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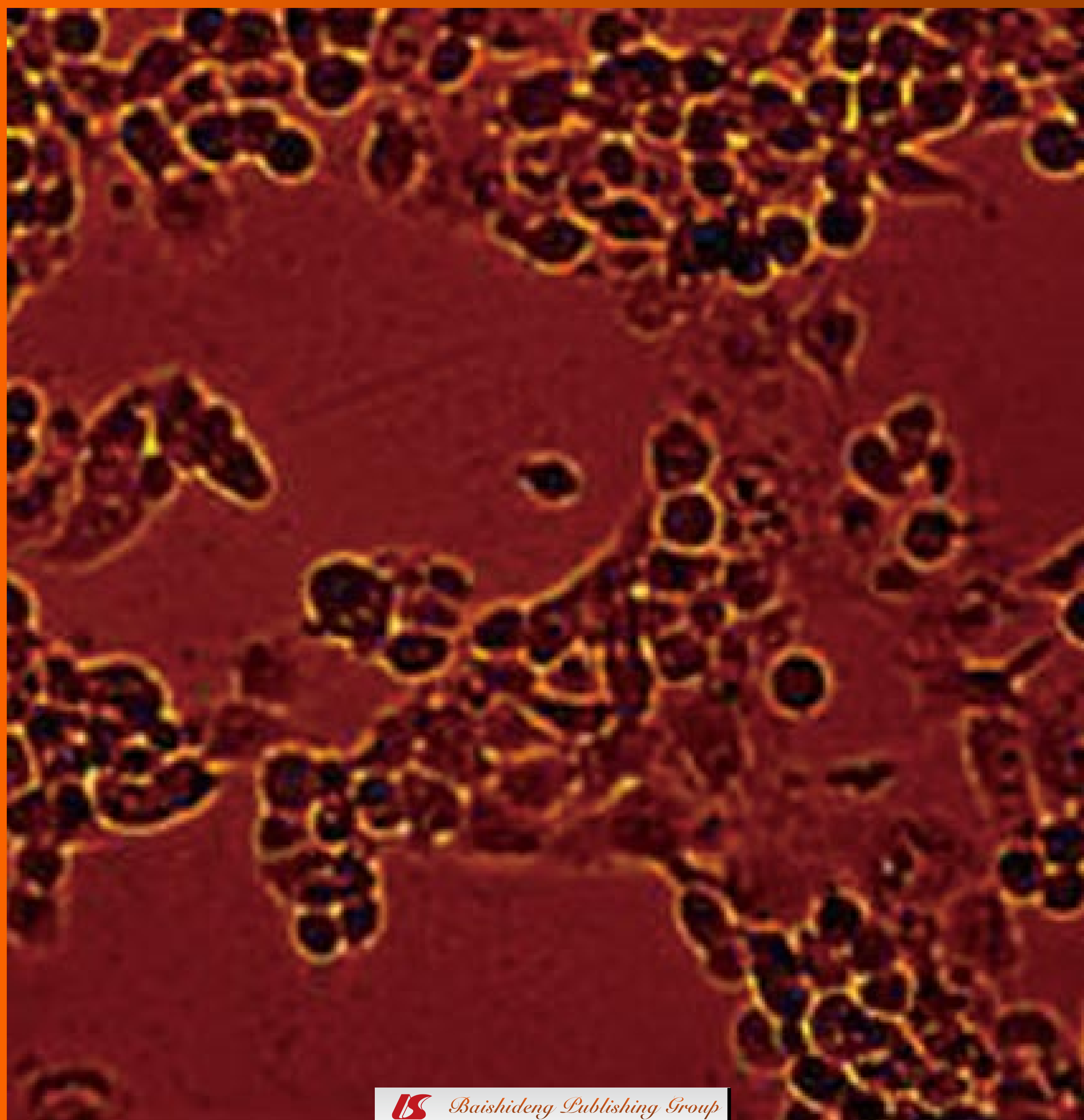

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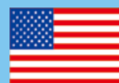

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The major task of *WJSC* is to rapidly report original articles and comprehensive reviews on basic laboratory investigations of stem cells and their application in clinical care and treatment of patients. *WJSC* is designed to cover all aspects of stem cells, including embryonic stem cells, neural stem cells, hematopoietic stem cells, mesenchymal stem cells, tissue-specific stem cells, cancer stem cells, the stem cell niche, stem cell genomics and proteomics, and translational and clinical research. In a word, papers published in *WJSC* will cover the biology, culture, and differentiation of stem cells from all stages of development from germ cell to embryo and adult.

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EDITORIAL

Mesenchymal stem cell-mediated cancer therapy: A dual-targeted strategy of personalized medicine

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Abstract

Cancer remains one of the leading causes of mortality and morbidity throughout the world. To a significant extent, current conventional cancer therapies are symptomatic and passive in nature. The major obstacle to the development of effective cancer therapy is believed to be the absence of sufficient specificity. Since the discovery of the tumor-oriented homing capacity of mesenchymal stem cells (MSCs), the application of specific anticancer gene-engineered MSCs has held great potential for cancer therapies. The dual-targeted strategy is based on MSCs' capacity of tumor-directed migration and incorporation and *in situ* expression of tumor-specific anticancer genes. With the aim of translating bench work into meaningful clinical applications, we describe the tumor tropism of MSCs and their use as therapeutic vehicles, the dual-targeted anticancer potential of engineered MSCs and a putative personalized strategy with anticancer gene-engineered MSCs.

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Key words: Mesenchymal stem cells; Gene therapy; Cancer therapy; Cytotherapy

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INTRODUCTION

Cancer is one of the top life-threatening diseases, accounting for an estimated one in four human deaths in all age groups in the Untied States in 2010[1]. Current conventional cancer therapies (surgery, chemotherapy and radiotherapy) are, to a significant extent, symptomatic and passive in nature. Despite improved treatment models, many tumors remain unresponsive to traditional therapy. When fatalities occur, the majority of cancer patients die from the recurrence of metastasis or therapy-related life-threatening complications. The major obstacle limiting the effectiveness of conventional therapies for cancer is their tumor specificity. Therefore, it is critical to explore efficient remedial strategies specifically targeting neoplasms.

Mesenchymal stem cells (MSCs) are the first type of stem cells to be utilized in clinical regenerative medicine. In addition to their capability of multipotent differentiation, MSCs show many other therapeutically advantageous features, such as easy acquisition, fast *ex vivo* expansion, the feasibility of autologous transplantation and a powerful paracrine function. More recently, the specific tumor-oriented migration and incorporation of MSCs have been demonstrated in various pre-clinical models,

revealing the potential for MSCs to be used as ideal vectors for delivering anticancer agents. With the discovery of specific anticancer genes and the revelation of MSCs' capacity of tumor-directed migration and incorporation, a new research field has been inspired with the aim of achieving efficient therapy for cancer using engineered MSCs. In the present review, following a general description of MSCs we describe the interactions of MSC with cancers and the dual-targeted anticancer potential of engineered MSCs. We also proposed a putative personalized strategy with anticancer gene-engineered MSCs to treat patients with cancers.

OVERVIEW OF MSCs

MSCs are a group of adult stem cells naturally found in the body. They were first identified in the stromal compartment of bone marrow by Friedenstein and colleagues in 1960s[2,3]. The exact nature and localization of MSCs *in vivo* remain poorly understood. In addition to bone marrow, MSCs have been shown to be present in a number of other adult and fetal tissues, including amniotic fluid, heart, skeletal muscle, adipose tissue, synovial tissue, pancreas, placenta, cord blood and circulating blood. It has been assumed that basically all organs containing connective tissue also contain MSCs[4]. Among adult stem cells, MSCs are the most studied and the best characterized stem cells. MSCs are primitive cells originating from the mesodermal germ layer and were classically described as giving rise to connective tissues, skeletal muscle cells, and cells of the vascular system. MSCs can differentiate into cells of the mesodermal lineage, such as bone, fat and cartilage cells, but they also have endodermic and neuroectodermic differentiation potential. Indeed, bone marrow-derived MSCs are a heterogeneous rather than homogeneous population[5]. As a result of their supposed capacity of self-renewal and differentiation, bone marrow-derived stromal cells were first considered as stem cells by Caplan and named MSCs[6], although there is some controversy regarding their nomenclature[7]. MSCs have generated considerable biomedical interest since their multilineage potential was first identified in 1999[8].

Owing to their easy acquisition, fast *ex vivo* expansion, and the feasibility of autologous transplantation, MSCs became the first type of stem cells to be utilized in the clinical regenerative medicine. MSCs can differentiate to several cell types and produce important growth factors and cytokines. They may provide important cues for cell survival in damaged tissues, with or without direct participation in long-term tissue repair[9]. MSCs also have the ability to modify the response of immune cells and are thereby associated with immune-related disorders, especially autoimmune diseases[10,11]. More detailed information on their characterization, tissue distribution and therapeutic potential is described in recent reviews[7,12].

