ISSN 2218-5836 (online)

World Journal of *Orthopedics*

World J Orthop 2012 November 18; 3(11): 167-198

Baishideng Publishing Group
www.wjgnet.com

WJO World Journal of Orthopedics

Contents

Monthly Volume 3 Number 11 November 18, 2012

World Journal of Orthopedics
Volume 3 Number 11 November 18, 2012

Contents

NAME OF JOURNAL
World Journal of Orthopedics

ISSN
ISSN 2218-5836 (online)

LAUNCH DATE
November 18, 2010

FREQUENCY
Monthly

EDITING
Editorial Board of *World Journal of Orthopedics*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893
E-mail: wjo@wjgnet.com
http://www.wjgnet.com

EDITOR-IN-CHIEF
Bao-Gan Peng, MD, PhD, Professor, Department of Spinal Surgery, General Hospital of Armed Police Force, 69 Yongding Road, Beijing 100039, China

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Orthopedics
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893
E-mail: wjo@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpg@baishideng.com
http://www.wjgnet.com

PUBLICATION DATE
November 18, 2012

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2218-5836/g_info_20100722172650.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

WJO World Journal of Orthopedics

A peer-reviewed, online, open-access journal of orthopedics

Editorial Board

2010-2014

The *World Journal of Orthopedics* Editorial Board consists of 261 members, representing a team of worldwide experts in orthopedics. They are from 30 countries, including Argentina (1), Australia (14), Austria (2), Belgium (1), Brazil (3), Canada (5), China (18), Croatia(2), Denmark (1), Egypt (2), Finland (2), France(2), Germany (10), Greece (5), Hungary (1), India (9), Iran (2), Ireland (1), Israel (5), Italy (19), Japan (14), Morocco (1), Netherlands (10), Norway (2), Portugal (1), Serbia (3), Singapore (3), South Korea (12), Spain (4), Sri Lanka (1), Sweden (3), Switzerland (6), Tunisia (3), Turkey (1), Tunisia (1), United Kingdom (6), and United States (69).

Yrjö T Konttinen, *Helsinki*

France

Federico Canavese, *Clermont-Ferrand*
Yiou Eric, *Orsay Cedex*

Germany

Annegret Mündermann, *Radolfzell*
Beat Knechtle, *Gallen*
Heinz LOHRER, *Frankfurt Am Main*
Olaf Lorbach, *Homburg*
Stefan Grote, *Munich*
Karsten Knobloch, *Hannover*
Philipp Kobbe, *Aachen*
Volker Schöffl, *Bamberg*
Arndt P Schulz, *Lübeck*
Lars V Baron von Engelhardt, *Bochum*

Greece

George C Babis, *Attiki*
Marios Georgios Lykissas, *Ioannina*
Lazaros I Sakkas, *Larissa*
Nikolaos G Papadimitriou, *Thessaloniki*
Konstantinos N Malizos, *Larissa*

Hungary

Andor Sebestyén, *Pécs*

India

Antony Gomes, *Calcutta*
Kunal Sharan, *Lucknow*
Divya Vohora, *New Delhi*
Devdatta Suhas Neogi, *Mumbai*
Mohamed Shafi, *Tamil Nadu State*
Pankaj Kumar, *Andhra Pradesh*
Pramod V Lokhande, *Pune*
Vidyadhara Srinivasa, *Karnataka*
Vaibhav Bagaria, *Ghaziabad*

Iran

Hossein Negahban, *Ahvaz*
Sayed Javad Mousavi, *Tehran*

Ireland

Joseph S Butler, *Dublin*

Israel

Alexander Blankstein, *Ramat Hasharon*
Dror Lakstein, *Haifa*
Itzhak Binderman, *Tel Aviv*
Nahum Rosenberg, *Haifa*
Youssef Maher Masharawi, *Tel Aviv*

Italy

Alessandro Geraci, *Feltre*
Angelo Cacchio, *L'Aquila*
Costantino Errani, *Bologna*
Claudia Mazzà, *Roma*
Donatella Lippi, *Florence*
Giuseppe M Campo, *Messina*
Giuseppe Banfi, *Milano*
Patrizia D'Amelio, *Torino*
Marco Crostelli, *Rome*
Marcello Maggio, *Parma*
Marco Giuseppe Angelo Teli, *Bergamo*
Monica Mattioli-Belmonte, *Ancona*
Marco Monticone, *Lissone*
Pasquale De Negri, *Rionero in Vulture*
Andrea Giusti, *Genova*
Alberto Gobbi, *Milan*
Raoul Saggini, *Chieti*
Saverio Affatato, *Bologna*
Tomaso Villa, *Milano*

Japan

Akio Sakamoto, *Fukuoka*
Jun Iwamoto, *Tokyo*
Kanji Mori, *Otsu*
Makoto Makishima, *Tokyo*
Ryuichi Morishita, *Suita*
Shuichi Kaneyama, *Kobe*
Tadahiko Yotsumoto, *Osaka*
Toru Yamaguchi, *Izumo-shi*
Toshimasa Uemura, *Ibaraki*
Hideki Nagashima, *Yonago*
Hisataka Yasuda, *Nagahama*
Yasuharu Nagano, *Saitama*
Yoichi Aota, *Yokohama*
Yuichi Kasai, *Tsu city*

Morocco

Abdellah El Maghraoui, *Rabat*

Netherlands

Claudine JC Lamoth, *Groningen*
Barend J van Royen, *Amsterdam*
Doug King, *Lower Hutt*
Paul C Jutte, *Groningen*
PE Huijsmans, *Hague*
PM van der Kraan, *Nijmegen*
Michel van den Bekerom, *Amsterdam*
JJ Verlaan, *Utrecht*
Rob GHH Nelissen, *Leiden*
Taco Gosens, *Tilburg*

Norway

Jan Oxholm Gordeladze, *Oslo*
Gunnar Knutsen, *Tromsø*

Portugal

João F Mano, *Guimarães*

Serbia

Radica Dunjic, *Belgrade*
Miroslav Z Milankov, *Novi Sad*
Zoran Vukasinovic, *Belgrade*

Singapore

Anselm Mak, *Singapore*
Dongan Wang, *Singapore*
V Prem Kumar, *Singapore*

South Korea

Dae-Geun Jeon, *Seoul*
Seok Woo Kim, *Gyeonggi*
Sang-Hun Ko, *Ulsan*
Sung-Uk Kuh, *Seoul*
Jaebeom Lee, *Miryang*
Yong Seuk Lee, *Suwon*
Hyun Woo Kim, *Seoul*
Jae Taek Hong, *Suwon*
Jong-Beom Park, *Kyunggi-do*
Kook Jin Chung, *Seoul*
Kyu Hyun Yang, *Seoul*
Sang Ki Lee, *Daejeon*

Spain

Antonio Herrera, *Zaragoza*
Daniel Hernandez Vaquero, *Aviles*
Francisco J Blanco, *A Coruña*
Nuria Vilaboa, *Madrid*

Sri Lanka

Janaka Lenora, *Galle*

Sweden

Jan G Jakobsson, *Stockholm*
Anna Nordström, *Umeå*

Switzerland

Achim Elfering, *Bern*
Benjamin Gantenbein, *Bern*

Nicola A Maffiuletti, *Zurich*
Michael Hirschmann, *Basel*
Elyazid Mouhsine, *Lausanne*
Peter Fennema, *Baar*

Thailand

Boonsin Tangtrakulwanich, *Songkla*
Prachya Kongtawelert, *Chiang Mai*
Sittisak Honsawek, *Bangkok*

Tunisia

Lamia Rezgui-Marhoul, *Tunis*

Turkey

Akmer Mutlu, *Ankara*
Bulent Daglar, *Ankara*
Kemal NAS, *Diyarbakır*
Salih Özgöçmen, *Kayseri*
Serdar Kahraman, *İstanbul*

United Kingdom

Henry DE Atkinson, *London*
Vikas Khanduja, *Cambridge*
Ali Mobasheri, *Sutton Bonington*
Charles Anthony Willis-Owen, *London*
Vikki Wylde, *Bristol*
Tosan Okoro, *Bangor*

United States

Srino Bharam, *New York*
Craig R Bottoni, *Honolulu*
Lavjay Butani, *Sacramento*
Chaoyang Chen, *Detroit*
Ock K Chun, *Storrs*
Christopher J Colloca, *Chandler*
Nabanita S Datta, *Detroit*
Paul E Di Cesare, *Sacramento*
Matthew B Dobbs, *Saint Louis*
Evan F Fkman, *Columbia*
Joel J Gagnier, *Ann Arbor*
Federico P Girardi, *New York*
David L Helfet, *New York*
Johnny Huard, *Pittsburgh*
Stefan Judex, *Stony Brook*
Monroe Laborde, *New Orleans*
Bingyun Li, *Morgantown*
Subburaman Mohan, *Loma Linda*
Arash Momeni, *Palo Alto*
Nader D Nader, *Buffalo*
John Nyland, *Louisville*
Karin Grävare Silbernagel, *Newark*
David H Song, *Chicago*
Nelson F SooHoo, *Los Angeles*
SPA Stawicki, *Columbus*
Ann Marie Swank, *Louisville*
R Shane Tubbs, *Birmingham*
Victoria M Virador, *Bethesda*
Savio LY Woo, *Pittsburgh*
Masayoshi Yamaguchi, *Atlanta*
Feng-Chun Yang, *Indianapolis*
Subhashini Yaturu, *Albany*
Hiroki Yokota, *Troy*
Charalampos Zalavras, *Los Angeles*
Chunfeng Zhao, *Rochester*
Anil Bhave, *Baltimore*
John Elias, *Akron*
Clark Dickin, *Muncie*
John S Early, *Dallas*
James S Harrop, *Philadelphia*
Adam H Hsieh, *Maryland*
Aditya V Maheshwari, *New York*
Zong-Ming Li, *Cleveland*
Richard M Lovering, *Baltimore*
Lyle Joseph Micheli, *Boston*
Ming Pei, *Morgantown*
Juan A Pretell – Mazzini, *Philadelphia*
David M Selkowitz, *Pomona*
Hassan Serhan, *Raynham*
Subhashini Yaturu, *Albany*
Sorin Siegler, *Pennsylvania*
Aaron David Sciascia, *Lexington*
Jeffrey C Wang, *Santa Monica*
David Andrew Spiegel, *Philadelphia*
Inna Belfer, *Pittsburgh*
Angie Botto-van Bemden, *Lauderdale*
Quan-Jun Cui, *Virginia*
Scott D Daffner, *Morgantown*
B Sonny Bal, *Columbia*
Beril Gok, *Baltimore*
Ashraf S Gorgey, *Richmond*
Kee D Kim, *Sacramento*
Brian Michael Haus, *Boston*
Dror Paley, *Pittsburgh*
Bing Wang, *Pittsburgh*
Wen-Bao Wang, *New York*
Li-Qun Zhang, *Chicago*
Nigel Zheng, *Charlotte*

World Journal of Orthopedics

Online Submissions: http://www.wjgnet.com/esps/
wjo@wjgnet.com
doi:10.5312/wjo.v3.i11.167

World J Orthop 2012 November 18; 3(11): 167-174
ISSN 2218-5836 (online)

TOPIC HIGHLIGHT

Hisataka Yasuda, PhD, *Series Editor*

Molecular mechanisms of triggering, amplifying and targeting RANK signaling in osteoclasts

Yukiko Kuroda, Koichi Matsuo

Yukiko Kuroda, Koichi Matsuo, Laboratory of Cell and Tissue Biology, School of Medicine, Keio University, Tokyo 160-8582, Japan
Author contributions: Kuroda Y and Matsuo K performed literature review and prepared the manuscript.
Supported by Grants from MEXT Japan, No. 23790265 (to Kuroda Y) and No. 21390425 (to Matsuo K)
Correspondence to: Koichi Matsuo, MD, PhD, Professor, Laboratory of Cell and Tissue Biology, School of Medicine, Keio University, Tokyo 160-8582, Japan. matsuo@z7.keio.jp
Telephone: +81-3-33531211 Fax: +81-3-33530530
Received: June 20, 2012 Revised: October 25, 2012
Accepted: November 1, 2012
Published online: November 18, 2012

Abstract

Osteoclast differentiation depends on receptor activator of nuclear factor-κB (RANK) signaling, which can be divided into triggering, amplifying and targeting phases based on how active the master regulator nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) is. The triggering phase is characterized by immediate-early RANK signaling induced by RANK ligand (RANKL) stimulation mediated by three adaptor proteins, tumor necrosis factor receptor-associated factor 6, Grb-2-associated binder-2 and phospholipase C (PLC)γ2, leading to activation of IκB kinase, mitogen-activated protein kinases and the transcription factors nuclear factor (NF)-κB and activator protein-1 (AP-1). Mice lacking NF-κB p50/p52 or the AP-1 subunit c-Fos (encoded by *Fos*) exhibit severe osteopetrosis due to a differentiation block in the osteoclast lineage. The amplification phase occurs about 24 h later in a RANKL-induced osteoclastogenic culture when Ca^{2+} oscillation starts and the transcription factor NFATc1 is abundantly produced. In addition to Ca^{2+} oscillation-dependent nuclear translocation and transcriptional auto-induction of NFATc1, a Ca^{2+} oscillation-independent, osteoblast-dependent mechanism stabilizes NFATc1 protein in differentiating osteoclasts. Osteoclast precursors lacking PLCγ2, inositol-1,4,5-trisphosphate receptors, regulator of G-protein signaling 10, or NFATc1 show an impaired transition from the triggering to amplifying phases. The final targeting phase is mediated by activation of numerous NFATc1 target genes responsible for cell-cell fusion and regulation of bone-resorptive function. This review focuses on molecular mechanisms for each of the three phases of RANK signaling during osteoclast differentiation.

Key words: Receptor activator of nuclear factor-κB ligand; Tumor necrosis factor receptor-associated factor 6; c-Fos; Nuclear factor of activated T-cells cytoplasmic 1; Immunoreceptor tyrosine-based activation motif; Ca^{2+} oscillation

Peer reviewer: Shao-Hung Hung, MD, PhD, Associate Professor, Department of Orthopedic Surgery, Fooyin University Hospital, No. 5, Chun-Sun Rd., Tungkang PingTung 928, Taiwan, China

Kuroda Y, Matsuo K. Molecular mechanisms of triggering, amplifying and targeting RANK signaling in osteoclasts. *World J Orthop* 2012; 3(11): 167-174 Available from: URL: http://www.wjgnet.com/2218-5836/full/v3/i11/167.htm DOI: http://dx.doi.org/10.5312/wjo.v3.i11.167

INTRODUCTION

Osteoclasts are bone-resorbing cells derived from hematopoietic precursor cells[1-3]. Macrophage-colony stimulating factor (M-CSF) stimulation up-regulates expression of receptor activator of nuclear factor-κB (RANK, encoded by *Tnfrsf11a*) in the osteoclast precursor cell[4]. RANK, a type I transmembrane receptor with a C-terminal cytosolic tail, is responsible for osteoclast differentiation and

function. RANK signaling is induced by RANK ligand (RANKL, encoded by *Tnfsf11*), which is a type Ⅱ transmembrane protein (i.e., with a cytoplasmic N-terminus and an extracellular C-terminus). Mice with genetic deletion of *Tnfrsf11a* or *Tnfsf11* lack osteoclasts and exhibit severe osteopetrosis[5,6]. In humans, mutations in genes encoding RANK or RANKL are associated with osteoclast poor, autosomal recessive osteopetrosis[7,8]. RANK signaling is also modified by osteoprotegerin (encoded by *Tnfrsf11b*), a soluble decoy receptor of RANK that blocks RANKL binding to RANK[9,10].

RANK is a member of the tumor necrosis factor receptor (TNFR) superfamily consisting of 616 and 625 amino acid residues in human and mouse, respectively[11]. RANKL is produced by osteoblasts and osteocytes[12,13] and binds in a trimeric form to RANK, initiating signaling[14,15]. Like other TNFR superfamily members, RANK lacks intrinsic enzymatic activity and transduces intracellular signals by recruiting adaptor proteins including TNFR-associated factors (TRAFs), activating nuclear factor (NF)-κB and downstream mitogen activated protein kinase (MAPK) and Akt signaling[16-18]. RANK exhibits one of the longest cytoplasmic tails of any TNFR superfamily protein, and this domain is responsible for the osteoclast-specific signaling pathway[19,20].

Spatio-temporal control of signaling downstream of RANK[21] is divided into three phases in this review (Figure 1). In the triggering phase, NF-κB, activator protein-1 (AP-1), and MAPKs are rapidly activated within an hour of RANKL stimulation in a culture system[22]. Then, during the amplifying phase, nuclear factor of activated T-cells cytoplasmic 1 (NFATc1, encoded by *Nfatc1*) begins to accumulate approximately 24 h after RANKL stimulation as cytosolic Ca^{2+} levels begin to oscillate[23]. Finally, in the targeting phase, RANK signaling regulates multinucleation and bone resorptive function mainly through activation of NFATc1 target genes. Concerted action of RANK and its adaptor proteins as well as immunoreceptors and other co-stimulatory molecules drive these phases. Here we review literature relevant to the molecular mechanism of RANK signaling at each phase during osteoclast differentiation.

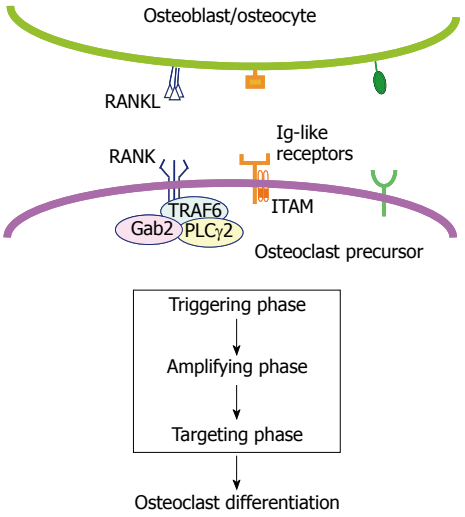

Figure 1 Three phases of receptor activator of nuclear factor-κB signaling during osteoclast differentiation. Osteoclast differentiation downstream of receptor activator of nuclear factor-κB (RANK) signaling is divided into triggering, amplifying and targeting phases, based on the nuclear factor of activated T-cells cytoplasmic 1 activation state. ITAM: Immunoreceptor tyrosine-based activation motif; TRAF6: Tumor necrosis factor receptor-associated factor 6; Gab2: Grb-2-associated binder-2; PLCγ2: Phospholipase C γ2; RANKL: RANK ligand; Ig: Immunoglobulin.

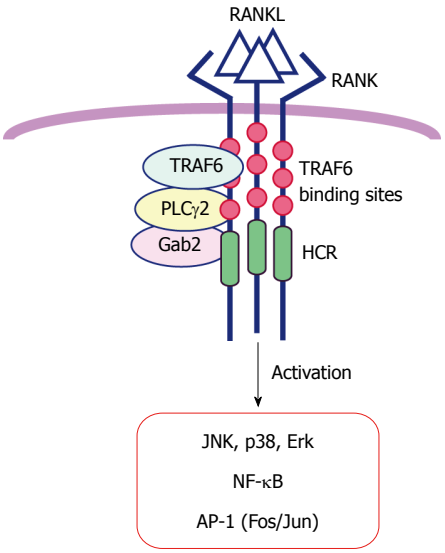

Figure 2 Triggering phase. Trimerization of receptor activator of nuclear factor-κB (RANK) by binding of RANK ligand (RANKL) immediately activates mitogen-activated protein kinases (MAPKs), nuclear factor (NF)-κB, and activator protein-1 (AP-1). An adaptor molecule complex including tumor necrosis factor receptor-associated factor 6 (TRAF6), Grb-2-associated binder-2 (Gab2) and phospholipase C (PLC)γ2 on TRAF6 binding sites of RANK is essential to induce the triggering phase. HCR: Highly conserved domain in RANK; JNK: c-Jun N-terminal kinase; Erk: Extracellular signal-regulated kinase.

TRIGGERING PHASE

Once homotrimeric RANKL forms complex with its receptor RANK[14,15], a cascade of downstream signaling is initiated. RANK recruits adaptor proteins to specific motifs in its C-terminal cytoplasmic tail, which contains three TRAF6 binding sites near the transmembrane domain, the a highly conserved domain in RANK (HCR) motif, and two binding sites for TRAF2 or TRAF5 near the C-terminus[20] (Figure 2). These motifs have been analyzed using various mutant RANK proteins[16,22]. Inoue and colleagues generated a CD40/RANK chimeric receptor carrying the N-terminal extracellular domain of human CD40 (*TNFRSF5*) and the cytoplasmic tail of mouse RANK (*Tnfrsf11a*), which can be specifically activated by anti-CD40 antibody and found that TRAF6 binding sites, but not the HCR, are essential for RANK signaling in the immediate-early phase[16,22]. At least three molecules, TRAF6, Grb-2-associated binder-2 (Gab2) and phospholipase C (PLC)γ2, function as adaptor molecules for RANK. TRAF6 is a really interesting new gene (RING) E3 ubiquitin ligase and Lys63-linked

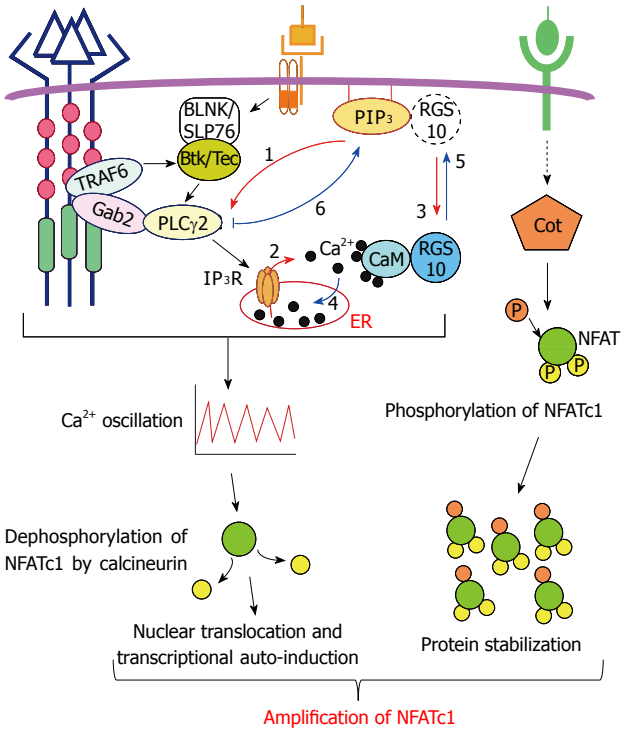

Figure 3 Amplifying phase. Both Ca^{2+} oscillation-dependent and -independent nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) amplification are induced. Highly conserved domain in receptor activator of nuclear factor-κB (RANK)-mediated RANK signaling and immunoreceptor tyrosine-based activation motif (ITAM) signaling lead to continuous phospholipase C (PLC)γ2 activation. Regulator of G-protein signaling 10 (RGS10) determines the Ca^{2+} oscillation pattern through control of PLCγ2 by competitive binding of Ca^{2+}/calmodulin and phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3). Sustained Ca^{2+} oscillation contributes to NFATc1 amplification mediated by transcriptional auto-induction. In the Ca^{2+} oscillation-independent pathway, Cot kinase enhances NFATc1 stabilization through direct phosphorylation and contributes to its accumulation. ER: Endoplasmic reticulum; BLNK: B cell linker protein; SLP76: Src homology 2 domain-containing leukocyte protein of 76 kD; TRAF6: Tumor necrosis factor receptor-associated factor 6; Gab2: Grb-2-associated binder-2; IP_3R: IP_3 receptor; CaM: Calmodulin; Btk: Bruton's tyrosine kinase.

auto-ubiquitination is necessary for the signal transduction to activate IκB kinase and NF-κB during osteoclast differentiation[24]. Mutational analysis of PLCγ2 revealed that catalytic activity of PLCγ2 is dispensable at the triggering phase but necessary for the amplifying phase of RANK signaling[25] (see below). These adaptor proteins activate diverse signaling molecules, phosphatidylinositol-3 kinase, Akt kinase, and MAPKs including c-Jun N-terminal kinase, p38, and extracellular-regulated kinase, leading to activation of the dimeric transcription factors NF-κB and AP-1[20,25,26]. Production of reactive oxygen species *via* a RANKL-TRAF6-Rac1-nicotinamide adenine dinucleotide phosphate oxidase-dependent pathway is also required for MAPK activation and osteoclastogenesis[27]. In osteoclast lineage cells, NF-κB and AP-1 are composed of two molecules among p65, RelB, p50 and p52 for NF-κB, and c-Fos (also Fra-1, Fra-2 or FosB) and c-Jun (also JunB or JunD) for AP-1. Double knockout mice lacking both p50 and p52 and single knockout mice lacking c-Fos lack osteoclasts and exhibit severe osteopetrosis[28-32]. Mice overexpressing dominant negative c-Jun also develop osteopetrosis[33]. These studies demonstrate the importance of NF-κB and AP-1 activation by RANK signaling in osteoclast differentiation.

