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Review

Immune signals in the context of secondary osteoporosis

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Summary. Bone homeostasis is maintained by a balance between bone resorption by osteoclasts and bone formation by osteoblasts, and alterations in bone metabolism can lead to diseases such as osteoporosis. Inter-cellular and intra-cellular signaling, originating from the immune system, the largest source of cell-derived regulatory signals, are involved in these processes. Immune-competent cells such as macrophages and lymphocytes deliver cell-cell signaling through soluble factors such as cytokines and through direct contact with the cells. Such immunological signals to the bone are transmitted primarily through osteoblasts or direct stimulation of osteoclasts to induce osteoclast maturation or bone resorption, which may in turn lead to the disequilibrium of bone metabolism. Inflammatory diseases such as rheumatoid arthritis are good examples of such a process, in which immunological signals play a central role in the pathogenesis of the accompanying secondary osteoporosis. We will achieve a better understanding of the pathogenesis of bone metabolism in osteoporosis through immune signaling, and thereby develop improved therapeutic strategies for these conditions.

Key words: Bone metabolism, Osteoporosis, Inflammation, Immunity, Adhesion

Immune signals and bone metabolism

The two major processes of bone remodeling, bone formation and resorption, are tightly regulated by soluble factors, systemic hormones and cellular adhesion (Manolagas et al., 1995, 2000; Teitelbaum, 2000; Duong and Rodan, 2001; Mundy et al., 2001; Raisz and Seeman, 2001; Takeda and Karsenty, 2001). Bone homeostasis is maintained by a balance between bone resorption by osteoclasts and bone formation by osteoblasts, synthesizing bone matrix proteins. However, osteoblasts also regulate osteoclast maturation by soluble factors and by cognate interaction, resulting in bone resorption. Such alterations in bone metabolism can lead to diseases including osteoporosis. The disequilibrium of bone metabolism is also brought about by immune signals, which consist of immune-competent cells such as macrophages and lymphocytes, the largest and most predominant source of cell-derived regulatory signals in the body. For example, the major source of interleukin-1 (IL-1) is macrophages; various chemokines are from activated lymphocytes; and dendritic cells express multiple co-stimulatory ligands, and thereby, the immune system has a wide range of effects on many systems, including bone metabolism.

Cell functions are regulated by soluble factors such as cytokines and hormones, and by cell-cell signaling mediated through functional molecules on the cell surface, such as adhesion molecules. The expression and function of adhesion molecules are regulated via intracellular signals stimulated by cytokines, whereas adhesion molecules not only function as glue but also transduce extracellular information into cytoplasmic organelles, resulting in cell activation and cytokine production. Such a two-directional cross-talk among adhesion molecules and cytokines appears to be significant for various pathological processes, including secondary osteoporosis.

Adhesion-dependent osteoclast maturation by osteoblasts

Bone remodeling is initiated by bone resorption by activated osteoclasts. The maturation and activation of osteoclast precursors are mediated by receptor activator of nuclear factor-κB ligand (RANKL), which is expressed on the cell membrane of osteoblasts and bone marrow stromal cells (Manolagas, 2000; Mundy et al., 2001).

Human osteoblasts express the intercellular adhesion molecule (ICAM)-1 and interactions between ICAM-1 on osteoblasts with integrin leukocyte function-associated antigen (LFA)-1 ($\alpha_L\beta_2$ on monocytes play

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pivotal roles in osteoclastogenesis (Tanaka et al., 1995). Osteoblasts and stromal cells adhere with high affinity to osteoclast precursors, peripheral blood monocytes and the osteoclast precursor-like cell line FLG29.1, bearing the integrin $ß_2$. RANKL expressed on osteoblasts provides essential signals to osteoclast precursors for their maturation. For the sake of binding of RANKL on osteoblasts to RANK on precursors, high affinity adhesion of these two committing cells is required, since RANKL does not mediate high affinity adhesion, though RANKL functions on the surface of the cell membrane. Actually, the adhesion of osteoblasts with osteoclast precursors is not inhibited by the functioning anti-RANKL antibody, whereas the adhesion is completely abolished by anti-$ß_2$ antibody (Tanaka et al., 2000a). Furthermore, ICAM-1-positive osteoblasts efficiently adhere to the precursors and induce osteoclast maturation in a co-culture system, whereas ICAM-1-negative cells do not.