Recently, the specific tumor-oriented migration and incorporation of MSCs have been demonstrated in various pre-clinical models, demonstrating the potential for MSCs to be used as ideal carriers for anticancer agents[13]. In addition to bone marrow-derived MSCs cells obtained from other tissues, such as adipose tissue, can also be potentially used as anticancer gene vehicles for cancer therapy[14,15]. As discussed in the following section, MSCs possess both pro- and anti-cancer properties[16]. It is not an overstatement to describe MSCs as a "double-edged sword" in their interaction with tumors. However, if MSCs are suitably engineered with anticancer genes they could be employed as a valuable "single-edged sword" against cancers.

TUMOR-TROPIC CAPACITIES OF MSCs

The first evidence of the tropism of MSCs to tumors was demonstrated by implantation of rat MSCs into rats bearing syngeneic gliomas[17]. Since then, an increasing number of studies have verified MSC tropism toward primary and metastatic tumor locations. Tumors can be characterized as "wounds that never heal", serving as a continuous source of cytokines, chemokines and other inflammatory mediators[18]. These signals are capable of recruiting respondent cell types including MSCs. Tumor-directed migration and incorporation of MSCs were evidenced in a number of pre-clinical studies *in vitro* using transwell migration assays and *in vivo* using animal tumor models. The homing capacity of MSCs has been demonstrated with almost all tested human cancer cell lines, such as lung cancer[19], malignant glioma[20-22], Kaposi's sarcoma[23], breast cancer[24,25], colon carcinoma[26], pancreatic cancer[27,28], melanoma[29] and ovarian cancer[24]. High frequency of MSC migration and incorporation was observed in *in vitro* co-culture and *in vivo* xenograft tumors respectively. These findings were consistent, independent of tumor type, immuno-competence, and the route of MSC delivery. The tropism of MSCs for tumor microenvironment is obvious, but the molecular mechanisms underlying the tumor-directed migration of MSCs have not been fully elucidated. The preconditions for this phenomenon are the production of chemo-attractant molecules from tumor tissue and the expression of corresponding receptors in MSCs[12]. The complex multistep process by which leukocytes migrate to peripheral sites of inflammation has been proposed as a paradigm. The possible pathways and prospective models have been summarized in recent reviews[7,13,30].

Although it is undisputable that MSCs migrate and integrate toward tumor tissues, their fate and function inside the tumor seems ambiguous and sometimes paradoxical, attributable to the complexities of both MSCs and tumor microenvironments. In order to make pertinent use of MSCs, it is essential to understand their advantages and disadvantages with regard to tumorigenesis. Native MSCs have been shown to suppress tumor growth in models of glioma[17], Kaposi's sarcoma[23], malignant melanoma[31], Lewis lung carcinoma[31], and colon carcinoma[32]. The release of soluble factors by MSCs has also

Table 1 Mesenchymal stem cells as cellular vehicles for targeting cancer

Anticancer agent	Anticancer mechanism	Tumor model	Route of MSC admi-nistration	Species: MSC/tumor/host	Ref.
CX3CL1	Immunostimulatory	Lung	iv	Mouse/mouse/mouse	[40]
CD	Prodrug converting	Prostate	sc/iv	Human/human/mouse	[41]
		Colon	sc/iv	Human/human/mouse	[14]
HSV-tk		Glioma	it	Rat/rat/rat	[42]
		Pancreas	iv	Mouse/mouse/mouse	[28]
IFNα	Immunostimulatory and apoptosis indcing	Melanoma	iv	Mouse/mouse/mouse	[43]
		Glioma	it/ic	Mouse/mouse/mouse	[44]
IFNβ		Breast	sc/iv	Human/human/mouse	[29,45]
		Pancreas	ip	Human/human/mouse	[27]
IL2		Glioma	it/ic	Rat/rat/rat	[17]
IL7	Immunostimulatory	Glioma	it	Rat/rat/rat	[46]
IL12	Activates cytotoxic lymphocyte and NK cells	Melanoma	iv	Mouse/mouse/mouse	[47]
		Hepatoma	iv	Mouse/mouse/mouse	[47]
		Breast	iv	Mouse/mouse/mouse	[47]
IL18	Immunostimulatory	Glioma	it	Rat/rat/rat	[48]
NK4	Inhibits angiogenesis	Colon	iv	Mouse/mouse/mouse	[49]
TRAIL	Induces apoptosis	Glioma	it	Human/human/mouse	[20]
		Glioma	ic	Human/human/mouse	[50]
		Glioma	iv	Human/human/mouse	[22,51]
		Lung	iv	Human/human/mouse	[52]
		Breast, lung	sc/iv	Human/human/mouse	[53]
		Colon	sc	Human/human/mouse	[54,55]
		Pancreas	iv	Human/human/mouse	[56]

CD: Cytosine deaminase; CX3CL1: Chemokine fractalkine; HSV-tk: Herpes simplex virus-thymidine kinase; ic: Intracerebral; IFN: Interferon; IL: Interleukin; ip: Intraperitoneal; it: Intratumoral; iv: Intravenous; NK: Natural killer; sc: Subcutaneous; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand.

been shown to reduce tumor growth and progression of glioma[17], melanoma and lung carcinoma models[31], and conditioned media from MSCs have been sown to cause the downregulation of NFκB in hepatoma and breast cancer cells resulting in a decrease in their *in vitro* proliferation[33]. While the precise mechanism underlying the intrinsic antitumor properties of MSCs has not been fully investigated, it is presumably related to the down-regulation of Akt, NFκB and Wnt signaling pathways[13]. On the other hand, several studies have demonstrated that MSCs can augment tumor growth[34-36]. Promotion of tumor growth is possibly mediated by MSC production of immunosuppressive factors and by the contribution of MSCs to tumor stroma and tumor vascularization. The intrinsic anti- and pro-tumorigenic effects of MSCs were summarized in our recent review[12].

MSCs AS THERAPEUTIC VEHICLES

Since the discovery of their tumor-directed homing capacity, MSCs have been considered as ideal therapeutic vehicles to deliver anticancer agents. In addition to their tumor-homing properties, MSCs are also easily transduced by integrating vectors due to their high levels of amphotropic receptors[37] and offer long-term gene expression without alteration of phenotype[38,39]. To date, a number of anticancer genes have been successfully engineered into MSCs, which then demonstrate anticancer effects in various carcinoma models. Table 1 summarizes experimental models using MSCs as therapeutic vehicles to deliver anticancer agents.

MSCs can also be utilized to deliver prodrug-converting enzymes. A pioneer example is the combination of herpes simplex virus-thymidine kinase (HSV-tk) (Table 1) gene-engineered MSCs and systemic administration of ganciclovir[57]. Within tumors, HSV-tk is released by engineered MSCs and converts (phosphorylates) the prodrug ganciclovir into its toxic form, thereby inhibiting DNA synthesis and leading to cell death. In addition, there is a substantial bystander effect that leads to the death of neighboring cells. This therapeutic regimen has been successfully employed in glioma[58] and pancreatic cancer[28] experimental models. MSCs have been used to deliver another prodrug-converting enzyme, cytosine deaminase. Following systemic administration, the prodrug 5-fluorocytosine is converted into the highly toxic active drug 5-fluorouracil in tumors. This system has shown therapeutic effectiveness in animal cancer models, such as melanoma[59], colon carcinoma[14], and prostate cancer[41].

The methods of MSC administration has been classified as directional, semi-directional and systemic[60]. For MSC-based cancer therapy, MSCs have been delivered to a variety of tumor models using a number of methods. Systemic delivery methods include intravenous (iv)[29] and intra-arterial[61] injection, whereas, intratumoral implantation[17], intraperitoneal[62] and intracerebral[61] injections, and intratracheal administration[63] are respectively considered as directional and semi-directional deliveries. The selection of delivery route for MSCs is based on consideration of factors, such as the type, location and stage of

cancer, and the feasibility of surgical interventions.

MULTIPLE ANTICANCER EFFECTS OF ENGINEERED MSCs

As described above, the major limitation on the effectiveness of conventional therapies for cancer treatment is their lack of tumor specificity. Advanced drug targeting of tumor cells is often impossible when treating highly invasive and infiltrative tumors, because of the high level of migration and invasiveness of tumor cells. Uncontrolled drug distribution in the body, resulting in insufficient concentration at the tumor site and toxic concentration on normal cells, is the cause of anticancer inefficacy, and is often the direct cause of side effects and sometimes life-threatening complications. Targeting solid tumors with antitumor gene therapy has also been hindered by systemic toxicity, low efficiency of delivery and nominal temporal expression. However, MSC-mediated anticancer therapies can overcome these limitations, mainly through preferentially homing to sites of primary and metastatic tumors and delivering antitumor agents. Anticancer gene-engineered MSCs are capable of specifically targeting and acting on tumors through multiple selections. The first selection is attributable to the tumor-directed migration and incorporation of MSCs. This phenomenon is independent of tumor type, immunocompetence, and the route of MSC delivery. In addition to the intrinsic anticancer effects of MSCs, the presence of MSCs in the tumor microenvironment allows the agents which are delivered by MSCs to exert their anticancer function locally and constantly. Therefore, the systemic and organ-specific side effects of anticancer agents can be greatly minimized by using this cell-based vector system.

The second level of selection lies in the cancer-specific agents being carried or expressed by MSCs. The research using MSCs as a vehicle for delivering agents to treat cancer has been greatly stimulated by the advances in study on specific anticancer genes. As indicated in Table 1, a number of anticancer genes have been engineered into MSCs, resulting in anticancer effects on various carcinoma models. In the tumor microenvironment, engineered MSCs could serve as a constant source of anticancer agent production, and locally release anticancer agents, which act on adjacent tumor cells thereby efficiently inducing tumor growth inhibition or apoptosis.