TRANSITION TO THE AMPLIFYING PHASE

The transition from triggering to amplifying phase requires induction of *Nfatc1* transcription, which allows cooperation with signaling downstream of immune receptors. NFAT was first identified in nuclear extracts of activated T-cells as a transcription factor that binds to the interleukin-2 (IL-2) promoter[34]. NFAT regulates not only differentiation and activation of immune cells but also the development of tissues such as skeletal muscle, cardiac valve, and bone[35]. Since the promoter of the osteoclast-specific tartrate-resistant acid phosphatase (*TRAP*) gene carries an evolutionarily conserved AP-1/NFAT binding element similar to the cooperative AP-1/NFAT binding site in the IL-2 promoter, it was hypothesized that c-Fos/AP-1 is required for NFAT function in osteoclasts[36]. It was demonstrated that *Nfatc1* itself is a major c-Fos target gene during osteoclast differentiation[37-39]. In cells lacking c-Fos, NF-κB activity is unexpectedly elevated[40], supporting the idea that *Fos* and *Nfatc1* induction is downstream of NF-κB p50 and p52 activation in RANK signaling[41]. It is likely that NF-κB, c-Fos/AP-1 and NFATc2 mediate basal expression of *Nfatc1* in preparation for the amplification phase[42].

In concert with RANK signaling, immunoglobulin-like receptors such as osteoclast-associated receptor (OSCAR) and the triggering receptor expressed in myeloid cells (TREM)-2 transduce *Nfatc1* induction signals[43,44]. Both are associated with adaptor proteins containing the immunoreceptor tyrosine-based activation motif (ITAM), such as DNAX-activation protein 12 or the Fc receptor common γ subunit[45]. After ITAM tyrosine phosphorylation, a complex containing the tyrosine kinases Bruton's tyrosine kinase and Tec and the adaptor molecules B cell linker protein and Src homology 2 domain-containing leukocyte protein of 76 kD may facilitate cooperation between RANK and ITAM signaling (Figure 3)[46]. This combined signaling selectively leads to PLCγ phosphorylation, suggesting that integration of RANK and ITAM signaling is required for efficient activation of PLCγ during the amplifying phase. Furthermore, following elevation of intracellular Ca^{2+} levels, prior to the beginning of Ca^{2+} oscillation, *Nfatc1* transcription is enhanced by Ca^{2+}/calmodulin-dependent kinase Ⅳ, which phosphorylates the cAMP response element-binding protein, inducing *Fos* expression[47].

AMPLIFYING PHASE

During the amplifying phase starting approximately 24 h after RANKL stimulation in osteoclastogenic cultures,

intracellular Ca^{2+} levels oscillate, and activate the Ca^{2+}/calmodulin-dependent phosphatase calcineurin, which dephosphorylates NFATc1 and induces its nuclear translocation. On the HCR of the RANK C-terminal tail, PLCγ2 forms a complex with the TRAF6 and Gab2 adapter proteins in a stimulation-dependent manner[20]. An HCR deletion mutant of CD40/RANK chimeric receptor does not alter NF-κB and MAPK activation in the triggering phase but abolishes Ca^{2+} oscillation, indicating that HCR-mediated signaling is indispensable for continuous PLCγ2 activation.

Both HCR-dependent RANK signaling and ITAM signaling lead to long-term induction of PLCγ2 catalytic activity. PLCγ2 increases intracellular Ca^{2+} levels by producing inositol-1,4,5-trisphosphate (IP_3). Since Ca^{2+} oscillation during osteoclast differentiation is abolished in IP_3 receptor (IP_3R) knockout cells, Ca^{2+} release from endoplasmic reticulum (ER) *via* IP_3Rs is required to generate Ca^{2+} oscillation[48]. The PLCγ family consists of PLCγ1, which is widely distributed, and PLCγ2, which is primarily limited to hematopoietic cells[49]. PLCγ2 null mice exhibit an osteopetrotic phenotype[25], indicating that PLCγ2, independent of PLCγ1, is required for osteoclastogenesis.

Intracellular Ca^{2+} levels (approximately 100 nmol/L) are 20 000-fold lower than outside the cell (approximately 2 mmol/L)[50]. Ca^{2+} oscillation in osteoclasts is tightly controlled by the regulator of G-protein signaling 10 (RGS10)[51]. RGS10 is competitively bound by phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3) and Ca^{2+}/calmodulin, and intracellular Ca^{2+} concentration shifts the balance between RGS10-PIP_3 and RGS10-Ca^{2+}/calmodulin complexes[51] (Figure 3). PIP_3 is required for membrane localization and subsequent activation of PLCγ2. As the first peak formation of Ca^{2+} oscillation, PLCγ2 activation induces transient release of Ca^{2+} from the ER, elevating intracellular Ca^{2+} concentration (Figure 3, arrows 1 and 2). RGS10 forms a complex with the Ca^{2+}/calmodulin complex and increases levels of free PIP_3, further activating PLCγ2 until the intracellular Ca^{2+} level reaches its peak (Figure 3, arrow 1 and 3). Empty ER Ca^{2+} stores reload through sooth endoplasmic reticular Ca^{2+} ATPase, decreasing intracellular Ca^{2+}, increasing RGS10-PIP_3, and reducing PLCγ2 activity (Figure 3, arrows 4-6). A repeat of these processes may generate Ca^{2+} oscillation through oscillatory regulation of PLCγ2 activation[51]. RGS10 knockout mice exhibit severe osteopetrosis caused by a defect in osteoclasts *in vivo*, indicating that Ca^{2+} oscillation is a crucial mechanism underlying NFATc1 activation and amplification during osteoclast differentiation[51].

NFATc1 is also activated by an osteoblast-induced Ca^{2+} oscillation-independent pathway. When osteoclast precursors are co-cultured with osteoblasts, osteoblasts increase NFATc1 levels in osteoclast precursors, and promote osteoclast differentiation even in the presence of the calcineurin inhibitor FK506. Furthermore, wild-type osteoblasts induce differentiation of osteoclast precursors derived from IP_3R type 2 and type 3 double knockout mice without detectable RANKL-induced Ca^{2+} oscillation[48]. Indeed, Cot (cancer osaka thyroid) serine/threonine kinase, also known as tumor progression locus 2, is activated by cell-cell interaction of osteoclasts with osteoblasts and promotes Ca^{2+} oscillation/calcineurin-independent osteoclastogenesis[52]. Furthermore, Cot increases NFATc1 protein levels through phosphorylation-dependent protein stabilization thereby amplifying NFATc1 activity in the absence of Ca^{2+} oscillation. Cot likely phosphorylates residues that differ from those targeted by calcineurin-mediated dephosphorylation required for nuclear translocation. At present, the identity of osteoblast-derived molecules that activate Cot in osteoclasts is unknown, but Cot-mediated NFATc1 stabilization clearly contributes to osteoclastogenesis *in vivo*. Collectively, NFATc1 amplification is achieved by both upregulated expression and enhanced stability.

TARGETING PHASE

NFATc1 induction and amplification regulate mRNA levels of target genes driving osteoclast differentiation, fusion and function. While forced NFATc1 expression directs osteoclast differentiation, NFATc1-deficient embryonic stem cells fail to differentiate into osteoclasts following RANKL-stimulation[23,42].

In osteoclast differentiation, the immunoglobulin-like receptor OSCAR, but not TREM-2, is an NFATc1 target gene[53,54]. During differentiation, positive regulators of NFATc1 are enhanced while negative regulators are suppressed. The transcriptional repressor B-lymphocyte-induced maturation protein-1 (Blimp1) is induced by RANKL-stimulation and down-regulates three negative regulators: the v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B; interferon regulatory factor-8; and B cell lymphoma 6. All of these proteins repress *Nfatc1* transcription[55-58] (Figure 4). Evidence showing that Blimp1 is a direct NFATc1 target[55] suggests that NFATc1 maintains expression of itself *via* NFATc1/Blimp1 signaling.

NFATc1 target genes encode proteins crucial for osteoclast cell-cell fusion such as a dendritic cell-specific transmembrane protein (DC-STAMP), vacuolar proton pump subunit Atp6v0d2 and the c-Src substrate Tks5 (tyrosine kinase substrate with five SH3 domains)[59-62]. Tks5 appears to be required not only for fusion but for circumferential podosome (actin ring or sealing ring) formation. Following Tks5 knockdown in osteoclasts, multinucleation is abolished although mononuclear osteoclasts still express and amplify NFATc1 in the presence of M-CSF and RANKL[62]. Furthermore, defects of c-Src knockout osteoclasts can be partially rescued by expression of a form of Tks5 carrying glutamate substitutions that mimic constitutive phosphorylation at c-Src phosphorylation target tyrosines[62]. The c-Src-Tks5 axis illustrates an additional signaling pathway induced by

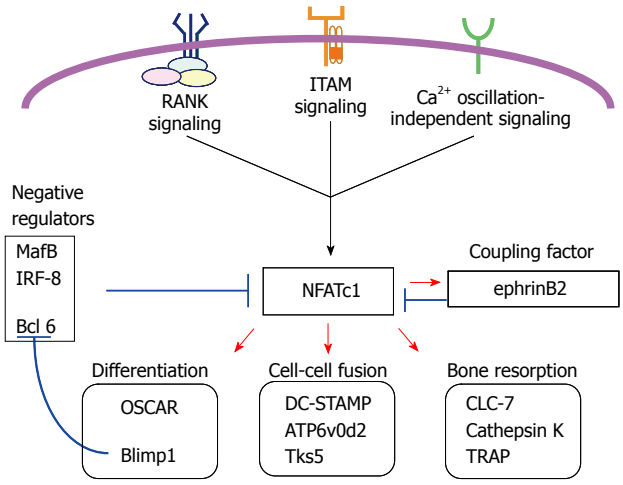

Figure 4 Targeting phase. Negative regulation of nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) is indicated by blue lines and activation of its targets, shown in boxes, is indicated by red arrows. RANK: Receptor activator of nuclear factor-κB; ITAM: Immunoreceptor tyrosine-based activation motif; OSCAR: Osteoclast-associated receptor; Blimp1: B-lymphocyte-induced maturation protein-1; DC-STAMP: Dendritic cell-specific transmembrane protein; TRAP: Tartrate-resistant acid phosphatase; IRF-8: Interferon regulatory factor-8; MafB: V-maf musculoaponeurotic fibrosarcoma oncogene family, protein B; Bcl6: B cell lymphoma 6; CLC: Chloride channel.

RANK signaling beyond NFATc1. In conjunction with ITAM-bearing proteins, c-Src also phosphorylates the tyrosine kinase Syk when integrin $\alpha_v\beta_3$ is activated by adhesion to bone matrix, in particular, vitronectin[63,64]. In these c-Src-Syk signaling components, integrin β_3 and c-Src are NFATc1 target gene products[65,66], suggesting that NFATc1 target genes include those critical for osteoclast-adhesion.

To resorb bone, osteoclasts secrete acid hydrogen chrolide and various hydrolases. Several NFATc1 target genes encode proteins required for acidification and proteolysis, such as the CLC-7 chloride channel (*Clc7*)[66], a late endosomal/lysosomal chloride channel localizing in ruffled borders, cathepsin K[67], which degrades collagens, and TRAP[23,39], which dephosphorylates the bone matrix phosphoproteins osteopontin and bone sialoprotein. Mice lacking *Clc7* or the V0-ATPase subunit a3 show severe osteopetrosis reminiscent of osteoclast-rich osteopetrosis in humans[68,69]. These mice show TRAP-positive osteoclasts with apparently normal NFATc1 amplification. Expression of the calcitonin receptor depends on NFATc1[23,39,53,70], and calcitonin receptor signaling inhibits both osteoclast formation and function independently of transcriptional regulation by RANK signaling[71].

Finally, NFATc1 induces transcription of ephrinB2[72]. Eph receptors and ephrin ligands are increasingly recognized as important in bone biology[73]. Reverse signaling into ephrinB2-expressing osteoclast lineage cells suppresses osteoclast differentiation by downregulating c-Fos and NFATc1, while forward signaling into receptor EphB4-expressing osteoblast lineage cells enhances osteoblastic differentiation and bone formation. Therefore, ephrinB2 is considered as a coupling factor inducible by RANK signaling[73].

In conclusion, RANK signaling appears to be a straightforward transcriptional cascade of "NF-κB/c-Fos induces NFATc1 induces target genes". Numerous signaling molecules including receptors, adaptors, kinases and lipases reinforce this cascade. Oscillation of intracellular Ca^{2+} levels drives the cascade, but a Ca^{2+} oscillation-independent mechanism also contributes to amplification of NFATc1 activity. RANK signaling stimulates the cell-cell fusion machinery (specifically, DC-STAMP and Tks5) and activates proteins located on or secreted from the osteoclast ruffled border (CLC-7 and cathepsin K, respectively). Numerous questions remain unanswered about RANK signaling, such as whether and how RANK signaling is connected to microRNA control[74-77] or to long noncoding RNAs (such as competing endogenous RNAs, or ceRNAs)[78]. Components of the RANK signaling pathway will continue to provide not only topics for investigation but novel therapeutic targets to prevent osteoporosis and other bone loss diseases.

ACKNOWLEDGMENTS

We thank Tsukasa Oikawa, Ichiro Takada and Elise Lamar for critical reading of the manuscript.

REFERENCES

1 **Teitelbaum SL**. Bone resorption by osteoclasts. *Science* 2000; **289**: 1504-1508
2 **Karsenty G**, Wagner EF. Reaching a genetic and molecular understanding of skeletal development. *Dev Cell* 2002; **2**: 389-406
3 **Asagiri M**, Takayanagi H. The molecular understanding of osteoclast differentiation. *Bone* 2007; **40**: 251-264
4 **Arai F**, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, Miyata T, Anderson DM, Suda T. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. *J Exp Med* 1999; **190**: 1741-1754
5 **Dougall WC**, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. RANK is essential for osteoclast and lymph node development. *Genes Dev* 1999; **13**: 2412-2424
6 **Kong YY**, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* 1999; **402**: 304-309
7 **Sobacchi C**, Frattini A, Guerrini MM, Abinun M, Pangrazio A, Susani L, Bredius R, Mancini G, Cant A, Bishop N, Grabowski P, Del Fattore A, Messina C, Errigo G, Coxon FP, Scott DI, Teti A, Rogers MJ, Vezzoni P, Villa A, Helfrich MH. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat Genet* 2007; **39**: 960-962
8 **Guerrini MM**, Sobacchi C, Cassani B, Abinun M, Kilic SS, Pangrazio A, Moratto D, Mazzolari E, Clayton-Smith J, Orchard P, Coxon FP, Helfrich MH, Crockett JC, Mellis D, Vellodi A, Tezcan I, Notarangelo LD, Rogers MJ, Vezzoni

P, Villa A, Frattini A. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am J Hum Genet* 2008; **83**: 64-76

9 **Simonet WS**, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309-319

10 **Yasuda H**, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 1998; **95**: 3597-3602

11 **Anderson DM**, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 1997; **390**: 175-179

12 **Nakashima T**, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, Bonewald LF, Kodama T, Wutz A, Wagner EF, Penninger JM, Takayanagi H. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat Med* 2011; **17**: 1231-1234

13 **Xiong J**, Onal M, Jilka RL, Weinstein RS, Manolagas SC, O'Brien CA. Matrix-embedded cells control osteoclast formation. *Nat Med* 2011; **17**: 1235-1241

14 **Douni E**, Rinotas V, Makrinou E, Zwerina J, Penninger JM, Eliopoulos E, Schett G, Kollias G. A RANKL G278R mutation causing osteopetrosis identifies a functional amino acid essential for trimer assembly in RANKL and TNF. *Hum Mol Genet* 2012; **21**: 784-798

15 **Liu C**, Walter TS, Huang P, Zhang S, Zhu X, Wu Y, Wedderburn LR, Tang P, Owens RJ, Stuart DI, Ren J, Gao B. Structural and functional insights of RANKL-RANK interaction and signaling. *J Immunol* 2010; **184**: 6910-6919

16 **Wong BR**, Josien R, Lee SY, Vologodskaia M, Steinman RM, Choi Y. The TRAF family of signal transducers mediates NF-kappaB activation by the TRANCE receptor. *J Biol Chem* 1998; **273**: 28355-28359

17 **Galibert L**, Tometsko ME, Anderson DM, Cosman D, Dougall WC. The involvement of multiple tumor necrosis factor receptor (TNFR)-associated factors in the signaling mechanisms of receptor activator of NF-kappaB, a member of the TNFR superfamily. *J Biol Chem* 1998; **273**: 34120-34127

18 **Wong BR**, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, Choi Y. TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. *Mol Cell* 1999; **4**: 1041-1049

19 **Gohda J**, Akiyama T, Koga T, Takayanagi H, Tanaka S, Inoue J. RANK-mediated amplification of TRAF6 signaling leads to NFATc1 induction during osteoclastogenesis. *EMBO J* 2005; **24**: 790-799

20 **Taguchi Y**, Gohda J, Koga T, Takayanagi H, Inoue J. A unique domain in RANK is required for Gab2 and PLC-gamma2 binding to establish osteoclastogenic signals. *Genes Cells* 2009; **14**: 1331-1345

21 **Takayanagi H**. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol* 2007; **7**: 292-304

22 **Kobayashi N**, Kadono Y, Naito A, Matsumoto K, Yamamoto T, Tanaka S, Inoue J. Segregation of TRAF6-mediated signaling pathways clarifies its role in osteoclastogenesis. *EMBO J* 2001; **20**: 1271-1280

23 **Takayanagi H**, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev Cell* 2002; **3**: 889-901

24 **Lamothe B**, Besse A, Campos AD, Webster WK, Wu H, Darnay BG. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of I kappa B kinase activation. *J Biol Chem* 2007; **282**: 4102-4112

25 **Mao D**, Epple H, Uthgenannt B, Novack DV, Faccio R. PLC-gamma2 regulates osteoclastogenesis via its interaction with ITAM proteins and GAB2. *J Clin Invest* 2006; **116**: 2869-2879

26 **Wada T**, Nakashima T, Oliveira-dos-Santos AJ, Gasser J, Hara H, Schett G, Penninger JM. The molecular scaffold Gab2 is a crucial component of RANK signaling and osteoclastogenesis. *Nat Med* 2005; **11**: 394-399

27 **Lee NK**, Choi YG, Baik JY, Han SY, Jeong DW, Bae YS, Kim N, Lee SY. A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* 2005; **106**: 852-859

28 **Wang ZQ**, Ovitt C, Grigoriadis AE, Möhle-Steinlein U, Rüther U, Wagner EF. Bone and haematopoietic defects in mice lacking c-fos. *Nature* 1992; **360**: 741-745

29 **Grigoriadis AE**, Wang ZQ, Cecchini MG, Hofstetter W, Felix R, Fleisch HA, Wagner EF. c-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. *Science* 1994; **266**: 443-448

30 **Franzoso G**, Carlson L, Xing L, Poljak L, Shores EW, Brown KD, Leonardi A, Tran T, Boyce BF, Siebenlist U. Requirement for NF-kappaB in osteoclast and B-cell development. *Genes Dev* 1997; **11**: 3482-3496

31 **Iotsova V**, Caamaño J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. *Nat Med* 1997; **3**: 1285-1289

32 **Matsuo K**, Owens JM, Tonko M, Elliott C, Chambers TJ, Wagner EF. Fosl1 is a transcriptional target of c-Fos during osteoclast differentiation. *Nat Genet* 2000; **24**: 184-187

33 **Ikeda F**, Nishimura R, Matsubara T, Tanaka S, Inoue J, Reddy SV, Hata K, Yamashita K, Hiraga T, Watanabe T, Kukita T, Yoshioka K, Rao A, Yoneda T. Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-regulated osteoclast differentiation. *J Clin Invest* 2004; **114**: 475-484

34 **Shaw JP**, Utz PJ, Durand DB, Toole JJ, Emmel EA, Crabtree GR. Identification of a putative regulator of early T cell activation genes. *Science* 1988; **241**: 202-205

35 **Hogan PG**, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* 2003; **17**: 2205-2232

36 **Matsuo K,** Tonko M, Wagner EF. Why do c-Fos deficient mice lack osteoclasts? *J Bone Miner Res* 1996; **11** Suppl 1: M377

37 **Ishida N**, Hayashi K, Hoshijima M, Ogawa T, Koga S, Miyatake Y, Kumegawa M, Kimura T, Takeya T. Large scale gene expression analysis of osteoclastogenesis in vitro and elucidation of NFAT2 as a key regulator. *J Biol Chem* 2002; **277**: 41147-41156

38 **Takayanagi H**, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, Yokochi T, Oda H, Nakamura K, Ida N, Wagner EF, Taniguchi T. RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta. *Nature* 2002; **416**: 744-749

39 **Matsuo K**, Galson DL, Zhao C, Peng L, Laplace C, Wang KZ, Bachler MA, Amano H, Aburatani H, Ishikawa H, Wagner EF. Nuclear factor of activated T-cells (NFAT) rescues osteoclastogenesis in precursors lacking c-Fos. *J Biol Chem* 2004; **279**: 26475-26480

