop.2013.130254]
14 **Hu J**, Cao Y, Xie Y, Wang H, Fan Z, Wang J, Zhang C, Wang J, Wu CT, Wang S. Periodontal regeneration in swine after cell injection and cell sheet transplantation of human dental pulp stem cells following good manufacturing practice. *Stem Cell Res Ther* 2016; **7**: 130 [PMID: 27613503 DOI: 10.1186/s13287-016-0362-8]
15 **Oshima M**, Inoue K, Nakajima K, Tachikawa T, Yamazaki H, Isobe T, Sugawara A, Ogawa M, Tanaka C, Saito M, Kasugai S, Takano-Yamamoto T, Inoue T, Tezuka K, Kuboki T, Yamaguchi A, Tsuji T. Functional tooth restoration by next-generation bio-hybrid implant as a bio-hybrid artificial organ replacement therapy. *Sci Rep* 2014; **4**: 6044 [PMID: 25116435 DOI: 10.1038/srep06044]
16 **Duan X**, Tu Q, Zhang J, Ye J, Sommer C, Mostoslavsky G, Kaplan D, Yang P, Chen J. Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. *J Cell Physiol* 2011; **226**: 150-157 [PMID: 20658533 DOI: 10.1002/jcp.22316]
17 **Hynes K**, Menicanin D, Han J, Marino V, Mrozik K, Gronthos S, Bartold PM. Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. *J Dent Res* 2013; **92**: 833-839 [PMID: 23884555 DOI: 10.1177/0022034513498258]
18 **Su F**, Liu SS, Ma JL, Wang DS, E LL, Liu HC. Enhancement of periodontal tissue regeneration by transplantation of osteoprotegerin-engineered periodontal ligament stem cells. *Stem Cell Res Ther* 2015; **6**: 22 [PMID: 25888745 DOI: 10.1186/s13287-015-0023-3]
19 **Pan J**, Deng J, Luo Y, Yu L, Zhang W, Han X, You Z, Liu Y. Thermosensitive Hydrogel Delivery of Human Periodontal Stem Cells Overexpressing Platelet-Derived Growth Factor-BB Enhances Alveolar Bone Defect Repair. *Stem Cells Dev* 2019; **28**: 1620-1631 [PMID: 31663419 DOI: 10.1089/scd.2019.0184]
20 **Chung VH**, Chen AY, Kwan CC, Chen PK, Chang SC. Mandibular alveolar bony defect repair using bone morphogenetic protein 2-expressing autologous mesenchymal stem cells. *J Craniofac Surg* 2011; **22**: 450-454 [PMID: 21403565 DOI: 10.1097/SCS.0b013e3182077de9]
21 **Tan Z**, Zhao Q, Gong P, Wu Y, Wei N, Yuan Q, Wang C, Liao D, Tang H. Research on promoting periodontal regeneration with human basic fibroblast growth factor-modified bone marrow mesenchymal stromal cell gene therapy. *Cytotherapy* 2009; **11**: 317-325 [PMID: 19308772 DOI: 10.1080/14653240902824757]
22 **Cao Y**, Liu Z, Xie Y, Hu J, Wang H, Fan Z, Zhang C, Wang J, Wu CT, Wang S. Adenovirus-mediated transfer of hepatocyte growth factor gene to human dental pulp stem cells under good manufacturing practice improves their potential for periodontal regeneration in swine. *Stem Cell Res Ther* 2015; **6**: 249 [PMID: 26670567 DOI: 10.1186/s13287-015-0244-5]
23 **Zheng B**, Jiang J, Chen Y, Lin M, Du Z, Xiao Y, Luo K, Yan F. Leptin Overexpression in Bone Marrow Stromal Cells Promotes Periodontal Regeneration in a Rat Model of Osteoporosis. *J Periodontol* 2017; **88**: 808-818 [PMID: 28440742 DOI: 10.1902/jop.2017.170042]
24 **Qiu W**, Wu BL, Fang FC. Overview of noncoding RNAs involved in the osteogenic differentiation of periodontal ligament stem cells. *World J Stem Cells* 2020; **12**: 251-265 [PMID: 32399134 DOI: 10.4252/wjsc.v12.i4.251]
25 **Muhammad SA**, Nordin N, Fakurazi S. Regenerative potential of secretome from dental stem cells: a systematic review of preclinical studies. *Rev Neurosci* 2018; **29**: 321-332 [PMID: 29220331 DOI: 10.1515/revneuro-2017-0069]
26 **Nagata M**, Iwasaki K, Akazawa K, Komaki M, Yokoyama N, Izumi Y, Morita I. Conditioned Medium from Periodontal Ligament Stem Cells Enhances Periodontal Regeneration. *Tissue Eng Part A* 2017; **23**: 367-377 [PMID: 28027709 DOI: 10.1089/ten.TEA.2016.0274]
27 **Qiu J**, Wang X, Zhou H, Zhang C, Wang Y, Huang J, Liu M, Yang P, Song A. Enhancement of periodontal tissue regeneration by conditioned media from gingiva-derived or periodontal ligament-derived mesenchymal stem cells: a comparative study in rats. *Stem Cell Res Ther* 2020; **11**: 42 [PMID: 32014015 DOI: 10.1186/s13287-019-1546-9]
28 **Inukai T**, Katagiri W, Yoshimi R, Osugi M, Kawai T, Hibi H, Ueda M. Novel application of stem cell-derived factors for periodontal regeneration. *Biochem Biophys Res Commun* 2013; **430**: 763-768 [PMID: 23206704 DOI: 10.1016/j.bbrc.2012.11.074]
29 **Kawai T**, Katagiri W, Osugi M, Sugimura Y, Hibi H, Ueda M. Secretomes from bone marrow-derived mesenchymal stromal cells enhance periodontal tissue regeneration. *Cytotherapy* 2015; **17**: 369-381 [PMID: 25595330 DOI: 10.1016/j.jcyt.2014.11.009]
30 **Chew JRJ**, Chuah SJ, Teo KYW, Zhang S, Lai RC, Fu JH, Lim LP, Lim SK, Toh WS. Mesenchymal stem cell exosomes enhance periodontal ligament cell functions and promote periodontal regeneration. *Acta Biomater* 2019; **89**: 252-264 [PMID: 30878447 DOI: 10.1016/j.actbio.2019.03.021]
31 **Kawai M**, Kataoka YH, Sonobe J, Yamamoto H, Inubushi M, Ishimoto T, Nakano T, Maruyama H, Miyazaki JI, Yamamoto T, Bessho K, Ohura K. Non-surgical model for alveolar bone regeneration by bone morphogenetic protein-2/7 gene therapy. *J Periodontol* 2018; **89**: 85-92 [PMID: 28820323 DOI: 10.1902/jop.2017.170328]
32 **Jin Q**, Anusaksathien O, Webb SA, Printz MA, Giannobile WV. Engineering of tooth-supporting structures by delivery of PDGF gene therapy vectors. *Mol Ther* 2004; **9**: 519-526 [PMID: 15093182 DOI: 10.1016/j.ymthe.2004.01.016]
33 **Tsuchiya S**, Chiba M, Kishimoto KN, Nakamura H, Tsuchiya M, Hayashi H. Transfer of the bone

morphogenetic protein 4 gene into rat periodontal ligament by in vivo electroporation. *Arch Oral Biol* 2017; **74**: 123-132 [PMID: 27940045 DOI: 10.1016/j.archoralbio.2016.11.013]
34 **Plonka AB**, Khorsand B, Yu N, Sugai JV, Salem AK, Giannobile WV, Elangovan S. Effect of sustained PDGF nonviral gene delivery on repair of tooth-supporting bone defects. *Gene Ther* 2017; **24**: 31-39 [PMID: 27824330 DOI: 10.1038/gt.2016.73]
35 **Maeda H**, Tomokiyo A, Fujii S, Wada N, Akamine A. Promise of periodontal ligament stem cells in regeneration of periodontium. *Stem Cell Res Ther* 2011; **2**: 33 [PMID: 21861868 DOI: 10.1186/scrt74]
36 **Seo BM**, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; **364**: 149-155 [PMID: 15246727 DOI: 10.1016/S0140-6736(04)16627-0]
37 **Chai Y**, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development* 2000; **127**: 1671-1679 [PMID: 10725243]
38 **Mitsiadis TA**, Graf D. Cell fate determination during tooth development and regeneration. *Birth Defects Res C Embryo Today* 2009; **87**: 199-211 [PMID: 19750524 DOI: 10.1002/bdrc.20160]
39 **Tomokiyo A**, Wada N, Maeda H. Periodontal Ligament Stem Cells: Regenerative Potency in Periodontium. *Stem Cells Dev* 2019; **28**: 974-985 [PMID: 31215350 DOI: 10.1089/scd.2019.0031]
40 **Huang CY**, Pelaez D, Dominguez-Bendala J, Garcia-Godoy F, Cheung HS. Plasticity of stem cells derived from adult periodontal ligament. *Regen Med* 2009; **4**: 809-821 [PMID: 19903001 DOI: 10.2217/rme.09.55]
41 **Maeda H**, Wada N, Tomokiyo A, Monnouchi S, Akamine A. Prospective potency of TGF-β1 on maintenance and regeneration of periodontal tissue. *Int Rev Cell Mol Biol* 2013; **304**: 283-367 [PMID: 23809439 DOI: 10.1016/B978-0-12-407696-9.00006-3]
42 **Song M**, Kim H, Choi Y, Kim K, Chung C. Skeletal myogenic differentiation of human periodontal ligament stromal cells isolated from orthodontically extracted premolars. *Korean J Orthod* 2012; **42**: 249-254 [PMID: 23173118 DOI: 10.4041/kjod.2012.42.5.249]
43 **Tomokiyo A**, Maeda H, Fujii S, Wada N, Shima K, Akamine A. Development of a multipotent clonal human periodontal ligament cell line. *Differentiation* 2008; **76**: 337-347 [PMID: 18021259 DOI: 10.1111/j.1432-0436.2007.00233.x]
44 **Huang L**, Liang J, Geng Y, Tsang WM, Yao X, Jhanji V, Zhang M, Cheung HS, Pang CP, Yam GH. Directing adult human periodontal ligament-derived stem cells to retinal fate. *Invest Ophthalmol Vis Sci* 2013; **54**: 3965-3974 [PMID: 23661377 DOI: 10.1167/iovs.13-11910]
45 **Takahashi M**, Okubo N, Chosa N, Takahashi N, Ibi M, Kamo M, Mizuki H, Ishisaki A, Kyakumoto S. Fibroblast growth factor-1-induced ERK1/2 signaling reciprocally regulates proliferation and smooth muscle cell differentiation of ligament-derived endothelial progenitor cell-like cells. *Int J Mol Med* 2012; **29**: 357-364 [PMID: 22108586 DOI: 10.3892/ijmm.2011.847]
46 **Lee JS**, An SY, Kwon IK, Heo JS. Transdifferentiation of human periodontal ligament stem cells into pancreatic cell lineage. *Cell Biochem Funct* 2014; **32**: 605-611 [PMID: 25187163 DOI: 10.1002/cbf.3057]
47 **Vasanthan P**, Jayaraman P, Kunasekaran W, Lawrence A, Gnanasegaran N, Govindasamy V, Musa S, Kasim NH. Generation of functional hepatocyte-like cells from human deciduous periodontal ligament stem cells. *Naturwissenschaften* 2016; **103**: 62 [PMID: 27379400 DOI: 10.1007/s00114-016-1387-7]
48 **Wada N**, Menicanin D, Shi S, Bartold PM, Gronthos S. Immunomodulatory properties of human periodontal ligament stem cells. *J Cell Physiol* 2009; **219**: 667-676 [PMID: 19160415 DOI: 10.1002/jcp.21710]
49 **Trubiani O**, Zalzal SF, Paganelli R, Marchisio M, Giancola R, Pizzicannella J, Bühring HJ, Piattelli M, Caputi S, Nanci A. Expression profile of the embryonic markers nanog, OCT-4, SSEA-1, SSEA-4, and frizzled-9 receptor in human periodontal ligament mesenchymal stem cells. *J Cell Physiol* 2010; **225**: 123-131 [PMID: 20458727 DOI: 10.1002/jcp.22203]
50 **Tomokiyo A**, Yoshida S, Hamano S, Hasegawa D, Sugii H, Maeda H. Detection, Characterization, and Clinical Application of Mesenchymal Stem Cells in Periodontal Ligament Tissue. *Stem Cells Int* 2018; **2018**: 5450768 [PMID: 30224921 DOI: 10.1155/2018/5450768]
51 **Kawanabe N**, Murata S, Murakami K, Ishihara Y, Hayano S, Kurosaka H, Kamioka H, Takano-Yamamoto T, Yamashiro T. Isolation of multipotent stem cells in human periodontal ligament using stage-specific embryonic antigen-4. *Differentiation* 2010; **79**: 74-83 [PMID: 19945209 DOI: 10.1016/j.diff.2009.10.005]
52 **Ng TK**, Chen CB, Xu C, Xu Y, Yao X, Huang L, Liang JJ, Cheung HS, Pang CP, Huang Y. Attenuated regenerative properties in human periodontal ligament-derived stem cells of older donor ages with shorter telomere length and lower SSEA4 expression. *Cell Tissue Res* 2020; **381**: 71-81 [PMID: 32043210 DOI: 10.1007/s00441-020-03176-y]
53 **Zhou LL**, Liu W, Wu YM, Sun WL, Dörfer CE, Fawzy El-Sayed KM. Oral Mesenchymal Stem/Progenitor Cells: The Immunomodulatory Masters. *Stem Cells Int* 2020; **2020**: 1327405 [PMID: 32184830 DOI: 10.1155/2020/1327405]
54 **Kassebaum NJ**, Smith AGC, Bernabé E, Fleming TD, Reynolds AE, Vos T, Murray CJL, Marcenes W; GBD 2015 Oral Health Collaborators. Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors. *J Dent Res* 2017; **96**: 380-387 [PMID: 28792274 DOI: 10.1177/0022034517693566]
55 **Iwata T**, Yamato M, Tsuchioka H, Takagi R, Mukobata S, Washio K, Okano T, Ishikawa I. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. *Biomaterials* 2009; **30**: 2716-2723 [PMID: 19201461 DOI: 10.1016/j.biomaterials.2009.01.032]
56 **Park JY**, Jeon SH, Choung PH. Efficacy of periodontal stem cell transplantation in the treatment of advanced periodontitis. *Cell Transplant* 2011; **20**: 271-285 [PMID: 20719084 DOI: 10.3727/096368910X519292]
57 **Yu N**, Oortgiesen DA, Bronckers AL, Yang F, Walboomers XF, Jansen JA. Enhanced periodontal tissue regeneration by periodontal cell implantation. *J Clin Periodontol* 2013; **40**: 698-706 [PMID: 23675871 DOI: 10.1111/jcpe.12113]
58 **Ding G**, Liu Y, Wang W, Wei F, Liu D, Fan Z, An Y, Zhang C, Wang S. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. *Stem Cells* 2010; **28**: 1829-1838 [PMID: 20979138 DOI:

10.1002/stem.512]
59 **Yan XZ**, Yang F, Jansen JA, de Vries RB, van den Beucken JJ. Cell-Based Approaches in Periodontal Regeneration: A Systematic Review and Meta-Analysis of Periodontal Defect Models in Animal Experimental Work. *Tissue Eng Part B Rev* 2015; **21**: 411-426 [PMID: 25929285 DOI: 10.1089/ten.TEB.2015.0049]
60 **Bassir SH**, Wisitrasameewong W, Raanan J, Ghaffarigarakani S, Chung J, Freire M, Andrada LC, Intini G. Potential for Stem Cell-Based Periodontal Therapy. *J Cell Physiol* 2016; **231**: 50-61 [PMID: 26058394 DOI: 10.1002/jcp.25067]
61 **Tsumanuma Y**, Iwata T, Washio K, Yoshida T, Yamada A, Takagi R, Ohno T, Lin K, Yamato M, Ishikawa I, Okano T, Izumi Y. Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. *Biomaterials* 2011; **32**: 5819-5825 [PMID: 21605900 DOI: 10.1016/j.biomaterials.2011.04.071]
62 **Iwasaki K**, Akazawa K, Nagata M, Komaki M, Honda I, Morioka C, Yokoyama N, Ayame H, Yamaki K, Tanaka Y, Kimura T, Kishida A, Watabe T, Morita I. The Fate of Transplanted Periodontal Ligament Stem Cells in Surgically Created Periodontal Defects in Rats. *Int J Mol Sci* 2019; **20** [PMID: 30621073 DOI: 10.3390/ijms20010192]
63 **Xu J**, Wang W, Kapila Y, Lotz J, Kapila S. Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. *Stem Cells Dev* 2009; **18**: 487-496 [PMID: 18593336 DOI: 10.1089/scd.2008.0113]
64 **Hidaka T**, Nagasawa T, Shirai K, Kado T, Furuichi Y. FGF-2 induces proliferation of human periodontal ligament cells and maintains differentiation potentials of STRO-1(+)/CD146(+) periodontal ligament cells. *Arch Oral Biol* 2012; **57**: 830-840 [PMID: 22244620 DOI: 10.1016/j.archoralbio.2011.12.003]
65 **Wada N**, Maeda H, Hasegawa D, Gronthos S, Bartold PM, Menicanin D, Fujii S, Yoshida S, Tomokiyo A, Monnouchi S, Akamine A. Semaphorin 3A induces mesenchymal-stem-like properties in human periodontal ligament cells. *Stem Cells Dev* 2014; **23**: 2225-2236 [PMID: 24380401 DOI: 10.1089/scd.2013.0405]
66 **Hasegawa D**, Hasegawa K, Kaneko H, Yoshida S, Mitarai H, Arima M, Tomokiyo A, Hamano S, Sugii H, Wada N, Kiyoshima T, Maeda H. MEST regulates the stemness of human periodontal ligament stem cells. *Stem Cells International* 2020; **2020**: 15 [DOI: 10.1155/2020/9672673]
67 **Hamano S**, Tomokiyo A, Hasegawa D, Yoshida S, Sugii H, Mitarai H, Fujino S, Wada N, Maeda H. Extracellular Matrix from Periodontal Ligament Cells Could Induce the Differentiation of Induced Pluripotent Stem Cells to Periodontal Ligament Stem Cell-Like Cells. *Stem Cells Dev* 2018; **27**: 100-111 [PMID: 29160151 DOI: 10.1089/scd.2017.0077]
68 **Xu H**, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, Ueda T, Gee P, Nishikawa M, Nomura M, Kitaoka F, Takahashi T, Okita K, Yoshida Y, Kaneko S, Hotta A. Targeted Disruption of HLA Genes *via* CRISPR-Cas9 Generates iPSCs with Enhanced Immune Compatibility. *Cell Stem Cell* 2019; **24**: 566-578.e7 [PMID: 30853558 DOI: 10.1016/j.stem.2019.02.005]

World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2020 September 26; 12(9): 1032-1049

DOI: 10.4252/wjsc.v12.i9.1032

ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Human mesenchymal stem cells derived from umbilical cord and bone marrow exert immunomodulatory effects in different mechanisms

Yunejin Song, Jung-Yeon Lim, Taekyu Lim, Keon-Il Im, Nayoun Kim, Young-Sun Nam, Young-Woo Jeon, Jong Chul Shin, Hyun Sun Ko, In Yang Park, Seok-Goo Cho

ORCID number: Yunejin Song 0000-0002-9379-422X; Jung-Yeon Lim 0000-0001-5903-8810; Taekyu Lim 0000-0002-3427-6705; Keon-Il Im 0000-0003-2811-9815; Nayoun Kim 0000-0001-8317-2921; Young-Sun Nam 0000-0002-9327-5745; Young-Woo Jeon 0000-0003-3362-8200; Jong Chul Shin 0000-0003-0360-2309; Hyun Sun Ko 0000-0001-6310-6206; In Yang Park 0000-0002-9458-2886; Seok-Goo Cho 0000-0002-5429-4839.

Author contributions: Song Y and Lim JY conceptualized the original idea, designed the experiments and wrote the manuscript; Song Y, Lim JY, Lim T, Im KI, Kim N, Nam YS and Jeon YW isolated the stem cells, performed the experiments and analyzed the data; Shin JC, Ko HS and Park IY provided human resources; All authors approved the final version of the article.

Supported by Korean Health Technology R&D Project, No. HI16C2178.

Institutional review board statement: This study was reviewed and approved by the Catholic University of Korea Catholic Medical Center Institutional Review Board.

Yunejin Song, Jung-Yeon Lim, Keon-Il Im, Nayoun Kim, Young-Sun Nam, Young-Woo Jeon, Seok-Goo Cho, Institute for Translational Research and Molecular Imaging, College of Medicine, The Catholic University of Korea, Seoul 06591, South Korea

Yunejin Song, Jung-Yeon Lim, Keon-Il Im, Nayoun Kim, Young-Sun Nam, Young-Woo Jeon, Seok-Goo Cho, Laboratory of Immune Regulation, Convergent Research Consortium for Immunologic Disease, College of Medicine, The Catholic University of Korea, Seoul 06591, South Korea

Yunejin Song, Department of Biomedicine and Health Sciences, College of Medicine, The Catholic University of Korea, Seoul 06591, South Korea

Jung-Yeon Lim, Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

Taekyu Lim, Division of Hematology Oncology, Department of Internal Medicine, Veterans Health Service Medical Center, Seoul 05368, South Korea

Young-Woo Jeon, Seok-Goo Cho, Department of Hematology, Catholic Blood and Marrow Transplantation Center, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul 06591, South Korea

Jong Chul Shin, Department of Obstetrics and Gynecology, CHA Bundang Medical Center, CHA University, Seongnam 13496, South Korea

Hyun Sun Ko, In Yang Park, Department of Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea, Seoul 06591, South Korea

Seok-Goo Cho, Division of Hematology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul 06591, South Korea

Corresponding author: Seok-Goo Cho, MD, PhD, Professor, Division of Hematology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Banpodaero 222, Seocho-Gu, Seoul 06591, South Korea. chosg@catholic.ac.kr

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by Institutional Animal care and Use Committee in School of Medicine, The Catholic University of Korea; approval number: CUMC-2018-0270-01.

Conflict-of-interest statement: The authors have nothing to disclose.

Data sharing statement: The dataset supporting the conclusions of this article are included within in the article.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Manuscript source: Unsolicited manuscript

Received: March 20, 2020
Peer-review started: March 20, 2020
First decision: April 25, 2020
Revised: June 20, 2020
Accepted: July 19, 2020
Article in press: July 19, 2020
Published online: September 26, 2020

P-Reviewer: Aydin M
S-Editor: Zhang L
L-Editor: Filipodia
P-Editor: Xing YX

Abstract

BACKGROUND

Mesenchymal stem cells (MSCs) are an attractive tool to treat graft-versus-host disease because of their unique immunoregulatory properties. Although human bone marrow-derived MSCs (BM-MSCs) were the most widely used MSCs in cell therapy until recently, MSCs derived from human umbilical cords (UC-MSCs) have gained popularity as cell therapy material for their ethical and noninvasive collection.

AIM

To investigate the difference in mechanisms of the immunosuppressive effects of UC-MSCs and BM-MSCs.

METHODS

To analyze soluble factors expressed by MSCs, such as indolamine 2,3-dioxygenase, cyclooxygenase-2, prostaglandin E2 and interleukin (IL)-6, inflammatory environments *in vitro* were reconstituted with combinations of interferon-gamma (IFN-γ), tumor necrosis factor alpha and IL-1β or with IFN-γ alone. Activated T cells were cocultured with MSCs treated with indomethacin and/or anti-IL-10. To assess the ability of MSCs to inhibit T helper 17 cells and induce regulatory T cells, induced T helper 17 cells were cocultured with MSCs treated with indomethacin or anti-IL-10. Xenogeneic graft-versus-host disease was induced in NOG mice (NOD/Shi-*scid*/IL-2Rγ^{null}) and UC-MSCs or BM-MSCs were treated as cell therapies.

RESULTS

Our data demonstrated that BM-MSCs and UC-MSCs shared similar phenotypic characteristics and immunomodulation abilities. BM-MSCs expressed more indolamine 2,3-dioxygenase after cytokine stimulation with different combinations of IFN-γ, tumor necrosis factor alpha-α and IL-1β or IFN-γ alone. UC-MSCs expressed more prostaglandin E2, IL-6, programmed death-ligand 1 and 2 in the *in vitro* inflammatory environment. Cyclooxygenase-2 and IL-10 were key factors in the immunomodulatory mechanisms of both MSCs. In addition, UC-MSCs inhibited more T helper 17 cells and induced more regulatory T cells than BM-MSCs. UC-MSCs and BM-MSCs exhibited similar effects on attenuating graft-versus-host disease.

CONCLUSION

UC-MSCs and BM-MSCs exert similar immunosuppressive effects with different mechanisms involved. These findings suggest that UC-MSCs have distinct immunoregulatory functions and may substitute BM-MBSCs in the field of cell therapy.

Key Words: Mesenchymal stem cells; Graft-versus-host disease; Umbilical cord; Cell therapy; Xenogeneic mouse model; Immunomodulation

Core Tip: Mesenchymal stem cells (MSCs) are a therapeutic approach to treat graft-versus-host disease because of their unique immunomodulatory abilities. Here, we compared and analyzed the differences and similarities between umbilical cord-derived MSCs and bone marrow-derived MSCs due to the growing needs for new sources of MSCs. We suggest that umbilical cord-derived MSCs and bone marrow-derived MSCs exhibit similar immunosuppression by different mechanisms, and umbilical cord-derived MSCs have the potentials to substitute bone marrow-derived MSCs as cell therapy products.