Additional selection can be achieved by modifying the vector construction according to organ-specific protein expression. For example, pancreas- or insulinoma-specific anticancer gene-bearing vectors can be made by employing an insulin promoter. Similarly, the unique expression of albumin by hepatocytes, neurotransmitter expression by neurons and surfactant expression by pulmonary alveoli can also be used to construct organ-specific expression vectors. If engineered with organ-specific vectors, MSCs express anticancer proteins only when they home to tumors located in the corresponding organs or to metastatic sites with the same type of cells.

It is worth noting that different types of viral vectors have been used to deliver the targeted genes in cancer gene therapy, including retrovirus, lentivirus, adenovirus and poxvirus. Retrovirus-induced oncogenesis remains the major concern in relation to retrovirus use in clinical applications. The targeted sites of integration, the most crucial factor associated with oncogenicity, are distinct for different retroviruses. In addition to insertional effects on protein-coding genes, insertional activation of non-coding sequences, such as microRNAs, should be carefully examined[64]. One effective and novel approach in the virus-mediated treatment of cancer is the use of conditionally replicating adenoviruses (CRAds), which can replicate in tumor cells but not in normal cells. Upon lysis of infected tumor cells, CRAds are released and can infect neighboring tumor cells. The current approaches targeting CRAds specifically to cancer cells were described in a recent review[65]. More recently, this field was further stimulated by the clinical report of Breitbach *et al*[66] who constructed a multi-mechanistic cancer-targeted oncolytic poxvirus and successfully applied it to patients with cancers. However, anti-viral immunity may theoretically attenuate the efficiency of viral vector-mediated therapy. As described in the following section, MSC-mediated gene therapy has unique advantages especially in terms of tumor-targeting selections.

ADVANTAGES OF MSC-MEDIATED THERAPY AND PUTATIVE PERSONALIZED MEDICINE

Intrinsic strength for transplantation

The greatest benefit of MSCs in clinical application is their suitability for autologous transplantation. Autotransplantation of MSCs has been used in a numerous clinical studies, most of most of them in regenerative medicine applications, such as myocardial infarction[67,68], traumatic brain injury[60], and liver disease[69]. MSCs also represent an advantageous cell type for allogeneic transplantation. A number of different studies have demonstrated that MSCs avoid allogeneic rejection in humans and in different animal models[70,71]. MSCs are immune-privileged, characterized by low expression of MHC-I with no expression of MHC-II and co-stimulatory molecules such as CD80, CD86 and CD40[72]. Due to their limited immunogenicity, MSCs are poorly recognized by HLA-incompatible hosts. This opens up a much broader range of uses for MSCs in transplantation, compared to cells from autologous sources only.

Possible pre-determination of carcinoma sensitivity to anticancer agents

Pre-determination of the sensitivity of particular carcinoma to any given anticancer agents is a critical step in developing personalized medicine. During *ex vivo* expansion, MSCs can be engineered with a variety of anti-

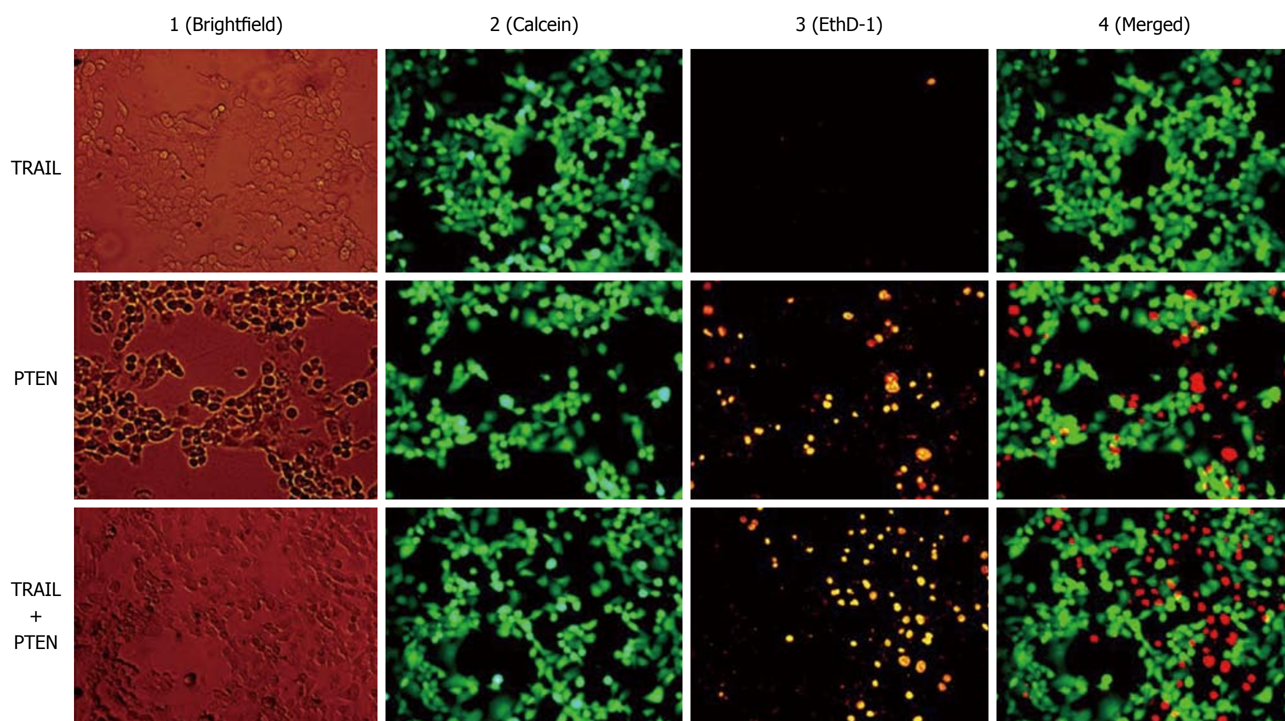

Figure 1 Tumor necrosis factor-related apoptosis-inducing ligand- and/or phosphatase and tensin homolog-induced apoptosis in Panc-1 cells. Panc-1 cells were pre-detected for death receptors and showed negative expression of death receptor 4 and 5. The cells were plated on day 0 and transfected with TRAIL- and/or phosphatase and tensin homolog (PTEN)-bearing vectors on day 1. Apoptosis was assessed on day 3 with a live/dead assay. The top two rows represent the cells transfected with TRAIL or PTEN individually, and the bottom row shows the cells with co-transfection of two vectors. Column 1: Whole population of cells which were still attached to the surface during the assessment; Column 2: Live cells stained with calcein show green; Column 3: Dead cells stained with EthD-1 show red; column 4: Merged images. Original magnification, × 400. TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; PTEN: Phosphatase and tensin homolog.

cancer agents and assessed *in vitro*. Transwell co-culture and/or real-time monitoring techniques can be applied to this detection. The cells isolated from clinical tumor biopsy are the most practically meaningful targets. Once a sensitive anticancer agent is selected, engineered MSCs can be prepared on a large scale for the treatment.

Potential synergistic effect of multiple anticancer agents

In addition to the therapeutic specificity of anticancer agents, the development of drug resistance of tumor cells is another factor contributing to inefficient cancer therapy. Since the beginning of cancer chemotherapy the frequent lack of drug response in solid tumors has been a major problem. In nearly 50% of all cancer cases, resistance to chemotherapy already exists before drug treatment starts (intrinsic resistance), and in a large proportion of remaining cases drug resistance develops during the treatment (acquired resistance)[73]. The mechanisms contributing to multidrug resistance phenotype and the challenges facing molecular targeted therapy were discussed in a recent review[74]. MSC-based cancer therapy is capable of providing multiple anticancer agents synchronously, which may potentiate therapeutic efficiency. For example, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has gained attention in cancer gene therapy because of its capacity to induce apoptosis specifically in tumor cells. Cancer specificity is determined by the differential expression of death receptors (DR4 and DR5). Dominant expression of DR4 and/or DR5 is a determinant factor for the sensitivity of target tumor cells to TRAIL. The expression of death receptors varies with tumor type and stage as well as the therapy utilized, and the sensitivity of tumor cells to TRAIL is not particularly consistent even under apparently identical conditions[75,76]. Recent work of our group demonstrates that TRAIL-insensitive Panc-1 cell can be suppressed by transducing a death receptor-independent anticancer gene phosphatase and tensin homolog (PTEN) (Figure 1). PTEN is a phosphatidylinositol phosphatate phosphatase and is frequently inactivated in human cancers[77]. Loss of PTEN function is associated with constitutive survival signaling through the phosphatidylinositol-3 kinas/Akt pathway. PTEN has also been demonstrated to sensitize tumor cells to death receptor-mediated apoptosis induced by TRAIL[78,79] and non-receptor mediated apoptosis induced by a kinase inhibitor staurosporine and chemotherapeutic agents mitoxantrone and etoposide[78]. The MSC-mediated therapeutic spectrum can be dramatically broadened by using multiple anticancer gene-engineered MSCs, and theoretically, a synergistic effect can be achieved *via* application of multiple anticancer agents simultaneously.