40 **Ray N**, Kuwahara M, Takada Y, Maruyama K, Kawaguchi T, Tsubone H, Ishikawa H, Matsuo K. c-Fos suppresses

systemic inflammatory response to endotoxin. *Int Immunol* 2006; **18**: 671-677
41 **Yamashita T**, Yao Z, Li F, Zhang Q, Badell IR, Schwarz EM, Takeshita S, Wagner EF, Noda M, Matsuo K, Xing L, Boyce BF. NF-kappaB p50 and p52 regulate receptor activator of NF-kappaB ligand (RANKL) and tumor necrosis factor-induced osteoclast precursor differentiation by activating c-Fos and NFATc1. *J Biol Chem* 2007; **282**: 18245-18253
42 **Asagiri M**, Sato K, Usami T, Ochi S, Nishina H, Yoshida H, Morita I, Wagner EF, Mak TW, Serfling E, Takayanagi H. Autoamplification of NFATc1 expression determines its essential role in bone homeostasis. *J Exp Med* 2005; **202**: 1261-1269
43 **Kim N**, Takami M, Rho J, Josien R, Choi Y. A novel member of the leukocyte receptor complex regulates osteoclast differentiation. *J Exp Med* 2002; **195**: 201-209
44 **Cella M**, Buonsanti C, Strader C, Kondo T, Salmaggi A, Colonna M. Impaired differentiation of osteoclasts in TREM-2-deficient individuals. *J Exp Med* 2003; **198**: 645-651
45 **Koga T**, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature* 2004; **428**: 758-763
46 **Shinohara M**, Koga T, Okamoto K, Sakaguchi S, Arai K, Yasuda H, Takai T, Kodama T, Morio T, Geha RS, Kitamura D, Kurosaki T, Ellmeier W, Takayanagi H. Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell* 2008; **132**: 794-806
47 **Sato K**, Suematsu A, Nakashima T, Takemoto-Kimura S, Aoki K, Morishita Y, Asahara H, Ohya K, Yamaguchi A, Takai T, Kodama T, Chatila TA, Bito H, Takayanagi H. Regulation of osteoclast differentiation and function by the CaMK-CREB pathway. *Nat Med* 2006; **12**: 1410-1416
48 **Kuroda Y**, Hisatsune C, Nakamura T, Matsuo K, Mikoshiba K. Osteoblasts induce Ca2+ oscillation-independent NFATc1 activation during osteoclastogenesis. *Proc Natl Acad Sci USA* 2008; **105**: 8643-8648
49 **Wilde JI**, Watson SP. Regulation of phospholipase C gamma isoforms in haematopoietic cells: why one, not the other? *Cell Signal* 2001; **13**: 691-701
50 **Clapham DE**. Calcium signaling. *Cell* 2007; **131**: 1047-1058
51 **Yang S**, Li YP. RGS10-null mutation impairs osteoclast differentiation resulting from the loss of [Ca2+]i oscillation regulation. *Genes Dev* 2007; **21**: 1803-1816
52 **Kuroda Y**, Hisatsune C, Mizutani A, Ogawa N, Matsuo K, Mikoshiba K. Cot kinase promotes Ca2+ oscillation/calcineurin-independent osteoclastogenesis by stabilizing NFATc1 protein. *Mol Cell Biol* 2012; **32**: 2954-2963
53 **Kim Y**, Sato K, Asagiri M, Morita I, Soma K, Takayanagi H. Contribution of nuclear factor of activated T cells c1 to the transcriptional control of immunoreceptor osteoclast-associated receptor but not triggering receptor expressed by myeloid cells-2 during osteoclastogenesis. *J Biol Chem* 2005; **280**: 32905-32913
54 **Kim K**, Kim JH, Lee J, Jin HM, Lee SH, Fisher DE, Kook H, Kim KK, Choi Y, Kim N. Nuclear factor of activated T cells c1 induces osteoclast-associated receptor gene expression during tumor necrosis factor-related activation-induced cytokine-mediated osteoclastogenesis. *J Biol Chem* 2005; **280**: 35209-35216
55 **Nishikawa K**, Nakashima T, Hayashi M, Fukunaga T, Kato S, Kodama T, Takahashi S, Calame K, Takayanagi H. Blimp1-mediated repression of negative regulators is required for osteoclast differentiation. *Proc Natl Acad Sci USA* 2010; **107**: 3117-3122
56 **Kim K**, Kim JH, Lee J, Jin HM, Kook H, Kim KK, Lee SY, Kim N. MafB negatively regulates RANKL-mediated osteoclast differentiation. *Blood* 2007; **109**: 3253-3259
57 **Zhao B**, Takami M, Yamada A, Wang X, Koga T, Hu X, Tamura T, Ozato K, Choi Y, Ivashkiv LB, Takayanagi H, Kamijo R. Interferon regulatory factor-8 regulates bone metabolism by suppressing osteoclastogenesis. *Nat Med* 2009; **15**: 1066-1071
58 **Miyauchi Y**, Ninomiya K, Miyamoto H, Sakamoto A, Iwasaki R, Hoshi H, Miyamoto K, Hao W, Yoshida S, Morioka H, Chiba K, Kato S, Tokuhisa T, Saitou M, Toyama Y, Suda T, Miyamoto T. The Blimp1-Bcl6 axis is critical to regulate osteoclast differentiation and bone homeostasis. *J Exp Med* 2010; **207**: 751-762
59 **Yagi M**, Ninomiya K, Fujita N, Suzuki T, Iwasaki R, Morita K, Hosogane N, Matsuo K, Toyama Y, Suda T, Miyamoto T. Induction of DC-STAMP by alternative activation and downstream signaling mechanisms. *J Bone Miner Res* 2007; **22**: 992-1001
60 **Lee SH**, Rho J, Jeong D, Sul JY, Kim T, Kim N, Kang JS, Miyamoto T, Suda T, Lee SK, Pignolo RJ, Koczon-Jaremko B, Lorenzo J, Choi Y. v-ATPase V0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. *Nat Med* 2006; **12**: 1403-1409
61 **Kim K**, Lee SH, Ha Kim J, Choi Y, Kim N. NFATc1 induces osteoclast fusion via up-regulation of Atp6v0d2 and the dendritic cell-specific transmembrane protein (DC-STAMP). *Mol Endocrinol* 2008; **22**: 176-185
62 **Oikawa T**, Oyama M, Kozuka-Hata H, Uehara S, Udagawa N, Saya H, Matsuo K. Tks5-dependent formation of circumferential podosomes/invadopodia mediates cell-cell fusion. *J Cell Biol* 2012; **197**: 553-568
63 **Zou W**, Kitaura H, Reeve J, Long F, Tybulewicz VL, Shattil SJ, Ginsberg MH, Ross FP, Teitelbaum SL. Syk, c-Src, the alphavbeta3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption. *J Cell Biol* 2007; **176**: 877-888
64 **Chambers TJ**, Fuller K. How are osteoclasts induced to resorb bone? *Ann N Y Acad Sci* 2011; **1240**: 1-6
65 **Crotti TN**, Flannery M, Walsh NC, Fleming JD, Goldring SR, McHugh KP. NFATc1 regulation of the human beta3 integrin promoter in osteoclast differentiation. *Gene* 2006; **372**: 92-102
66 **Song I**, Kim JH, Kim K, Jin HM, Youn BU, Kim N. Regulatory mechanism of NFATc1 in RANKL-induced osteoclast activation. *FEBS Lett* 2009; **583**: 2435-2440
67 **Matsumoto M**, Kogawa M, Wada S, Takayanagi H, Tsujimoto M, Katayama S, Hisatake K, Nogi Y. Essential role of p38 mitogen-activated protein kinase in cathepsin K gene expression during osteoclastogenesis through association of NFATc1 and PU.1. *J Biol Chem* 2004; **279**: 45969-45979
68 **Frattini A**, Orchard PJ, Sobacchi C, Giliani S, Abinun M, Mattsson JP, Keeling DJ, Andersson AK, Wallbrandt P, Zecca L, Notarangelo LD, Vezzoni P, Villa A. Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis. *Nat Genet* 2000; **25**: 343-346
69 **Scimeca JC**, Franchi A, Trojani C, Parrinello H, Grosgeorge J, Robert C, Jaillon O, Poirier C, Gaudray P, Carle GF. The gene encoding the mouse homologue of the human osteoclast-specific 116-kDa V-ATPase subunit bears a deletion in osteosclerotic (oc/oc) mutants. *Bone* 2000; **26**: 207-213
70 **Anusaksathien O**, Laplace C, Li X, Ren Y, Peng L, Goldring SR, Galson DL. Tissue-specific and ubiquitous promoters direct the expression of alternatively spliced transcripts from the calcitonin receptor gene. *J Biol Chem* 2001; **276**: 22663-22674
71 **Granholm S**, Lundberg P, Lerner UH. Calcitonin inhibits osteoclast formation in mouse haematopoetic cells independently of transcriptional regulation by receptor activator of NF-{kappa}B and c-Fms. *J Endocrinol* 2007; **195**: 415-427
72 **Zhao C**, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishi-

waki T, Suda T, Matsuo K. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab* 2006; **4**: 111-121

73 **Matsuo K**, Otaki N. Bone cell interactions through Eph/ephrin: bone modeling, remodeling and associated diseases. *Cell Adh Migr* 2012; **6**: 148-156

74 **Lian JB**, Stein GS, van Wijnen AJ, Stein JL, Hassan MQ, Gaur T, Zhang Y. MicroRNA control of bone formation and homeostasis. *Nat Rev Endocrinol* 2012; **8**: 212-227

75 **Sugatani T**, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. *J Biol Chem* 2009; **284**: 4667-4678

76 **Mizoguchi F**, Izu Y, Hayata T, Hemmi H, Nakashima K, Nakamura T, Kato S, Miyasaka N, Ezura Y, Noda M. Osteoclast-specific Dicer gene deficiency suppresses osteoclastic bone resorption. *J Cell Biochem* 2010; **109**: 866-875

77 **Sugatani T**, Vacher J, Hruska KA. A microRNA expression signature of osteoclastogenesis. *Blood* 2011; **117**: 3648-3657

78 **Cesana M**, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 2011; **147**: 358-369

S- Editor Huang XZ **L- Editor** A **E- Editor** Xiong L

World Journal of Orthopedics

Online Submissions: http://www.wjgnet.com/esps/
wjo@wjgnet.com
doi:10.5312/wjo.v3.i11.175

World J Orthop 2012 November 18; 3(11): 175-181
ISSN 2218-5836 (online)

TOPIC HIGHLIGHT

Hisataka Yasuda, PhD, *Series Editor*

New roles of osteoblasts involved in osteoclast differentiation

Teruhito Yamashita, Naoyuki Takahashi, Nobuyuki Udagawa

Teruhito Yamashita, Naoyuki Takahashi, Institute for Oral Science, Matsumoto Dental University, 1780 Gobara, Hirooka, Shiojiri, Nagano 399-0781, Japan
Nobuyuki Udagawa, Department of Biochemistry, Matsumoto Dental University, 1780 Gobara, Hirooka, Shiojiri and Nagano 399-0781, Japan
Author contributions: Yamashita T, Takahashi N and Udagawa N wrote the manuscript; Yamashita T and Takahashi N drew the figures and revised the manuscript.
Correspondence to: Teruhito Yamashita, PhD, Institute for Oral Science, Matsumoto Dental University, 1780 Gobara, Hirooka, Shiojiri, Nagano 399-0781, Japan. teru@po.mdu.ac.jp
Telephone: +81-263-512235 Fax:+81-51-2632223
Received: June 28, 2012 Revised: October 21, 2012
Accepted: November 1, 2012
Published online: November 18, 2012

Abstract

Bone-resorbing osteoclasts are formed from a monocyte/macrophage lineage under the strict control of bone-forming osteoblasts. So far, macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor-κB ligand (RANKL), and osteoprotegerin (OPG) produced by osteoblasts play major roles in the regulation of osteoclast differentiation. Recent studies have shown that osteoblasts regulate osteoclastogenesis through several mechanisms independent of M-CSF, RANKL, and OPG production. Identification of osteoclast-committed precursors *in vivo* demonstrated that osteoblasts are involved in the distribution of osteoclast precursors in bone. Interleukin 34 (IL-34), a novel ligand for c-Fms, plays a pivotal role in maintaining the splenic reservoir of osteoclast-committed precursors in M-CSF deficient mice. IL-34 is also able to act as a substitute for osteoblast-producing M-CSF in osteoclastogenesis. Wnt5a, produced by osteoblasts, enhances osteoclast differentiation by upregulating RANK expression through activation of the non-canonical Wnt pathway. Semaphorin 3A produced by osteoblasts inhibits RANKL-induced osteoclast differentiation through the suppression of immunoreceptor tyrosine-based activation motif signals. Thus, recent findings show that osteoclast differentiation is tightly regulated by osteoblasts through several different mechanisms. These newly identified molecules are expected to be promising targets of therapeutic agents in bone-related diseases.

Key words: Osteoclast; Osteoblast; Receptor activator of nuclear factor-κB ligand; Wnt5a; Semaphorin 3A; Interleukin 34

Peer reviewer: Wing-Hoi Cheung, Research Associate Professor, Department of Orthopaedics and Traumatology, the Chinese University of Hong Kong, Shatin, NT, Hong Kong, China

Yamashita T, Takahashi N, Udagawa N. New roles of osteoblasts involved in osteoclast differentiation. *World J Orthop* 2012; 3(11): 175-181 Available from: URL: http://www.wjgnet.com/2218-5836/full/v3/i11/175.htm DOI: http://dx.doi.org/10.5312/wjo.v3.i11.175

INTRODUCTION

Bone is continuously destroyed and reformed in vertebrates to maintain bone volume and calcium homeostasis. Osteoblasts and osteoclasts are specialized cells responsible for bone formation and bone resorption, respectively. Osteoblasts produce bone matrix proteins and take charge of mineralization of the tissue. Osteoclasts are multinucleated cells responsible for bone resorption. It has been well established that osteoclasts are formed from monocyte/macrophage lineage precursors under the strict regulation of osteoblasts, osteocytes, and bone marrow stromal cells (referred to as "osteoblasts" in this review).

Osteoblasts express two cytokines essential for osteoclast differentiation, macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-

κB ligand (RANKL)[1,2] (Figure 1). Experiments using an osteopetrotic op/op mouse model have established that the osteoblast product M-CSF is crucial for osteoclast differentiation[3]. The *M-CSF* gene in op/op mice cannot code functionally active M-CSF protein[4]. Administration of recombinant M-CSF to op/op mice restores impaired bone resorption[5]. RANKL is a member of the tumor necrosis factor (TNF) family (TNF superfamily member 11)[6,7]. The expression of RANKL by osteoblasts is inducible. Osteoblasts express RANKL as a membrane-associated form in response to stimuli of bone resorption-stimulating factors such as 1α,25-dihydroxyvitamin D$_3$ [1α,25(OH)$_2$D$_3$], parathyroid hormone, prostaglandin E$_2$, and interleukin (IL)-11[2]. Osteoclast precursors express c-Fms (M-CSF receptor) and RANK (RANKL receptor) and differentiate into osteoclasts in the presence of M-CSF and RANKL. RANKL stimulation strongly induced the expression of nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), a pivotal transcription factor for osteoclast development, in osteoclast precursors[8]. Osteoblasts also produce osteoprotegerin (OPG, TNFRSF11B), a soluble decoy receptor for RANKL[9,10]. OPG inhibits osteoclastogenesis by blocking the RANKL-RANK interaction[1,2]. Both RANKL-deficient mice[11] and RANK-deficient mice[12] develop severe osteopetrosis with no osteoclasts in bone. In contrast, OPG-deficient mice[13,14] exhibit severe trabecular and cortical bone porosity with enhanced osteoclastic bone resorption.

Discovery of the RANKL-RANK signal in osteoclastogenesis has clarified the cause of some bone diseases in humans. Loss-of-function mutations in the *OPG* gene cause Juvenile Paget's disease and idiopathic hyperphosphatasia[15-17]. Gain-of-function mutations in the *RANK* gene induce familial expansile osteolysis, familial Paget' s disease of bone, and expansile skeletal hyperphosphatasia[18]. Osteopetrosis due to few osteoclasts is caused by loss-of-function mutations in the *RANK* gene[19] and *RANKL* gene[20]. The phenotypes of these bone diseases in humans support the concept that *RANKL* expressed by osteoblasts stimulates osteoclast differentiation of precursors through the receptor RANK. Thus, the RANKL-RANK axis is the central pathway for osteoclastogenesis. Recent *in vivo* studies have also shown that osteoblasts regulate osteoclastogenesis through several mechanisms independent of M-CSF, RANKL and OPG production.

In this review article, we focus on the new roles of osteoblasts in osteoclast differentiation. First, osteoblasts are involved in the distribution of osteoclast precursors in bone. Second, osteoblast-producing M-CSF can be replaced by IL-34 in osteoclastogenesis. Third, osteoblasts produce Wnt5a, which enhances osteoclast differentiation through the upregulation of RANK expression. Lastly, osteoblast-producing semaphorins regulate osteoclast formation in the presence of RANKL signaling. Overall, these findings remind us of the importance of osteoblasts in osteoclast development.

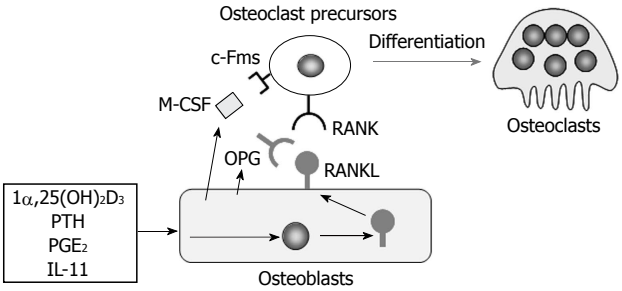

Figure 1 Regulation of osteoclast differentiation by osteoblasts through macrophage colony-stimulating factor, receptor activator of nuclear factor-κB ligand, and osteoprotegerin production. Osteoblasts express two cytokines essential for osteoclast differentiation, macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-κB ligand (RANKL). Osteoblasts constitutively express M-CSF. On the other hand, osteoblasts express RANKL as a membrane-associated form in response to bone resorption-stimulating factors such as 1α,25-dihydroxyvitamin D3 [1α,25(OH)$_2$D$_3$], parathyroid hormone (PTH), prostaglandin E$_2$ (PGE$_2$), and interleukin 11 (IL-11). Osteoclast precursors express c-Fms (M-CSF receptor) and RANK (RANKL receptor) and differentiate into osteoclasts in the presence of M-CSF and RANKL. Osteoblasts also produce osteoprotegerin (OPG), which inhibits osteoclastogenesis by blocking the RANKL-RANK interaction.

CHARACTERISTICS OF OSTEOCLAST PRECURSORS *IN VIVO*

Recent attempts to identify osteoclast precursors *in vivo* established a new role of osteoblasts in osteoclast differentiation. Mizoguchi *et al*[21] showed that cells expressing both RANK and c-Fms detected near osteoblasts in bone directly differentiated into osteoclasts without cell cycle progression (Figure 2). To clarify the relationship between differentiation and proliferation of osteoclast precursors, BrdU and M-CSF were simultaneously administrated to op/op mice. M-CSF administration induced many osteoclasts in bone in op/op mice. Most of the nuclei of osteoclasts induced by M-CSF were BrdU negative. Similarly, when BrdU and RANKL were administrated to RANKL-deficient mice, osteoclasts were also induced in bone. Most nuclei of RANKL-induced osteoclasts were BrdU negative. These results suggest that both M-CSF and RANKL induce the differentiation of osteoclast precursors into osteoclasts without cell cycle progression. In these experiments, M-CSF and RANKL were intraperitoneally injected into op/op mice and RANKL-deficient mice, respectively. Nevertheless, osteoclasts were observed only on the surface of calcified bone, not in the surrounding soft tissues. These results also suggest that neither RANKL nor M-CSF expressed by osteoblasts is involved in the determination of the correct site for osteoclast formation. Using immunohistochemistry, RANK and c-Fms double-positive cells as osteoclast precursors were detected along the bone surface in RANKL-deficient mice. RANK and c-Fms double-positive cells were always observed near osteoblasts, did not express Ki67, a marker of cell proliferation, and possessed a relatively long life span. Therefore, RANK and c-Fms double-positive cells were named "cell

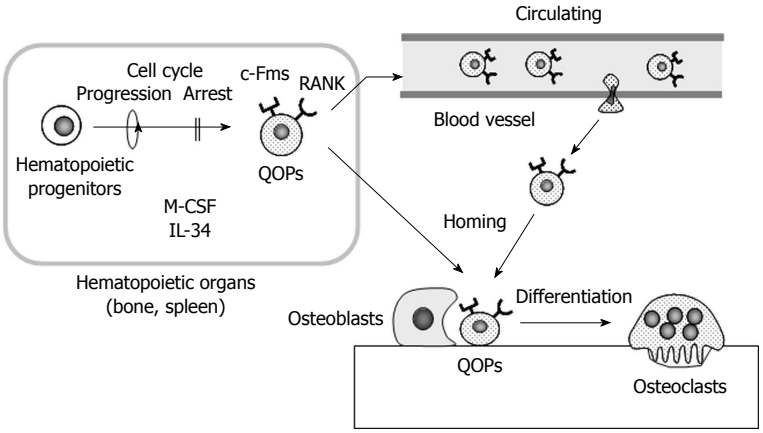

Figure 2 ***In vivo*** **dynamics of osteoclast precursors.** Cells expressing both receptor activator of nuclear factor-κB (RANK) and c-Fms are cell cycle-arrested quiescent osteoclast precursors (QOPs) *in vivo*. QOPs are detected in hematopoietic organs such as the spleen and bone. macrophage colony-stimulating factor (M-CSF) and/or interleukin 34 (IL-34) appear to be involved in the differentiation of hematopoietic progenitor cells into QOPs. Some QOPs circulate to find bone. Osteoblasts play a role in the homing of QOPs to bone. QOPs in bone differentiate into osteoclasts without cell cycle progression in response to M-CSF/IL-34 and RANK ligand.

cycle-arrested quiescent osteoclast progenitors (QOPs)" (Figure 2). QOPs were also isolated from bone marrow and peripheral blood[22]. Bone marrow-derived QOPs failed to express macrophage-associated markers such as F4/80 and CD11b. Bone marrow-derived QOPs showed no phagocytic activity and did not proliferate in response to M-CSF. They differentiated into osteoclasts, but not into dendritic cells. Therefore, it has been concluded that QOPs are committed osteoclast precursors[22].

ROLE OF IL-34 IN OSTEOCLASTOGENESIS

Recently, Lin *et al*[23] discovered IL-34 as a new ligand for c-Fms. The amino acid sequence of IL-34 was quite different from that of M-CSF, but IL-34 promoted macrophage colony formation similar to M-CSF. Chihara *et al*[24] reported that M-CSF and IL-34 used different signaling to induce the expression of several chemokines and suggested that they differentially regulated macrophage function. However, IL-34 as well as M-CSF, in combination with RANKL, induced osteoclast formation in mouse and human cell culture systems[25]. IL-34 was specifically expressed in splenic tissues, predominantly in the red pulp region. Recently, Nakamichi *et al*[26] showed that RANK and c-Fms double-positive QOPs did not exist in bone, but existed in the spleen of op/op mice (Figure 2). IL-34 was highly expressed in vascular endothelial cells in the spleen. Vascular endothelial cells in bone also expressed IL-34, but its expression level was much lower than that in the spleen, suggesting a role of IL-34 in the splenic maintenance of QOPs. Indeed, removal of the spleen (splenectomy) completely blocked M-CSF-induced osteoclast formation in op/op mice. Osteoclasts appeared in aged op/op mice with up-regulation of IL-34 expression in the spleen and bone. Splenectomy also blocked the age-associated appearance of osteoclasts[26]. These results suggest that IL-34 plays a pivotal role in maintaining the splenic reservoir of QOPs, which are transferred to bone in response to M-CSF administration in op/op mice (Figure 2). Recently, sphingosine-1-phosphate, a lipid mediator regulating immune cell trafficking, was shown to regulate the migration of osteoclast precursors[22,27]. Osteoblasts appear to help homing of QOPs to bone. Thus, osteoblasts determine the distribution of QOPs, which decide the correct sites of osteoclast formation.

WNT5A-RECEPTOR TYROSINE KINASE-LIKE ORPHAN RECEPTOR 2 SIGNALING AND OSTEOCLASTOGENESIS

Immunohistochemical analysis revealed that RANK expression in osteoclast precursors was much stronger than that in bone marrow and the spleen[21,28]. Recently, Maeda *et al*[29] reported that Wnt5a produced by osteoblasts promoted RANK expression in osteoclast precursors (Figure 3).

Wnt binds to two distinct receptor complexes: a complex of Frizzled and low density lipoprotein receptor-related protein 5/6 (LRP5/6) and another complex of Frizzled and receptor tyrosine kinase-like orphan receptors (Rors)[30]. The binding of Wnts to these Wnt receptors activates two classes of signaling pathways: a β-catenin-dependent (canonical) pathway and β-catenin-independent (non-canonical) pathway. The importance of the canonical pathway in bone metabolism has been emphasized by the identification of a link between bone mass and mutations in the *LRP5* gene. Loss-of-function mutations in *LRP5* reduced the number of osteoblasts and caused osteoporosis[31]. Glass *et al*[32] developed mice expressing a stabilized form of β-catenin in osteoblasts (β-catenin mutant mice) and reported that β-catenin mutant mice developed severe osteopetrosis due to the up-regulation of OPG expression. Thus, Wnt/β-catenin signaling is crucial in osteoblastogenesis and osteoclastogenesis. However, the role of the non-canonical Wnt pathway in bone resorption remains largely unknown.