Citation: Song Y, Lim JY, Lim T, Im KI, Kim N, Nam YS, Jeon YW, Shin JC, Ko HS, Park IY, Cho SG. Human mesenchymal stem cells derived from umbilical cord and bone marrow exert immunomodulatory effects in different mechanisms. *World J Stem Cells* 2020; 12(9): 1032-1049

URL: https://www.wjgnet.com/1948-0210/full/v12/i9/1032.htm
DOI: https://dx.doi.org/10.4252/wjsc.v12.i9.1032

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HSCT) is one of the most curative treatments for patients with hematological disorders, hematopoietic malignancies and immune diseases. Through HSCT, healthy hematopoietic donor cells can reconstruct the hematopoietic system of recipients. Despite the benefits of HSCT, there are risks of graft rejection, tissue toxicity, organ injury and leukemia relapse; among these risks, graft-versus-host disease (GVHD) is the major cause of death after transplantation[1]. In GVHD, alloreactive donor T cells recognize histocompatibility antigens in host cells as foreign and activate to produce inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-2 and interferon-gamma (IFN-γ)[2]. Although immunosuppressive drugs such as tacrolimus, cyclosporine and steroids are used in clinical therapy to lower GVHD mortality, a new therapeutic approach for GVHD following HSCT is needed.

Mesenchymal stem cells (MSCs) are multipotent stromal cells that have the ability to differentiate into different cell types, such as osteoblasts, chondrocytes and adipocytes[3]. Recently, MSCs have been studied for cell therapy in the field of transplantation. A major characteristic of MSCs is that they express low major histocompatibility complex II, suppressing T cells independent from the major histocompatibility complex identity between the donor and recipient[4]. Their immunosuppressive effects mainly occur through the production of soluble factors such as indolamine 2,3-dioxygenase (IDO), transforming growth factor-β, human leukocyte antigen (HLA)-G and hepatocyte growth factor[5-8]. While soluble factors are the primary mechanism by which MSCs exert immunosuppressive effects, direct cell-to-cell contact is also a contributing factor[9]. Cell-to-cell contact between $CD3^+$ T cells and MSCs can induce $CD4^+CD25^+$ regulatory T cells[10].

Traditionally, MSCs have been isolated from bone marrow, and bone marrow-derived MSCs (BM-MSCs) have been identified as a key candidate for cell therapy[11]. However, their clinical application is restricted by the invasive procedures by which they are obtained, their decrease in proliferation and differentiation capacity with age and the difficulty of using them to treat patients with hereditary diseases[12,13]. As a result, other adult and fetal tissues have been studied as alternative sources of stem cells, including adipose tissue, umbilical cord blood, umbilical cords, amniotic fluid and placental tissue[14,15]. Although the major source of MSCs in clinical trials is bone marrow, recent studies have suggested that the allogenicity of MSCs has no significant adverse impact on the engraftment of MSCs in wound healing[16]. It is better to use freshly isolated MSCs because the five major histocompatibility complex II molecules may increase during *in vitro* expansion[17,18]. Additionally, the proliferative capacity of umbilical cord-derived MSCs (UC-MSCs) is higher than that of BM-MSCs. Several studies have emphasized the potential of UC-MSCs as an alternative to BM-MSCs; however, detailed comparisons of UC-MSCs and BM-MSCs are lacking.

Therefore, we compared the similarities and differences between UC-MSCs and BM-MSCs. In particular, we (1) investigated the *in vitro* immunoregulatory properties of UC-MSCs; (2) explored the mechanisms of immunosuppressive effects; and (3) analyzed the efficacy of UC-MSC and BM-MSC therapies to treat xenogeneic GVHD induced in severely immunodeficient NOG mice.

MATERIALS AND METHODS

Mice

Eight- to ten-week-old female NOG mice (NOD/Shi-*scid*/IL-2Rγ^{null}) were purchased from the Central Institute for Experimental Animals (Kanagawa, Japan). Mice were maintained under pathogen-free conditions in an animal facility with controlled daylength (12L:12D), humidity (55% ± 5%) and temperature (22 ± 1 °C). The air in the facility passed through a HEPA filter system designed to exclude bacteria and viruses. Animals were fed mouse chow and tap water *ad libitum*.

Isolation and culturing of human MSCs

UCs were collected aseptically from full-term cesarean-section patients with informed consent. The UCs were transferred after collection, and the process was initiated within 24 h of delivery. The UC surface was rinsed with phosphate-buffered saline (Gibco–BRL) containing antibiotics and antifungal reagents (Antibiotic–Antimycotic, 100 ×; Gibco–BRL). In the explant method, the minced fragments were aligned and attached at regular intervals in 10-cm culture dishes. After the fragments were semi-dried and firmly attached to the bottom, culture medium was gently poured into the dishes. The cells were then washed with Alpha Minimum Essential Medium (Gibco) supplemented with 10% fetal bovine serum and seeded in 10-cm tissue culture dishes with culture medium as described above. The culture medium was refreshed once a week for 3-4 wk until fibroblast-like adherent cells reached 80%-90% confluence. The first-harvested master cells were defined as passage 0[19]. BM-MSCs (passage 2–3) were purchased from Catholic MASTER Cells (Seoul, South Korea) and maintained in Dulbecco's modified Eagle's medium (Gibco) supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin solution (Gibco).

Characterization of MSCs

Fluorescence-activated cell sorting flow cytometry analysis was performed using CD29 (TS2/16; eBioscience, San Diego, CA, United States), CD44 (IM7; eBioscience), CD105 (SN6; eBioscience), CD90 (5E10, eBioscience), CD73 (AD2, eBioscience), CD45 (2D1; eBioscience), CD31 (WM-59; eBioscience), CD34 (4H11; eBioscience), HLA-ABC (W6/32; eBioscience), HLA-DR (L243; eBioscience), C-C chemokine receptor type 1 (CCR1; 5F10B29; BioLegend, San Diego, CA, United States), CCR2 (K036C2; BioLegend), CCR7 (3D12; eBioscience), C-X-C chemokine receptor type 4 (CXCR4; 12G5; eBioscience) and CXCR5 (MU5UBEE; eBioscience) antibodies to confirm that the MSC phenotype was maintained after expansion in the culture. The samples were incubated with antibodies against each surface marker for 30 min, and this treatment was followed by fluorescence-activated cell sorting. Flow cytometry analysis was performed on a LSRFortessa (BD Pharmingen, San Diego, CA, United States).

Differentiation assay

One function of MSCs is to differentiate into osteoblasts, chondrocytes or adipocytes[20]. For adipogenic differentiation assays, MSCs were seeded into 24-well plates at a density of 1×10^4 per well and induced to differentiate into adipocytes using the Human MSC Functional Identification Kit (R and D Systems, Minneapolis, MN, United States) following the manufacturer's protocol. The medium was changed three times a week for 14-21 d. Adipocytes were detected using goat anti-mouse FABP4 polyclonal antibody.

For osteogenic and chondrogenic differentiation assays, MSCs were seeded into 12-well plates at the manufacturer-recommended density and induced to differentiate into osteoblasts or chondrocytes using StemPro Chondrogenesis/Osteogenesis Differentiation Kits (Gibco, United States) following the manufacturer's protocols. The medium was changed three times a week for 21 d. Osteoblasts and chondrocytes were stained using Alizarin Red S and Alcian Blue, respectively.

Real-time reverse-transcription polymerase chain reaction

Total RNA was extracted using TRIzol LS reagent (Invitrogen). Isolated total RNA (2 μg) was reverse transcribed into complementary DNA at 50 °C for 2 min, followed by 60 °C for 30 min. Quantitative polymerase chain reaction was performed using the FastStart DNA Master SYBR Green I kit and a LightCycler 480 Detection system (Bio-Rad, CA, United States), as specified by the manufacturer. The crossing point was defined as the maximum of the second derivative from the fluorescence curve. Negative controls were included and contained all elements of the reaction mixture, except for template DNA. For quantification, we report relative mRNA expression levels of specific genes obtained using the $2^{-\Delta Ct}$ method. For normalization, *GAPDH*, a housekeeping gene, was used. The following gene-specific primers were used: *Klf4* (forward: 5'-CGAACCCACACAGGTGAGAA-3'; reverse: 5'-GAGCGGGCGAATTTCCAT-3'), *OCT4* (forward: 5'-GGAGGAAGCTGACAACAATGAAA-3'; reverse: 5'-GGCCTGCACGAGGGTTT-3'), *Nanog* (forward: 5'-ACAACTGGCCGAAGAATAGCA-3'; reverse: 5'-GGTTCCCAGTCGGGTTCAC-3'), and *GAPDH* (forward: 5'-TGCCAAATATGATGACATCA-3'; reverse: 5'-GGAGTGGGTGTCGCTGTTG-3').

Luminex multiplex cytokine assay

Different cytokines (IDO, IL-6) in human cell culture supernatants were assessed with a Luminex MAGPIX instrument (Luminex, Austin, TX, United States) using the ProcartaPlex Human 4-plex immunoassay kit (Affymetrix, Santa Clara, CA, United States) according to the manufacturer's instructions as described below. Briefly, samples were mixed with antibody-linked polystyrene beads in 96-well filter-bottom plates and incubated at room temperature for 2 h on an orbital shaker at 500-600 rpm. The plate was inserted into a magnetic plate washer and washed twice, then incubated with biotinylated detection antibody for 30 min at room temperature. Samples were washed twice as above and resuspended in streptavidin-PE. Two additional washes were performed as before with 30 min incubation at room temperature. After wash step, Reading Buffer was added to the sample. Each sample was measured in duplicate. Plates were read using a MAGPIX instrument with xPONENT 4.2 software (Luminex). Cytokine concentrations were calculated using ProcartaPlex Analyst 1.0 software (Affymetrix).

Western blot analysis

MSCs protein extractions were prepared from 1×10^5 cells by homogenization in lysis buffer with a protease/phosphatase inhibitor cocktail (Cell Signaling, Danvers, MA, United States) and centrifuged for 15 min at 14000 rpm. The protein concentration in the supernatant was measured following the Bradford method (Bio-Rad). Protein samples were separated using sodium dodecyl sulfate gel electrophoresis and transferred onto a nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). Membranes were stained with primary antibodies specific to IDO, COX2, β-actin (Cell Signaling) or iNOS (R and D Systems). Then, horseradish peroxidase-conjugated secondary antibodies were added. Membranes were washed with Tris-buffered saline and Tween 20 solution, and the hybridized protein bands were detected using an enhanced chemiluminescence detection kit and Hyperfilm enhanced chemiluminescence reagents (Amersham Pharmacia Biotech).

Induction and treatment of xenogenic GVHD

Human peripheral blood was obtained from healthy volunteers with their consent, and peripheral blood mononuclear cells (PBMCs) were isolated with Ficoll-Hypaque (GE Healthcare +UK Ltd., United Kingdom) density centrifugation and washed in phosphate-buffered saline. Cells were resuspended in phosphate-buffered saline and injected through the tail vein into irradiated mice. The mice received a single dose of 200 cGy gamma irradiation from a linear accelerator before injection of human PBMCs on the same day. These animals were divided into three groups (n = 12 mice/group): GVHD, UC-MSC and BM-MSC. All animals were randomly allocated and coded. All animals were monitored for clinical symptoms of GVHD (weight loss, hunched posture, fur loss, reduced mobility, skin integrity) and mean serial weight measurements. The mice that showed severe inflammation in unintended body parts causing blindness and bowel obstruction, 20% weight loss compared to mice of same age, anorexia, quadriplegia and gait impairment were administered euthanasia by CO_2 exposure.

In vitro growth assay

MSCs at each passage were seeded at a density of 1×10^5 in 100 mm culture dishes. Culture dishes were incubated in 37 °C with 5% CO_2 until reaching 90% confluence and then counted.

In vitro proliferation assay

Human PBMCs (1×10^5/well) from healthy adult donors were plated in 96-well flat-bottomed plates (200 μL/well). Cells were then stimulated with 5 μg/mL phytohemagglutinin (Sigma-Aldrich) and/or MSCs (5×10^3, 1×10^4, 5×10^4, 1×10^5) for 72 h, followed by the incorporation of 1 μCi/mL [3H]-thymidine (GE Healthcare, Piscataway, NJ, United States) for the last 18 h of the indicated culture period. Radioactivity was measured using a Micro Beta instrument (Pharmacia Biotech, Piscataway, NJ, United States).

In other experiments, neutralizing antibodies for human IL-10 or transforming growth factor-β mAb (10 μg/mL; R and D Systems) and chemical antagonists for COX2 (indomethacin, 20 μM; Sigma-Aldrich), iNOS [N-nitro-L-arginine methyl ester (L-NAME), 1 mM; Sigma-Aldrich], heme oxygenase 1 inducer (hemin, 50 ng/mL; Sigma-Aldrich), selective A_{2B} adenosine receptor (alloxazine, 10 μM; Sigma-Aldrich),

and CD73 (α,β-methylene ADP [APCP], 100 μM; Sigma-Aldrich) were added into the coculture. All experiments were performed in quadruple and were repeated at least twice.

Flow cytometry analysis

PBMCs were immunostained using various combinations of the following fluorescence-conjugated antibodies: CD4 (RPA-T4; BioLegend), CD25 (BC96; eBioscience), Foxp3 (PCH101; eBioscience), IL-17 (eBio64DEC17; eBioscience) and IFN-γ (4S.B3; eBioscience). The cells were also intracellularly stained with the following antibodies: IL-17, IFN- γ and Foxp3. Before intracellular staining, cells were stimulated in culture medium containing 25 ng/mL phorbol myristate acetate (Sigma-Aldrich), 250 ng/mL ionomycin (Sigma-Aldrich) and 1 μL/mL GolgiSTOP (BD Pharmingen) in an incubator with 5% CO_2 at 37 °C for 4 h. Intracellular staining was conducted using an intracellular staining kit (eBioscience) according to the manufacturer's protocol. Flow cytometric analysis was performed on a LSRFortessa (BD Pharmingen).