Putative personalized medicine

MSCs can be acquired from the patients' own body,

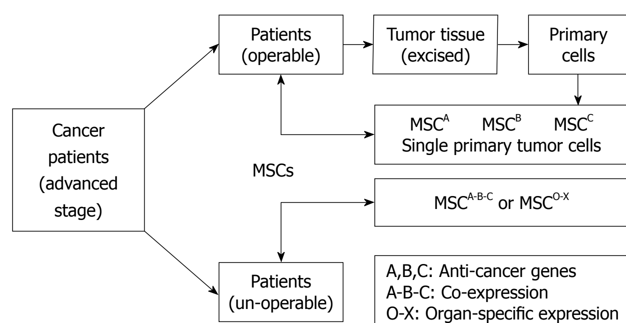

Figure 2 Putative personalized strategy for cancer therapy with engineered mesenchymal stem cells. Operable patients provide direct access to tumor tissue. Primary tumor cells can be isolated from excised tumor tissue and co-cultured with anti-cancer gene pre-engineered MSCs. The most sensitive type of engineered MSCs can be selected through transwell co-culture or other real-time monitoring systems. Useable amounts of MSCs can be obtained through large scale of transduction with selected anti-cancer gene. For patients not receiving surgical intervention (un-operable), multiple gene co-expression or organ-specific expression vectors can be used for MSC transduction. As explained in the text, MSCs can be obtained from patient's own body or other suitable allo-sources and transplanted accordingly. MSCs: mesenchymal stem cells.

quickly expanded *in vitro* and easily transduced with expression vectors. The exhibition of the powerful tumor-directed migration capacity of MSCs makes them suitable for use in anticancer therapies. Anticancer-engineered MSCs could be eventually used as an alterative treatment for cancer patients without the concern of rejection or other ethical problems. Since there is a great deal of variation amongst the cancer patient population with respect to degree of carcinogenic differentiation and preparation of human MSCs, it is unlikely that a single fixed therapeutic model will be found that would successfully perform on different types of cancers. In order to translate bench research into real clinical applications, it will be necessary to develop a specific personalized treatment for each individual patient. Figure 2 illustrated putative personalized strategy with anticancer gene-engineered MSCs.

CONCLUSION

There is a pressing clinical demand for more efficient remedies to replace existing symptomatic anticancer therapies. The extensive achievements of MSCs and anticancer agent studies have laid the foundation for the exploitation of MSC-based cancer therapies. MSCs possess powerful capabilities of tumor-directed migration and incorporation, giving them the potential of acting as optimal vehicles to deliver anticancer agents. Although MSCs have both positive and negative effects on tumor progression, profound anticancer effects have been demonstrated by using apropriately engineered MSCs. MSC-mediated anticancer therapy relies on tumor-specific selectivity provided by MSCs and MSC-carried anticancer agents. Homed directly at the tumor microenvironment, engineered MSCs are able to express and/or release anticancer agents constantly, acting on the adjacent tumor cells. To date, however, almost all of the available findings are confined to cell culture and/or animal cancer models, and more well-designed pre-clinical studies are definitely required before applying this strategy to real clinical settings.

In conclusion, the recent progress in both stem cell and anticancer gene studies has great potential for exploitation in new efficient cancer therapies. The combination of human MSCs and specific anticancer genes can selectively act upon targeted tumor cells. Further translational studies could lead to novel and effective treatments for cancer. Hopefully, the utilization of dual-targeted anticancer gene-engineered MSCs will be of great benefit to future cancer patients.

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ORIGINAL ARTICLE

Immunophenotyping of hematopoietic progenitor cells: Comparison between cord blood and adult mobilized blood grafts

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Abstract

AIM: To study the immunophenotype of hematopoietic progenitor cells from cord blood (CB) grafts (*n* = 39) in comparison with adult apheresis grafts (AG, *n* = 229) and pre-apheresis peripheral blood (PAPB) samples (*n* = 908) using flow cytometry analysis.

METHODS: First, we performed a qualitative analysis of CD34+ cell sub-populations in both CB and PAPB grafts using the standardized ISHAGE protocol and a wide panel of 20 monoclonal antibodies. Next, we studied some parameters, such as the age of mothers and the weight of newborns, which can influence the quality and the quantity of CD34+ cells from CB.

RESULTS: We found that the percentage of apoptotic cells was high in CB in comparison to PAPB (PAPB: 4.6% ± 2.6% *vs* CB: 53.4% ± 5.2%, $P < 0.001$). In CB, the weight of newborn and the age of the mother have the influence on CD34+ cells. The follow-up of Ag CD133 in the ISHAGE double platform protocol in association with CD45, CD34 and the 7'AAD shows an equal rate between the two cell populations CD133+CD45+CD34+ high and CD34+CD45+ high with a higher percentage. So, is the inclusion of Ac CD133 necessary in the present panel included in the ISHAGE method? Last part, we showed a significant presence of interferon γ in CB in comparison to PAPB, the annexin showing the high number of apoptotic cells in CB.

CONCLUSION: This study demonstrates that many different obstetric factors must be taken into account when processing and cryo-banking umbilical CB units for transplantation.

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Key words: Immunophenotyping; Hematopoietic progenitor; Cord blood; Mobilized blood

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INTRODUCTION

The global rise in the use of umbilical cord blood (UCB) as a transplant source has been amazing; over 20 000 transplants have already taken place alone[1-3]. It has become a real alternative to bone marrow (BM) and periph-

eral blood as a source of adult stem cells to treat multiple diseases.

UCB has become such a popular adult stem cell source for many reasons, not least because over 130 million births per annum worldwide represent the largest, easily available stem cell source. It also allows for storage of units from ethnic minorities not easily possible within BM registries[2,3]. This potentially allows for an increase in the rate of matched unrelated donor allogenic transplants[3]. It has also been found that there is a lower risk of graft *versus* host disease (GvHD) when transplanting UCB when compared to BM[3-6].

Although a valuable source of hematopoietic stem cells (HSCs), in order to bank UCB units suitable for transplantation effectively, samples need to be characterized and obstetric factors which impact upon UCB quality should be further examined. In this study, we compared two different parts of UCB: before placenta delivery (CB) and after placenta delivery (PlaB). For this comparison we used four different physiological parameters that pertain to either the baby or the mother and we compared levels of HSC CD34+. The four different parameters were: number of pregnancies of mothers, mother's age at delivery, newborn weight and newborn' s sex. Previous studies show that some patterns have already emerged. Birth weight impacted on HSC concentrations, especially mid-stage HSC[6-8]. When looking at mother's age, a previous study demonstrated that HSC concentration is greatly reduced as age increases[7]. Infant gender has previously been found to have an impact on HSC of UCB samples and newborn boys appear to have fewer stem cells than girls[8,9] whereas other works showed that the newborn's sex was not found to be significant to influence HSC in UCB. The number of pregnancies was also studied and seems to have an impact on HSC concentrations in UCB[7,8].

The principle aim of this study was to optimize UCB separation and cryopreservation by the characterization of these cellular groups. Several physiological factors were examined in order to determine the most suitable method. However, some of these findings appeared themselves to be of particular interest. In the last part of this work, variable levels of immaturity were detected on pre-apheresis peripheral blood (PAPB) and UCB populations using CD34, CD133 and CD45 antigens. In parallel, we analyzed some antigens to compare between these two HSC sources.

MATERIALS AND METHODS

Cells sources

PAPB samples (n = 190) were collected from patients from the Hematology Department at Aziza Othmana Hospital, the National Center of Bone Marrow Transplantation, Salah Azaiez Hospital, the Military Hospital and the National Blood center (Tunis, Tunisia). These patients, suffering from various conditions including: 34 acute myeloid leukemia, 24 acute lymphoblastic leukemia, 5 chronic myelocytic leukemia, 32 Medullar Aplasis, 31 multiple myelomas, 4 Diffuse Large Cell B Lymphomas, 13 Fanconi disease, 4 Gaucher disease, 6 Drepanocytosis, 2 β-Thalassemic, 24 Hodgkin's diseases, 6 Non Hodgkin' s diseases, 1 mantle cell lymphoma and 1 Kahler's disease were destined for autologous or allogenic HSC transplantation performed at the National Centre of Bone Marrow Transplantation. Blood samples were taken in tubes containing EDTA as anticoagulant (Vacutainer®, Becton-Dickinson). These patients consisted of 89 women and 98 men whose mean age was 39.5 years. We noted that these PAPB samples are collected from patients after treatment with conditioning factors such as as G-CSF.

Apheresis graft (AG) samples were collected in the same patients (n = 189) who were studied in PAPB.

Cord blood (CB) and PlaB samples were collected from women (n = 39) who had delivered at the Wassila Bourguiba maternity centre in Tunis and whose mean age (± SE) was 28.4 ± 4.4 years. After the umbilical cord was clamped off from the newborn, the CB was collected by aspiration using a syringe needle in a sterile tube containing EDTA as anticoagulant (Vacutainer®, Becton-Dickinson). Two collection methods were used by blood punction: (1) from the maternal end of the severed cord after vaginal delivery of the infant while the placenta was still in uterus (CB sample); and (2) from placenta-umbilical cord junction after placenta delivery (PlaB sample).