Maeda *et al*[29] showed that Wnt5a-receptor tyrosine kinase-like orphan receptor 2 (Ror2) signaling between osteoblasts and osteoclast precursors enhanced osteoclastogenesis. Ror2-deficient mice exhibited impaired osteoclastogenesis. A deficiency in Wnt5a, a ligand of Ror2, caused a similar defect in mice. Osteoblasts expressed Wnt5a, while osteoclast precursors expressed Ror2, a co-receptor of Wnt5a. Wnt5a enhanced RANK expression in osteoclast precursors through co-receptor Ror2 signaling. RANK promoter-driven luciferase activi-

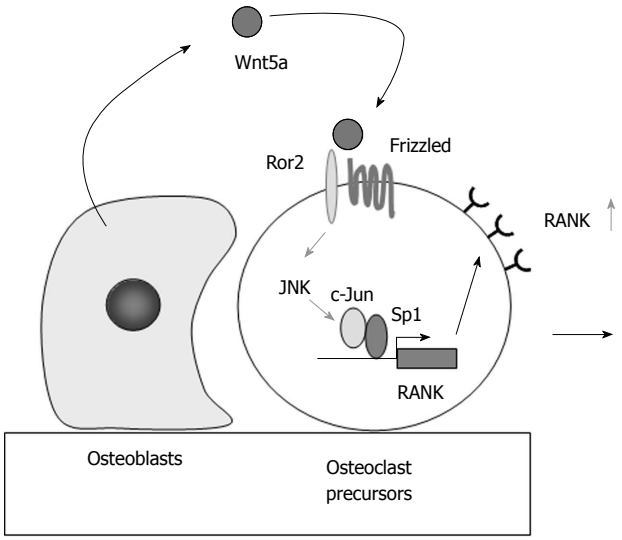

Figure 3 Role of Wnt5a-receptor tyrosine kinase-like orphan receptor 2 signaling in osteoclast precursors. Receptor activator of nuclear factor-κB (RANK) expression in osteoclast precursors is much stronger than that in bone marrow and the spleen. Osteoblasts express Wnt5a, while osteoclast precursors express receptor tyrosine kinase-like orphan receptor 2 (Ror2), a co-receptor of Wnt5a. Wnt5a produced by osteoblasts enhances RANK expression in osteoclast precursors through Ror2. Wnt5a up-regulates RANK expression through the recruitment of c-Jun to Sp1 sites of the RANK promoter. The up-regulation of RANK expression in osteoclast precursors increases their sensitivity to RANK ligand. JNK: c-Jun N-terminal kinase.

ty was upregulated by Wnt5a-Ror2 signaling *via* the c-Jun N-terminal kinase pathway (Figure 3). Wnt5a-induced recruitment of c-Jun to Sp1 sites up-regulated RANK expression in osteoclast precursors[29].

As described above, QOPs detected on bone surfaces strongly express RANK. Wnt5a secreted by osteoblasts appears to be involved in the up-regulation of RANK expression in QOPs through Ror2. The up-regulation of RANK by Wnt5a-Ror2 signals in osteoclast precursors must lower the threshold for RANKL-induced osteoclastogenesis. Atkins *et al*[33] reported that human peripheral blood monocytes expressing high surface levels of RANK were capable of responding rapidly to RANKL stimulation. Under physiological conditions, the up-regulation of RANK expression in osteoclast precursors must be an important requirement for RANKL-induced osteoclastogenesis.

IMMUNORECEPTOR TYROSINE-BASED ACTIVATION MOTIF SIGNALS AND SEMAPHORIN 3A

Koga *et al*[34] showed for the first time that osteoclastogenesis induced by RANKL also requires co-stimulatory receptor signaling through adaptors containing immunoreceptor tyrosine-based activation motifs (ITAMs), such as Fc receptor common γ (FcRγ) and DNAX-activating protein of 12 kDa (DAP12). RANK and ITAM signaling cooperated to induce NFATc1, the master transcription factor for osteoclastogenesis. FcRγ and DAP12 are adaptor molecules that associate with immunoglobulin-like receptors such as osteoclast-associated receptor (OSCAR) and triggering receptor expressed on myeloid cells 2 (TREM-2). OSCAR uses FcRγ, while TREM-2 associates with DAP12. Koga *et al*[34] showed that deficiencies in both FcRγ and DAP12 caused osteopetrotic phenotypes in mice. Because this pathway is crucial for the robust induction of NFATc1 that leads to osteoclastogenesis, these signals are called co-stimulatory signals for RANK in osteoclastogenesis. Recently, Barrow *et al*[35] reported that OSCAR bound to a specific motif of collagen and stimulated osteoclastogenesis. These series of experiments have established a new research area called "osteoimmunology"[36].

Recently, Hayashi *et al*[37] reported that semaphorin 3A (Sema3A) produced by osteoblasts suppressed osteoclast differentiation (Figure 4). Sema3A, a secreted axon guidance molecule, is highly expressed by osteoblasts. Neurophilin-1 (Nrp1), the receptor of Sema3A, is expressed by osteoclast precursors. Nrp1 usually forms a receptor complex with Plexin-A1 in bone marrow macrophages of osteoclast precursors. Using Plexin-A1-deficient mice, Takegahara *et al*[38] previously showed that Plexin-A1 interacted with TREM-2 and DAP12 to form the receptor complex for Sema6D, a transmembrane semaphorin. Sema6D stimulated osteoclast differentiation through the receptor complex Pleixn-A1/TREM-2/DAP12 in osteoclast precursors through ITAM signaling (Figure 4). These findings suggest that the Sema3A-Nrp1 axis inhibits osteoclast differentiation by sequestering Plexin-A1 from TREM-2 so as to suppress ITAM signaling. RANK-mediated signals rapidly down-regulated Nrp1 expression in osteoclast precursors. In the absence of Nrp1, Plexin-A1 easily forms the receptor complex for Sema6D or Sema6C. Thus, Sema3A produced by osteoblasts inhibits osteoclast differentiation (Figure 4). Hayashi *et al*[37] also showed that Sema3A and Nrp1 binding stimulated osteoblast differentiation through the canonical Wnt/β-catenin pathway. Administration of Sema3A to mice increased bone volume and expedited bone regeneration through the suppression of bone resorption and enhancement of bone formation[37]. These results suggest that Sema3A is a new therapeutic agent in bone and joint diseases.

THERAPEUTIC TARGETS IN THE OSTEOBLAST-OSTEOCLAST INTERACTION

Secreted OPG acts as a decoy receptor of RANKL to compete with RANK on the surface of osteoclast lineage cells. OPG is expressed in osteoblasts and inhibits both osteoclast formation and function. Therefore, biological agents such as an antibody against RANKL have been developed and successfully suppress bone loss. Denosumab, an anti-RANKL antibody, has achieved the most success in the treatment of osteoporosis, tumor-related bone disorders, and arthritis[39-41].

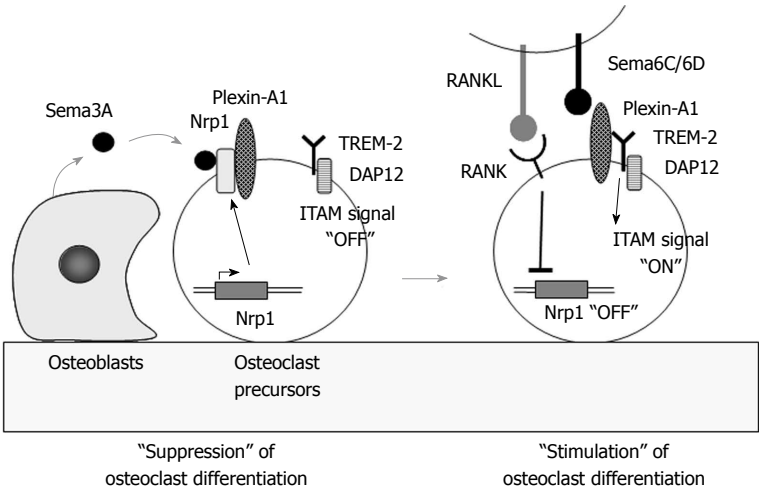

Figure 4 Role of semaphorin 3A in osteoclast differentiation. Semaphorin 3A (Sema3A) produced by osteoblasts usually binds to the receptor complex of Nrp1 and Plexin-A1 in osteoclast precursors. Sema3A inhibits differentiation of osteoclast precursors into osteoclasts through the suppression of immunoreceptor tyrosine-based activation motifs (ITAM) signaling. Receptor activator of nuclear factor-κB ligand (RANKL) stimulation rapidly suppresses Nrp1 expression in osteoclast precursors. Plexin-A1 then makes a complex with triggering receptor expressed on myeloid cells 2 (TREM-2) and DNAX-activating protein of 12 kDa (DAP12). Sema6D or 6C, transmembrane semaphorins, binds to the receptor complex and stimulates ITAM signals in osteoclast precursors to enhance RANK signaling.

Expression levels of Wnt5a and frizzled 5 are higher in the synovial tissue of rheumatoid arthritis (RA) patients than in those of osteoarthritis[42]. Wnt5a stimulated the production of pro-inflammatory cytokines such as IL-6 and IL-8 in synoviocytes. Treatment of RA patient-derived synoviocytes with antibodies against frizzled 5, one of the receptors for Wnt5a, diminished the production of RANKL[43]. These findings suggest that Wnt5a promotes pro-inflammatory cytokine production and enhances bone resorption through the production of RANKL in the pathogenesis of RA. In addition, Ror2, another receptor for Wnt5a, is expressed in osteoclast progenitors and co-stimulated non-canonical Wnt signaling[29]. Wnt5a enhanced osteoclast formation in mouse bone marrow macrophage cultures. Administration of glutathione-S-transferase-fused soluble Ror2 restored bone destruction caused by collagen-induced arthritis in mice[29]. These results suggest that Wnt5a is involved in bone destruction in chronic inflammatory diseases. Molecules involved in non-canonical Wnt signaling may be therapeutic targets for the treatment of patients suffering from RA and periodontitis.

The finding of Sema3A has invented a new concept that osteoblasts themselves express a bifunctional factor that induces osteoblastogenesis and inhibits osteoclastogenesis. Sema3A may inhibit bone loss without affecting the coupling system between osteoblasts and osteoclasts. Takayanagi and his colleagues[37] have demonstrated that administration of recombinant Sema3A increases bone volume and expedites bone regeneration in osteoporotic mice established by ovariectomy. Thus, Sema3A is a promising anabolic factor possessing an inhibitory activity on bone resorption.

In conclusion, the discovery of the RANKL-RANK interaction opened a new area in bone biology focusing on the molecular mechanisms of osteoclast development and function. This series of experiments concerning the RANKL-RANK interaction have established the concept that osteoblasts, through the expression of RANKL and M-CSF, tightly regulate the development of osteoclasts. Recent *in vivo* studies also indicate other aspects of osteoblasts in the regulation of osteoclastogenesis. Osteoblasts are involved in the decision of the place for osteoclast formation through taking care of osteoclast precursors. Osteoblasts produce some ligands for immunoglobulin-like receptors to induce ITAM-mediated co-stimulatory signals. Osteoblasts also produce Wnt5a, which stimulates RANK expression in osteoclast precursors though co-receptor Ror2 signaling. The up-regulation of RANK by Wnt5a-Ror2 signals in osteoclast precursors must enhance the sensitivity of QOPs to RANKL. Osteoblasts also produce Sema3A, which inhibits ITAM signals in osteoclast precursors. These findings provide a new concept that osteoblasts play several roles as an omnipotent conductor in osteoclastogenesis. In conformity with the new concept, we must come back to the osteoblast, which may be a promising target for therapeutic agents in the regulation of bone resorption in the near future.

REFERENCES

1 **Boyle WJ**, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature* 2003; **423**: 337-342
2 **Suda T**, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev* 1999; **20**: 345-357
3 **Tanaka S**, Takahashi N, Udagawa N, Tamura T, Akatsu T, Stanley ER, Kurokawa T, Suda T. Macrophage colony-stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. *J Clin Invest* 1993; **91**: 257-263
4 **Yoshida H**, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okamura H, Sudo T, Shultz LD, Nishikawa S. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature* 1990; **345**: 442-444
5 **Felix R**, Cecchini MG, Fleisch H. Macrophage colony stimulating factor restores in vivo bone resorption in the op/op osteopetrotic mouse. *Endocrinology* 1990; **127**: 2592-2594
6 **Yasuda H**, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci USA* 1998; **95**: 3597-3602

7 **Lacey DL**, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; **93**: 165-176

8 **Takayanagi H**, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev Cell* 2002; **3**: 889-901

9 **Simonet WS**, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309-319

10 **Yasuda H**, Shima N, Nakagawa N, Mochizuki SI, Yano K, Fujise N, Sato Y, Goto M, Yamaguchi K, Kuriyama M, Kanno T, Murakami A, Tsuda E, Morinaga T, Higashio K. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. *Endocrinology* 1998; **139**: 1329-1337

11 **Kong YY**, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; **397**: 315-323

12 **Li J**, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Scully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik P, Hsu H, Fletcher F, Dunstan CR, Lacey DL, Boyle WJ. RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. *Proc Natl Acad Sci USA* 2000; **97**: 1566-1571

13 **Bucay N**, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998; **12**: 1260-1268

14 **Mizuno A**, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, Gomibuchi T, Yano K, Shima N, Washida N, Tsuda E, Morinaga T, Higashio K, Ozawa H. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. *Biochem Biophys Res Commun* 1998; **247**: 610-615

15 **Cundy T**, Hegde M, Naot D, Chong B, King A, Wallace R, Mulley J, Love DR, Seidel J, Fawkner M, Banovic T, Callon KE, Grey AB, Reid IR, Middleton-Hardie CA, Cornish J. A mutation in the gene TNFRSF11B encoding osteoprotegerin causes an idiopathic hyperphosphatasia phenotype. *Hum Mol Genet* 2002; **11**: 2119-2127

16 **Chong B**, Hegde M, Fawkner M, Simonet S, Cassinelli H, Coker M, Kanis J, Seidel J, Tau C, Tüysüz B, Yüksel B, Love D. Idiopathic hyperphosphatasia and TNFRSF11B mutations: relationships between phenotype and genotype. *J Bone Miner Res* 2003; **18**: 2095-2104

17 **Whyte MP**, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH, Mumm S. Osteoprotegerin deficiency and juvenile Paget's disease. *N Engl J Med* 2002; **347**: 175-184

18 **Hughes AE**, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. *Nat Genet* 2000; **24**: 45-48

19 **Guerrini MM**, Sobacchi C, Cassani B, Abinun M, Kilic SS, Pangrazio A, Moratto D, Mazzolari E, Clayton-Smith J, Orchard P, Coxon FP, Helfrich MH, Crockett JC, Mellis D, Vellodi A, Tezcan I, Notarangelo LD, Rogers MJ, Vezzoni P, Villa A, Frattini A. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am J Hum Genet* 2008; **83**: 64-76

20 **Sobacchi C**, Frattini A, Guerrini MM, Abinun M, Pangrazio A, Susani L, Bredius R, Mancini G, Cant A, Bishop N, Grabowski P, Del Fattore A, Messina C, Errigo G, Coxon FP, Scott DI, Teti A, Rogers MJ, Vezzoni P, Villa A, Helfrich MH. Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat Genet* 2007; **39**: 960-962

21 **Mizoguchi T**, Muto A, Udagawa N, Arai A, Yamashita T, Hosoya A, Ninomiya T, Nakamura H, Yamamoto Y, Kinugawa S, Nakamura M, Nakamichi Y, Kobayashi Y, Nagasawa S, Oda K, Tanaka H, Tagaya M, Penninger JM, Ito M, Takahashi N. Identification of cell cycle-arrested quiescent osteoclast precursors in vivo. *J Cell Biol* 2009; **184**: 541-554

22 **Muto A**, Mizoguchi T, Udagawa N, Ito S, Kawahara I, Abiko Y, Arai A, Harada S, Kobayashi Y, Nakamichi Y, Penninger JM, Noguchi T, Takahashi N. Lineage-committed osteoclast precursors circulate in blood and settle down into bone. *J Bone Miner Res* 2011; **26**: 2978-2990

23 **Lin H**, Lee E, Hestir K, Leo C, Huang M, Bosch E, Halenbeck R, Wu G, Zhou A, Behrens D, Hollenbaugh D, Linnemann T, Qin M, Wong J, Chu K, Doberstein SK, Williams LT. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science* 2008; **320**: 807-811

24 **Chihara T**, Suzu S, Hassan R, Chutiwitoonchai N, Hiyoshi M, Motoyoshi K, Kimura F, Okada S. IL-34 and M-CSF share the receptor Fms but are not identical in biological activity and signal activation. *Cell Death Differ* 2010; **17**: 1917-1927

25 **Chen Z**, Buki K, Vääräniemi J, Gu G, Väänänen HK. The critical role of IL-34 in osteoclastogenesis. *PLoS One* 2011; **6**: e18689

26 **Nakamichi Y**, Mizoguchi T, Arai A, Kobayashi Y, Sato M, Penninger JM, Yasuda H, Kato S, DeLuca HF, Suda T, Udagawa N, Takahashi N. Spleen serves as a reservoir of osteoclast precursors through vitamin D-induced IL-34 expression in osteopetrotic op/op mice. *Proc Natl Acad Sci USA* 2012; **109**: 10006-10011

27 **Ishii M**, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, Proia RL, Germain RN. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature* 2009; **458**: 524-528

28 **Arai A**, Mizoguchi T, Harada S, Kobayashi Y, Nakamichi Y, Yasuda H, Penninger JM, Yamada K, Udagawa N, Takahashi N. Fos plays an essential role in the upregulation of RANK expression in osteoclast precursors within the bone microenvironment. *J Cell Sci* 2012; **125**: 2910-2917

29 **Maeda K**, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T, Kikuchi Y, Takada I, Kato S, Kani S, Nishita M, Marumo K, Martin TJ, Minami Y, Takahashi N. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat Med* 2012; **18**: 405-412

30 **Gordon MD**, Nusse R. Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factors. *J Biol Chem* 2006; **281**: 22429-22433

31 **Gong Y**, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Jüppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A,

LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 2001; **107**: 513-523

32 **Glass DA**, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005; **8**: 751-764

33 **Atkins GJ**, Kostakis P, Vincent C, Farrugia AN, Houchins JP, Findlay DM, Evdokiou A, Zannettino AC. RANK Expression as a cell surface marker of human osteoclast precursors in peripheral blood, bone marrow, and giant cell tumors of bone. *J Bone Miner Res* 2006; **21**: 1339-1349

34 **Koga T**, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature* 2004; **428**: 758-763

35 **Barrow AD**, Raynal N, Andersen TL, Slatter DA, Bihan D, Pugh N, Cella M, Kim T, Rho J, Negishi-Koga T, Delaisse JM, Takayanagi H, Lorenzo J, Colonna M, Farndale RW, Choi Y, Trowsdale J. OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12-deficient humans and mice. *J Clin Invest* 2011; **121**: 3505-3516

36 **Takayanagi H**. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol* 2007; **7**: 292-304

37 **Hayashi M**, Nakashima T, Taniguchi M, Kodama T, Kumanogoh A, Takayanagi H. Osteoprotection by semaphorin 3A. *Nature* 2012; **485**: 69-74

38 **Takegahara N**, Takamatsu H, Toyofuku T, Tsujimura T, Okuno T, Yukawa K, Mizui M, Yamamoto M, Prasad DV, Suzuki K, Ishii M, Terai K, Moriya M, Nakatsuji Y, Sakoda S, Sato S, Akira S, Takeda K, Inui M, Takai T, Ikawa M, Okabe M, Kumanogoh A, Kikutani H. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. *Nat Cell Biol* 2006; **8**: 615-622

39 **Cummings SR**, San Martin J, McClung MR, Siris ES, Eastell R, Reid IR, Delmas P, Zoog HB, Austin M, Wang A, Kutilek S, Adami S, Zanchetta J, Libanati C, Siddhanti S, Christiansen C. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009; **361**: 756-765

40 **Stopeck AT**, Lipton A, Body JJ, Steger GG, Tonkin K, de Boer RH, Lichinitser M, Fujiwara Y, Yardley DA, Viniegra M, Fan M, Jiang Q, Dansey R, Jun S, Braun A. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. *J Clin Oncol* 2010; **28**: 5132-5139

41 **Cohen SB**, Dore RK, Lane NE, Ory PA, Peterfy CG, Sharp JT, van der Heijde D, Zhou L, Tsuji W, Newmark R. Denosumab treatment effects on structural damage, bone mineral density, and bone turnover in rheumatoid arthritis: a twelve-month, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial. *Arthritis Rheum* 2008; **58**: 1299-1309

42 **Sen M**, Lauterbach K, El-Gabalawy H, Firestein GS, Corr M, Carson DA. Expression and function of wingless and frizzled homologs in rheumatoid arthritis. *Proc Natl Acad Sci USA* 2000; **97**: 2791-2796

43 **Sen M**, Chamorro M, Reifert J, Corr M, Carson DA. Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synoviocyte activation. *Arthritis Rheum* 2001; **44**: 772-781

S- Editor Huang XZ **L- Editor** A **E- Editor** Xiong L

World Journal of Orthopedics

Online Submissions: http://www.wjgnet.com/esps/
wjo@wjgnet.com
doi:10.5312/wjo.v3.i11.182

World J Orthop 2012 November 18; 3(11): 182-189
ISSN 2218-5836 (online)

REVIEW

Management of postoperative spinal infections

Vishal Hegde, Dennis S Meredith, Christopher K Kepler, Russel C Huang

Vishal Hegde, Department of Orthopedic Surgery, Weill Cornell Medical College, New York, NY 10021, United States
Dennis S Meredith, Russel C Huang, Department of Orthopedic Surgery, Spine and Scoliosis Service, Hospital for Special Surgery, Weill Cornell Medical Center, New York, NY 10021, United States
Christopher K Kepler, Department of Orthopedic Surgery, Rothman Institute, Thomas Jefferson University, Philadelphia, PA 19107, United States
Author contributions: Hegde V, Meredith DS, Kepler CK and Huang RC contributed to this paper.
Correspondence to: Vishal Hegde, Research Fellow, Department of Orthopedic Surgery, Weill Cornell Medical College, 420 East 70th Street Apt No. 5K-2, New York, NY 10021, United States. vvh2001@med.cornell.edu
Telephone: +1-212-6061172 Fax:+1-212-7721061
Received: April 3, 2012 Revised: October 21, 2012
Accepted: November 1, 2012
Published online: November 18, 2012

Abstract

Postoperative surgical site infection (SSI) is a common complication after posterior lumbar spine surgery. This review details an approach to the prevention, diagnosis and treatment of SSIs. Factors contributing to the development of a SSI can be split into three categories: (1) microbiological factors; (2) factors related to the patient and their spinal pathology; and (3) factors relating to the surgical procedure. SSI is most commonly caused by *Staphylococcus aureus*. The virulence of the organism causing the SSI can affect its presentation. SSI can be prevented by careful adherence to aseptic technique, prophylactic antibiotics, avoiding myonecrosis by frequently releasing retractors and preoperatively optimizing modifiable patient factors. Increasing pain is commonly the only symptom of a SSI and can lead to a delay in diagnosis. C-reactive protein and magnetic resonance imaging can help establish the diagnosis. Treatment requires acquiring intra-operative cultures to guide future antibiotic therapy and surgical debridement of all necrotic tissue. A SSI can usually be adequately treated without removing spinal instrumentation. A multidisciplinary approach to SSIs is important. It is useful to involve an infectious disease specialist and use minimum serial bactericidal titers to enhance the effectiveness of antibiotic therapy. A plastic surgeon should also be involved in those cases of severe infection that require repeat debridement and delayed closure.

Key words: Surgical site infection; Spine surgery; Discitis; Postoperative infection

Peer reviewers: Pramod V Lokhande, Professor, Department of Orthopedics, SKN Medical College and Hospital, Pune, India; Yasuaki Tokuhashi, MD, Professor, Chairman, Department of Orthopedic Surgery, Nihon University School of Medicine, 30-1 Oyaguchi-kamicho, Itabashi-ku, Tokyo 173-8610, Japan; Hwan Tak Hee, Associate Professor, Department of Orthopaedic Surgery, National University Hospital, 1E Kent Ridge Road, Singapore 119228, Singapore

Hegde V, Meredith DS, Kepler CK, Huang RC. Management of postoperative spinal infections. *World J Orthop* 2012; 3(11): 182-189 Available from: URL: http://www.wjgnet.com/2218-5836/full/v3/i11/182.htm DOI: http://dx.doi.org/10.5312/wjo.v3.i11.182

INTRODUCTION

Postoperative surgical site infection (SSI) in the lumbar spine is a relatively frequent complication of invasive spine procedures. The management of a SSI can be costly due to its potentially devastating consequences, including lost productivity during prolonged treatment and recovery, increased morbidity, the need for subsequent reoperation and even death. With the rise in prevalence of antibiotic-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), the prevention and treatment of SSIs has become even more difficult, particularly in those patients with spinal instrumentation.

This review describes the factors that contribute to the development of a SSI and strategies for their prevention, the range of presentations of SSIs, and the challenges that arise during diagnosis and treatment.