Statistical analysis

Statistical significance was determined using Student's two-tailed *t*-test. In all analyses, *P* values less than 0.05 were considered to indicate statistical significance.

Ethics

All procedures involving animals were performed in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals and the Guidelines and Policies for Rodent Experimentation provided by the Institutional Animal Care and Use Committee of the School of Medicine of the Catholic University of Korea (Seoul, South Korea). The protocols used in this study were approved by the institutional review board of The Catholic University of Korea (CUMC-2018-0270-01).

RESULTS

Characteristics of MSCs

We analyzed the expression of surface proteins for immunophenotypic characterization of culture-expanded UC-MSCs and BM-MSCs. All culture-expanded MSCs showed similar spindle-shaped morphologies (data not shown) and were uniformly positive for CD29, CD44, CD105, CD73, CD90 and HLA-ABC but negative for CD45, CD31, CD34 and HLA-DR (Figure 1A).

We used flow cytometry to examine the cell surface expression of chemokine receptors. Neither UC-MSCs nor BM-MSCs expressed CCR2. Both MSC types expressed CCR1, CCR7, CXCR4 and CXCR5 (Figure 1B).

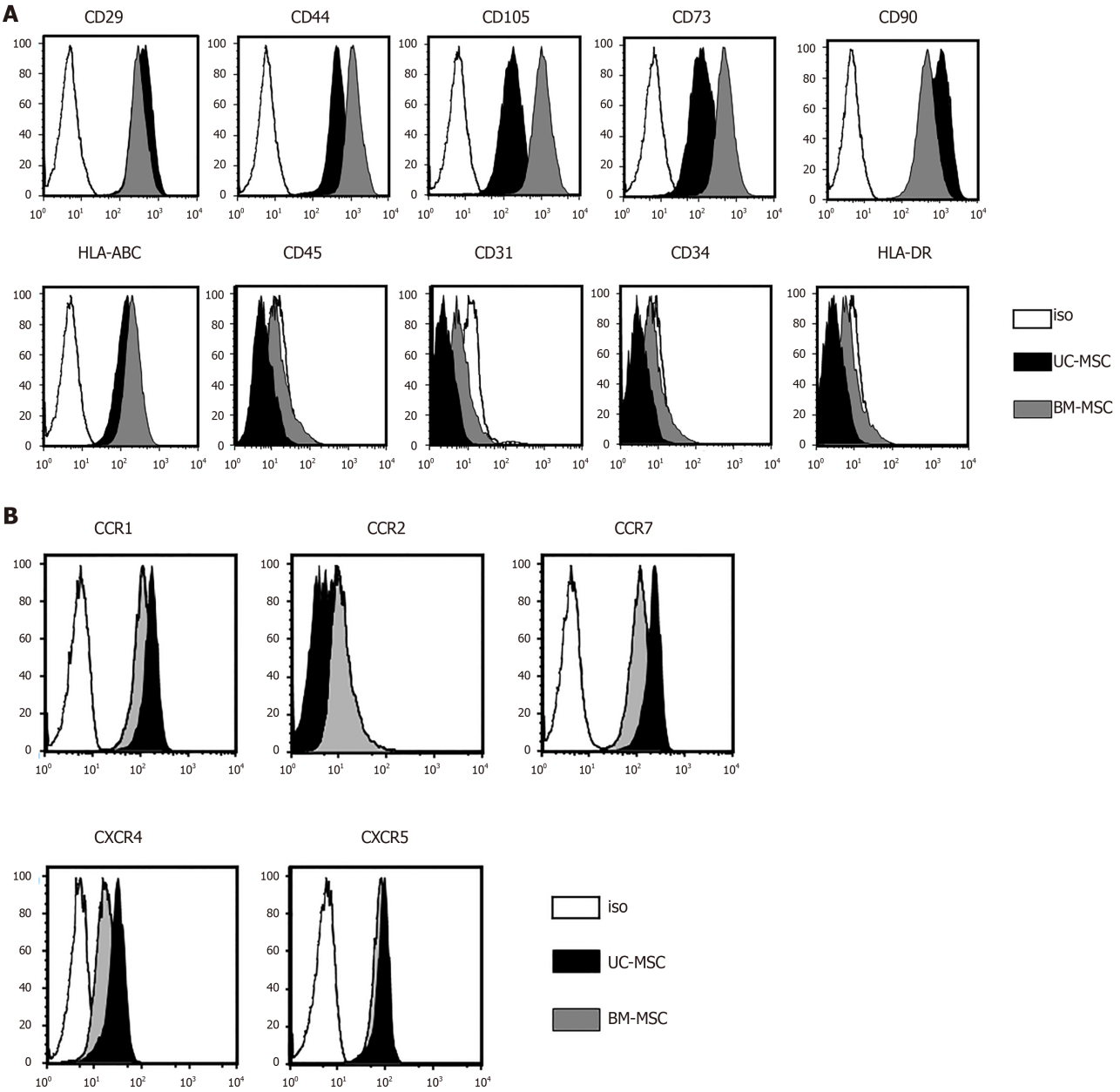
For adipogenic induction, BM-MSCs formed lipid vacuoles after 7 d and UC-MSCs after 14 d. After they changed morphology, adipocytes were detected by goat anti-mouse FABP4 polyclonal antibody under a fluorescence microscope. For osteogenic induction, both MSCs lost their spindle shape and became irregularly shaped. After 21 d, differentiated osteocytes were stained using Alizarin Red S. Chondrocytes were induced in the micromass cultures with chondrocyte differentiation medium for 21 d. Chondrogenic-induced cells were stained with Alcian Blue. These data showed that both UC-MSCs and BM-MSCs have the potential to differentiate into adipocytes, osteocytes and chondrocytes (Figure 1C). Previous reports have suggested that donor age affects the proliferation rate of bone marrow-derived MSCs; as donors get older, the proliferation rate tends to decline[21]. We found that this trend also occurred with UC-MSCs (data not shown).

To compare the growth rates of each passage in UC-MSCs and BM-MSCs, we seeded 1 × 10^5 cells in 100-mm culture dishes and cultured until reaching 90% confluence. We compared the number of cells detached from the dish. The growth rate increased with passage number; however, UC-MSC growth increased more rapidly than that of BM-MSC (Figure 1D).

We then compared the expression levels of pluripotency transcription factors by quantitative real-time polymerase chain reaction. The expression levels of *Klf4* and *OCT4* were similar. The expression levels of *Nanog* in UC-MSCs and BM-MSCs were not statistically different (Figure 1E).

Immunomodulatory effects of MSCs with IFN-γ stimulation

The immunosuppressive effects of MSCs are enhanced when stimulated by pro-


A
CD29
CD44
CD105
CD73
CD90
HLA-ABC
CD45
CD31
CD34
HLA-DR
iso
UC-MSC
BM-MSC
B
CCR1
CCR2
CCR7
CXCR4
CXCR5
iso
UC-MSC
BM-MSC

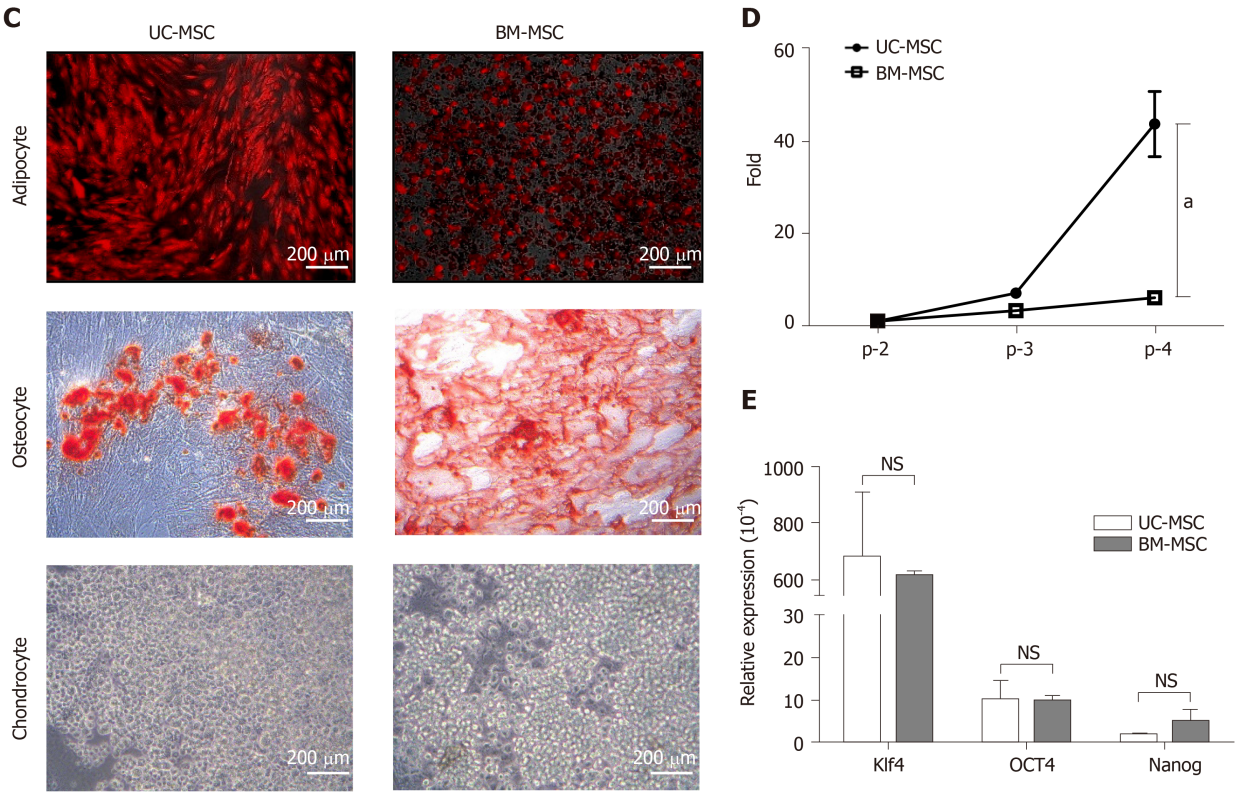


Figure 1 Characterization of umbilical cord-derived mesenchymal stem cells and bone marrow-derived mesenchymal stem cells. A: Surface protein expression was analyzed using flow cytometry. Mesenchymal stem cells (MSCs) were assessed at the fourth passage; B: Chemokine receptor expression was analyzed at passages 3–7 by flow cytometry; C: Representative images of umbilical cord-derived MSCs and bone marrow-derived MSCs differentiation into adipocytes (bone marrow-derived MSCs: day 7; umbilical cord-derived MSCs: day 21), chondrocytes (day 21) and osteocytes (day 14). Scale bars: 200 μm. Adipocytes were detected by goat anti-mouse FABP4 polyclonal antibody under a fluorescence microscope. Osteoblasts and chondrocytes were stained with Alizarin Red S and Alcian Blue, respectively and observed under a light microscope; D: Growth rate of MSCs at different passages; and E: Pluripotency transcription factor expression levels of cultured MSCs measured by quantitative real-time polymerase chain reaction (target, *GAPDH*), D and E: Bone marrow-derived MSCs. Statistical analysis was performed by Student's *t*-tests, $^{a}P < 0.05$ *vs* indicated group. iso: Isotype; Klf4: Kruppel‐like factor 4; Nanog: Nanog homeobox; NS: No significance; OCT4: Octamer-binding transcription factor 4; UC-MSC: Umbilical cord-derived mesenchymal stem cells; BM-MSCs: Bone marrow-derived mesenchymal stem cells.

inflammatory cytokine IFN-γ[5]. Therefore, we created inflammatory conditions using different concentrations of IFN-γ and examined the expression levels of soluble factors representing the immunosuppressive capacity of MSCs. Stimulated MSCs were detached and lysed to analyze IDO by western blot. The IDO levels of both UC-MSCs and BM-MSCs increased in a dose-dependent manner with IFN-γ. However, BM-MSCs expressed more IDO than UC-MSCs (Figure 2A). We collected cultured medium to assess the IDO concentrations. The concentration of IFN-γ was positively correlated with IDO levels produced by both UC-MSCs and BM-MSCs; however, IDO in BM-MSC culture supernatant was significantly higher than that of UC-MSCs (Figure 2B).

Next, UC-MSCs and BM-MSCs were assessed for their expression of inflammatory mediators. When stimulated with IFN-γ, COX2 in UC-MSCs decreased in a dose-dependent manner, although BM-MSCs did not express COX2 with or without IFN-γ (Figure 2C). Also, IFN-γ stimulation did not affect expression of iNOS in both UC-MSCs and BM-MSCs (Figure 2C). According to recent studies, prostaglandin E2 (PGE2) is considered a major immunomodulatory factor of MSCs[22]. Therefore, we measured the concentrations of PGE2 and IL-6, which is increased by PGE2 in cultured medium[23]. PGE2 was notably secreted in UC-MSCs (Figure 2D). Interestingly, IL-6 levels were significantly high in UC-MSCs, and IFN-γ stimulation did not affect IL-6 levels (Figure 2E).