Numbers of CD34+ cells were derived from either the flow cytometry assessed per cent CD34+ cells within the nucleated cells and/or the white blood cell count from a hematology cell analyzer. All the samples were processed within 1 h of collection.

Flow cytometric analysis

Four-color flow cytometric analysis was performed with Cell Quest Pro (Becton Dickinson), as follows. Whole blood cells were stained with appropriate amounts of fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, peridinin-chlorophyll-a protein- and allophycocyanin (APC) conjugated to the following monoclonal antibodies at 4℃ for 20 min: mouse anti-human- anti-CD34-FITC, anti-CD45-FITC, anti-CD133- APC, anti-CD19-PE, anti-CD38-FITC, anti-CD11c-FITC, anti-CD25-FITC, anti-CD117-PE, anti-HLA-DR-FITC, - anti-CD7-FITC, anti-CD33-PE, anti-CD20-FITC, anti-CD15-FITC, anti-CD56-PE and anti- CD10-PE.

All McAbss were obtained from Becton Dickinson. The intracellular-antigens were: anti-TdT-PE, anti-IL_2-PE, anti-IL_4-PE and anti-interferon (IFN)α-FITC. The cell viability marker 7'AAD was used to exclude dead or apoptotic cells or DNA colorants to mark the cells and exclude the fragments.

Red blood cells were then lysed with FACS lysing solution (Becton-Dickinson, San Diego, CA). The cells were washed twice and suspended in phosphate-buffered saline. Analysis was performed on cells within the mononuclear light scatter and side scatter and on CD34-positive cells by CD34-positive staining and side scatter.

A total of at least 100 000 events were analyzed for each sample.

Hematological cell counts

The total number of CD34+ cells from PAPB, AG or CB samples was measured by direct flow cytometry. The results were expressed as %CD34+ cells and the number of CD34+ cells/μL.

Statistical analysis

Means were compared statistically using the STATGRAPHICS Centurion XVI program, version 16.0.08. A one-way analysis of variance and Newman-Keuls multiple range test were carried out to determine any significant difference between group values at $P < 0.05$. Values are mean ± SE.

RESULTS

Viability study of different CD34+ sources

The cell viability determined by the 7'AAD staining of each biological sample (Figure 1) allowed us to distinguish three cell populations: (1) living cells (7'AAD^{neg}), (2) dead cells (7'AAD^{pos}); and (3) apoptotic cells (7'AAD^{low}).

Thus, for PAPB samples mean percentage of living cells was 82.0% ± 11.9% that of dead cells 13.4% ± 12.4% and that of apoptotic cells 4.6% ± 2.6%. For AG samples, this percentage was 49% ± 14.4%, 33.5% ± 13.4% and 18.3% ± 8.4% respectively. For CB samples, this percentage was 40.7% ± 2.7%, 1.1% ± 0.37% and 53.4% ± 5.2%, respectively ($P = 0.0003$).

Enumeration of CD34+ cells in PAPB, AG and CB samples by flow cytometry

From all the 190 PAPB samples, the mean CD34$^+$ cell counts in PAPB was 80.5 ± 5.8 × 10^6 per kg. Forty-five per cent of patients had counts less than 50 cells/μL, 25% between 50 and 100 cells/μL, 18 % between 100 and 200 cells/μL and 12% above 200 cells/μL.

From 189 AG, mean CD34+ cell count was 5 ± 0.3 × 10^6 per kg in collected grafts. Only one aphaeresis was performed in 52% of the patients, with a mean of (2500 ± 181.9 CD34$^+$ cells/μL), while 36% of the patients were collected after two aphaeresis (1365.4 ± 128.5 CD34$^+$ cells/μL), and only 10% of patients had more than 2 aphaeresis (978 ± 217 CD34+ cells/μL).

From 39 CB samples, mean CD34$^+$ cell count was 16.0 ± 3.5/μL, while from PlaB samples this mean was 14.57 ± 2.9/μL. No Statistical significance between CB and PlaB samples for CD34$^+$ was found ($P = 0.31$).

The most important feature with CB samples and PlaB samples was a much wider variation rate of CD34$^+$ cell counts compared to PAPB and AG samples. This finding led us to investigate several factors that could affect CB and PlaB CD34$^+$ cell content, including those related to the mother such as age, parity, blood group and newborn's sex.

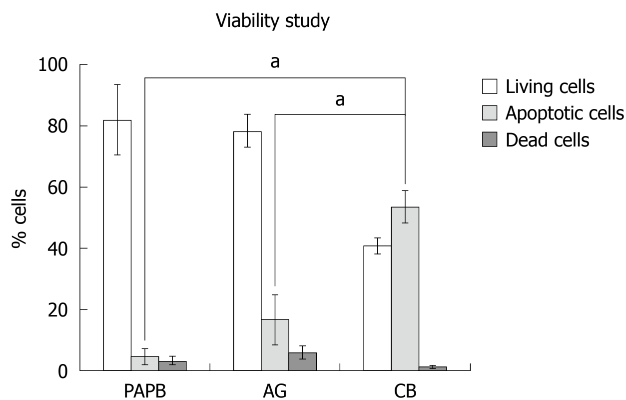

Figure 1 Profile of apoptotic cells in CD34+ cells from three different sources. The averages and SE of samples from independent biological replicates are shown: $^aP = 0.005$, in a Kurskal wallis test. PAPB: Pre-apheresis peripheral blood; AG: Apheresis graft; CB: Cord blood.

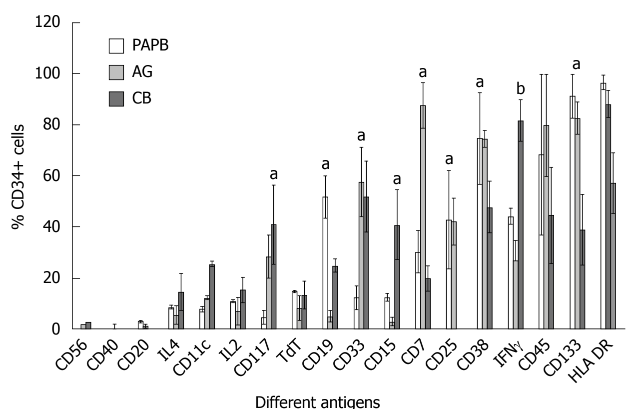

Figure 2 Comparison of the antigenic profile of CD34+ cells from three different sources: pre-apheresis peripheral blood, apheresis graft and cord blood. Results are expressed as mean percentage ± SE of positive cells. $^aP < 0.05$, $^bP < 0.0001$. PAPB: Pre-apheresis peripheral blood; AG: Apheresis graft; CB: Cord blood; IFN: Interferon.

Immunophenotypic profile of CD34+ cells from PAPB, AG and CB samples

We used a panel of 20 different McAbs to determine precisely the different subpopulations of CD34+ cells present in PAPB, AG, CB and PlaB samples.

We compared three sources of CD34$^+$ cells (PAPB, AG and CB), the samples were gated on 100% living CD45$^+$CD34$^+$ cells.

Some antigens were strongly expressed on CD34$^+$ cells in PAPB in comparison to CB such as CD133$^+$ (91.3% ± 8.6% in PAPB *vs* 39% ± 13.8%, $P = 0.04$) and CD38 (74.8% ± 17.9% in PAPB *vs* 47.8% ± 10.1%, $P < 0.05$). Other antigens were also strongly expressed but little statistical significance in expression was found such as: HLA-DR+ (96.7% ± 3% in PAPB *vs* 57.3% ± 11.8% in CB) and CD45+ (68.5% ± 31.4% in PAPB *vs* 44.6% ± 18.7% in CB, $P > 0.05$) (Figure 2).

The intracellular antigen IFNγ appeared in CB in a higher percentage than PAPB and AG (81.7% ± 8.1% in CB *vs* 27.1% ± 7.7% in AG and 44.3% ± 3% in PAPB,