PATHOGENESIS AND PREVENTION

Although multifactorial, the various risk factors that contribute to the development of a SSI can be broadly divided into three categories: (1) microbiological; (2) patient/host; and (3) procedure-related. Understanding the contribution of these risk factors to SSIs enhances measures aimed at the prevention of this common yet dreadful complication.

Microbiological factors

The most common organism causing a SSI is *Staphylococcus aureus* (*S. aureus*), although other reported causative organisms include *Staphylococcus epidermidis* (*S. epidermidis*), *Enterococcus faecalis, Pseudomonas* spp., *Enterobacter cloacae,* and *Proteus mirabilis*[1,2]. Trauma patients are more likely to present with infections due to gram-negative bacteria, which may result from hematogenous spread in the setting of urosepsis, frequently in patients with neurological injury related to their trauma[3]. Recently, a consecutive series of 3218 patients undergoing posterior lumbar-instrumented arthrodesis was reviewed by Koutsoumbelis *et al*[4]. In this series, 34% of SSIs demonstrated positive cultures for MRSA, indicating an increasing prevalence of this organism.

When addressing microbiological factors that contribute to SSIs, it is important to emphasize that meticulous adherence to aseptic technique is the key component of SSI prevention[5]. One intervention that the bulk of available evidence has suggested may decrease the rate of SSI after spinal surgery is the use of prophylactic antibiotics[6]. Antibiotic prophylaxis has brought the incidence of SSI following lumbar discectomy down to < 1%[1,7-12]. In fact, one report by Transfeldt *et al*[13] showed a decrease in the SSI rate from 7% to 3.6% following elective spinal arthrodesis with the use of routine antibiotic prophylaxis. When choosing an antibiotic, one with good efficacy against common strains of *S. aureus* and *S. epidermidis* should be used due to the higher frequency of infection with these bacteria. A first-generation cephalosporin such as cefazolin is popular, as it also quickly reaches peak serum concentrations and has a more benign side effect profile than other antibiotics. If a patient is at high risk for colonization with MRSA, we recommend combining vancomycin with cefazolin, as vancomycin alone has relatively low efficacy against non-methicillin resistant strains of *Staphylococcus* spp. Yet for those patients with allergies to penicillin or cephalosporins, vancomycin alone can be used. Risk factors for colonization with MRSA include antibiotic use within 3 mo before admission, hospitalization during the past 12 mo, diagnosis of skin or soft-tissue infection at admission, and human immunodeficiency virus infection[14,15]. Bacterial antibiotic resistance continues to be an evolving problem and these recommendations may need to be modified based on regional bacterial susceptibilities or if common pathogens in SSIs develop widespread resistance to these antibiotics in the future.

Patient/host factors

Several patient-related risk factors have been reported for SSIs including: diabetes mellitus, obesity, alcohol abuse, smoking, advanced age, corticosteroid use, malnutrition and hospitalization greater than one week[16-40]. Koutsoumbelis *et al*[4] also identified coronary artery disease, osteoporosis and chronic obstructive pulmonary disease as independent risk factors for SSIs. Although the exact mechanism by which these factors increase the likelihood of a SSI is not definitively known, it is clear that an inability of the host to heal the surgical wound and mount an inflammatory response sufficient to eradicate the infectious organisms leads to their growth. Obese patients have a large layer of adipose tissue with poor vascular perfusion that may become necrotic following wound closure, creating a nidus for infection[2,16,20,24,41,42]. Smoking and diabetes both predispose patients to infection through microvascular damage and subsequent induction of tissue ischemia[23,24,41-43]. Advancing age increases the likelihood of the presence of other comorbidities and is associated with immunosenescence, a phenomenon by which the immune response gradually wanes and becomes ineffective.

The pathology that patients present with also influences susceptibility to infection. Patients with traumatic spine injury, especially those with a concomitant neurological injury, have infection rates of up to 10%[2-4,16-40,43-47]. Such patients may have additional injuries to the viscera or appendicular skeleton and usually have a greater degree of soft-tissue injury than patients undergoing elective surgery, which contributes to tissue hypoxia. Trauma patients are in a catabolic state and are more likely to have protein-calorie malnutrition. Prolonged stays in intensive care units lead to increased exposure to antibiotic resistant bacteria, which may increase the severity of a SSI and make treatment more difficult. Those factors that cause trauma patients to have a higher risk of developing SSIs also apply to patients with spinal neoplasms. In addition, these patients may also undergo systemic chemotherapy or radiation to the surgical site, leading to immunosuppression and delayed healing, and consequently increasing their susceptibility to infection.

Modifiable risk factors should be mitigated preoperatively to minimize the risk of postoperative infection. A nutrition consult should be obtained in patients after significant polytrauma, with catabolic processes due to neoplasm, or otherwise at significant risk for malnutrition. Blood sugar should be closely controlled in diabetic patients.

Procedure-related factors

The length and complexity of the index surgical procedure has a significant impact on the incidence of SSIs.

Although the risk of a SSI is < 1% for lumbar discectomy, the risk is higher following spinal arthrodesis, particularly with posterior instrumentation. This is likely due to increased dead space, longer duration of surgery and the potential for adherence of biofilm to metal implants. Following elective thoracic or lumbar spinal arthrodesis, reported rates of SSI from individual surgeons or institutions ranges from 1.9% to 4.4% in the last ten years[41,42,48-50]. The most recent National Nosocomial Infections Surveillance report in 2004 cited the infection rate following spinal arthrodesis as 2.1%[51]. The risk of SSI is less common after anterior spinal arthrodesis and is not greater for a combined anterior/posterior arthrodesis than for a posterior arthrodesis alone[44], except for when it is a staged procedure done under separate anesthesia[48]. Devices such as an operating microscope or headlamp and loupe magnification can create a source of bacterial shedding onto the surgical field, although increased contamination from these devices has not been shown to directly increase infection risk[8,9,52-54]. There is also some limited evidence that minimally invasive surgery may decrease the risk of a SSI. A recent systematic review of single cohort studies comparing minimally invasive transforaminal interbody fusion (TLIF) to open TLIF showed a significant decrease in SSI rates from 4% to 0.6%[55-57]. In addition, it has recently been shown that the risk of returning to the operating room (OR) to treat a SSI increases along with the surgical invasiveness index of the primary spine surgery[58].

The study by Koutsoumbelis *et al*[4] reported an overall incidence of SSIs of 2.6%. Their study identified four procedure related risk factors: (1) longer duration of surgery; (2) intra-operative blood loss/need for transfusion; (3) incidental durotomy; and (4) greater than ten people in the OR, specifically cautioning against extraneous nurses. Previous studies have also identified increased operative time, multilevel surgery, revision surgery, and an increased number of people in the OR as important predisposing factors for a SSI[1,2,16,41,42,45,46,48,49]. However, this is the first time incidental durotomy has been identified as a risk factor for SSI[47]. It is unclear how and to what extent incidental durotomy and an increased number of people in the OR increase the likelihood of a SSI. Both may increase the risk of contamination of the surgical field directly, or be indicative of a longer and/or more complex surgical procedure.

Modifications to procedural technique can assist in the prevention of a SSI. It is important to frequently release retractors to prevent myonecrosis, avoid excessive use of electrocautery during subperiosteal dissection of muscle, and debride necrotic appearing muscle at the conclusion of the case. This will prevent the retention of devitalized necrotic tissue, which is a potential nidus for infection. Although the use of this technique in the lumbar spine has not yet been investigated, the addition of vancomycin powder to posterior cervical incisions prior to closure has been shown to decrease SSIs[59,60]. At our institution, patients undergoing multi-level decompression and/or posterior spinal arthrodesis routinely receive antibiotic irrigation and closed suction drains postoperatively. Existing investigations have not shown that these interventions provide a significant benefit, although they have been underpowered to detect a change in infection rate, a rare event[61-63]. Evidence for the use of vertical laminar flow systems to decrease the risk of SSI in the OR is limited[64].

Recently, Dipaola *et al*[65] created a predictive model to stratify patients with spinal SSIs into those needing single *vs* multiple irrigation and debridements. To develop the model, risk factors from all three categories (microbiological, patient/host and procedure related), were analyzed. It was found that positive MRSA cultures and concomitant infections at sites other than the spine or bacteremia were strong predictors of the need for multiple irrigation and debridements. In addition, diabetes, location of surgery in the posterior lumbar spine, presence of instrumentation and the use of bone graft material other than autogenous bone graft were also more likely to result in multiple irrigation and debridements. In the future, this predictive model may help stratify patients with SSIs, enabling surgeons to adapt their index surgery and SSI treatment strategies accordingly.

CLINICAL PRESENTATION AND DIAGNOSIS

The diagnosis of a SSI requires the synthesis of all available data, as there is no one pathognomonic sign or symptom to indicate its presence. The most common symptom of a SSI in the early postoperative period is increasing pain at the surgical site. Signs on exam include tenderness to palpation, peri-incisional erythema, induration and drainage. A particular concern is a patient with constitutional symptoms such as fever and chills, and in the case of a severe infection: hypotension, lethargy and confusion from sepsis. Such an infection is an absolute indication for emergent irrigation and debridement, but presents rarely. In the setting of a revision surgery, latent infection from organisms such as *Propionibacterium acnes* must always be considered and routine cultures sent, as the presentation may be limited to vague complaints of pain with evidence of hardware loosening or pseudoarthrosis.

Imaging

Except in the setting of latent infections or discitis, plain radiographs of the spine are not particularly useful to diagnose an early SSI. Patients with latent infections may have lucency around instrumentation, while those with discitis may show loss of disk height and end plate erosion. Along those lines, computed tomography (CT) can be used in these patients to assess bony destruction and implant loosening three-dimensionally. Bone scan is not useful in these patients, as it will commonly show increased uptake due to the reactive bone at the surgical

site post-operatively[66]. Gadolinium enhanced magnetic resonance imaging (MRI) is the best radiologic modality to use when a SSI is suspected. Progressive marrow signal changes, rim enhancing fluid collections, ascending or descending epidural collections and bony destruction are all indicative of infection on MRI.

When interpreting MRI results, confounding factors such as time from index procedure should be taken into account, as tissue edema from a non-infectious cause can be confused with an infectious process. Infection typically occurs between three days and three months postoperatively and takes several days to become established. In the immediate post-operative period (< 6 wk), it has been shown that diffuse, spotty, linear intervertebral disk enhancement, with two thin bands paralleling the endplates, as well as annular enhancement at the surgical curette site are common findings and do not indicate that an infection is developing. Type 1 changes of adjacent endplates, such as decreased signal intensity on T1 imaging and edema of the vertebral marrow adjacent to the disc, are also common post-operatively. Vertebral osteomyelitis is typically recognized by endplate changes similar to these Type 1 changes, and is described as a diffuse, irregular area of non-anatomic high signal intensity in the disc. Contrast is valuable in differentiating between the two entities, as osteomyelitis shows circumferential enhancement of the disc, while the postoperative state will only produce subtle linear areas of enhancement[67,68].

Laboratory tests

Measurement of acute phase reactants is very useful when diagnosing an infection. C-reactive protein (CRP) has been shown to be more sensitive than erythrocyte sedimentation rate (ESR) for detecting a SSI, as CRP levels only stay elevated for two weeks postoperatively before decreasing, while it may take up to six weeks for ESR levels to normalize. For this reason, time since index surgery is important when interpreting levels of acute phase reactants. Persistent elevation of CRP is an early indicator of an infection. In addition, preoperative measurement of CRP levels in high-risk patients with associated medical co-morbidities that may confound a postoperative CRP measurement can be useful as a baseline for detection of early infection postoperatively[69]. White blood cell count, although routinely obtained, is an unreliable indicator of a SSI. It may remain normal despite a SSI or may be normally elevated in the post-operative period. When attempting to identify the causative organism in a SSI, intra-operative tissue cultures are the gold standard. Superficial cultures, from either the skin or drainage, are not reliable due to the likelihood of contamination by skin flora. Alternatively, some authors have proposed wound aspiration as a method for detecting early infections[70].

TREATMENT OF SSI

The timing and location of the infection dictates treatment. The timing of a SSI can be classified as early, late or latent, and location is either limited to the disc, or superficial or deep to the fascia.

Posterior spinal infections

Superficial extrafascial SSIs, such as cellulitis or subcutaneous abscesses, are usually managed with Ⅳ antibiotics and/or surgical incision and drainage, which can often be performed at the bedside. Subfascial wound infections rarely respond to antibiotic treatment alone and require surgical debridement and removal of all necrotic tissue with closure over drains. Epidural abscesses can be managed medically when small. However, surgical drainage is typically required for large collections, small collections that progress despite antibiotic therapy, and decompression of the dural sac in the event of a neurological deficit. Paraspinal epidural abscesses, such as a psoas abscess, may respond to medical treatment when small. However, CT-guided aspiration and drainage is often required for large collections[32]. A SSI in an immunocompromised host or with a particularly virulent organism may require multiple irrigation and debridements.

Patients with a SSI and spinal instrumentation present similarly to those without instrumentation, but pose unique challenges. The use of MRI in patients with instrumentation requires specialized protocols for suppression of metal artifact, such as the metal artifact reduction sequence described by Chang *et al*[71], without which the MRI is of limited value[72,73]. Thorough surgical debridement of all necrotic tissue and irrigation with large amounts of normal saline is crucial[74]. Loose bone graft material should be removed if unincorporated, as dead bone will only serve as a nidus for continued infection. Loose pedicle screws and other non-essential spinal instrumentation should be removed, but essential instrumentation should be maintained if possible to avoid the creation of instability or the loss of deformity correction. Interbody and posterior segmental instrumentation can usually be left in place early on, as several authors have reported high success rates using this hardware-preservation strategy in the management of early SSIs[1,4,75-78]. Patients with a late infection and solid fusion can have their instrumentation removed during surgical debridement to help clear the infection[79]. Unfortunately, these patients are at an increased risk of developing a pseudoarthrosis and must be monitored with serial imaging studies[80].

As multiple debridements are often necessary when treating a SSI, involving a plastic surgeon early on can facilitate optimal wound management[81,82]. The debridement of soft tissue required to treat a SSI may result in a significant soft tissue defect. Such defects may be definitively closed with a muscle flap, or heal by secondary intention using a vacuum-dressing. We recommend that patients who require multiple surgical debridements have antibiotic impregnated polymethylmethacrylate (PMMA) beads placed into the wound during early debridements, permitting high local antibiotic concentrations despite

poor tissue vascularity. PMMA beads have been shown to decrease the development of infection after wound contamination, and have been documented to decrease both acute infection rates and osteomyelitis after compound limb fractures[83-85].

Postprocedure discitis

With a reported incidence ranging from 0.2% to 2.75%, postprocedure discitis is an infrequent complication of spine surgery[86-89]. A vague complaint of low back pain is commonly the only indication that a patient may be suffering from postprocedure discitis, which can lead to a delay in diagnosis. Especially concerning are those patients with a history of increasing low back pain following surgery. For these patients, bracing can be used for comfort. Image guided percutaneous aspiration of the disc to identify the causative organism and guide antibiotic treatment is very effective[90]. Most of these cases can be treated with six weeks of IV antibiotics, usually resulting in spontaneous fusion of the disk space[91-93].

Surgery is indicated in those patients whose infection has progressed on MRI despite appropriate antibiotic therapy, with deformity due to progressive destruction of the vertebral bodies, or with severe pain or neurological deficits due to progression of the infection into the spinal canal. For early postoperative discitis with minimal involvement of the vertebral bodies, percutaneous transforaminal endoscopic debridement is an effective and minimally invasive option that has been shown to bring immediate pain reduction and good clinical results[94]. Otherwise, anterior only or posterior only approaches for debridement and fusion may be sufficient, depending on the location of the infection and the extent of debridement and resulting instability[95-97]. Many surgeons prefer to use autologous bone graft as an interbody spacer to minimize the risk of recurrent infection. If performed, harvesting of the bone graft should be performed prior to opening the spinal wound to minimize the risk of graft donor site SSI. When performing a surgical discectomy, as much of the disk as possible should be removed to prevent recurrent infection, as the adult intervertebral disk is avascular.

Postoperative antibiotic therapy

Infectious disease specialists are routinely involved in the selection and monitoring of antibiotic therapy at our institution. For implanted spinal instrumentation, the protocol our institution uses is based on previous experience with SSIs following total joint replacement[98-100]. Intravenous antibiotics are chosen based on the type of causative organism and its sensitivity profile. Dosage is monitored by the trough serum bactericidal titer (SBT), which indicates the amount of bactericidal activity in the patient's serum at the trough level between antibiotic doses. The trough SBT should be maintained at a minimum of 1:2[101]. This ensures that at a trough level, there is at least twice the minimum concentration of antibiotic in the serum that is required for bactericidal activity. Using the SBT to monitor antibiotic therapy improves its efficacy, even in cases with resistant organisms. Antibiotics are continued for six weeks postoperatively, although recent recommendations advise eight weeks of total IV antibiotic therapy for patients with resistant organisms such as MRSA[102]. Patients are subsequently maintained on oral suppressive antibiotics. The patient's health status, success in achieving spinal fusion and causative organism influence the choice between lifetime oral antibiotic suppression to prevent recurrent infection and removal of instrumentation.

CONCLUSION

SSI is a common but challenging complication, particularly after instrumented spinal arthrodesis. Using meticulous aseptic technique, intra-operative irrigation, prophylactic antibiotics and optimizing patient factors preoperatively are key to preventing a SSI. In patients who still develop an infection despite efforts at prevention, timely diagnosis and treatment is critical. Instrumentation can be retained while still successfully clearing an early infection, although following fusion, instrumentation can be removed if lifetime oral antibiotic suppression is either not indicated or undesirable. Involving a plastic surgeon early on in the process is useful for closure of complex soft tissue defects.

REFERENCES

1 **Weinstein MA**, McCabe JP, Cammisa FP. Postoperative spinal wound infection: a review of 2,391 consecutive index procedures. *J Spinal Disord* 2000; **13**: 422-426
2 **Massie JB**, Heller JG, Abitbol JJ, McPherson D, Garfin SR. Postoperative posterior spinal wound infections. *Clin Orthop Relat Res* 1992; (284): 99-108
3 **Rechtine GR**, Bono PL, Cahill D, Bolesta MJ, Chrin AM. Postoperative wound infection after instrumentation of thoracic and lumbar fractures. *J Orthop Trauma* 2001; **15**: 566-569
4 **Koutsoumbelis S**, Hughes AP, Girardi FP, Cammisa FP, Finerty EA, Nguyen JT, Gausden E, Sama AA. Risk factors for postoperative infection following posterior lumbar instrumented arthrodesis. *J Bone Joint Surg Am* 2011; **93**: 1627-1633
5 **Lister J.** Antiseptic Principle in the practice of surgery. *Brit Med J* 1867; **2:** 246-248
6 **Barker FG**. Efficacy of prophylactic antibiotic therapy in spinal surgery: a meta-analysis. *Neurosurgery* 2002; **51**: 391-400; discussion 400-401
7 **Bassewitz HL,** Fischgrund JS, Herkowitz HN. Postoperative spine infections. *Semin Spine Surg* 2000; **12:** 203-211
8 **Wilson DH**, Harbaugh R. Microsurgical and standard removal of the protruded lumbar disc: a comparative study. *Neurosurgery* 1981; **8**: 422-427
9 **Stolke D**, Sollmann WP, Seifert V. Intra- and postoperative complications in lumbar disc surgery. *Spine* (Phila Pa 1976) 1989; **14**: 56-59
10 A Methodological Systematic Review on Surgical Site Infections Following Spinal Surgery: Part 2: Prophylactic Treatments. *Spine* (Phila Pa 1976) 2012; **37**: 2034-2045
11 **Kanayama M**, Hashimoto T, Shigenobu K, Oha F, Togawa D. Effective prevention of surgical site infection using a Centers for Disease Control and Prevention guideline-based antimicrobial prophylaxis in lumbar spine surgery. *J Neuro-*

surg Spine 2007; **6**: 327-329
12 **Khan IU**, Janjua MB, Hasan S, Shah S. Surgical site infection in lumbar surgeries, pre and postoperative antibiotics and length of stay: a case study. *J Ayub Med Coll Abbottabad* 2009; **21**: 135-138
13 **Transfeldt EE,** Lonstein JE. Wound infections in elective reconstructive spinal surgery. *Orthopaedic Transactions* 1985; **9:** 128-129
14 **Hidron AI**, Kourbatova EV, Halvosa JS, Terrell BJ, McDougal LK, Tenover FC, Blumberg HM, King MD. Risk factors for colonization with methicillin-resistant Staphylococcus aureus (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage. *Clin Infect Dis* 2005; **41**: 159-166
15 **Slover J**, Haas JP, Quirno M, Phillips MS, Bosco JA. Cost-effectiveness of a Staphylococcus aureus screening and decolonization program for high-risk orthopedic patients. *J Arthroplasty* 2011; **26**: 360-365
16 **Wimmer C**, Gluch H, Franzreb M, Ogon M. Predisposing factors for infection in spine surgery: a survey of 850 spinal procedures. *J Spinal Disord* 1998; **11**: 124-128
17 **Klein JD**, Hey LA, Yu CS, Klein BB, Coufal FJ, Young EP, Marshall LF, Garfin SR. Perioperative nutrition and postoperative complications in patients undergoing spinal surgery. *Spine* (Phila Pa 1976) 1996; **21**: 2676-2682
18 **Carreon LY**, Puno RM, Dimar JR, Glassman SD, Johnson JR. Perioperative complications of posterior lumbar decompression and arthrodesis in older adults. *J Bone Joint Surg Am* 2003; **85-A**: 2089-2092
19 **Cassinelli EH**, Eubanks J, Vogt M, Furey C, Yoo J, Bohlman HH. Risk factors for the development of perioperative complications in elderly patients undergoing lumbar decompression and arthrodesis for spinal stenosis: an analysis of 166 patients. *Spine* (Phila Pa 1976) 2007; **32**: 230-235
20 **Patel N**, Bagan B, Vadera S, Maltenfort MG, Deutsch H, Vaccaro AR, Harrop J, Sharan A, Ratliff JK. Obesity and spine surgery: relation to perioperative complications. *J Neurosurg Spine* 2007; **6**: 291-297
21 **Peng CW**, Bendo JA, Goldstein JA, Nalbandian MM. Perioperative outcomes of anterior lumbar surgery in obese versus non-obese patients. *Spine J* 2009; **9**: 715-720
22 **Simpson JM**, Silveri CP, Balderston RA, Simeone FA, An HS. The results of operations on the lumbar spine in patients who have diabetes mellitus. *J Bone Joint Surg Am* 1993; **75**: 1823-1829
23 **Friedman ND**, Sexton DJ, Connelly SM, Kaye KS. Risk factors for surgical site infection complicating laminectomy. *Infect Control Hosp Epidemiol* 2007; **28**: 1060-1065
24 **Capen DA**, Calderone RR, Green A. Perioperative risk factors for wound infections after lower back fusions. *Orthop Clin North Am* 1996; **27**: 83-86
25 **Thalgott JS**, Cotler HB, Sasso RC, LaRocca H, Gardner V. Postoperative infections in spinal implants. Classification and analysis--a multicenter study. *Spine* (Phila Pa 1976) 1991; **16**: 981-984
26 **Schuster JM**, Rechtine G, Norvell DC, Dettori JR. The influence of perioperative risk factors and therapeutic interventions on infection rates after spine surgery: a systematic review. *Spine* (Phila Pa 1976) 2010; **35**: S125-S137
27 A Methodological Systematic Review on Surgical Site Infections Following Spinal Surgery: Part 1: Risk Factors. *Spine* (Phila Pa 1976) 2012; **37**: 2017-2033
28 **Abdul-Jabbar A**, Takemoto S, Weber MH, Hu SS, Mummaneni PV, Deviren V, Ames CP, Chou D, Weinstein PR, Burch S, Berven SH. Surgical site infection in spinal surgery: description of surgical and patient-based risk factors for postoperative infection using administrative claims data. *Spine* (Phila Pa 1976) 2012; **37**: 1340-1345
29 **Gelalis ID**, Arnaoutoglou CM, Politis AN, Batzaleksis NA, Katonis PG, Xenakis TA. Bacterial wound contamination during simple and complex spinal procedures. A prospective clinical study. *Spine J* 2011; **11**: 1042-1048
30 **Lazennec JY**, Fourniols E, Lenoir T, Aubry A, Pissonnier ML, Issartel B, Rousseau MA. Infections in the operated spine: update on risk management and therapeutic strategies. *Orthop Traumatol Surg Res* 2011; **97**: S107-S116
31 **Rao SB**, Vasquez G, Harrop J, Maltenfort M, Stein N, Kaliyadan G, Klibert F, Epstein R, Sharan A, Vaccaro A, Flomenberg P. Risk factors for surgical site infections following spinal fusion procedures: a case-control study. *Clin Infect Dis* 2011; **53**: 686-692
32 **Pull ter Gunne AF**, Mohamed AS, Skolasky RL, van Laarhoven CJ, Cohen DB. The presentation, incidence, etiology, and treatment of surgical site infections after spinal surgery. *Spine* (Phila Pa 1976) 2010; **35**: 1323-1328
33 **Chen S**, Anderson MV, Cheng WK, Wongworawat MD. Diabetes associated with increased surgical site infections in spinal arthrodesis. *Clin Orthop Relat Res* 2009; **467**: 1670-1673
34 **Stambough JL**, Beringer D. Postoperative wound infections complicating adult spine surgery. *J Spinal Disord* 1992; **5**: 277-285
35 **Calderone RR**, Larsen JM. Overview and classification of spinal infections. *Orthop Clin North Am* 1996; **27**: 1-8
36 **Lim MR**, Lee JY, Vaccaro AR. Surgical infections in the traumatized spine. *Clin Orthop Relat Res* 2006; **444**: 114-119
37 **Pull ter Gunne AF**, van Laarhoven CJ, Cohen DB. Incidence of surgical site infection following adult spinal deformity surgery: an analysis of patient risk. *Eur Spine J* 2010; **19**: 982-988
38 **Schwarzkopf R**, Chung C, Park JJ, Walsh M, Spivak JM, Steiger D. Effects of perioperative blood product use on surgical site infection following thoracic and lumbar spinal surgery. *Spine* (Phila Pa 1976) 2010; **35**: 340-346
39 **Kanafani ZA**, Dakdouki GK, El-Dbouni O, Bawwab T, Kanj SS. Surgical site infections following spinal surgery at a tertiary care center in Lebanon: incidence, microbiology, and risk factors. *Scand J Infect Dis* 2006; **38**: 589-592
40 **Malone DL**, Genuit T, Tracy JK, Gannon C, Napolitano LM. Surgical site infections: reanalysis of risk factors. *J Surg Res* 2002; **103**: 89-95
41 **Olsen MA**, Nepple JJ, Riew KD, Lenke LG, Bridwell KH, Mayfield J, Fraser VJ. Risk factors for surgical site infection following orthopaedic spinal operations. *J Bone Joint Surg Am* 2008; **90**: 62-69
42 **Pull ter Gunne AF**, Cohen DB. Incidence, prevalence, and analysis of risk factors for surgical site infection following adult spinal surgery. *Spine* (Phila Pa 1976) 2009; **34**: 1422-1428
43 **Goodson WH**, Hung TK. Studies of wound healing in experimental diabetes mellitus. *J Surg Res* 1977; **22**: 221-227
44 **Blam OG**, Vaccaro AR, Vanichkachorn JS, Albert TJ, Hilibrand AS, Minnich JM, Murphey SA. Risk factors for surgical site infection in the patient with spinal injury. *Spine* (Phila Pa 1976) 2003; **28**: 1475-1480
45 **Levi AD**, Dickman CA, Sonntag VK. Management of postoperative infections after spinal instrumentation. *J Neurosurg* 1997; **86**: 975-980
46 **Maragakis LL**, Cosgrove SE, Martinez EA, Tucker MG, Cohen DB, Perl TM. Intraoperative fraction of inspired oxygen is a modifiable risk factor for surgical site infection after spinal surgery. *Anesthesiology* 2009; **110**: 556-562
47 **Cammisa FP**, Girardi FP, Sangani PK, Parvataneni HK, Cadag S, Sandhu HS. Incidental durotomy in spine surgery. *Spine* (Phila Pa 1976) 2000; **25**: 2663-2667
48 **Fang A**, Hu SS, Endres N, Bradford DS. Risk factors for infection after spinal surgery. *Spine* (Phila Pa 1976) 2005; **30**: 1460-1465
49 **Olsen MA**, Mayfield J, Lauryssen C, Polish LB, Jones M, Vest J, Fraser VJ. Risk factors for surgical site infection in spinal surgery. *J Neurosurg* 2003; **98**: 149-155