In addition, when anti-$ß_2$ antibody is added to the co-culture system containing murine spleen cells and osteoblasts in the presence of IL-1, osteoclast formation and subsequent bone resorption are inhibited. Similar inhibition is observed in bone organ cultures by the calcium release assay (Tanaka et al., 1995, 2000a; Okada et al., 2002). These results imply that high affinity adhesion of osteoblasts/stromal cells and osteoclast precursors via ICAM-1 and $ß_2$ integrin mediates juxtacrine stimulation by promoting efficient binding of RANKL on osteoblasts to RANK on osteoclasts (Fig. 1).

Indirect regulation of osteoclast maturation by soluble factors

Bone metabolism is tightly regulated by soluble factors such as cytokines, hormones and growth factors and prostaglandins (PGs). PGs produced in a large amount in inflamed tissue as well as bone, are known to play a pivotal role in osteoclast maturation and bone resorption. PGs are synthesized by the action of cyclooxygenase (COX), existing as the constitutive enzyme COX-1 and the inducible enzyme COX-2, which are induced by inflammatory cytokines. We observed a clear decrease in bone metabolic turnover in COX-$2^{-/-}$ mice, associated with down-regulation of osteoclast formation due to reduced RANKL expression and up-regulation of granulocyte macrophage-colony stimulating factor (GM-CSF). Furthermore, GM-CSF induces differentiation of tissue macrophages from hematopoietic stem cells, while GM-CSF inhibits osteoclast differentiation from the precursors (Okada et al., 2000). Thus, COX-2/PGs and GM-CSF affect osteoclast maturation (Fig. 2).

Contrarily to PGs, inflammatory cytokines such as IL-1 and TNF-α are indirectly involved in osteoclast maturation. The strong stimuli for the induction of RANKL and ICAM-1 on osteoblasts are brought about by IL-1 and TNF-α, both of which transduce intracellular signals by activating a transcription factor NF-κB. ICAM-1-bearing osteoblasts can induce osteoclast maturation and bone resorption, processes which are strongly potentiated by IL-1-mediated induction of ICAM-1 and RANKL on osteoblasts/stromal cells. Thus, pro-inflammatory stimuli such as IL-1 and TNF-α centrally create the disequilibrium in bone metabolism that favors bone resorption (Fig. 1) (Tanaka et al., 2000a; Okada et al., 2002).

Thus, COX-2-dependent PGs directly induce osteoclast differentiation, whereas IL-1 and TNF-α indirectly induce a juxtacrine stimulation of osteoclasts, which is mediated by ICAM-1 and RANKL induced on osteoblasts by the stimuli. It is noteworthy that both PGE2 and IL-1 are produced abundantly in the inflamed synovium of rheumatoid arthritis (RA) and, thereby, play a central role in both peri-articular osteoporosis and systemic osteoporosis.

Indirect regulation of osteoclast maturation by adhesion

Stimulation of osteoblasts through cell adhesion may be similar to stimulation by inflammatory cytokines such as IL-1. For example, activated T-cells bind to osteoblasts via LFA-1/ICAM-1, and this binding induces

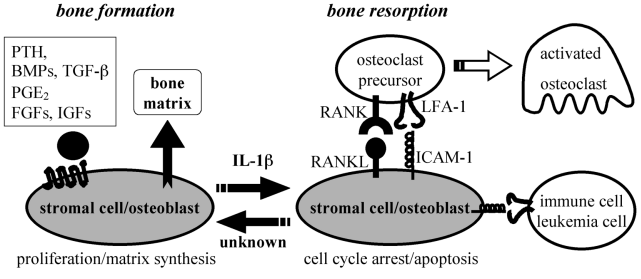

Fig. 1. Inflammatory signals in the context of differential functions of osteoblasts. ICAM-1 on osteoblasts binds to $ß_2$ integrin on osteoclasts with a high affinity and mediates juxtacrine stimulation by promoting efficient binding between RANKL on osteoblasts and RANK on osteoclasts. Inflammatory cytokines such as IL-1 and direct cell adhesion to osteoblasts induce the expression of ICAM-1 and RANKL on osteoblasts, resulting in osteoclast maturation.