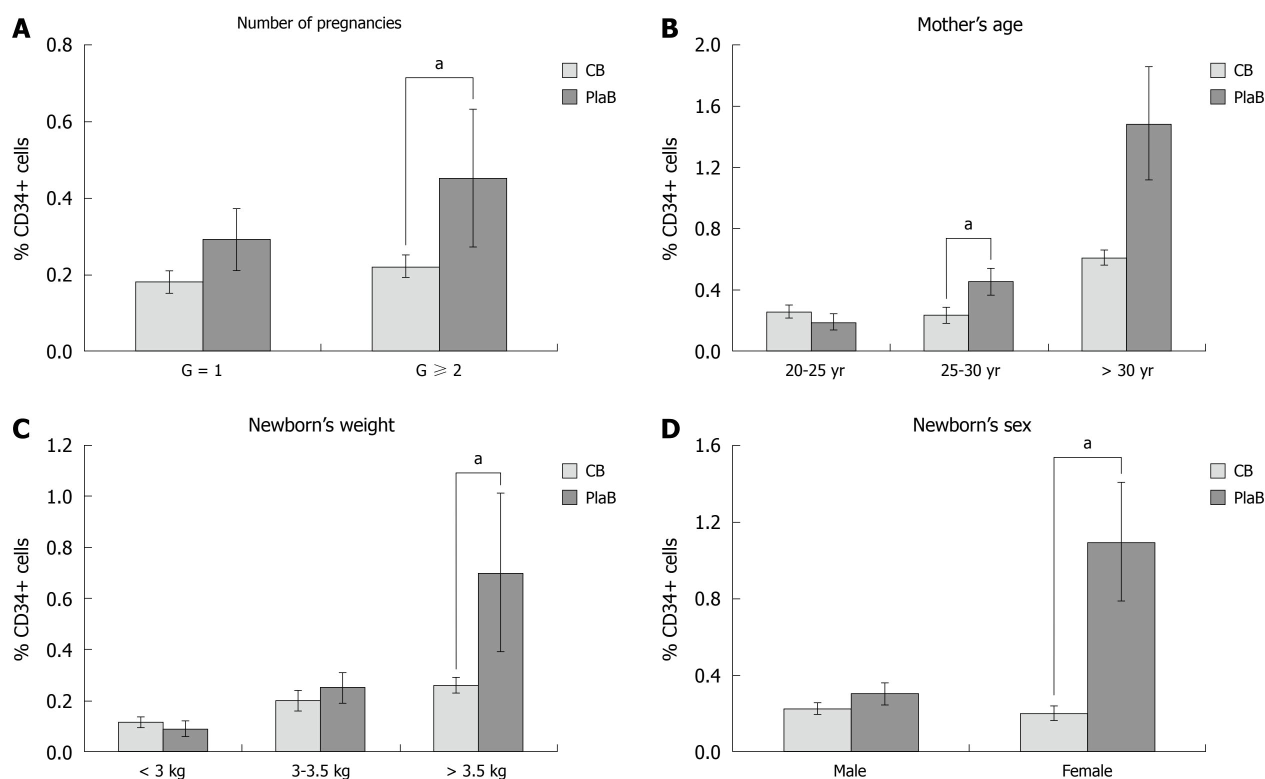

Figure 3 Variation of the incidence of hematopoietic stem cell CD34+ in relation to the number of pregnancies (A), the mother's age (B), the newborn's weight (C) and sex (D): Comparison between cord blood and placenta delivery. Data showed here represent average ± SE. $^aP < 0.05$. G: Gestation; CB: Cord blood; PlaB: Placenta delivery.

P < 0.0001). Also the same statistical result was found for CD33$^+$ antigen: (52% ± 13.8% in CB and 57.7% ± 13.6% in AG *vs* only 12.5% ± 4.6%, *P* = 0.01) (Figure 2).

Other antigens were moderately expressed like CD25 with a higher percentage in PAPB in comparison to CB (43% ± 19.1% in PAPB and 42.2% ± 9.1% in AG *vs* 0% in CB, *P* = 0.01), CD19 (51.9% ± 8.3% in PAPB *vs* 5.14% ± 2.2% in AG and 25% ± 2.5% in CB, *P* < 0.05) and CD7 with a low occurrence in CB (30.4% ± 8.2% in PAPB and 87.8% ± 8.9% in AG *vs* 20% ± 5% in CB, *P* = 0.01) whereas CD15 presented a high percentage in CB (41% ± 13.8% in CB *vs* 3% ± 1.6% in AG and 12.5% ± 1.3%, *P* = 0.01), also CD33 presented a statistically significant level in CB (52% ± 13.8%) and AG (57.7% ± 13.6%) compared with the low percentage in PAPB (12.5% ± 4.6%, *P* = 0.01).

Finally, a few antigens were practically absent in the three different CD34$^+$ sources such as: CD11c, CD20, CD10 and CD56 (Figure 2).

Comparable results were found for CD117 antigen which was expressed in higher proportions in CB than in PAPB with mean values of (41.0% ± 15.4% in CB *vs* 4.84% ± 2.7%, *P* = 0.01) (Figure 2).

Analysis of the factors that could influence CD34+ cell content in CB and placental blood: Number of pregnancies, mother's age, newborn's weight and newborn's gender

In this study, many parameters that can impact on CD34+ cells from CB were analyzed. For every parameter we compared two different parts of the umbilical cord: CB is the part near to the newborn and PlaB is part of placenta after delivery.

The number of pregnancies ranged from 1 to 3. This parameter appears to have an impact on CB CD34+ cell counts. Thus, CBs from multiparous women (G > 2) displayed higher CD34+ cell counts than that from primiparous women (G = 1) (0.22% ± 0.03% *vs* 0.18% ± 0.03%). Figure 3A illustrates the relationship between the number of pregnancies and CD34+ cell counts in CB and in PlaB. This difference was statistically clear in multiparous women (0.45% ± 0.18% *vs* 0.29% ± 0.08%, *P* = 0.02) (Figure 3A).

The relationship between mother's age at delivery and CD34+ cell counts was also examined (Figure 3B). The age range of the 39 women studied was between 19-39 years with a mean age of 28.26 ± 4.4 years.

Mothers under 25 years presented (CB: 0.21% ± 0.04% *vs* PlaB: 0.18% ± 0.05%, *P* = 0.35), and over 30 years presented (CB: 0.26% ± 0.05% *vs* PlaB: 0.84% ± 0.56%, *P* = 0.1), these results were not statistically significant. Those mothers aged between 25-30 years tended to have lower CD34+ cell counts (CB: 0.2% ± 0.05% *vs* PlaB: 0.45% ± 0.09%, *P* = 0.02) with a significant difference.

Newborn's weight appeared to influence CD34+ counts in CB samples. Thus, CD34+ counts were higher with newborns above 3.5 kg body weight than with newborns beneath 3 kg body weight (mean values 0.26% ±

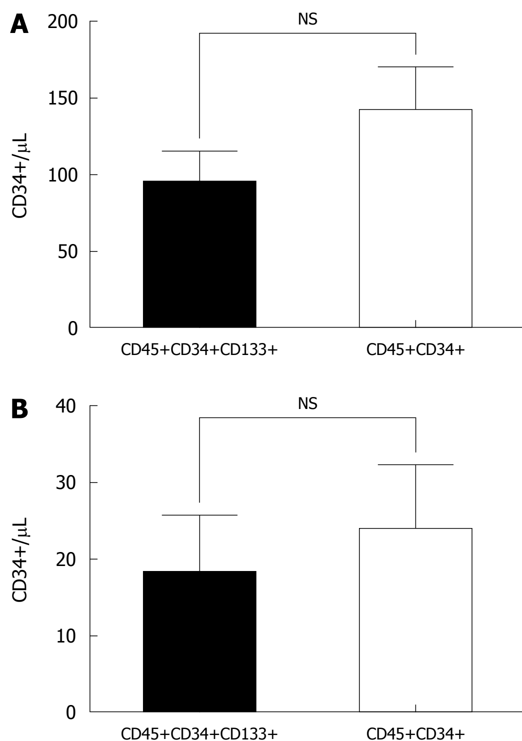

Figure 4 Quantitative study of antigenic marker CD133 in pre-apheresis peripheral blood (A) and in cord blood (B): The population CD34+CD45+ CD133+ is present at a similar level to the population CD34+CD45+. NS: No statistical significance.

0.03% *vs* 0.11% ± 0.02%). The difference between the UCB sites (CB and PlaB) was statistically significant only for body weight superior to 3.5 kg (0.7% ± 0.31%) (P = 0.05) (Figure 3C).

Newborn's gender presented a significant difference: female CD34+% samples differed between PlaB and CB (CB: 0.19% ± 0.04% *vs* PlaB: 0.74% ± 0.36%, P = 0.02) whereas the male samples did not show a significant difference between the two sources (Figure 3D).

Another parameter was studied: mother's blood group, but it had no statistically significant influence on CD34+ cell counts.

A quantitative study by flow cytometry of CD133 antigen in CB, PAPB and AG in association with CD34

In this part of study, we were interested in the CD34+ CD45+CD133+ cell population. We analyzed different antigen expression with the objective of determining the phenotype and studying the level of immaturity between PAPB and CB.

Firstly, we studied the presence of CD133 and used the panel of Abs to detect HSCs with the antigen CD34. We showed that the number of positive cells CD133+, CD45+, CD34+, 7'AAD- was equivalent to the number of CD45+ CD34+ cells. This result for PAPB: (CD133+ CD45+CD34+; 94.7 ± 18.9 cells/µL *vs* CD45+CD34+; 142.1 ± 25.5 cells/µL, P > 0.05) (Figure 4A).

In the case of CB, the values were lower than PAPB, but the same result is obtained: equivalent numbers of positive cells (CD133+CD45+CD34+; 18.0 ± 7.7 cells/µL *vs* CD45+CD34+; 23.7 ± 8.6 cells/µL, P > 0.05) (Figure 4B).