50 **Glassman SD**, Dimar JR, Puno RM, Johnson JR. Salvage of instrumental lumbar fusions complicated by surgical wound infection. *Spine* (Phila Pa 1976) 1996; **21**: 2163-2169
51 National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control* 2004; **32**: 470-485
52 **Weiner BK**, Kilgore WB. Bacterial shedding in common spine surgical procedures: headlamp/loupes and the operative microscope. *Spine* (Phila Pa 1976) 2007; **32**: 918-920
53 **Bible JE**, O'Neill KR, Crosby CG, Schoenecker JG, McGirt MJ, Devin CJ. Microscope sterility during spine surgery. *Spine* (Phila Pa 1976) 2012; **37**: 623-627
54 **Shiono Y**, Watanabe K, Hosogane N, Tsuji T, Ishii K, Nakamura M, Toyama Y, Chiba K, Matsumoto M. Sterility of posterior elements of the spine in posterior correction surgery. *Spine* (Phila Pa 1976) 2012; **37**: 523-526
55 **Ahn DK**, Park HS, Choi DJ, Kim TW, Chun TH, Yang JH, Kim DG. The difference of surgical site infection according to the methods of lumbar fusion surgery. *J Spinal Disord Tech* 2012; **25**: E230-E234
56 **O'Toole JE**, Eichholz KM, Fessler RG. Surgical site infection rates after minimally invasive spinal surgery. *J Neurosurg Spine* 2009; **11**: 471-476
57 **Parker SL**, Adogwa O, Witham TF, Aaronson OS, Cheng J, McGirt MJ. Post-operative infection after minimally invasive versus open transforaminal lumbar interbody fusion (TLIF): literature review and cost analysis. *Minim Invasive Neurosurg* 2011; **54**: 33-37
58 **Cizik AM**, Lee MJ, Martin BI, Bransford RJ, Bellabarba C, Chapman JR, Mirza SK. Using the spine surgical invasiveness index to identify risk of surgical site infection: a multivariate analysis. *J Bone Joint Surg Am* 2012; **94**: 335-342
59 **O'Neill KR**, Smith JG, Abtahi AM, Archer KR, Spengler DM, McGirt MJ, Devin CJ. Reduced surgical site infections in patients undergoing posterior spinal stabilization of traumatic injuries using vancomycin powder. *Spine J* 2011; **11**: 641-646
60 **Sweet FA**, Roh M, Sliva C. Intrawound application of vancomycin for prophylaxis in instrumented thoracolumbar fusions: efficacy, drug levels, and patient outcomes. *Spine* (Phila Pa 1976) 2011; **36**: 2084-2088
61 **Kanayama M**, Oha F, Togawa D, Shigenobu K, Hashimoto T. Is closed-suction drainage necessary for single-level lumbar decompression?: review of 560 cases. *Clin Orthop Relat Res* 2010; **468**: 2690-2694
62 **Payne DH**, Fischgrund JS, Herkowitz HN, Barry RL, Kurz LT, Montgomery DM. Efficacy of closed wound suction drainage after single-level lumbar laminectomy. *J Spinal Disord* 1996; **9**: 401-403
63 **Brown MD**, Brookfield KF. A randomized study of closed wound suction drainage for extensive lumbar spine surgery. *Spine* (Phila Pa 1976) 2004; **29**: 1066-1068
64 **Gruenberg MF**, Campaner GL, Sola CA, Ortolan EG. Ultraclean air for prevention of postoperative infection after posterior spinal fusion with instrumentation: a comparison between surgeries performed with and without a vertical exponential filtered air-flow system. *Spine* (Phila Pa 1976) 2004; **29**: 2330-2334
65 **Dipaola CP**, Saravanja DD, Boriani L, Zhang H, Boyd MC, Kwon BK, Paquette SJ, Dvorak MF, Fisher CG, Street JT. Postoperative infection treatment score for the spine (PITSS): construction and validation of a predictive model to define need for single versus multiple irrigation and debridement for spinal surgical site infection. *Spine J* 2012; **12**: 218-230
66 **Thakkar RS**, Malloy JP, Thakkar SC, Carrino JA, Khanna AJ. Imaging the postoperative spine. *Radiol Clin North Am* 2012; **50**: 731-747
67 **Yu SJ**, Terriere LC. Metabolism of [14C]hydroprene (ethyl 3,7,11-trimethyl-2,4-dodecadienoate) by microsomal oxidases and esterases from three species of diptera. *J Agric Food Chem* 1977; **25**: 1076-1080
68 **Van Goethem JW**, Parizel PM, Jinkins JR. Review article: MRI of the postoperative lumbar spine. *Neuroradiology* 2002; **44**: 723-739
69 **Kang BU**, Lee SH, Ahn Y, Choi WC, Choi YG. Surgical site infection in spinal surgery: detection and management based on serial C-reactive protein measurements. *J Neurosurg Spine* 2010; **13**: 158-164
70 **Sponseller PD**, LaPorte DM, Hungerford MW, Eck K, Bridwell KH, Lenke LG. Deep wound infections after neuromuscular scoliosis surgery: a multicenter study of risk factors and treatment outcomes. *Spine* (Phila Pa 1976) 2000; **25**: 2461-2466
71 **Chang SD**, Lee MJ, Munk PL, Janzen DL, MacKay A, Xiang QS. MRI of spinal hardware: comparison of conventional T1-weighted sequence with a new metal artifact reduction sequence. *Skeletal Radiol* 2001; **30**: 213-218
72 **Olsen RV**, Munk PL, Lee MJ, Janzen DL, MacKay AL, Xiang QS, Masri B. Metal artifact reduction sequence: early clinical applications. *Radiographics* 2000; **20**: 699-712
73 **Eustace S**, Goldberg R, Williamson D, Melhem ER, Oladipo O, Yucel EK, Jara H. MR imaging of soft tissues adjacent to orthopaedic hardware: techniques to minimize susceptibility artefact. *Clin Radiol* 1997; **52**: 589-594
74 **Watanabe M**, Sakai D, Matsuyama D, Yamamoto Y, Sato M, Mochida J. Risk factors for surgical site infection following spine surgery: efficacy of intraoperative saline irrigation. *J Neurosurg Spine* 2010; **12**: 540-546
75 **Sierra-Hoffman M**, Jinadatha C, Carpenter JL, Rahm M. Postoperative instrumented spine infections: a retrospective review. *South Med J* 2010; **103**: 25-30
76 **Pappou IP**, Papadopoulos EC, Sama AA, Girardi FP, Cammisa FP. Postoperative infections in interbody fusion for degenerative spinal disease. *Clin Orthop Relat Res* 2006; **444**: 120-128
77 **Gerometta A**, Rodriguez Olaverri JC, Bitan F. Infections in spinal instrumentation. *Int Orthop* 2012; **36**: 457-464
78 **Ahmed R**, Greenlee JD, Traynelis VC. Preservation of spinal instrumentation after development of postoperative bacterial infections in patients undergoing spinal arthrodesis. *J Spinal Disord Tech* 2012; **25**: 299-302
79 **Viola RW**, King HA, Adler SM, Wilson CB. Delayed infection after elective spinal instrumentation and fusion. A retrospective analysis of eight cases. *Spine* (Phila Pa 1976) 1997; **22**: 2444-250; discussion 2444-2450
80 **Weiss LE**, Vaccaro AR, Scuderi G, McGuire M, Garfin SR. Pseudarthrosis after postoperative wound infection in the lumbar spine. *J Spinal Disord* 1997; **10**: 482-487
81 **Mitra A**, Mitra A, Harlin S. Treatment of massive thoracolumbar wounds and vertebral osteomyelitis following scoliosis surgery. *Plast Reconstr Surg* 2004; **113**: 206-213
82 **Dumanian GA**, Ondra SL, Liu J, Schafer MF, Chao JD. Muscle flap salvage of spine wounds with soft tissue defects or infection. *Spine* (Phila Pa 1976) 2003; **28**: 1203-1211
83 **Stall AC**, Becker E, Ludwig SC, Gelb D, Poelstra KA. Reduction of postoperative spinal implant infection using gentamicin microspheres. *Spine* (Phila Pa 1976) 2009; **34**: 479-483
84 **Seligson D**, Mehta S, Voos K, Henry SL, Johnson JR. The use of antibiotic-impregnated polymethylmethacrylate beads to prevent the evolution of localized infection. *J Orthop Trauma* 1992; **6**: 401-406
85 **Ostermann PA**, Seligson D, Henry SL. Local antibiotic therapy for severe open fractures. A review of 1085 consecutive cases. *J Bone Joint Surg Br* 1995; **77**: 93-97
86 **Silber JS**, Anderson DG, Vaccaro AR, Anderson PA, McCormick P. Management of postprocedural discitis. *Spine J* 2002; **2**: 279-287
87 **Lindholm TS**, Pylkkänen P. Discitis following removal of intervertebral disc. *Spine* (Phila Pa 1976) 1982; **7**: 618-622

88 **Tyler KL**. Acute pyogenic diskitis (spondylodiskitis) in adults. *Rev Neurol Dis* 2008; **5**: 8-13

89 **Tronnier V**, Schneider R, Kunz U, Albert F, Oldenkott P. Postoperative spondylodiscitis: results of a prospective study about the aetiology of spondylodiscitis after operation for lumbar disc herniation. *Acta Neurochir* (Wien) 1992; **117**: 149-152

90 **Chew FS**, Kline MJ. Diagnostic yield of CT-guided percutaneous aspiration procedures in suspected spontaneous infectious diskitis. *Radiology* 2001; **218**: 211-214

91 **Ozuna RM**, Delamarter RB. Pyogenic vertebral osteomyelitis and postsurgical disc space infections. *Orthop Clin North Am* 1996; **27**: 87-94

92 **Carragee EJ**. Pyogenic vertebral osteomyelitis. *J Bone Joint Surg Am* 1997; **79**: 874-880

93 **Visuri T**, Pihlajamäki H, Eskelin M. Long-term vertebral changes attributable to postoperative lumbar discitis: a retrospective study of six cases. *Clin Orthop Relat Res* 2005; (433): 97-105

94 **Ito M**, Abumi K, Kotani Y, Kadoya K, Minami A. Clinical outcome of posterolateral endoscopic surgery for pyogenic spondylodiscitis: results of 15 patients with serious comorbid conditions. *Spine* (Phila Pa 1976) 2007; **32**: 200-206

95 **Przybylski GJ**, Sharan AD. Single-stage autogenous bone grafting and internal fixation in the surgical management of pyogenic discitis and vertebral osteomyelitis. *J Neurosurg* 2001; **94**: 1-7

96 **Pee YH**, Park JD, Choi YG, Lee SH. Anterior debridement and fusion followed by posterior pedicle screw fixation in pyogenic spondylodiscitis: autologous iliac bone strut versus cage. *J Neurosurg Spine* 2008; **8**: 405-412

97 **Kuklo TR**, Potter BK, Bell RS, Moquin RR, Rosner MK. Single-stage treatment of pyogenic spinal infection with titanium mesh cages. *J Spinal Disord Tech* 2006; **19**: 376-382

98 **Toulson C**, Walcott-Sapp S, Hur J, Salvati E, Bostrom M, Brause B, Westrich GH. Treatment of infected total hip arthroplasty with a 2-stage reimplantation protocol: update on "our institution's" experience from 1989 to 2003. *J Arthroplasty* 2009; **24**: 1051-1060

99 **Westrich GH**, Walcott-Sapp S, Bornstein LJ, Bostrom MP, Windsor RE, Brause BD. Modern treatment of infected total knee arthroplasty with a 2-stage reimplantation protocol. *J Arthroplasty* 2010; **25**: 1015-1021, 1015-1021

100 **Volin SJ**, Hinrichs SH, Garvin KL. Two-stage reimplantation of total joint infections: a comparison of resistant and non-resistant organisms. *Clin Orthop Relat Res* 2004; : 94-100

101 **Weinstein MP**, Stratton CW, Hawley HB, Ackley A, Reller LB. Multicenter collaborative evaluation of a standardized serum bactericidal test as a predictor of therapeutic efficacy in acute and chronic osteomyelitis. *Am J Med* 1987; **83**: 218-222

102 **Liu C**, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, Kaplan SL, Karchmer AW, Levine DP, Murray BE, J Rybak M, Talan DA, Chambers HF. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children: executive summary. *Clin Infect Dis* 2011; **52**: 285-292

S- Editor Huang XZ **L- Editor** A **E- Editor** Xiong L

World Journal of Orthopedics

Online Submissions: http://www.wjgnet.com/esps/
wjo@wjgnet.com
doi:10.5312/wjo.v3.i11.190

World J Orthop 2012 November 18; 3(11): 190-198
ISSN 2218-5836 (online)

REVIEW

Energy metabolism and the skeleton: Reciprocal interplay

Patrizia D'Amelio, Anna Panico, Elena Spertino, Giovanni Carlo Isaia

Patrizia D'Amelio, Anna Panico, Elena Spertino, Giovanni Carlo Isaia, Department of Surgical and Medical Disciplines, Gerontology Section, University of Torino, 10126 Torino, Italy
Author contributions: All authors contributed equally to this article.
Correspondence to: Elena Spertino, MD, Department of Surgical and Medical Disciplines, Gerontology Section, University of Torino, Cso Bramante 88, 10126 Torino, Italy. elenaspertino@gmail.com
Telephone: +39-11-6337140 Fax: +39-11-6961045
Received: March 14, 2011 Revised: July 31, 2012
Accepted: October 20, 2012
Published online: November 18, 2012

Abstract

The relation between bone remodelling and energy expenditure is an intriguing, and yet unexplained, challenge of the past ten years. In fact, it was only in the last few years that the skeleton was found to function, not only in its obvious roles of body support and protection, but also as an important part of the endocrine system. In particular, bone produces different hormones, like osteocalcin (OC), which influences energy expenditure in humans. The undercarboxylated form of OC has a reduced affinity for hydroxyapatite; hence it enters the systemic circulation more easily and exerts its metabolic functions for the proliferation of pancreatic β-cells, insulin secretion, sensitivity, and glucose tolerance. Leptin, a hormone synthesized by adipocytes, also has an effect on both bone remodelling and energy expenditure; in fact it inhibits appetite through hypothalamic influence and, in bone, stimulates osteoblastic differentiation and inhibits apoptosis. Leptin and serotonin exert opposite influences on bone mass accrual, but several features suggest that they might operate in the same pathway through a sympathetic tone. Serotonin, in fact, acts *via* two opposite pathways in controlling bone remodelling: central and peripheral. Serotonin product by the gastrointestinal tract (95%) augments bone formation by osteoblast, whereas brain-derived serotonin influences low bone mineral density and its decrease leads to an increase in bone resorption parameters. Finally, amylin (AMY) acts as a hormone that alters physiological responses related to feeding, and plays a role as a growth factor in bone. *In vitro* AMY stimulates the proliferation of osteoblasts, and osteoclast differentiation. Here we summarize the evidence that links energy expenditure and bone remodelling, with particular regard to humans.

Key words: Leptin; Osteocalcin; Serotonin; Amylin; Bone mass; Energy metabolism

Peer reviewer: Belinda R Beck, PhD, Associate Professor, School of Physiotherapy and Exercise Science, Gold Coast Campus, Griffith University, Qld 4222, Australia

D'Amelio P, Panico A, Spertino E, Isaia GC. Energy metabolism and the skeleton: Reciprocal interplay. *World J Orthop* 2012; 3(11): 190-198 Available from: URL: http://www.wjgnet.com/2218-5836/full/v3/i11/190.htm DOI: http://dx.doi.org/10.5312/wjo.v3.i11.190

INTRODUCTION

Every part of the human body communicates and cooperates with each other in a specific way, and with unique functions, and bone is not an exception. The skeleton was considered for a long time just a "stone" with movement function, a reserve of minerals, and the home of the hematopoietic system; only in recent years has the idea that it is in deep contact with other systems, such as the immune and cardiovascular systems, been developed[1,2]. More recently, the skeleton's ability to regulate energy expenditure has been described, and bone is now also considered as an endocrine organ.

An important feature of hormonal regulation is that there are some cells, controlled by a feedback loop, that produce hormones; these hormones send specific signals to other cells and are responsible for several functions in the human organism. Bone is a target for different hormones that regulate both bone metabolism and re-

modeling through a central control. The fact that energy metabolism affects bone mass accrual by acting through a neuronal relay on one cell type, the osteoblasts, raised the testable hypothesis that, in turn, the osteoblasts might secrete one or more hormones affecting energy metabolism[3].

The skeleton, in particular, secretes two hormones: fibroblast growth factor 23 and osteocalcin (OC); OC is an osteoblast-specific protein that influences pancreatic β-cell proliferation, insulin expression, secretion, sensitivity, and energy expenditure[4]. Mice knock-out for OC, created by Ducy *et al*[5], appear mildly hyperglycaemic and have slightly increased visceral fat; the opposite phenotype null for the *Esp* gene, which encodes a tyrosine phosphatase that hampers glucose metabolism by inhibiting OC functions, instead displays improved glucose tolerance. An even more intimate relationship between skeleton and energy metabolism was demonstrated by recent genetic experiments that found that leptin, an adipocyte-derived hormone, inhibits insulin secretion by decreasing the production of undercarboxylated osteocalcin, and is also involved in osteoblast differentiation[6]. Moreover, serotonin, which is produced by neurons of the brainstem and by the enterochromaffin cells of the duodenum, controls bone remodeling[7]. The relationship between energy expenditure and bone is still controversial; studies on humans are few, and the majority of data have been derived by animal models.

This review aims to summarize the evidence linking energy metabolism and skeleton, with particular attention to humans.

CENTRAL CONTROL OF BONE MASS: LEPTIN

Data in animals

Leptin, the protein product of the obese gene (*Ob* or *Lep*), is a hormone synthesized by adipocytes that signals available energy reserves to the brain, and thereby influences development, growth, metabolism, and reproduction. In mammals, leptin functions as a signal for fat reserves: circulating leptin fluctuates in proportion to fat mass, and acts on the hypothalamus to suppress food intake[8].

When adipose tissue is abundant, leptin levels rise as a result energy expenditure and sympathetic activity increases. In comparison, when adipocyte mass decreases, energy expenditure, temperature, and reproductive function are down-regulated. As proof of this fact, *Ob-/Ob-* mice are obese, hypogonadal, and diabetic[9]. *Ob* genes were recently isolated from several fish and two amphibian species. While vertebrate leptins largely differ in their primary amino acid sequences, they have similar tertiary structures and potencies when tested *in vitro* on heterologous leptin receptors (LepRs)[8,10-12]. Leptin acts through a leptin receptor that is a member of the type I cytokine receptor family[13]. There are different isoforms of this receptor that are produced by alternative splicing of the transcript from the LepR gene, defined as: LepRa, LepRb, LepRc, LepRd and LepRf; these isoforms have in common an extracellular domain of 800 amino acids and a transmembrane domain of 34 amino acids, although the intracellular domain is variable and characteristic for each of the isoforms; in particular, LepRb seems to be suitable for all leptin actions[13-15]. In fact, in mammals, LepRb is highly expressed in the hypothalamus and at lower levels in several other tissues, including the liver, kidney, lung, stomach, pancreatic cells, and immune cells[16-20]. Leptin's role in energy balance/body weight control is mediated by LepRb expressed in the brain[21,22]. Leptin binds to LepRs in the plasma membrane of this specific cell, activating several intracellular signaling pathways[23].

Vertebrate LepRs signals *via* the Janus kinase (Jak) and signal transducer, and is the activator of the transcription (STAT) pathway. Three tyrosine residues located within the LepR cytoplasmic domain are phosphorylated by Jak2, and are constitutively associated with mouse LepRb at membrane-proximal residues located within the cytoplasmic domain and are required for the activation of SH2-containing tyrosine phosphatase-2, STAT5, and STAT3 signaling[8]. These tyrosines are conserved from fish to mammals, demonstrating their critical role in signaling by LepR.