PD-L1 is known to maintain cancer cells as an immunosuppressive factor, which is necessary for cancer to evade the immune system[24]. In contrast, PD-L1 may act as an immunomodulatory factor for MSCs, which are expected to suppress the immune system. PD-L1 is also needed for cell-to-cell contact between MSCs and T cells. We analyzed the expression of PD-L1 and PD-L2 in UC-MSCs and BM-MSCs with or without IFN-γ stimulation. Overall, UC-MSCs expressed higher levels of PD-L1 (Figure 2F) and PD-L2 (Figure 2G). We also found that PD-L2 expression increased

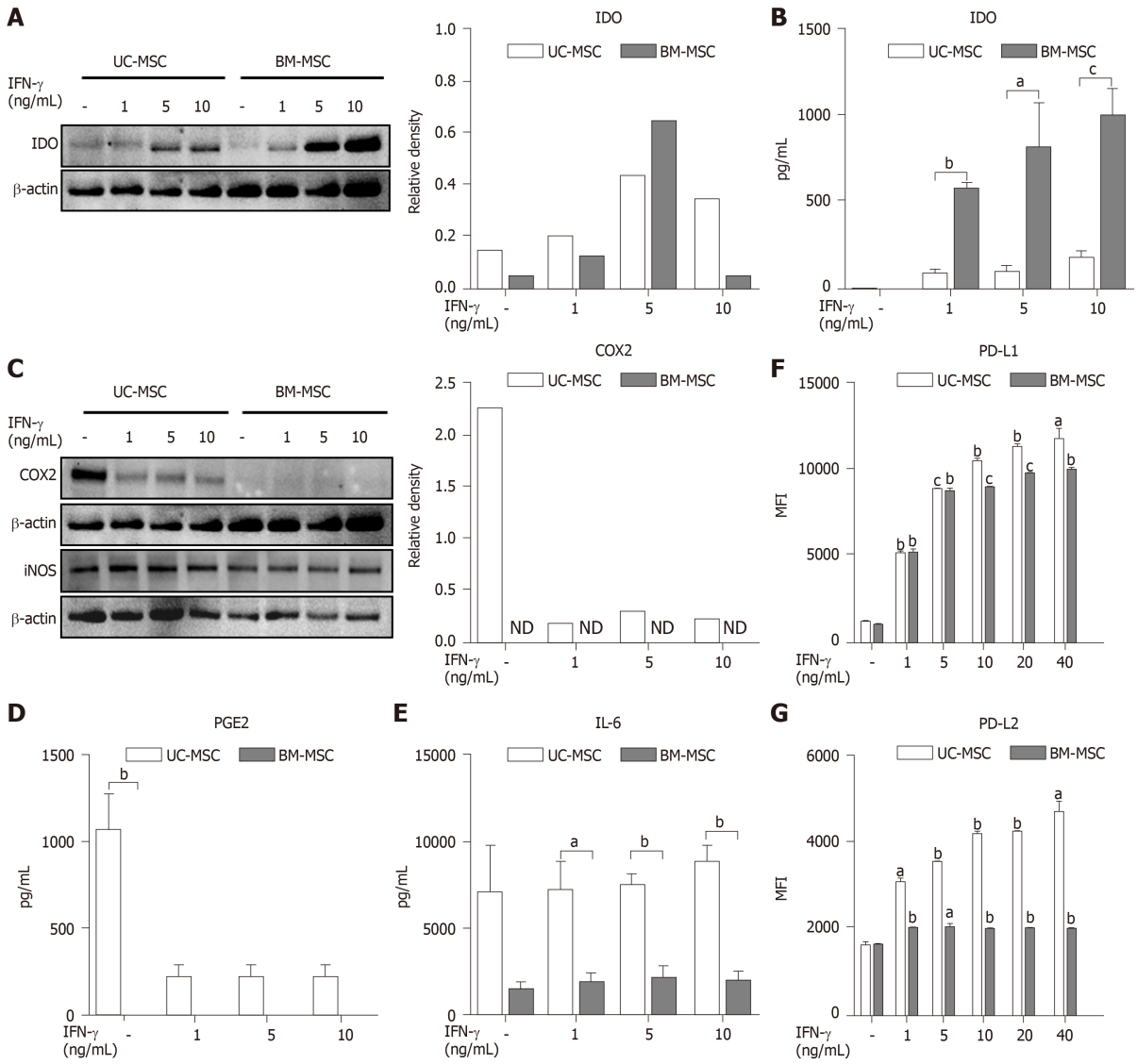


Figure 2 Immunomodulatory effects of interferon-gamma-stimulated mesenchymal stem cells. Umbilical cord-derived mesenchymal stem cells and bone marrow-derived mesenchymal stem cells were stimulated with 0, 1, 5 or 10 ng/mL of interferon-gamma in culture for 72 h. A and C: Expression of indoleamine 2,3-dioxygenase (A) and cyclooxygenase-2 (C) of interferon-gamma-stimulated mesenchymal stem cells measured by western blotting; B, D and E: Expression of indoleamine 2,3-dioxygenase (B), prostaglandin E2 (D), and interleukin-6 (E) in cultured medium measured by ELISA; F and G: Expression of PD-L1 (F) and PD-L2 (G) after interferon-gamma stimulation analyzed by flow cytometry. Statistical analysis was performed by Student's *t*-tests, $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$ *vs* indicated group. B, D and E: $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$ *vs* first bar of each group (F and G). BM-MSC: Bone marrow-derived mesenchymal stem cells; IDO: Indoleamine 2,3-dioxygenase; IFN-γ: Interferon-gamma; IL-6: Interleukin 6; iNOS: Inducible nitric oxide synthase; MFI: Mean fluorescence intensity; ND: Not detected; PGE2: Prostaglandin E2; UC-MSC: Umbilical cord-derived mesenchymal stem cells.

upon IFN-γ treatment in UC-MSCs but was consistent in BM-MSCs (Figure 2G).

Immunomodulatory effects of MSCs with combinations of diverse cytokines

To enhance their immunomodulatory capacity, we stimulated MSCs with multiple combinations of IFN-γ, TNF-α and IL-1β. We found that UC-MSCs and BM-MSCs responded differently to the combinations. Western blot revealed that combinations of IFN-γ, TNF-α and IL-1β elevated the expression level of both IDOs. IFN-γ treatment alone did not increase IDO and COX2, but stimulation with TNF-α and IL-1β did (Figure 3A). Conversely, the protein levels in UC-MSCs were not significantly affected by stimulation (Figure 3A). Next, we analyzed PGE2 and IL-6 in cultured medium with combination stimulations. Interestingly, BM-MSCs, which did not secrete detectable amounts of PGE2 with IFN-γ, secreted high levels of PGE2 and IL-6 with combinations of IFN-γ and TNF-α. Moreover, combinations of cytokines enhanced the secreted levels of PGE2 and IL-6 (Figure 3B and 3C).

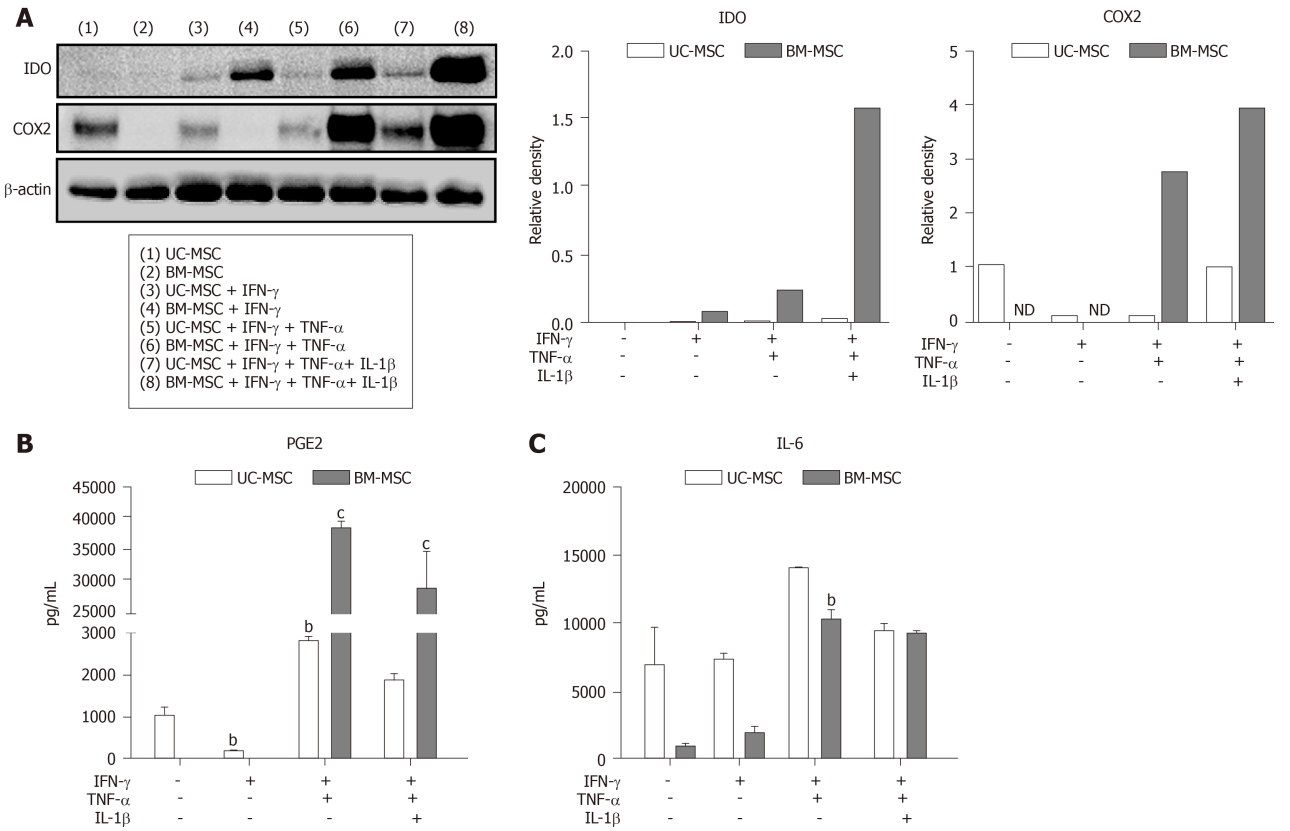


Figure 3 Immunomodulatory effects of mesenchymal stem cells treated with combinations of cytokines. Umbilical cord-derived mesenchymal stem cells and bone marrow-derived mesenchymal stem cells were stimulated with different combinations of interferon-gamma (5 ng/mL), tumor necrosis factor alpha (5 ng/mL), and/or IL-1β (5 ng/mL) for 72 h. A: Expression levels of indoleamine 2,3-dioxygenase and cyclooxygenase-2 of cytokine-stimulated mesenchymal stem cells measured by western blotting; B and C: Expression levels of prostaglandin E2 (B) and interleukin-6 (C) in culture medium measured by ELISA. Statistical analysis was performed by Student's *t*-tests, $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$ *vs* first bar of each group. (B and C) BM-MSC: Bone marrow-derived mesenchymal stem cells; IDO: Indoleamine 2,3-dioxygenase; IFN-γ: Interferon-gamma; IL-1β: Interleukin 1 beta; IL-6: Interleukin 6; ND: Not detected; PGE2: Prostaglandin E2; TNF-α: Tumor necrosis factor alpha; UC-MSC: Umbilical cord-derived mesenchymal stem cells.

T cell inhibition

To test the immunosuppressive effects of the MSCs *in vitro*, we cultured phytohemagglutinin stimulated-human PBMCs in the presence of either UC-MSCs or BM-MSCs. When activated T cells were cocultured with MSCs, there was a decrease in T cell proliferation. Also, the immunosuppressive effects of UC-MSCs increased as the MSC-to-T cell ratio increased (Figure 4A). In the case of BM-MSCs, the immunosuppressive effects also tended to increase as MSC-to-T cell ratio increased; however, suppression effects of 20:1 and 10:1 were not significantly different (Figure 4A). Next, we inhibited various soluble factors with the respective inhibitors and assessed the effects on immunosuppressive activity. In both MSCs, the proliferation of T cells was recovered when cells were treated with indomethacin, which inhibits the COX2 pathway. This indicated that COX2 plays a critical role in the immunomodulation of MSCs. Another factor that restored T cell proliferation was anti-IL-10 (inhibitor of IL-10) in UC-MSCs and L-NAME (inhibitor of iNOS) in BM-MSCs. These findings suggested that UC-MSCs and BM-MSCs may regulate T cells through different pathways (Figure 4B).