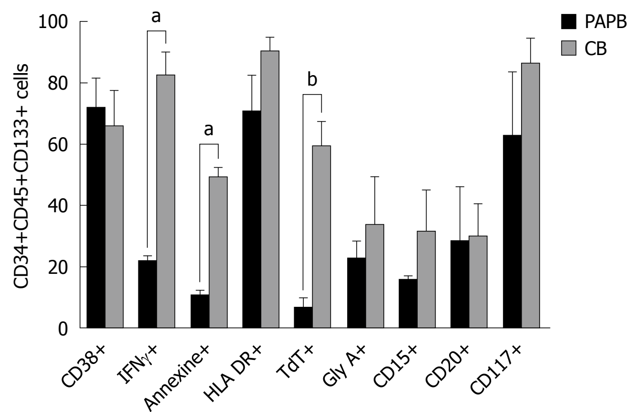

Figure 5 Comparison of the immunophenotyping of the population CD133+CD45+CD34+ cells in pre-apheresis peripheral blood and cord blood. ^{a}P < 0.05, ^{b}P < 0.01. PAPB: Pre-apheresis peripheral blood; CB: Cord blood; IFN: Interferon.

Secondly, different antigens were studied: CD38, IFNγ, Annexin, HLA-DR, TdT, GlyA, CD15, CD20 and CD117. We showed that the intracellular marker IFNγ was present in a higher percentage in CB samples comparedto PAPB (81.7% ± 7.5% *vs* 21.2% ± 1.7%, P < 0.05).

Also, we found a similar, statistically significant result with TdT antigen (58.7% ± 8.1% in CB *vs* 6.2% ± 3.3% in PAPB, P < 0.01).

Our data, using Annexin, show that CB contains a significantly higher percentage apoptotic cells compared to PAPB CD34+CD45+CD133+ cells. This result is in accordance with our results of viability with 7'AAD. Here, we found the same high percentage of apoptotic cells in CB (48.8% ± 3.3% in CB *vs* 10.2% ± 1.9%, P < 0.05) (Figure 5).

DISCUSSION

Reserachers have long been interested in determining the best immunophenotyping technique for identifying and counting HSC CD34+.

Our work focused on the qualitative and quantitative study of HSC CD34+ cells in three samples groups: PAPB, AG and CB. The use of the viability marker 7' AAD allowed us to conduct our study using flow cytometry. Thus, each time we observed three cell populations: living cells 7'-AAD^{neg}, apoptotic cells 7'-AAD^{dim} and dead cells7'-AAD^{pos}.

Our results showed that the viability of HSC CD34+ cells, in AG samples, was reduced in comparison with PAPB (33.5% of cells 7'AAD^{pos} for AG *vs* 13.5% only for PAPB). The number of apoptotic cells is higher in CB (59%). Our results are similar to those described by Shim *et al*[10] in 2006 who confirmed, using Annexin V (as an indicator of apoptosis), the very high level of apoptotic cells in CB. This apoptosis increases even more with cryopreservation. Several studies made by different authors inform us that 7'AAD is a very important marker in the qualitative and quantitative study of HSC

CD34+[11-17].

Keeney *et al*[18] have shown that the use of 7'AAD evaluates negatively the presence of cells CD34+/μL per sample, sometimes at 50%, suggesting that these cells are not viable and so not useful to graft; this has allowed for its integration into the routine enumerating protocol of HSC CD34+.

The qualitative analysis of HSC CD34+ in three samples types (PAPB, AG and CB) revealed differentiation markers expressed at a lower level in CB: CD133 (91.3% ± 8.6% in PAPB *vs* 39.0% ± 13.8% in CB): this was shown to be significant (P = 0.04).

According to the literature, the lower rate of antigen HLA-DR expression in CB has also been observed by other authors. It has, been suggested that this is an advantage in comparison with other sources of HSC CD34+ (BM) in engraftment capacity with a reduction of GvHD[19] reactions.

In our study, antigen CD133 appeared with a higher frequency in PAPB and AG than CB (91.3% ± 8.6% in PAPB *vs* 39.0% ± 13.8% in CB, P = 0.04). Our results agreed with those suggested by other authors. Indeed, in 2007, Tura *et al*[20] noted an expression percentage of phenotype CD34+ CD133+ of about 79% in PAPB, of 53% in CB and only 13% in BM. This significant difference between CB and PAPB seems to be related to the injection of growth factors like G-CSF.

The antigen CD45 is one of the most important factors in the detection of CSH CD34+ by means of different qualitative and quantitative protocols. This is why it was always included in our study panels. However, it was expressed with a weak intensity in comparison with other antigens (CD34, CD133) less than 10^2 on the logarithmic scale in FL1 and with variable frequencies according to the biological sample under examination: (68.56% ± 31.4% in PAPB, 80% ± 20% in AG) and a lesser rate in the case of CB: (44.66% ± 18.7%). However, this difference was not statistically significant in our study. CD45 is a surface marker restricted to the hematopoietic lineage in mature PB, with the exception of erythroid cells and platelets which causes the loss of this antigen during maturation. Ogata *et al*[21], suggest edthat cells (CD45+ CD34- CD38- Lin-) are probably less mature than cells (CD45+ CD34+ CD38- Lin-). Other authors[22-26] studied the co-expression of antigens CD34 and CD45 in different sources of HSC CD34+ and they noted that the ratio of CD34+ CD45+ cells appeared to be similar between CB and normal PB at 30% but it was higher in BM (60%). The level was higher still in PAPB. Indeed, we think that the level of CD34+ CD45+cells depends on conditioning and on the protocol used for this conditioning: (chemotherapy and G-CSF: 35%, chemotherapy only 20%).

The immunophenotyping of HSC CD34+ has allowed us to follow the expression frequency of the antigen CD33. Indeed, the latter was present at a lower level in PAPB (12.5% ± 4.6%) compared with CB (52.0% ± 13.8%, P = 0.01). It appeared also more highly expressed in AG samples (57.7% ± 13.6%). In 1997, the results of Sakabe *et al*[27] proved that CD34+ cells, in PAPB, which do not express antigens CD33, HLA-DR or CD38, contain all types of progenitors including CFU-Mix. On the contrary, the population ($CD34^+$ $CD33^+$) contains a large number of CFU-GM, unlike cells $CD34^+$ $CD38^-$ which contain few CFU-GM cells.

In this study, the antigen CD38 was strongly expressed on $CD34^+$ cells in PAPB compared to CB (74.8% ± 17.9% in PAPB *vs* 47.8% ± 10.1%, P < 0.05). Many researchers showed that the expression of CD38, multifunctional membrane co-enzyme, is a negative regulator in HSC. This phenomenon is still a potential research topic. The results of the percentage of cell population ($CD34^+$ $CD38^-$) and ($CD34^+$ $CD38^+$) seem contradictory from one team to another[28]. Many authors[2,3,29], note that ($CD34^+$ $CD38^-$) CB cells can proliferate rapidly in response to cytokine stimulation compared to those of BM.

In summary, our data shows that UCB contains a significantly higher percentage of these $CD34^+CD38^-$ primitive progenitors compared to PAPB $CD34^+$ cells. This suggests that $CD34^+$ stem cell populations taken from UCB have higher engraftment capacity than $CD34^+$cells from conditioned PB since they contain a higher percentage of pluripotent stem cells. This is in accordance with the successful clinical use of UCB even when a low number of cells was transplanted[2,3].

Recently, McKenzie *et al*[30] confirmed that the expression of antigen CD38 is not continuous in the hematopoietic hierarchy but has a high expression rate in cells involved in cell-cell interactions: $CD34^+$ $CD38^+$ cells have only the capacity for cell repopulation and producing progenitors in the short term. The expression of the marker CD38, beyond a certain level, becomes non-reversible and is associated with differentiation and the repopulation capacity.

Concerning Ag CD117 or c-kit, we mention in our results a low expression percentage in PAPB (4.84% ± 2.7%) compared to higher rates in CB (41.0% ± 15.4%, P = 0.01). Our results confirm the works of Sakabe *et al*[27] who found a 20% frequency of $CD34^+$ cells expressing Ag c-kit in PAPB, clearly lower than the cells of BM or those deriving from CB. Moreover, three cellular fractions have been identified: c-kit^{high}, c-kit^{low} and c-kit^-. The two populations $CD34^+$ c-kit^{high} and $CD34^+$c-kit^{low} are most abundant, at about 70%, enriched mainly by the BFU-E; while the $CD34^+$c-kit population is the least abundant, enriched by the CFU-GM.

In 2006, Machaliński *et al*[31] had the idea of following the expression potential of Ag CD117 according to the age of the donors: they reported that the Ag CD117 seemed significantly higher among the donors 35 years or younger compared with thoseover 35 years.

The multiparous factor of the mothers appears to have an impact on CB $CD34^+$ cell counts. Our CB data from multiparous women (G > 2) displayed higher $CD34^+$ cell counts than that from primiparous women (G = 1) (0.22% ± 0.03% *vs* 0.18% ± 0.03%).

Indeed, recently, different authors have suggested that this parameter has an impact on $CD34^+$ cells from CB.

Gajkowska *et al*[32] reported that the incidence of mononuclear cells, including that of CD34$^+$ cells, decreases significantly (P= 0.0001) with the increase of the number of gestations (from 1 to 7).

Concerning the newborn's weight, our results showed that CD34$^+$ counts were higher with newborns above 3.5 kg body weight than with newborns below 3 kg (mean values 0.26% ± 0.03% *vs* 0.11% ± 0.02%). The difference between the two sites of CB was statistically significant only for body weight over 3.5 kg (0.7% ± 0.31%, P = 0.05). Our results confirm those suggested by McGuckin *et al*[9].