Leptin can also be considered as a growth factor, with the ability to directly enhance the development of hemopoietic precursor cells, myoblast-like cells, and lung cells. Moreover, Kume *et al*[2] observed that leptin has angiogenic effects on vascular musculoskeletal endothelial cells. This could be critical during fetal development; in fact, leptin and its receptor are produced by the human placenta[1,24]. Both leptin and its receptors were found in murine cartilage and bone, especially in chondrocytes near the vascular system. This observation may explain the angiogenic properties of leptin[2]. In addition, leptin increases both the proliferation and differentiation of the chondrocyte population of skeletal growth centers in organ cultures through the insulin-like growth factors (IGF) and the regulation of receptor IGF expression[25,26].

Dixit *et al*[27] showed that leptin is a potent stimulator of growth hormone secretion, both at the central pituitary level and at the peripheral level, from lymphocytes. Experimentally, leptin has a positive effect on bone mass when infused intravenously, but a negative one after intracerebroventricular administration[28,29]. These opposite effects of leptin were brilliantly demonstrated by Thomas[30] using a parabiosis experiment. Further experiments demonstrated that leptin inhibits appetite through the arcuate nucleus, and bone mass through the ventromedial hypothalamus nucleus. These experiments indicate that hypothalamic integrity is required in bone regulation[29,31]. Different studies, using a human stromal cell line, demonstrated that cells of osteoblastic lineage are targets for leptin action, as they actively expressed both forms of leptin receptors[32-35].

Although leptin plays a critical role in starvation-induced T-cell-mediated immunosuppression, little is known about its role in B-cell homeostasis under starvation conditions. A Japanese study show the alteration of B-cell development in the bone marrow of fasted mice, characterized by a decrease in pro-B, pre-B, and immature B cells, and an increase in mature B cells. Interestingly, an intracerebroventricular leptin injection was sufficient to prevent the alteration of B-cell development in fasted mice[36].

Data in humans

In humans there are some reports linking leptin with bone mass, even if studies in humans are biased by confounding factors. Some data obtained using animal models were confirmed by human studies, and it is generally accepted that body weight is a major determinant of bone density; in fact, obesity is generally accompanied by increased bone strength and obese persons have stronger bones and lose bone tissue at a slower pace[37]. Serum leptin levels positively correlate with the mass of adipose tissue, and show a weak correlation with bone density in humans[38]. Clinical studies on animals and humans show that leptin access to the hypothalamic centre, which has a negative effect on appetite and bone mass, is limited by the blood brain barrier[37-39]. This access implies a saturable transport system involving the LepRa receptors (with a shorter intracellular domain than that of its effective receptor LepRb)[39]. Renal failure increases the leptin serum level above the concentration which may lead to saturation of leptin transport to the brain[38]. In fact, Ghazali *et al*[38] showed, in an hemodialysis population, that only when the serum leptin levels are above this threshold is there is a sparing effect in bone.

Stimulated by animal studies that describe the relationship between a lack of leptin in mice and low sympathetic tone, the pathway of leptin's indirect control of bone mass has also been investigated in humans[40]. Visitsunthorn *et al*[41] observed that human reflex sympathetic dystrophy is characterized by a rapid onset of osteoporosis in the affected region, with labile vasomotor activity, trophic skin changes, pain, and swelling, because of deregulated sympathetic tone. In some cases, β-blockers resolve reflex sympathetic dystrophy-associated symptoms and osteopenia. Outside the context of reflex sympathetic dystrophy, people receiving β-blockers experience 24%-32% reductions in the risk of fractures, as shown in several large studies[42-45]. Schlienger *et al*[46] suggest that use of β-blockers is associated with a reduced risk of fractures, taken alone or in combination with thiazide diuretics. Thomas[30] observed that, in human cell cultures, leptin induced activation of the mitogen-activated protein kinase cascade could be critical, because it stimulated both osteoblastic differentiation from bone marrow precursors and phosphorylation of peroxisome proliferator-activated receptor-γ, which has been shown to inhibit adipogenesis[47,48]. In addition, leptin could enhance osteoblastic activity by inhibiting apoptosis, stimulating mineralization, and inhibiting support of osteoclastogenesis, as shown in primary human osteoblast cultures[49]. Through direct positive effects on osteoblast differentiation, leptin might modulate bone remodelling. It has also recently been shown in human stromal cells that leptin inhibits the expression of the receptor activator of nuclear factor-κB-ligand, the major downstream cytokine controlling osteoclastogenesis[50].

Leptin serum levels have different effects in different human demographics. In premenopausal women, a higher proportion of fat and a higher leptin concentration are negatively associated with bone mass[51]. Interestingly, in postmenopausal women, leptin levels were significantly lower in women with vertebral fractures than those without, and an increase in fat mass negatively predicts fracture presence[52]. A recent study showed that obese children have altered bone turnover[53]. Conversely, Farooqi *et al*[54] reported in three obese children congenitally deficient in leptin, that whole-body bone mineral content (BMC) and bone mineral density (BMD) were normal for their age and gender, despite very high weight and advanced bone ossification. After leptin therapy administered for up to four years, BMC, BMD, and skeletal maturation increased normally, although weight and fat mass dramatically decreased, suggesting counteracting and beneficial effects of leptin therapy on the skeleton[54]. Although these different studies converge to support the role of leptin as a regulator of bone metabolism, understanding the complexity of its multiple pathways to the skeleton requires further investigation.

SEROTONIN AND ITS TWO IDENTITIES

Production and secretion

Serotonin plays a major role in controlling bone remodelling *via* two distinctly opposite pathways; in fact, it is synthesized by two different genes and plays an antagonist function on bone mass[55]. The major site (95%) of serotonin production is the gastrointestinal tract by the tryptophan hydroxylase (*Tph1*) gene[56]. The importance of gut-derived serotonin was identified recently, thanks to studies on the lipoprotein receptor-related proteins 5 (Lrp5) receptor, a member of the low density lipoprotein receptor family; the signal mediated by Lrp5 in an unknown cell type increase bone formation by osteoblasts[57]. Brain-derived serotonin produced by the *Tph2* gene also influences bone mass, and the severe low bone mass observed in the absence of Tph2 results from an effect on both bone resorption and formation, mediated by an increased sympathetic tone. In the brain, synthesis of serotonin by neurons which express the leptin receptor is negatively controlled by leptin through its effects on Tph2 expression[58]. Patients taking synthetic serotonin reuptake inhibitors chronically (a class of drugs increasing extracellular serotonin concentration throughout the body) have reduced bone mass[59].

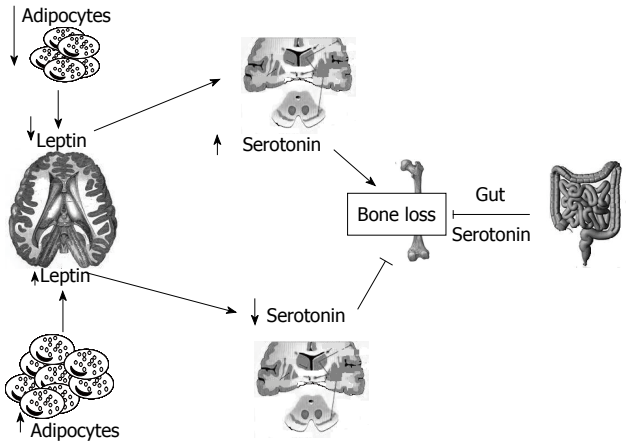

Figure 1 Schematic representation of the relationship between fat tissue, brain, gut, and bone mediated by leptin and serotonin.

Energy expenditure and serotonin

The signalling of serotonin to bone is attributed to different receptors: Htr1b signalling decreased bone formation, in contrast with Htr2c which inhibits the synthesis of epinephrine and has a decreased sympathetic tone; thus, this results in increased formation and decreased bone resorption[55]. The decrease in bone formation and the increase in bone reabsorption in Tph2-/-mice mirrors the phenotype of 2 adrenergic receptor knocked-out mice. This feature suggested that the bone phenotype of the mice lacking serotonin in the brain could be secondary to an increase in the sympathetic signal in osteoblasts[60].

Serotonin absence in the brain resulted in a phenotype with severe low bone mass, affecting axial and appendicular skeleton, while bone length and width were unaffected[7]. This was secondary to a decrease in bone formation parameters (osteoblast numbers and bone formation rate) and to an increase in bone resorption parameters (osteoclast surface and circulating levels of deoxypridinoline, a degradation product of type I collagen and a biomarker of bone resorption)[61]. Even if leptin and serotonin exert opposite influences on bone mass accrual, several features suggested that they might operate in the same pathway: for instance serotonin, like leptin, regulates bone mass through their action on sympathetic tone and requires ventromedial hypothalamic neuron integrity to achieve its functions[7]. This fact raised the prospect that axonal projections emanating from Tph2- expressing neurons reach arcuate nuclei to regulate these functions[7]. Analysis verified that neurons of the arcuate nuclei were target by serotoninergic innervation emanating from the brainstem, an observation confirmed in Tph2+/-mice by retrograde labelling of the projections reaching the serotoninergic neurons of the brainstem[7]. Experimental evidence supports the notion that the appetite phenotype of the Tph 2-/-mice was caused, at least in part, by an increase in melanocortin signaling mediated through the Htr1a and Htr2b receptors, and involves melanocortin signaling[7]. Several reasons led us to ask whether the appetite and energy expenditure phenotypes of the Ob-/-mice were serotonin dependent: the first is that the conjunction of a decrease in appetite and an increase in energy expenditure is the mirror image of what is seen in mice lacking leptin signaling, the second is that leptin inhibition of serotonin synthesis in the brainstem is the mechanism used by this hormone to inhibit bone mass accrual, and the third is that no molecular mechanism has been identified so far to explain the common control of bone mass and energy metabolism[7]. Figure 1 summarizes the relationships between leptin and serotonin.

Lrp5 and bone formation

One of the most studied regulators of bone remodelling is low-density lipoprotein (LDL)-Lrp5, which a loss of function mutation causes osteoporosis pseudoganglioma (OPPG), a rare disease characterized by decreased bone formation and blindness[62], while activating mutations causing high bone mass syndrome[57]. Lrp5 can enhance Wnt (the vertebrate homolog of Wingless in Drosophila) and canonical signaling in cultured cells. The blindness observed in OPPG patients and Lrp5-/-mice is caused by the deregulation of Wnt canonical signaling during eye development[63]. Binding of Wnt to Frizzled (Fz) receptors, expressed by osteoblasts, causes intracellular β-catenin stabilization. In cooperation with lymphoid enhancer factor/T cell factor transcription factors, β-catenin activates transcription of osteoprotegerin (OPG), a cytokine secreted by osteoblasts that decreases bone resorption.

Inactivation of Lrp5 and activation of β-catenin, the molecular node of Wnt signalling, affects different transcriptomes in osteoblasts[63]. Lastly, inactivation of Lrp5 in osteoblast progenitors does not influence bone homeostasis, whereas inactivation of canonical Wnt signaling does[64]. Taken together, these observations suggest that Lrp5 and canonical Wnt signaling use different mechanisms to regulate osteoblast functions. It is assumed that Lrp5 is a coreceptor for Wnt proteins[65]; as a result, OPPG and high bone mass syndrome are viewed as Wnt-related diseases[66]. Some observations, however, change this view. Firstly, there is no overt skeletal defect in Lrp5-/- embryos; secondly, a function gain mutation in Lrp5 does not cause bone tumors as the activation of Wnt signaling does in other organs[67]; and lastly, osteoblast-specific loss and a function gain mutation in β-catenin, the molecular node of canonical Wnt signaling, does not affect either bone formation or the expression of genes deregulated upon Lrp5 inactivation. Analyses of a microarray experiment comparing bones from Lrp5-/- and wild type littermate mice provided the completely unexpected clue that the gene most highly overexpressed in Lrp5 deficient bone was Tph1, for which expression in the gut is increased in the absence of Lrp5, as are serum serotonin levels in Lrp5 deficient patients or mice[68].

The only genes whose expression was decreased in Lrp-/-mice bones were the regulators of cell proliferation *CicD1, D2* and *E1*[69]. Lrp5-/-osteoblasts proliferated as well as wild-type cell *ex vivo*, and the discrepancy between the *in vivo* and *ex vivo* proliferation abilities of the

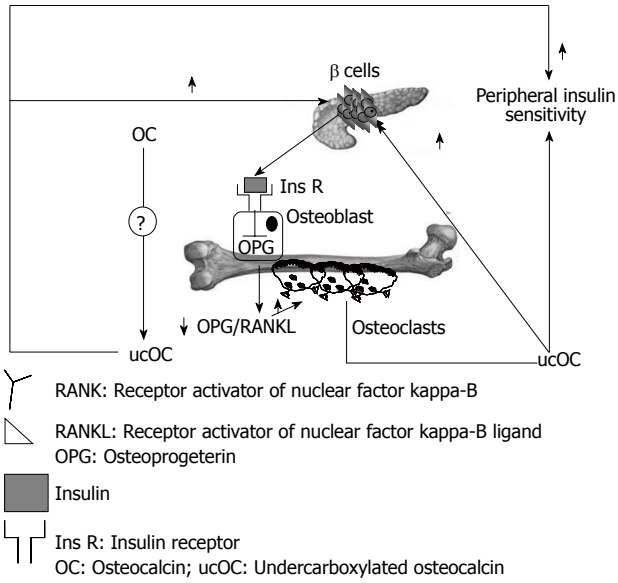

Figure 2 Schematic representation of the interaction between bone and glucose tolerance mediated by insulin and undercarboxylated osteocalcin.

Lrp5-/-osteoblasts indicated that Lrp5 loss of function mutations affected osteoblast proliferation through extracellular signals that can not originate from osteoblasts; in other words Lrp-5 related bone diseases may not originate from bones[69].

HOW BONE CONTROLS ENERGETIC METABOLISM: OC

OC is a 5 kDa protein produced by the skeleton and is the most prevalent non-collagenous protein in bone[70]. It has several features as a hormone, but moreover it represents one of the most important links between bone tissue and energy metabolism. OC is one of the very few osteoblasts-specific proteins, and it is subject to post-translational carboxylation on three or four glutamic residues, depending on the species.

Vitamin K is a co-factor for the enzyme glutamate carboxylase, required for carboxylation of the Gla-containing proteins in the coagulation cascade and for carboxylation of OC[71]. Lower dietary levels of vitamin K are associated with increased levels of undercarboxylated osteocalcin (ucOC), and vitamin K supplementation reduces ucOC[72]. Warfarin, an anti-coagulant which action is based on inhibition of the vitamin K dependent carboxylase, also regulates mRNA expression of OC, and this fact makes interpretation of warfarin treatment studies more complex[73]. Decarboxylation allows the molecule to tightly bind the calcium ions in hydroxyapatite[74-76]; ucOC has a reduced avidity for hydroxyapatite, and so it enters the systemic circulation more easily[77]. There is a feed forward regulation loop that links insulin, bone resorption, and OC. Insulin signaling in OPG expression and the decrease in the OPG/receptor activator of the nuclear factor-kappa B ligand ratio results in an increased acidification of the resorption lacunae. The acidic pH is sufficient to activate the OC molecules stored in the bone extracellular matrix. The ucOC promotes insulin sensitivity in peripheral organs and stimulates insulin secretion by pancreatic β-cells (Figure 2).

Work by Karsenty *et al*[77] suggested that bone could influence glucose homeostasis by acting as an endocrine organ; this concept came from the observation that mice which were OC deficient were not only fat, but also had higher blood glucose, lower serum insulin, impaired glucose-stimulated insulin secretion, and poor glucose tolerance as compared to wild type mice. These observations remained unexplained for some years until the same investigators, in the course of experiments in which they were ablating bone-specific proteins in mice, noted the opposite phenotype in mice null for *Esp* gene[78], which encodes an osteotesticular protein tyrosine phosphatase (OST-PTP) that hampers glucose metabolism by inhibiting OC endocrine functions.

When Esp-/- were bred, a considerable number of deaths in newborns were observed, which resulted from severe hypoglycemia[77]. Studies of surviving mice showed increased pancreatic cell size, β-cells number, circulating insulin levels and sensitivity, decreased body fat, and increased expression of insulin target genes in the liver and muscles[77]. This phenotype was identical in global knock-out and osteoblast specific Esp knock-out mice, and opposite to OC null mice. OC-/-mice have increased visceral fat and glucose intolerance, decreased insulin levels, islet cell proliferation, and insulin content, similarly to mice over-expressing OST-PTP in osteoblasts. These findings suggest osteoblasts as a source of a humoral factor that influences energy metabolism[77]. *In vivo*, OC can favor proliferation of pancreatic β-cells, insulin, adiponectin expression in β-cells, and adipocytes[77]. In humans, the insulin receptor is a substrate of OST-PTP, the protein encoded by Esp. This raised the testable hypothesis that PTP-1B expressed in human osteoblasts could be the functional human homologue of the *Esp* gene[77]. Elevated levels of both carboxylated and undercarboxylated forms of OC were associated with improved glucose tolerance in healthy men given an oral glucose load[77].

In older healthy men, serum OC concentrations were inversely associated with blood markers of the dysmetabolic phenotype and measures of adiposity[79]. There is no univocal explanation of how parathyroid hormone (PTH) influences glucose metabolism in humans and mice, but it has been observed that hyperparathyroidism could impair glucose tolerance through a different mechanism, such as an increased intracellular free calcium concentration (which decreases insulin sensitivity by decreasing insulin-dependent glucose transport)[80,81], or decreased plasma phosphate levels (which decrease insulin sensitivity, as insulin-dependent glucose uptake is closely related to phosphate uptake)[82], or down regulation of insulin receptors, or PTH *per se*[83]. The administration of intermittent subcutaneous PTH (1-34 Teriparatide or 1-84) has been recently available for osteoporosis treatment[84,85].

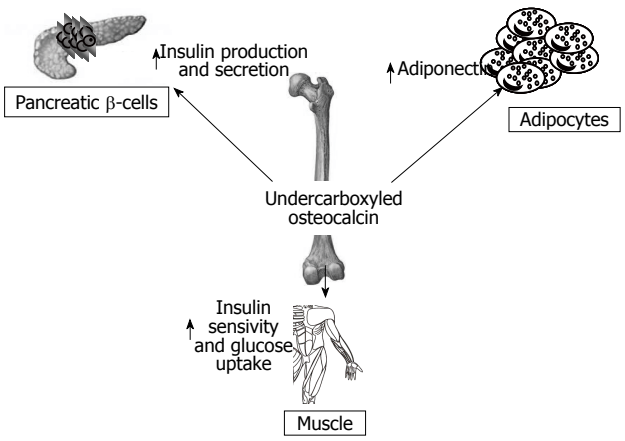

Figure 3 Schematic representation of the multiple interactions between pancreatic β-cells, adipocytes, muscle cells, and bone mediated by undercarboxylated osteocalcin.

The intermittent administration of this molecule in osteoporotic patients has an anabolic effect on the skeleton in contrast with the catabolic effect of continuous PTH excess in hyperparathyroidism. It has been previously reported that there is either an acute, subclinical adverse effect of PTH 1-34 on stimulated glucose levels[86] or no effect of this hormone on glucose tolerance[87].

It is known the ability of the treatment with intermittent PTH is to modify the secretion of OC from the osteoblasts and, at the time of writing, many studies been set to established if the secretion of ucOC is influenced by the therapy and if, consequently, therapy with PTH can interfere with the ability of the skeleton to regulate energetic metabolism. Schafer *et al*[88] investigated whether changes in ucOC during osteoporosis treatment with PTH are associated with changes in metabolic parameters. They found that not only the median total and undercarboxylated levels increased with PTH 1-84 treatment, but also the body weight and fat mass decreased, and this change was positively correlated with a change in adiponectin. Pittas *et al*[79] reported that in older adults, total serum OC was inversely associated with body fat, fasting glucose, and fasting insulin. In a cohort of men and postmenopausal women with type 2 diabetes mellitus, undercarboxylate osteocalcin inversely correlated with percentage trunk fat and haemoglobin A1c[89].

AMYLIN IN THE PERIPHERY

Amylin (AMY) is a 37-amino acid peptide that belongs to the calcitonin (CT) family and has evolutionary links with insulin. It is co-secreted with insulin by pancreatic β-cells and has been considered a partner peptide in the etiology of diabetes-associated complications and related conditions[90]. While the soluble monomeric form of AMY acts as a hormone that alters physiological responses related to feeding and acts as a growth factor, the less soluble and insoluble polymeric forms may contribute to the establishment of a pathophysiological pathway to overt diabetes[90]. Research into the potential effect of AMY on BMD followed the observation that a large number of diabetic people are osteopenic. *In vitro* AMY acted as a grow factor in bone for the proliferation of osteoblasts[91], and recently it was demonstrated that it also acts in osteoclast differentiation[92].

In foetal rat osteoblasts, intact AMY and 1-8 AMY stimulates cell proliferation, but AMY 8-37, COOH terminally deaminated AMY and reduced AMY, by acting in an antagonist manner[93]. In osteoblasts, AMY acts through a increase of cyclic adenosine monophosphate and the activation of mitogen-activated protein kinase and protein kinase C[93]. Data on humans are lacking in the literature; it is known that aging is associated with impairment of AMY release from pancreatic beta cells, but further studies are needed to verify this[94]. It is also known that aging is associated with an impairment of AMY release from pancreatic β-cells[95]. In previous studies it was demonstrated that there were significantly lower unreduced AMY plasma levels in patients with osteoporosis than in those with type Ⅱ DM and healthy controls[96].

More recently, the analyses of calcitonin-related gene-deletion mouse models have demonstrated that AMY is a factor that inhibits osteoclastogenesis and reduces the rate of osteolysis[97-99]. CT was shown to decrease osteoclast acidification and is also able to inhibit acid phosphatase secretion[100]. *CT* gene-related peptides α and β, produced by alternative splicing of the *CT* gene, have dual roles: prevention of bone reabsorption in hypercalcemic states and regulation of bone formation. On the other hand, there is an increase in the rate of bone formation that seems to contradict previous findings concerning the activity of osteoclasts[90].

CONCLUSION

Here we summarize numerous studies that demonstrate a deep interaction between the skeleton, glucose, and energy metabolism (Figure 3). Many studies show that bone shares hormonal and molecular pathways with glucose and fat metabolism. The skeleton is subjected to various influences from fat tissue and glucose metabolism and is able to regulate these two systems in turn. Bone must therefore be considered as an endocrine organ with multiple functions, and not only a support for muscles. In the recent years this role has been confirmed in humans, and some studies, although controversial, demonstrate a correlation between bone endocrine function, body fat distribution and percentage, and glucose metabolism.