Because IL-10 and PGE2 play important roles in the immunomodulatory effects of MSCs, we evaluated the effects of inhibiting PGE2 and/or IL-10 on the immunomodulatory functions of MSCs. Because PGE2 is produced *via* the COX2 pathway, we inhibited COX2 or/and IL-10 with indomethacin and anti-IL-10, respectively. In GVHD, the balance between T cell subpopulations, especially T helper cell 17 (Th17), Th1 and regulatory T cell (Treg) populations, is very important. Therefore, we investigated how the inhibition of these two factors affected MSCs to modulate Th17, Th1 and Treg populations. First, we cocultured phytohemagglutinin-activated T cells with MSCs and treated them with indomethacin and/or anti-IL-10 to assess the effects on IFN-γ-producing $CD4^{+}$ T cells. Next, we induced Th17 and cocultured them with MSCs in the presence of each inhibitor to identify changes in

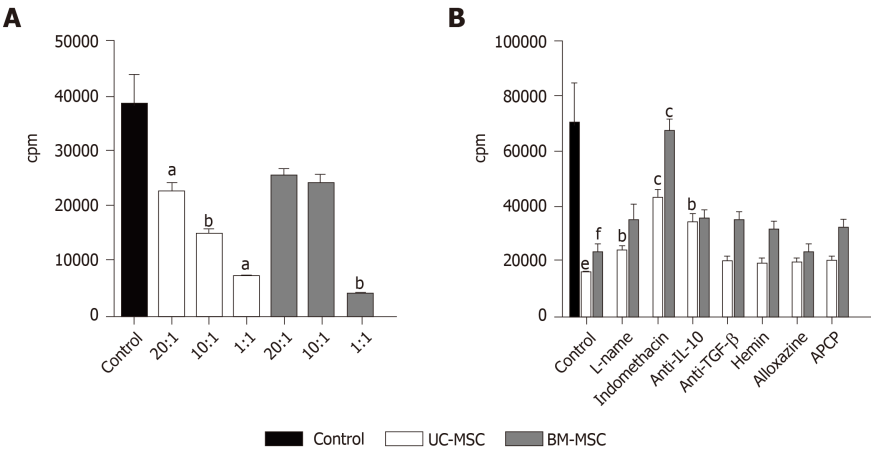


Figure 4 T cell inhibition by mesenchymal stem cells. T cell proliferation was inhibited by either umbilical cord-derived mesenchymal stem cells (MSCs) or bone marrow-derived MSCs in coculture. A: Human peripheral blood mononuclear cells (1×10^5/well) from heathy adult donors were stimulated with phytohemagglutinin (5 μg/mL) with or without MSCs (5×10^3, 1×10^4, 5×10^4, 1×10^5) for 72 h. In the final 18 h, ^{3}H-thymidine was added to the cultures, $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.0001$ *vs* control of each group, $^eP < 0.01$; $^fP < 0.0001$ *vs* control; and B: Human peripheral blood mononuclear cells were treated with L-NAME (iNOS inhibitor), indomethacin (cyclooxygenase-2 inhibitor), anti-IL-10, anti-transforming growth factor-β, hemin (heme oxygenase inducer), alloxazine (selective A_{2B} adenosine receptor antagonist), or adenosine 5'-(α,β-methylene)diphosphate (CD73 inhibitor) with or without MSCs for 72 h. In the final 18 h, ^{3}H-thymidine was added to the cultures. Statistical analysis was performed by Student's *t*-tests $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.0001$ *vs* control. Anti-IL-10: Anti-interleukin 10 antibody; Anti-TGF-β: Anti-transforming growth factor β antibody; APCP: Adenosine 5'-(α,β-methylene)diphosphate; BM-MSC: Bone marrow-derived mesenchymal stem cells; cpm: Count per minute; L-NAME: N-nitro-L-arginine methyl ester; UC-MSC: Umbilical cord-derived mesenchymal stem cells.

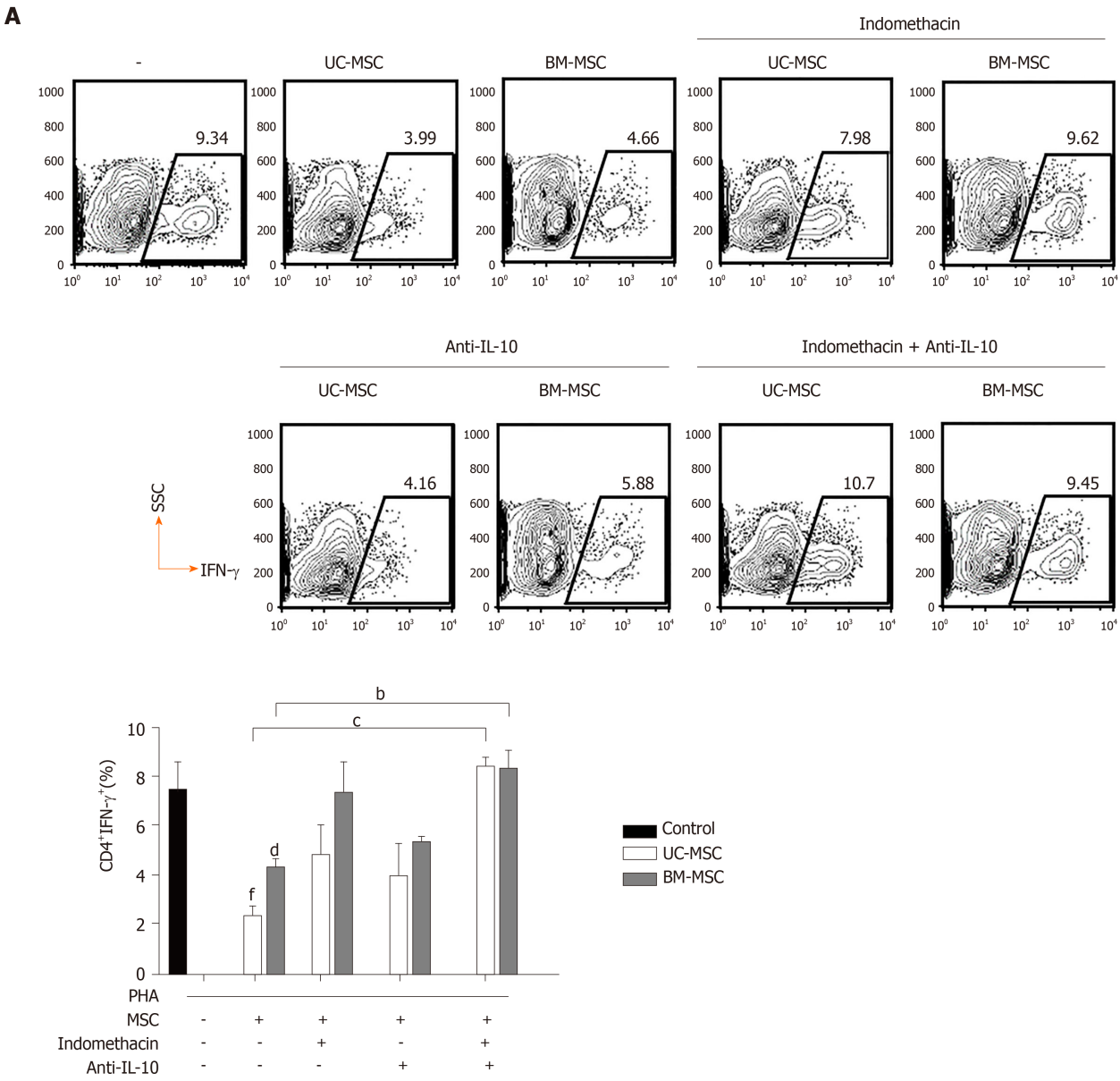
Th17 and Treg populations. In UC-MSCs, COX2 suppression increased the population of IFN-γ-producing $CD4^+$ T cells and decreased the population of Treg cells. COX2 inhibition did not affect the population of IL-17-producing $CD4^+$ T cells. In BM-MSCs, COX2 suppression increased the population of IFN-γ-producing and IL-17-producing $CD4^+$ T cells. However, Treg cells were not affected. Through IL-10 inhibition, we concluded that IL-10 was more critical for UC-MSCs than BM-MSCs to suppress Th1 cells (Figure 5A and 5B).

MSC therapy in a humanized GVHD mouse model

To assess the immunosuppressive activity of MSCs *in vivo*, we established a humanized GVHD mouse model in NOG mice that were exposed to a 200-cGy dose of radiation, and human PBMCs (2×10^7) were transferred intravenously to induce GVHD. At days 0, 7 and 14 after GVHD induction, mice were administered with UC-MSCs or BM-MSCs (1×10^5) for cell therapy. Mice were monitored at different time points after GVHD induction for survival, body weight and clinical GVHD scores. Figure 6 presents the combined results of two independent experiments under the same conditions. Most untreated GVHD-induced mice died between days 20 and 32. At 1 wk after the final death of untreated mice, survivors were treated with UC-MSCs (6/12) and BM-MSCs (8/12). MSC therapy significantly increased the survival rate (Figure 6A). Mice treated with MSCs also had less severe GVHD symptoms, such as declines in weight, activity, posture, fur texture and skin integrity (Figure 6B and 6C).

DISCUSSION

The use of MSCs to treat various diseases has been gaining popularity due to their immunosuppression abilities. Studies have shown that MSCs play a critical role in injury healing[16,25], Crohn's disease[26], systemic lupus erythematosus[27] and rheumatoid arthritis[28]. MSC therapy has been applied for many years to treat GVHD, which is a lethal disease occurring after allogeneic hematopoietic stem cell or organ transplantation. Several treatments, such as steroids with or without calcineurin inhibitors, are used to treat GVHD, but newer therapies are needed due to the poor prognosis of current methods. Since Le Blanc *et al*[11] first reported that adult BM-MSCs were effective in treating severe treatment-resistant grade IV acute GVHD, many clinical trials have confirmed the potential of MSC therapy for GVHD. Given these promising results, MSCs have been derived from various sources, such as adipose tissue, molar cells, amniotic fluid and umbilical cords. However, it remains unclear which source is best, or how MSCs of different sources regulate other cells[29]. Recently,


A
Indomethacin
-
UC-MSC
BM-MSC
UC-MSC
BM-MSC
9.34
3.99
4.66
7.98
9.62
Anti-IL-10
Indomethacin + Anti-IL-10
UC-MSC
BM-MSC
UC-MSC
BM-MSC
SSC
IFN-γ
4.16
5.88
10.7
9.45
b
c
d
f
CD4+IFN-γ+(%)
Control
UC-MSC
BM-MSC
PHA
MSC
Indomethacin
Anti-IL-10