The mother's age at the time of delivery indicated that the younger the mother, the higher the incidence of CD34$^+$ cells. In fact, mothers between 25-30 years tended to have lower CD34$^+$ cell counts (CB: 0.2% ± 0.05% *vs* PlaB: 0.45% ± 0.09%, P = 0.02) with a significant difference between the two sites of CB samples. We notice that similar results were described by McGuckin *et al*[9].

The newborn's sex seems to influence the number of CSH CD34$^+$ cells in CB. We have noticed in our series of samples a significant difference in females between the PlaB and CB (CB: 0.19% ± 0.04% *vs* PlaB: 0.74% ± 0.36%, P = 0.02) whereas there was not a significant difference in males between the two sources of CD34$^+$% cells. McGuckin *et al*[9] made the same observations as ours with additionally, the presence, among female babies, of a higher concentration of precocious cells (CD45^{+dim}/CD34$^-$/CD133$^+$) and late cells (CD45$^+$/CD34$^+$/CD133$^+$).

A great debate concerning the concentration of CSH CD34$^+$ cells in the placenta and the umbilical cord presents a potential research topic. The difference in blood sampling site in the CB shows, in a curious way, a clear variation of the percentage in CSH CD34$^+$. However, it is important to point out that the sampling in the cord is made before the delivery of the placenta. Through the different parameters mentioned in this work, our researches compared the variation of the rate of CSH CD34$^+$ only before and after delivery.

The mother's blood group is one parameter studied in our work, but our data showed a statistical difference. Galan *et al*[33] also described the possible effects of blood groups on the proliferation and the capacity for self-renewal of CD34+ CB cells. Indeed, the results of this study suggested that, following culture in the presence of growth factors, the proliferation of CD34+ cells with the phenotype O+ 23.2% (extremes from 21% to 25%) for the group (O) may be more important than the one perceived with the phenotypes A: 21% (from 15% to 33%) and B: 19% (from 12% to 30%) which seemed to be independent of the culture conditions.

There are other parameters we have not dealt with in our work such as the age of gestation. According to one study, the percentage of CD34+ cells, in relation to CD45+ cells or mononuclear cells, is inversely proportional to the age of gestation[9]. The frequency of these cells is significantly higher among the premature newborn than among the babies born at term[7]. The sharp change in the incidence of CD34+ cells seems to occur in the 40th gestation week with a low incidence before and a high incidence after[8]. Thus, do the best units of CB valid for a future graft, come from the eldest among the newborn and those born of mothers who are younger and have had fewer pregnancies? Can other parameters such as the placenta weight and vaginal delivery, have an effect on the incidence of CSH CD34+ of CB?

Finally, in the population (CD45$^+$CD34$^+$CD133$^+$), we showed the highest percentage of IFNγ in CB comparedto PAPB (P < 0.0001). The follow-up of the antigen CD133 by flow cytometry, in association with CD45, CD34 and 7'AAD shows that the total number, of positive cells (CD133$^+$ CD45$^+$CD34$^+$7'AAD$^-$) is equivalent to the total number of (CD45$^+$CD34$^+$7'AAD$^-$) cells. This equality of incidence of these two cellular populations implies that in order to detect the (CD34$^+$CD133^{+high}) cells, the CD34$^+$ cells should be targeted. Several studies have targeted the CD34$^+$CD133$^+$ cellular population. The same result was found in PAPB and CB.

The proportion of CD34+ cells which co-express the marker CD133 appears in a decreasing way in PAPB, CB and BM[24].

Other authors reported that after conditioning with G-CSF, the predominance of doubly positive populations (CD34+CD133+) is observed in a quantity similar to that of cells CD34+ with a low frequency of GvHD[22].

UCB raised great hope as an alternative source of transplantable hematopoietic stem/progenitor cell (HSPC) to adult BM or PAPB. CB HSPC has been used successfully in over 2000 HSC transplantations in a range of malignant and non-malignant diseases. In spite of the high level of apoptotic cells in CB shown in this study, it still offers a new source of cellular therapy.

Recently, cells expressing the CD133 antigen were considered a potent substitute to CD34+ cells. Phenotypic and functional studies revealed CB CD133+ populations contain higher levels of early HSPC than CB CD34+ harvested populations.

In summary, the simple maneuver of placing the new-born on the maternal abdomen after delivery and the method to collect the blood before or after cord clamping may significantly increase the influence of other parameters like the newborn's weight or the mother's age and therefore the absolute amount of blood progenitors required for engraftment success of UCB transplant without any harmful effects to the newborn.

We showed also that the population CD34+CD45+ was similar to the CD34+CD45+CD133+ positive cells in PAPB and CB. Our data also showed that in CD34+CD133+ cells there was an increased level of cells positive for IFNγ from CB compared to PAPB. In earlier studies, IFNγ was shown to act as an anti-proliferative and pro-inflammatory cytokine.

COMMENTS

Background

Studies of Immunophenotyping hematopoietic progenitor cells from cord blood (CB) and some parameters, such as the age of mothers and the weight of new-

borns, which can influence the quality and the quantity of CD34+ cells from CB: a comparison with adult mobilized blood grafts

Research frontiers

CSH CD34+ is a sort of natural biological material and it has been processed into many forms for medical us in celltherapy. The research hotspot is to demonstrate that many different obstetric factors must be taken into account when processing and cryo-banking umbilical CB (UCB) units for transplantation.

Innovations and breakthroughs

In the previous application of CB CSH CD34+ in celltherapy in human, medicine particularly in the hematopoietic reconstructions it was found that the simple maneuver of placing the new-born on the maternal abdomen after delivery and the method to collect the blood before or after cord clamping may significantly increase the influence of other parameters like the newborn`s weight or the mother`s age and therefore the absolute amount of blood progenitors required for engraftment success of UCB transplant without any harmful effects to the newborn.

Applications

The study results suggest that the CB CSH CD34 + is a potential therapeutic material that could be used as a transplant source. It has become a real alternative to bone marrow (BM) and peripheral blood (PB) as a source of adult stem cells to treat multiple diseases.

Terminology

The most important feature with CB samples and placenta delivery (PlaB) samples was a much wider variation rate of $CD34^+$ cell counts compared to pre-apheresis peripheral blood (PAPB) and apheresis grafts samples. This finding led us to investigate several factors that could affect CB and PlaB $CD34^+$ cell content, including those related to the mother such as age, parity, blood group and newborn's sex.

Peer review

This is a descriptive study in which authors analyze and optimize UCB separation and cryopreservation by the characterization of these cellular groups. Several physiological factors were examined in order to determine the most suitable method by flow cytometry using monoclonal antibodies. The results are interesting and suggest that the UCB raised great hope as an alternative source of transplantable hematopoietic stem/progenitor cell (HSPC) to adult BM or PAPB. CB HSPC and a potential therapeutic substance that could be used in medicine regenerative.

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MEETINGS

Events Calendar 2011

March 26, 2011
Stem Cell Agency Governance Subcommittee Meeting, Crowne Plaza SFO, 1177 Airport Blvd, Burlingame, CA, United States

January 29-February 2, 2011
LabAutomation2011, Palm Springs, CA, United States

February 4, 2011
7th annual Swiss Stem Cell Network meeting, Swiss Federal Institute of Technology in Lausanne, Switzerland

March 1, 2011
The 6th Annual Stem Cell Summit, 11 Fulton Street, New York City, NY, United States

March 22, 2011
StemCONN 2011, Farmington, CT, United States

March 27-31, 2011
SBS 17th Annual Conference and Exhibition, Orlando, FL, United States

April 6-8, 2011
EMBO Conference-Advances in Stem Cell Research: Development, Regeneration and Disease, Institut Pasteur, Paris, France

April 7-10, 2011
2011 CSHL Meeting on Stem Cell Engineering & Cell Therapy, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

April 25-26, 2011
International Conference on Stem Cell Research, Hotel Equatorial Penang, Malaysia

April 27, 2011
6th Annual Wisconsin Stem Cell Symposium, BioPharmaceutical Technology Center, Madison, WI, United States

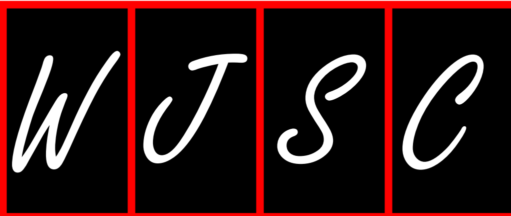
May 9-11, 2011
The World Stem Cells and Regenerative Medicine Congress 2011, Victoria Park Plaza, London, United Kingdom

May 23-24, 2011
The 4th Annual Israeli Stem Cell Meeting, Beit Sourasky, Chaim Sheba Medical Center, Israel

May 26-27, 2011
7th annual Stem Cell Research & Therapeutics Conference, Boston, MA, United States

September 20-24, 2011
2011 CSHL Meeting on Stem Cell Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States

October 2011
3rd Annual World Stem Cells & Regenerative Medicine Congress Asia 2011, Seoul, South Korea



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INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Stem Cells (*World J Stem Cells*, *WJSC*, online ISSN 1948-0210, DOI: 10.4252), is a monthly, open-access (OA), peer-reviewed journal supported by an editorial board of 284 experts in stem cell from 28 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

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Aims and scope

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

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14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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