REFERENCES

1 **Sierra-Honigmann MR**, Nath AK, Murakami C, García-Cardeña G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. *Science* 1998; **281**: 1683-1686

2 **Kume K**, Satomura K, Nishisho S, Kitaoka E, Yamanouchi K, Tobiume S, Nagayama M. Potential role of leptin in endochondral ossification. *J Histochem Cytochem* 2002; **50**: 159-169

3 **Clemens TL**, Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. *J Bone Miner Res* 2011; **26**: 677-680
4 **Fukumoto S**, Martin TJ. Bone as an endocrine organ. *Trends Endocrinol Metab* 2009; **20**: 230-236
5 **Ducy P**, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G. Increased bone formation in osteocalcin-deficient mice. *Nature* 1996; **382**: 448-452
6 **Shi Y**, Yadav VK, Suda N, Liu XS, Guo XE, Myers MG, Karsenty G. Dissociation of the neuronal regulation of bone mass and energy metabolism by leptin in vivo. *Proc Natl Acad Sci USA* 2008; **105**: 20529-20533
7 **Yadav VK**, Oury F, Suda N, Liu ZW, Gao XB, Confavreux C, Klemenhagen KC, Tanaka KF, Gingrich JA, Guo XE, Tecott LH, Mann JJ, Hen R, Horvath TL, Karsenty G. A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. *Cell* 2009; **138**: 976-989
8 **Denver RJ**, Bonett RM, Boorse GC. Evolution of leptin structure and function. *Neuroendocrinology* 2011; **94**: 21-38
9 **Tartaglia LA**, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; **83**: 1263-1271
10 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432
11 **Doyon C,** Trudeau VL, Hibbert BM, Howes LA, Moon TW, mRNA analysis in flattened fauna: obtaining gene-sequence information from road-kill and game-hunting samples. *Can J Zool* 2003; **81**: 692-698
12 **Doyon C**, Drouin G, Trudeau VL, Moon TW. Molecular evolution of leptin. *Gen Comp Endocrinol* 2001; **124**: 188-198
13 **Tartaglia LA**. The leptin receptor. *J Biol Chem* 1997; **272**: 6093-6096
14 **Löllmann B**, Grüninger S, Stricker-Krongrad A, Chiesi M. Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and, e in different mouse tissues. *Biochem Biophys Res Commun* 1997; **238**: 648-652
15 **Chua SC**, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science* 1996; **271**: 994-996
16 **Ikejima K**, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, Lang T, Fukuda T, Yamashina S, Kitamura T, Sato N. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology* 2002; **122**: 1399-1410
17 **Fei H**, Okano HJ, Li C, Lee GH, Zhao C, Darnell R, Friedman JM. Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proc Natl Acad Sci USA* 1997; **94**: 7001-7005
18 **Sobhani I**, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Laigneau JP, Attoub S, Lehy T, Henin D, Mignon M, Lewin MJ. Leptin secretion and leptin receptor in the human stomach. *Gut* 2000; **47**: 178-183
19 **Covey SD**, Wideman RD, McDonald C, Unniappan S, Huynh F, Asadi A, Speck M, Webber T, Chua SC, Kieffer TJ. The pancreatic beta cell is a key site for mediating the effects of leptin on glucose homeostasis. *Cell Metab* 2006; **4**: 291-302
20 **Lord GM**, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; **394**: 897-901
21 **Cohen P**, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P, Friedman JM. Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 2001; **108**: 1113-1121
22 **Villanueva EC**, Myers MG. Leptin receptor signaling and the regulation of mammalian physiology. *Int J Obes (Lond)* 2008; **32 Suppl 7**: S8-12
23 **Liongue C**, Ward AC. Evolution of Class I cytokine receptors. *BMC Evol Biol* 2007; **7**: 120
24 **Masuzaki H**, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T, Mise H, Nishimura H, Yoshimasa Y, Tanaka I, Mori T, Nakao K. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med* 1997; **3**: 1029-1033
25 **Maor G**, Rochwerger M, Segev Y, Phillip M. Leptin acts as a growth factor on the chondrocytes of skeletal growth centers. *J Bone Miner Res* 2002; **17**: 1034-1043
26 **Nakajima R**, Inada H, Koike T, Yamano T. Effects of leptin to cultured growth plate chondrocytes. *Horm Res* 2003; **60**: 91-98
27 **Dixit VD**, Mielenz M, Taub DD, Parvizi N. Leptin induces growth hormone secretion from peripheral blood mononuclear cells via a protein kinase C- and nitric oxide-dependent mechanism. *Endocrinology* 2003; **144**: 5595-5603
28 **Ducy P**, Amling M, Takeda S, Priemel M, Schilling AF, Beil FT, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell* 2000; **100**: 197-207
29 **Takeda S**, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell* 2002; **111**: 305-317
30 **Thomas T**. The complex effects of leptin on bone metabolism through multiple pathways. *Curr Opin Pharmacol* 2004; **4**: 295-300
31 **Balthasar N,** Coppari R, McMinn J, Liu SM, Lee CE, Tang V, Kenny CD, McGovern RA, Chua SC Jr, Elmquist JK, Lowell BB.. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron* 2004; **42**: 983-991
32 **Balthasar N**, Coppari R, McMinn J, Liu SM, Lee CE, Tang V, Kenny CD, McGovern RA, Chua SC, Elmquist JK, Lowell BB. Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. *Neuron* 2004; **42**: 983-991
33 **Reseland JE**, Syversen U, Bakke I, Qvigstad G, Eide LG, Hjertner O, Gordeladze JO, Drevon CA. Leptin is expressed in and secreted from primary cultures of human osteoblasts and promotes bone mineralization. *J Bone Miner Res* 2001; **16**: 1426-1433
34 **Cornish J**, Callon KE, Bava U, Lin C, Naot D, Hill BL, Grey AB, Broom N, Myers DE, Nicholson GC, Reid IR. Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. *J Endocrinol* 2002; **175**: 405-415
35 **Lee YJ**, Park JH, Ju SK, You KH, Ko JS, Kim HM. Leptin receptor isoform expression in rat osteoblasts and their functional analysis. *FEBS Lett* 2002; **528**: 43-47
36 **Tanaka M**, Suganami T, Kim-Saijo M, Toda C, Tsuiji M, Ochi K, Kamei Y, Minokoshi Y, Ogawa Y. Role of central leptin signaling in the starvation-induced alteration of B-cell development. *J Neurosci* 2011; **31**: 8373-8380
37 **Caro JF**, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, Considine RV. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 1996; **348**: 159-161
38 **Ghazali A**, Grados F, Oprisiu R, Bunea D, Morinière P, El Esper N, El Esper I, Brazier M, Souberbielle JC, Fournier A, Thomas T. Bone mineral density directly correlates with elevated serum leptin in haemodialysis patients. *Nephrol Dial Transplant* 2003; **18**: 1882-1890
39 **Burguera B**, Couce ME, Curran GL, Jensen MD, Lloyd RV, Cleary MP, Poduslo JF. Obesity is associated with a decreased leptin transport across the blood-brain barrier in

rats. *Diabetes* 2000; **49**: 1219-1223
40 **Confavreux CB**. Bone: from a reservoir of minerals to a regulator of energy metabolism. *Kidney Int Suppl* 2011; (121): S14-S19
41 **Visitsunthorn U**, Prete P. Reflex sympathetic dystrophy of the lower extremity: a complication of herpes zoster with dramatic response to propranolol. *West J Med* 1981; **135**: 62-66
42 **Pasco JA**, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Nicholson GC. Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. *J Bone Miner Res* 2004; **19**: 19-24
43 **Reid IR**, Gamble GD, Grey AB, Black DM, Ensrud KE, Browner WS, Bauer DC. Beta-Blocker use, BMD, and fractures in the study of osteoporotic fractures. *J Bone Miner Res* 2005; **20**: 613-618
44 **Arai M**, Nagasawa T, Koshihara Y, Yamamoto S, Togari A. Effects of beta-adrenergic agonists on bone-resorbing activity in human osteoclast-like cells. *Biochim Biophys Acta* 2003; **1640**: 137-142
45 **Turker S**, Karatosun V, Gunal I. Beta-blockers increase bone mineral density. *Clin Orthop Relat Res* 2006; **443**: 73-74
46 **Schlienger RG**, Kraenzlin ME, Jick SS, Meier CR. Use of beta-blockers and risk of fractures. *JAMA* 2004; **292**: 1326-1332
47 **Lai CF**, Chaudhary L, Fausto A, Halstead LR, Ory DS, Avioli LV, Cheng SL. Erk is essential for growth, differentiation, integrin expression, and cell function in human osteoblastic cells. *J Biol Chem* 2001; **276**: 14443-14450
48 **Hu E**, Kim JB, Sarraf P, Spiegelman BM. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. *Science* 1996; **274**: 2100-2103
49 **Gordeladze JO**, Drevon CA, Syversen U, Reseland JE. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. *J Cell Biochem* 2002; **85**: 825-836
50 **Burguera B**, Hofbauer LC, Thomas T, Gori F, Evans GL, Khosla S, Riggs BL, Turner RT. Leptin reduces ovariectomy-induced bone loss in rats. *Endocrinology* 2001; **142**: 3546-3553
51 **Blum M**, Harris SS, Must A, Naumova EN, Phillips SM, Rand WM, Dawson-Hughes B. Leptin, body composition and bone mineral density in premenopausal women. *Calcif Tissue Int* 2003; **73**: 27-32
52 **Yamauchi M**, Sugimoto T, Yamaguchi T, Nakaoka D, Kanzawa M, Yano S, Ozuru R, Sugishita T, Chihara K. Plasma leptin concentrations are associated with bone mineral density and the presence of vertebral fractures in postmenopausal women. *Clin Endocrinol* (*Oxf*) 2001; **55**: 341-347
53 **Dimitri P**, Wales JK, Bishop N. Fat and bone in children: differential effects of obesity on bone size and mass according to fracture history. *J Bone Miner Res* 2010; **25**: 527-536
54 **Farooqi IS**, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 2002; **110**: 1093-1103
55 **Ducy P**, Karsenty G. The two faces of serotonin in bone biology. *J Cell Biol* 2010; **191**: 7-13
56 **Gershon MD**, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 2007; **132**: 397-414
57 **Boyden LM**, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP. High bone density due to a mutation in LDL-receptor-related protein 5. *N Engl J Med* 2002; **346**: 1513-1521
58 **Yadav VK**, Karsenty G. Leptin-dependent co-regulation of bone and energy metabolism. *Aging* (*Albany NY*) 2009; **1**: 954-956
59 **Richards JB**, Papaioannou A, Adachi JD, Joseph L, Whitson HE, Prior JC, Goltzman D. Effect of selective serotonin reuptake inhibitors on the risk of fracture. *Arch Intern Med* 2007; **167**: 188-194
60 **Elefteriou F**, Yang X. Genetic mouse models for bone studies--strengths and limitations. *Bone* 2011; **49**: 1242-1254
61 **Eyre DR**, Dickson IR, Van Ness K. Collagen cross-linking in human bone and articular cartilage. Age-related changes in the content of mature hydroxypyridinium residues. *Biochem J* 1988; **252**: 495-500
62 **Going JJ**. Extraction of DNA from microdissected archival tissues. *Methods Mol Med* 2001; **39**: 291-298
63 **Glass DA**, Bialek P, Ahn JD, Starbuck M, Patel MS, Clevers H, Taketo MM, Long F, McMahon AP, Lang RA, Karsenty G. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005; **8**: 751-764
64 **Day TF**, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. *Dev Cell* 2005; **8**: 739-750
65 **Ueda T**, Oji Y, Naka N, Nakano Y, Takahashi E, Koga S, Asada M, Ikeba A, Nakatsuka S, Abeno S, Hosen N, Tomita Y, Aozasa K, Tamai N, Myoui A, Yoshikawa H, Sugiyama H. Overexpression of the Wilms' tumor gene WT1 in human bone and soft-tissue sarcomas. *Cancer Sci* 2003; **94**: 271-276
66 **Krishnan V**, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. *J Clin Invest* 2006; **116**: 1202-1209
67 **Moon SH**, Park SR, Kim H, Kwon UH, Kim KH, Kim HS, Lee HM. Biologic modification of ligamentum flavum cells by marker gene transfer and recombinant human bone morphogenetic protein-2. *Spine (Phila Pa 1976)* 2004; **29**: 960-965
68 **Saarinen A**, Saukkonen T, Kivelä T, Lahtinen U, Laine C, Somer M, Toiviainen-Salo S, Cole WG, Lehesjoki AE, Mäkitie O. Low density lipoprotein receptor-related protein 5 (LRP5) mutations and osteoporosis, impaired glucose metabolism and hypercholesterolaemia. *Clin Endocrinol (Oxf)* 2010; **72**: 481-488
69 **Yadav VK**, Ryu JH, Suda N, Tanaka KF, Gingrich JA, Schütz G, Glorieux FH, Chiang CY, Zajac JD, Insogna KL, Mann JJ, Hen R, Ducy P, Karsenty G. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell* 2008; **135**: 825-837
70 **Lian JB**, Gundberg CM. Osteocalcin. Biochemical considerations and clinical applications. *Clin Orthop Relat Res* 1988; (226): 267-291
71 **Berkner KL**. The vitamin K-dependent carboxylase. *Annu Rev Nutr* 2005; **25**: 127-149
72 **Sokoll LJ**, Sadowski JA. Comparison of biochemical indexes for assessing vitamin K nutritional status in a healthy adult population. *Am J Clin Nutr* 1996; **63**: 566-573
73 **Barone LM**, Aronow MA, Tassinari MS, Conlon D, Canalis E, Stein GS, Lian JB. Differential effects of warfarin on mRNA levels of developmentally regulated vitamin K dependent proteins, osteocalcin, and matrix GLA protein in vitro. *J Cell Physiol* 1994; **160**: 255-264
74 **Hauschka PV**, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiol Rev* 1989; **69**: 990-1047
75 **Hoang QQ**, Sicheri F, Howard AJ, Yang DS. Bone recognition mechanism of porcine osteocalcin from crystal structure. *Nature* 2003; **425**: 977-980
76 **Frazão C**, Simes DC, Coelho R, Alves D, Williamson MK, Price PA, Cancela ML, Carrondo MA. Structural evidence of a fourth Gla residue in fish osteocalcin: biological implications. *Biochemistry* 2005; **44**: 1234-1242
77 **Lee NK**, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007; **130**: 456-469
78 **Morrison DF**, Mauro LJ. Structural characterization and

chromosomal localization of the mouse cDNA and gene encoding the bone tyrosine phosphatase, mOST-PTP. *Gene* 2000; **257**: 195-208
79 **Pittas AG**, Harris SS, Eliades M, Stark P, Dawson-Hughes B. Association between serum osteocalcin and markers of metabolic phenotype. *J Clin Endocrinol Metab* 2009; **94**: 827-832
80 **Taylor WH**, Khaleeli AA. Coincident diabetes mellitus and primary hyperparathyroidism. *Diabetes Metab Res Rev* 2001; **17**: 175-180
81 **Yamaguchi T,** Sugimoto T. [Calcium homeostasis and diabetes mellitus]. *Clin Calcium* 2006; **16**: 1270-1275
82 **Nowicki M,** Fliser D, Fode P, Ritz E. Changes in plasma phosphate levels influence insulin sensitivity under euglycemic conditions. *J Clin Endocrinol Metab* 1996; **81**: 156-159
83 **Prager R,** Kovarik J, Schernthaner G, Woloszczuk W, Willvonseder R. Peripheral insulin resistance in primary hyperparathyroidism. *Metabolism* 1983; **32**: 800-805
84 **Greenspan SL,** Bone HG, Ettinger MP, Hanley DA, Lindsay R, Zanchetta JR, Blosch CM, Mathisen AL, Morris SA, Marriott TB. Effect of recombinant human parathyroid hormone (1-84) on vertebral fracture and bone mineral density in postmenopausal women with osteoporosis: a randomized trial. *Ann Intern Med* 2007; **146**: 326-339
85 **Neer RM,** Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* 2001; **344**: 1434-1441
86 **Anastasilakis A,** Goulis DG, Koukoulis G, Kita M, Slavakis A, Avramidis A. Acute and chronic effect of teriparatide on glucose metabolism in women with established osteoporosis. *Exp Clin Endocrinol Diabetes* 2007; **115**: 108-111
87 **Anastasilakis AD,** Efstathiadou Z, Plevraki E, Koukoulis GN, Slavakis A, Kita M, Avramidis A. Effect of exogenous intermittent recombinant human PTH 1-34 administration and chronic endogenous parathyroid hormone excess on glucose homeostasis and insulin sensitivity. *Horm Metab Res* 2008; **40**: 702-707
88 **Schafer AL,** Sellmeyer DE, Schwartz AV, Rosen CJ, Vittinghoff E, Palermo L, Bilezikian JP, Shoback DM, Black DM. Change in undercarboxylated osteocalcin is associated with changes in body weight, fat mass, and adiponectin: parathyroid hormone (1-84) or alendronate therapy in postmenopausal women with osteoporosis (the PaTH study). *J Clin Endocrinol Metab* 2011; **96**: E1982-E1989
89 **Kanazawa I,** Yamaguchi T, Yamauchi M, Yamamoto M, Kurioka S, Yano S, Sugimoto T. Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus. *Osteoporos Int* 2011; **22**: 187-194
90 **Wookey PJ,** Lutz TA, Andrikopoulos S. Amylin in the periphery II: An updated mini-review. *ScientificWorldJournal* 2006; **6**: 1642-1655
91 **Cornish J,** Callon KE, Cooper GJ, Reid IR. Amylin stimulates osteoblast proliferation and increases mineralized bone volume in adult mice. *Biochem Biophys Res Commun* 1995; **207**: 133-139
92 **Cornish J,** Callon KE, Bava U, Kamona SA, Cooper GJ, Reid IR. Effects of calcitonin, amylin, and calcitonin gene-related peptide on osteoclast development. *Bone* 2001; **29**: 162-168
93 **Cornish J,** Naot D. Amylin and adrenomedullin: novel regulators of bone growth. *Curr Pharm Des* 2002; **8**: 2009-2021
94 **Bronský J,** Průsa R, Nevoral J. The role of amylin and related peptides in osteoporosis. *Clin Chim Acta* 2006; **373**: 9-16
95 **Dechenes CJ,** Verchere CB, Andrikopoulos S, Kahn SE. Human aging is associated with parallel reductions in insulin and amylin release. *Am J Physiol* 1998; **275**: E785-E791
96 **Bronský J,** Průsa R. Amylin fasting plasma levels are decreased in patients with osteoporosis. *Osteoporos Int* 2004; **15**: 243-247
97 **Dacquin R,** Davey RA, Laplace C, Levasseur R, Morris HA, Goldring SR, Gebre-Medhin S, Galson DL, Zajac JD, Karsenty G. Amylin inhibits bone resorption while the calcitonin receptor controls bone formation in vivo. *J Cell Biol* 2004; **164**: 509-514
98 **Cornish J,** Callon KE, King AR, Cooper GJ, Reid IR. Systemic administration of amylin increases bone mass, linear growth, and adiposity in adult male mice. *Am J Physiol* 1998; **275**: E694-E699
99 **Alam AS,** Moonga BS, Bevis PJ, Huang CL, Zaidi M. Amylin inhibits bone resorption by a direct effect on the motility of rat osteoclasts. *Exp Physiol* 1993; **78**: 183-196
100 **Chambers TJ,** Fuller K, Darby JA. Hormonal regulation of acid phosphatase release by osteoclasts disaggregated from neonatal rat bone. *J Cell Physiol* 1987; **132**: 90-96

S- Editor Huang XZ **L- Editor** Roemmele A **E- Editor** Lu YJ

World Journal of Orthopedics

Online Submissions: http://www.wjgnet.com/esps/
wjo@wjgnet.com
www.wjgnet.com

World J Orthop 2012 November 18; 3(11): I
ISSN 2218-5836 (online)

Acknowledgments to reviewers of *World Journal of Orthopedics*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

Charles Anthony Willis-Owen, BM, BCh, MA, MFSEM, FRCS (Tr and Ortho), 25 Copenhagen Gardens, London W4 5NN, United Kingdom

Patrizia D Amelio, MD, PhD, Department of Surgical and Medical Disciplines, Section of Gerontology, University of Torino, Cso AM Dogliotti 14, 10126 Torino, Italy

Shang-You Yang, Associate Professor, Department of Biological Sciences, Wichita State University, 1845 Fairmount St., Wichita, KS 67260, United States

Vassilis Paschalis, PhD, Department of Physical Education and Sport Science, University of Thessaly, Karies, 42100 Trikala, Greece

Yong Hu, PhD, Department of Orthopaedics and Traumatology, the University of Hong Kong, 12 Sandy Bay Road, Hong Kong, China

World Journal of Orthopedics

Online Submissions: http://www.wjgnet.com/esps/
wjo@wjgnet.com
www.wjgnet.com

World J Orthop 2012 November 18; 3(11): I
ISSN 2218-5836 (online)

Events Calendar 2012

January 12-15, 2012
ICJR 4th Annual Winter Hip and Knee Course
Colorado, CO, United States

January 23-25, 2012
8th Middle East Orthopaedics Conference "Future Challenges and Directions in Orthopedic Surgery"
Dubai, United Arab Emirates

February 7-11, 2012
American Academy of Orthopaedic Surgeons
San Diego, CA, United States

February 14-15, 2012
7th National Conference: Orthopaedics and Sports Medicine 2012 London, United Kingdom

February 16-19, 2012
Orthopaedic MRI and Small Parts
Scottsdale, AZ, United States

March 4-8, 2012
The 30th Annual Emergencies in Medicine Conference
Utah, UT, United States

March 8-10, 2012
ICJR 2nd Annual Advances in Orthopaedic Trauma and Arthroplasty Course
Florida, FL, United States

March 17-24, 2012
Orthopaedics and Sports Medicine for Primary Care Practitioners
Honolulu, HI, United States

May 2-6, 2012
Association of Bone and Joint Surgeons
Charleston, SC, United States

May 4-5, 2012
8th Annual Spine Care Conference for the Primary Care Practitioner
Sacramento, CA, United States

May 9-11, 2012
Pan-African Orthopaedics Conference 2012
Nasrec, South Africa

May 11-21, 2012
Rheumatology and Orthopaedics
Civitavecchia, Italy

July 8-15, 2012
Practice Management and Technology for The 21st Century Practice
Lauderdale, FL, United States

August 13-15, 2012
International Conference and Exhibition on Orthopedics
Chicago, IL, United States

September 14-21, 2012
Orthopaedics and Sports Medicine
Barcelona, Spain

September 21-25, 2012
36th Annual UC Davis Fingers to the Toes Comprehensive Review of Primary Care Orthopaedics
Lake Tahoe, CA, United States

World Journal of Orthopedics

Online Submissions: http://www.wjgnet.com/esps/
wjo@wjgnet.com
www.wjgnet.com

World J Orthop 2012 November 18; 3(11): I-V
ISSN 2218-5836 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Orthopedics (*World J Orthop*, *WJO*, online ISSN 2218-5836, DOI: 10.5312) is a monthly peer-reviewed, online, open-access (OA), journal supported by an editorial board consisting of 261 experts in orthopedics from 30 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

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJO* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJO* is an OA journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJO* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The aim of *WJO* is to report rapidly new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of orthopedics. *WJO* covers diagnostic imaging, arthroscopy, evidence-based medicine, epidemiology, nursing, sports medicine, therapy of bone and spinal diseases, bone trauma, osteoarthropathy, bone tumors and osteoporosis, minimally invasive therapy, traditional medicine, and integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of applied and basic research in fields related to orthopedics, such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs.

Columns

The columns in the issues of *WJO* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide Guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Articles: To report innovative and original findings in orthopedics; (9) Brief Articles: To briefly report the novel and innovative findings in orthopedics; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJO*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of orthopedics; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research orthopedics.

Name of journal

World Journal of Orthopedics

ISSN

ISSN 2218-5836 (online)

Editor-in-chief

Editorial Office

World Journal of Orthopedics
Editorial Department: Room 903, Building D,
Ocean International Center,

No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjo@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893

Indexed and Abstracted in
PubMed Central, PubMed, Digital Object Identifier and Directory of Open Access Journals.

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statisital review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJO* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions
Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2218-5836/g_info_20100722172650.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjo@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins.

Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJO*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…"; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2218-5836/g_info_list.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc*. It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multicurve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc*., in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that...".

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

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]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

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

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

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

Books

Personal author(s)

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

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

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

Conference proceedings

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

Conference paper

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pres-

sure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2218-5836/g_info_20100724204625.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H. pylori*, *E coli*, *etc*.

Examples for paper writing

Editorial: http://www.wjgnet.com/2218-5836/g_info_20100723140942.htm

Frontier: http://www.wjgnet.com/2218-5836/g_info_20100723141035.htm

Topic highlight: http://www.wjgnet.com/2218-5836/g_info_20100723141239.htm

Observation: http://www.wjgnet.com/2218-5836/g_info_20100723141532.htm

Guidelines for basic research: http://www.wjgnet.com/2218-5836/g_info_20100723142040.htm

Guidelines for clinical practice: http://www.wjgnet.com/2218-5836/g_info_20100723142248.htm

Review: http://www.wjgnet.com/2218-5836/g_info_20100723145519.htm

Original articles: http://www.wjgnet.com/2218-5836/g_info_20100723145856.htm

Brief articles: http://www.wjgnet.com/2218-5836/g_info_20100723150253.htm

Case report: http://www.wjgnet.com/2218-5836/g_info_20100723150420.htm

Letters to the editor: http://www.wjgnet.com/2218-5836/g_info_20100723150642.htm

Book reviews: http://www.wjgnet.com/2218-5836/g_info_20100723150839.htm

Guidelines: http://www.wjgnet.com/2218-5836/g_info_20100723150924.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade B certificate (for non-native speakers of English), should be submitted to the online system via the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2218-5836/g_info_20100724204516.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2218-5836/g_info_20100724204306.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJO will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJO is an international, peer-reviewed, OA, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.