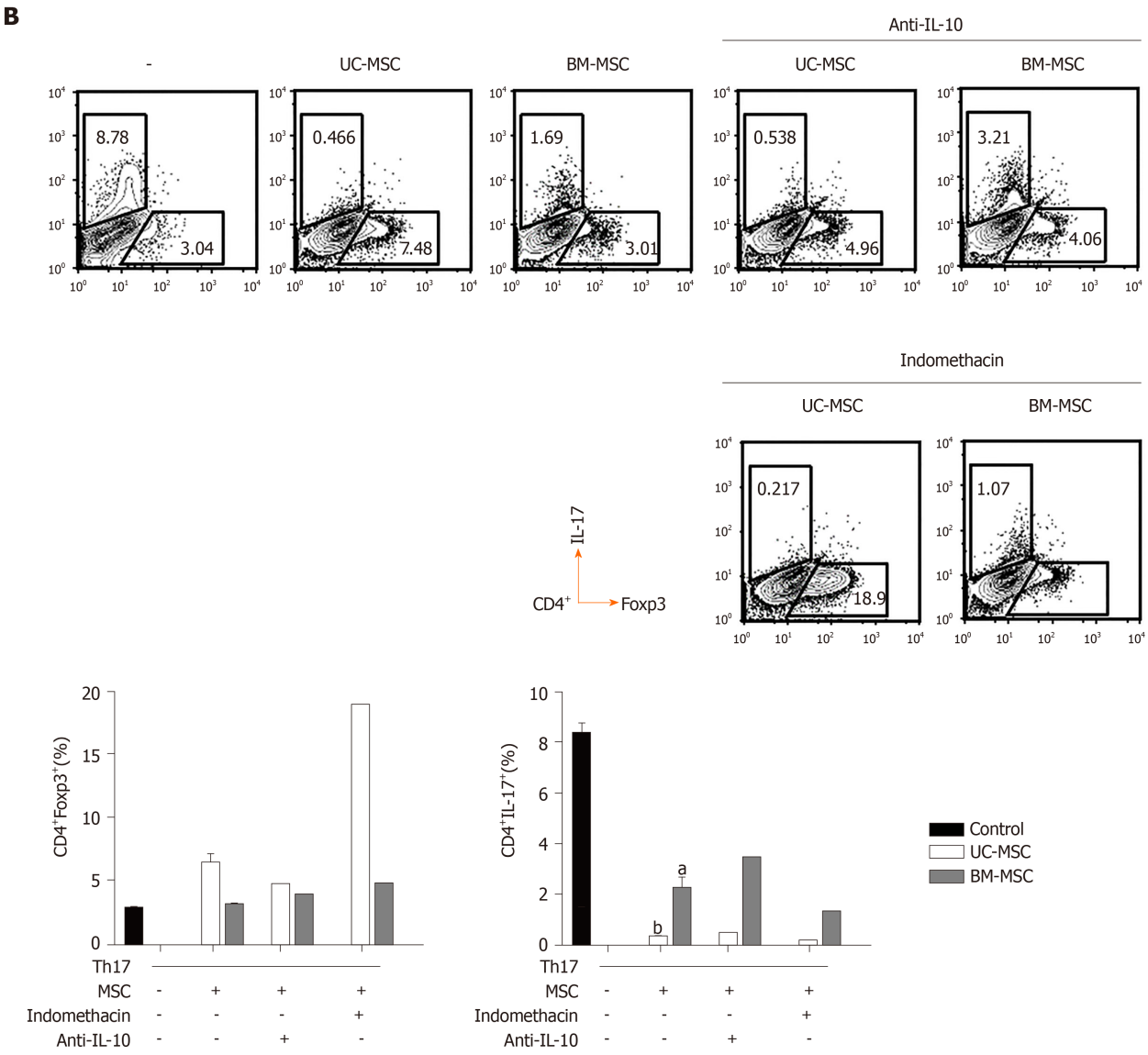


Figure 5 Roles of prostaglandin E2 and interleukin-10 in the immunomodulatory functions of mesenchymal stem cells. A: Human peripheral blood mononuclear cells were stimulated with phytohemagglutinin (10 μg/mL) and cocultured with mesenchymal stem cells treated with indomethacin (20 μM) and/or anti- interleukin (IL)-10 (5 μg/mL). Then, interferon-gamma-producing $CD4^+$ T cells were analyzed by flow cytometry, $^aP < 0.05$; $^bP < 0.01$ *vs* first bar; B: Human peripheral blood mononuclear cells were treated with anti-interferon-gamma (10 μg/mL), anti-IL-4 (10 μg/mL), IL-6 (10 ng/mL), IL-23 (5 ng/mL), IL-1β (10 ng/mL), tumor necrosis factor alpha-α (5 ng/mL) and transforming growth factor-β (2 ng/mL) to induce T helper 17 cells. Induced T helper 17 cells were cocultured with mesenchymal stem cells treated with indomethacin (20 μM) and/or anti-IL-10 (5 μg/mL). Populations of $CD4^+Foxp3^+$ T cells and $CD4^+IL\text{-}17^+$ T cells were analyzed by flow cytometry. Statistical analysis was performed by Student's *t*-tests, $^aP < 0.05$; $^bP < 0.01$; $^cP < 0.0001$ *vs* indicated group, $^dP < 0.05$; $^fP < 0.0001$ *vs* phytohemagglutinin control. Anti-IL-10: Anti-interleukin 10 antibody; BM-MSC: Bone marrow-derived mesenchymal stem cells; IFN-γ: Interferon-gamma; IL-17: Interleukin 17; MSC: Mesenchymal stem cells; PHA: Phytohemagglutinin; Th17: T helper 17; UC-MSC: Umbilical cord-derived mesenchymal stem cells.

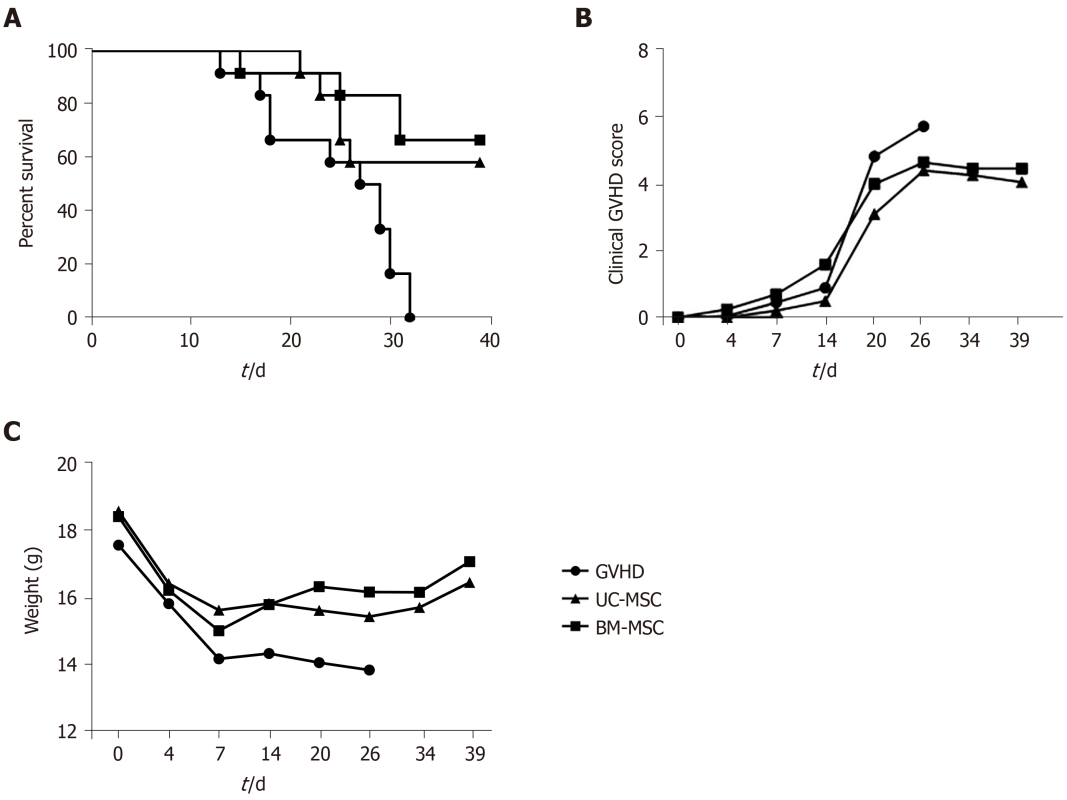


Figure 6 Inhibitory effects of mesenchymal stem cells therapy on graft-versus-host disease severity. A-C: NOG mice were administered 200 cGy of total body irradiation before transplantation of human peripheral blood mononuclear cells (2×10^7). At days 0, 7 and 14 after transplantation, mice were administered with umbilical cord-derived mesenchymal stem cells or bone marrow-derived mesenchymal stem cells (1×10^5). All NOG mice were monitored for survival (A), clinical signs of graft-versus-host disease (B) and weight (C). BM-MSC: Bone marrow-derived mesenchymal stem cells; GVHD: Graft-versus-host disease; UC-MSC: Umbilical cord-derived mesenchymal stem cells.

UC-MSCs have gained attention for their ease of collection from a young source.

In this study, we compared UC-MSCs and BM-MSCs not only to find the difference in degree of immunoregulation but also to discover the difference in factors affecting immunoregulation. Specifically, we compared the phenotypic and functional features of human UC-MSCs and human BM-MSCs. Our findings demonstrated that UC-MSCs and BM-MSCs share similar phenotypic characteristics and immunomodulation capacities. Furthermore, we suggest that UC-MSCs and BM-MSCs may suppress activated immune systems *via* different pathways.

Early studies emphasized the critical roles of chemokines and their receptors in the migration of MSCs to sites of injury. For example, interactions between stromal cell-derived factor-1 and CXCR4 and between monocyte chemoattractant protein-1 and CCR2 regulate the migration of MSCs *in vitro*[30,31]. In this study, our data showed that UC-MSCs and BM-MSCs share similar immunophenotypic characteristics and chemokine receptors. In addition, they possess similar abilities to differentiate into adipocytes, osteocytes and chondrocytes. Moreover, the expression levels of pluripotency transcription factors such as *Klf4*, *OCT4* and *Nanog* are similar between UC-MSCs and BM-MSCs. Our results provide evidence that UC-MSCs and BM-MSCs share similar phenotypic characteristics.

MSCs have enhanced immunosuppressive effects under acute inflammatory conditions, especially in the presence of the pro-inflammatory cytokine IFN-γ[5]. As expected, exposure to IFN-γ increased the expressions of IDO in both UC-MSCs and BM-MSCs. However, BM-MSCs produced more IDO than UC-MSCs with stimulation by IFN-γ.

Previous studies have claimed that combinations of cytokines upregulate IDO and COX2 levels in MSCs. For instance, upregulated IDO activity of TNF-α- and IFN-γ-activated MSCs differentiated monocytes into IL-10-secreting M2 immunosuppressive macrophages, which suppress T cell proliferation[32]. Another study observed that the induction of IDO in MSCs was regulated by IFN-β and IFN-γ[33]. Finally, IL-1β has also been shown to induce COX2[34]. In this study, we treated MSCs with diverse combinations of cytokines and assessed factors representing the immunosuppressive activity of MSCs. BM-MSCs expressed more upregulated IDO and COX2. However, UC-MSCs secreted high levels of IL-6 compared to BM-MSCs. UC-MSCs expressed low levels of IL-6 even without stimulation and high levels with cytokine stimulation. Paradoxically, while IL-6 is mostly known as a pro-inflammatory cytokine, it is also an anti-inflammatory cytokine in terms of maintaining the immunosuppressive capacity of MSCs[35]. When MSCs are differentiated, IL-6 is downregulated, which leads to loss of immune privilege in MSCs[36]. At the same time, IL-6 is claimed as a critical factor for maintaining the stemness of MSCs. Thus, the role of IL-6 in MSCs is to enhance MSC proliferation, protect MSCs from apoptosis and inhibit differentiation[37]. Based on our findings, we speculate that high levels of IL-6 in UC-MSCs are involved in suppressive effects.

Li *et al*[38] suggested that overexpression of IL-6 in tumor tissue enhanced PD-L1 expression, suggesting that the high levels of IL-6 in UC-MSCs might be related to the higher levels of PD-L1 and PD-L2 in UC-MSCs than BM-MSCs. In tumor tissue, PD-L1 acts as a ligand of PD-1 expressed in T cells, inhibiting anti-tumor effects[39]. Therefore, much like PD-L1 in tumor tissue suppresses T cells *via* PD-1, UC-MSCs may inhibit T cell proliferation through the PD-1/PD-L1 pathway.

Although the Th1-mediated response is considered a main contributor to the induction of GVHD, Th17 cells are also capable of developing lethal GVHD[40]. Also, it is well known that imbalances between Th17 and Treg cells affect the severity of GVHD[41]. In this study, we demonstrated that UC-MSCs and BM-MSCs effectively suppressed Th1 and Th17 cells and induced Treg cells.

In conclusion, we have demonstrated that UC-MSCs and BM-MSCs share similar phenotypic characteristics and immunomodulation abilities. Moreover, cytokine stimulation enhances the immunomodulation of both MSCs, although UC-MSCs and BM-MSCs exhibit different tendencies toward expression of IDO, COX2, PGE2, IL-6, PD-L1 and PD-L2. These findings suggested that UC-MSCs and BM-MSCs may induce immunosuppression *via* different pathways. High expression of IDO and COX2 in BM-MSCs suggests that these two factors are involved in T cell suppression. Meanwhile, high expression of IL-6, PD-L1 and PD-L2 in UC-MSCs suggests that UC-MSCs inhibited T cell proliferation through cell-to-cell contact *via* the PD-1/PD-L1 pathway. Finally, we demonstrated that treating a GVHD mouse model with MSCs improved the clinical score, survival and weight loss of irradiated mice. Interestingly, the mechanisms by which UC-MSCs and BM-MSCs alleviated GVHD seemed to differ, but their ability to attenuate GVHD in a mouse model was overall similar. Taken together, these findings underscore the need for future studies to identify the

mechanisms of immunosuppression of different types of MSCs and further studies in mechanisms of MSCs may allow advanced MSC therapy in clinical use.

ARTICLE HIGHLIGHTS

Research background

Mesenchymal stem cells (MSCs) are a promising therapeutic approach to treat graft-versus-host disease (GVHD) because of their immunoregulatory properties. Until recently, human bone marrow-derived MSCs (BM-MSCs) were used widely as cell therapy sources. However, the needs for new source of MSCs are growing due to the invasive collection method and decrease in donors. Among many other adult and fetal tissues, human umbilical cord has emerged as a promising source of MSCs because of their ethical and noninvasive collection.

Research motivation

Although several studies have pointed out the potential of human umbilical cord-derived MSCs (UC-MSCs), the difference in immunomodulatory effects and mechanisms of BM-MSCs and UC-MSCs should be examined in greater detail.

Research objectives

In this study, we aim to investigate the difference in mechanisms of the immunosuppressive effects of UC-MSCs and BM-MSCs.

Research methods

Western blot, quantitative real-time polymerase chain reaction and luminex multiplex cytokine assay were employed to examine the expression of soluble factors after MSCs were primed with different combinations of interferon-gamma, tumor necrosis factor alpha and interleukin (IL)-1β, or interferon-gamma alone. Human peripheral blood mononuclear cells stimulated with phytohemagglutinin were cocultured with MSCs to examine the immunosuppressive effects of the MSCs *in vitro*. Several inhibitors of soluble factors were used to identify which soluble factors played critical roles in the immunomodulation of MSCs. Lastly, xenogeneic GVHD was induced in NOG mice (NOD/Shi-scid/IL-2Rγnull) and UC-MSCs or BM-MSCs were used as cell therapies.

Research results

BM-MSCs and UC-MSCs shared similar phenotypic characteristics and immunosuppressive effects. COX2 and IL-10 were key factors in the immunomodulatory mechanisms of both MSCs. However, upon *in vitro* cytokine stimulation, BM-MSCs expressed more indolamine 2,3-dioxygenase, and UC-MSCs expressed more prostaglandin E2, IL-6, PD-L1 and PD-L2. UC-MSCs and BM-MSCs established different T cell subpopulations when cultured with stimulated T cells. UC-MSCs inhibited more T helper 17 cells and induced more regulatory T cells than BM-MSCs. In a humanized GVHD mouse model, UC-MSCs and BM-MSCs showed comparable effects in attenuating GVHD.

Research conclusions

Our data provides a deeper understanding in similarities and differences between UC-MSCs and BM-MSCs. This study demonstrated that UC-MSCs and BM-MSCs exhibited similar immunosuppression in different mechanisms. Also, this study introduced that UC-MSCs have the potential to substitute for BM-MSCs as cell therapy products.

Research perspectives

In summary, we have demonstrated that UC-MSCs and BM-MSCs exhibit different tendencies toward expression of proteins known to contribute to immunosuppression although they share similar phenotypic characteristics and immunomodulation abilities. Our data also suggest that UC-MSCs and BM-MSCs induced immunosuppression through different pathways underscoring the need for future studies to identify detailed mechanisms of MSCs derived from different sources.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Veterans Health Service Medical Center for supporting this experiment. The authors would like to acknowledge Hyeon Woo Yim (The Catholic University of Korea, South Korea) for his assistance of statistical analysis.

REFERENCES

1 **Shlomchik WD**. Graft-versus-host disease. *Nat Rev Immunol* 2007; **7**: 340-352 [PMID: 17438575 DOI: 10.1038/nri2000]
2 **Imamura M**, Hashino S, Kobayashi H, Kubayashi S, Hirano S, Minagawa T, Tanaka J, Fujii Y, Kobayashi M, Kasai M. Serum cytokine levels in bone marrow transplantation: synergistic interaction of interleukin-6, interferon-gamma, and tumor necrosis factor-alpha in graft-versus-host disease. *Bone Marrow Transplant* 1994; **13**: 745-751 [PMID: 7920309]
3 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]
4 **Majumdar MK**, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR, Mosca JD. Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci* 2003; **10**: 228-241 [PMID: 12595759 DOI: 10.1159/000068710]
5 **Krampera M**, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, Romagnani P, Maggi E, Romagnani S, Annunziato F. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells* 2006; **24**: 386-398 [PMID: 16123384 DOI: 10.1634/stemcells.2005-0008]
6 **Kögler G**, Radke TF, Lefort A, Sensken S, Fischer J, Sorg RV, Wernet P. Cytokine production and hematopoiesis supporting activity of cord blood-derived unrestricted somatic stem cells. *Exp Hematol* 2005; **33**: 573-583 [PMID: 15850835 DOI: 10.1016/j.exphem.2005.01.012]
7 **Selmani Z**, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, Borg C, Saas P, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. *Stem Cells* 2008; **26**: 212-222 [PMID: 17932417 DOI: 10.1634/stemcells.2007-0554]
8 **Di Nicola M**, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002; **99**: 3838-3843 [PMID: 11986244 DOI: 10.1182/blood.v99.10.3838]
9 **Krampera M**, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood* 2003; **101**: 3722-3729 [PMID: 12506037 DOI: 10.1182/blood-2002-07-2104]
10 **Di Ianni M**, Del Papa B, De Ioanni M, Moretti L, Bonifacio E, Cecchini D, Sportoletti P, Falzetti F, Tabilio A. Mesenchymal cells recruit and regulate T regulatory cells. *Exp Hematol* 2008; **36**: 309-318 [PMID: 18279718 DOI: 10.1016/j.exphem.2007.11.007]
11 **Le Blanc K**, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004; **363**: 1439-1441 [PMID: 15121408 DOI: 10.1016/s0140-6736(04)16104-7]
12 **Atala A**. Recent developments in tissue engineering and regenerative medicine. *Curr Opin Pediatr* 2006; **18**: 167-171 [PMID: 16601497 DOI: 10.1097/01.mop.0000193294.94646.be]
13 **Stenderup K**, Justesen J, Clausen C, Kassem M. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. *Bone* 2003; **33**: 919-926 [PMID: 14678851 DOI: 10.1016/j.bone.2003.07.005]
14 **Kern S**, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* 2006; **24**: 1294-1301 [PMID: 16410387 DOI: 10.1634/stemcells.2005-0342]
15 **Zheng YB**, Gao ZL, Xie C, Zhu HP, Peng L, Chen JH, Chong YT. Characterization and hepatogenic differentiation of mesenchymal stem cells from human amniotic fluid and human bone marrow: a comparative study. *Cell Biol Int* 2008; **32**: 1439-1448 [PMID: 18782626 DOI: 10.1016/j.cellbi.2008.08.015]
16 **Chen L**, Tredget EE, Liu C, Wu Y. Analysis of allogenicity of mesenchymal stem cells in engraftment and wound healing in mice. *PLoS One* 2009; **4**: e7119 [PMID: 19771171 DOI: 10.1371/journal.pone.0007119]
17 **Tarte K**, Gaillard J, Lataillade JJ, Fouillard L, Becker M, Mossafa H, Tchirkov A, Rouard H, Henry C, Splingard M, Dulong J, Monnier D, Gourmelon P, Gorin NC, Sensebé L; Société Française de Greffe de Moelle et Thérapie Cellulaire. Clinical-grade production of human mesenchymal stromal cells: occurrence of aneuploidy without transformation. *Blood* 2010; **115**: 1549-1553 [PMID: 20032501 DOI: 10.1182/blood-2009-05-219907]
18 **Bocelli-Tyndall C**, Zajac P, Di Maggio N, Trella E, Benvenuto F, Iezzi G, Scherberich A, Barbero A, Schaeren S, Pistoia V, Spagnoli G, Vukcevic M, Martin I, Tyndall A. Fibroblast growth factor 2 and platelet-derived growth factor, but not platelet lysate, induce proliferation-dependent, functional class II major histocompatibility complex antigen in human mesenchymal stem cells. *Arthritis Rheum* 2010; **62**: 3815-3825 [PMID: 20824797 DOI: 10.1002/art.27736]
19 **He H**, Nagamura-Inoue T, Tsunoda H, Yuzawa M, Yamamoto Y, Yorozu P, Agata H, Tojo A. Stage-specific embryonic antigen 4 in Wharton's jelly-derived mesenchymal stem cells is not a marker for proliferation and multipotency. *Tissue Eng Part A* 2014; **20**: 1314-1324 [PMID: 24279891 DOI: 10.1089/ten.TEA.2013.0333]

20 **Wang Y**, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol* 2014; **15**: 1009-1016 [PMID: 25329189 DOI: 10.1038/ni.3002]

21 **Stolzing A**, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. *Mech Ageing Dev* 2008; **129**: 163-173 [PMID: 18241911 DOI: 10.1016/j.mad.2007.12.002]

22 **Kopper O**, Giladi O, Golan-Lev T, Benvenisty N. Characterization of gastrulation-stage progenitor cells and their inhibitory crosstalk in human embryoid bodies. *Stem Cells* 2010; **28**: 75-83 [PMID: 19921748 DOI: 10.1002/stem.260]

23 **Cho JS**, Han IH, Lee HR, Lee HM. Prostaglandin E2 Induces IL-6 and IL-8 Production by the EP Receptors/Akt/NF-κB Pathways in Nasal Polyp-Derived Fibroblasts. *Allergy Asthma Immunol Res* 2014; **6**: 449-457 [PMID: 25229003 DOI: 10.4168/aair.2014.6.5.449]

24 **Juneja VR**, McGuire KA, Manguso RT, LaFleur MW, Collins N, Haining WN, Freeman GJ, Sharpe AH. PD-L1 on tumor cells is sufficient for immune evasion in immunogenic tumors and inhibits CD8 T cell cytotoxicity. *J Exp Med* 2017; **214**: 895-904 [PMID: 28302645 DOI: 10.1084/jem.20160801]

25 **Chen L**, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One* 2008; **3**: e1886 [PMID: 18382669 DOI: 10.1371/journal.pone.0001886]

26 **Ciccocioppo R**, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, Minelli A, Alvisi C, Vanoli A, Calliada F, Dionigi P, Perotti C, Locatelli F, Corazza GR. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut* 2011; **60**: 788-798 [PMID: 21257987 DOI: 10.1136/gut.2010.214841]

27 **Wang D**, Zhang H, Liang J, Li X, Feng X, Wang H, Hua B, Liu B, Lu L, Gilkeson GS, Silver RM, Chen W, Shi S, Sun L. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years of experience. *Cell Transplant* 2013; **22**: 2267-2277 [PMID: 24388428 DOI: 10.3727/096368911X582769c]

28 **Lim JY**, Im KI, Lee ES, Kim N, Nam YS, Jeon YW, Cho SG. Enhanced immunoregulation of mesenchymal stem cells by IL-10-producing type 1 regulatory T cells in collagen-induced arthritis. *Sci Rep* 2016; **6**: 26851 [PMID: 27246365 DOI: 10.1038/srep26851]

29 **Wolf D**, von Lilienfeld-Toal M, Wolf AM, Schleuning M, von Bergwelt-Baildon M, Held SA, Brossart P. Novel treatment concepts for graft-versus-host disease. *Blood* 2012; **119**: 16-25 [PMID: 21948301 DOI: 10.1182/blood-2011-08-339465]

30 **Son BR**, Marquez-Curtis LA, Kucia M, Wysoczynski M, Turner AR, Ratajczak J, Ratajczak MZ, Janowska-Wieczorek A. Migration of bone marrow and cord blood mesenchymal stem cells in vitro is regulated by stromal-derived factor-1-CXCR4 and hepatocyte growth factor-c-met axes and involves matrix metalloproteinases. *Stem Cells* 2006; **24**: 1254-1264 [PMID: 16410389 DOI: 10.1634/stemcells.2005-0271]

31 **Dwyer RM**, Potter-Beirne SM, Harrington KA, Lowery AJ, Hennessy E, Murphy JM, Barry FP, O'Brien T, Kerin MJ. Monocyte chemotactic protein-1 secreted by primary breast tumors stimulates migration of mesenchymal stem cells. *Clin Cancer Res* 2007; **13**: 5020-5027 [PMID: 17785552 DOI: 10.1158/1078-0432.Ccr-07-0731]

32 **François M**, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. *Mol Ther* 2012; **20**: 187-195 [PMID: 21934657 DOI: 10.1038/mt.2011.189]

33 **Croitoru-Lamoury J**, Lamoury FM, Caristo M, Suzuki K, Walker D, Takikawa O, Taylor R, Brew BJ. Interferon-γ regulates the proliferation and differentiation of mesenchymal stem cells via activation of indoleamine 2,3 dioxygenase (IDO). *PLoS One* 2011; **6**: e14698 [PMID: 21359206 DOI: 10.1371/journal.pone.0014698]

34 **Martin M**, Neumann D, Hoff T, Resch K, DeWitt DL, Goppelt-Struebe M. Interleukin-1-induced cyclooxygenase 2 expression is suppressed by cyclosporin A in rat mesangial cells. *Kidney Int* 1994; **45**: 150-158 [PMID: 8127004 DOI: 10.1038/ki.1994.18]

35 **Scheller J**, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 2011; **1813**: 878-888 [PMID: 21296109 DOI: 10.1016/j.bbamcr.2011.01.034]

36 **Li P**, Li SH, Wu J, Zang WF, Dhingra S, Sun L, Weisel RD, Li RK. Interleukin-6 downregulation with mesenchymal stem cell differentiation results in loss of immunoprivilege. *J Cell Mol Med* 2013; **17**: 1136-1145 [PMID: 23802625 DOI: 10.1111/jcmm.12092]

37 **Pricola KL**, Kuhn NZ, Haleem-Smith H, Song Y, Tuan RS. Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. *J Cell Biochem* 2009; **108**: 577-588 [PMID: 19650110 DOI: 10.1002/jcb.22289]

38 **Li J**, Xu J, Yan X, Jin K, Li W, Zhang R. Targeting Interleukin-6 (IL-6) Sensitizes Anti-PD-L1 Treatment in a Colorectal Cancer Preclinical Model. *Med Sci Monit* 2018; **24**: 5501-5508 [PMID: 30087314 DOI: 10.12659/msm.907439]

39 **Blank C**, Brown I, Peterson AC, Spiotto M, Iwai Y, Honjo T, Gajewski TF. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. *Cancer Res* 2004; **64**: 1140-1145 [PMID: 14871849 DOI: 10.1158/0008-5472.can-03-3259]

40 **Carlson MJ**, West ML, Coghill JM, Panoskaltsis-Mortari A, Blazar BR, Serody JS. In vitro-differentiated TH17 cells mediate lethal acute graft-versus-host disease with severe cutaneous and pulmonary pathologic manifestations. *Blood* 2009; **113**: 1365-1374 [PMID: 18957685 DOI: 10.1182/blood-2008-06-162420]

41 **Malard F**, Bossard C, Brissot E, Chevallier P, Guillaume T, Delaunay J, Mosnier JF, Moreau P, Grégoire M, Gaugler B, Mohty M. Increased Th17/Treg ratio in chronic liver GVHD. *Bone Marrow Transplant* 2014; **49**: 539-544 [PMID: 24419519 DOI: 10.1038/bmt.2013.215]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com