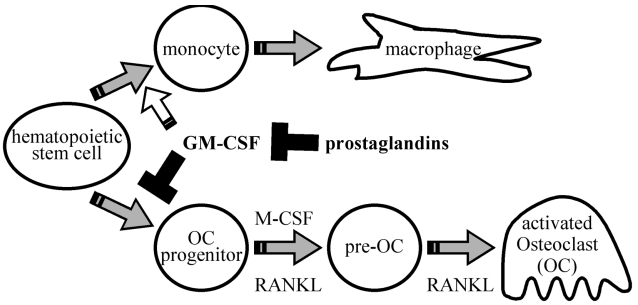

Fig. 2. Possible roles of GM-CSF in the formation of new osteoclasts. PGs may act on hematopoietic cells to inhibit GM-CSF expression, resulting in increased entry of progenitor cells into the osteoclastic differentiation pathway.

the expression of IL-1 and RANKL on osteoblasts via intracellular signals, which leads to osteoclast maturation and bone resorption (Tanaka et al., 1995, 1998a,b). Thus, stimulation of osteoblasts via cell adhesion transmits outside-in signaling, resulting in the disequilibrium in bone metabolism that favors bone resorption.

Osteoblasts are surrounded by and encounter extracellular matrix proteins including type I collagen and fibronectin mainly through $ß_1$ integrin. Although osteoblasts highly express $ß_1$ integrins, their relevance to the intracellular signaling and functions in osteoblasts remains unclear. It is noteworthy that the engagement of $ß_1$ integrin on osteoblasts by a specific antibody or ligand matrices up-regulates ICAM-1 and RANKL expression on osteoblasts and induces osteoclast formation via tyrosine kinase, especially FAK (Nakayamada et al., 2003). Although several papers have reported that ß1 integrin-mediated adhesion to bone matrix induces proliferation, differentiation and bone matrix-synthesis of osteoblasts, our novel findings suggest that $ß_1$ integrin/FAK-mediated signaling on osteoblasts could be involved in ICAM-1- and RANKL-dependent osteoclast maturation.

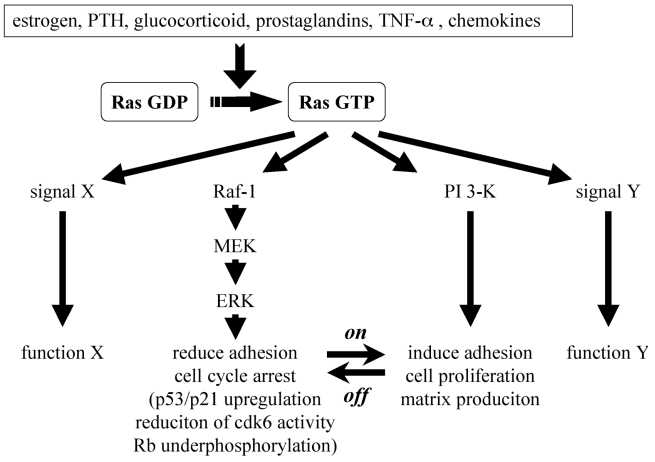

Fig. 3. Regulation of osteoblast functions by intracellular signaling by H-Ras. The H-Ras/Raf/MAPK signaling pathway is involved in the cell cycle regulation seen in ICAM-1-positive osteoblasts, implying the differential regulation of cell functions based on differences in second signaling of H-Ras.

Differential functions of osteoblast regulated by different signaling

During bone remodeling, about half of the osteoblasts differentiate into osteocytes and are mobilized, while the remainder undergo apoptosis (Weinstein and Manolagas, 2000). Although the potential importance of the balance between survival and apoptosis of osteoblasts during bone remodeling is well accepted, the precise mechanism remains unclear. Interestingly, although ICAM-1-positive osteoblasts intensively commit with osteoclast maturation by the juxtacrine stimulation, the majority of the cells represent cell-cycle arrest at the G0/G1 phase (Tanaka et al., 2000a). Actually, ICAM-1-bearing cells possess the increased cytoplasmic expression of the cell cycle inhibitors p21 and p53, decreased cyclin-dependent kinase (cdk)-6 activity and result in apoptosis, showing propidium iodide^{low}/annexin V^{high} and TUNEL-positive. In contrast, ICAM-1-negative osteoblasts produce bone matrix proteins and are highly proliferative with the high level of the cdk-6 activity.

Such a regulation of the cell-cycle appears to be regulated by H-Ras, a small guanine nucleotide-binding regulatory protein (G protein). G proteins are involved in signaling, serving as the "hub" for various second signal transduction events and regulate numerous cell functions (Liu et al., 1999; Tanaka et al., 1999, 2000b; Fujimoto et al., 2001). Introduction of the mutant-form H-Ras^{V12}Y35S gene, which efficiently activates Raf-1/mitogen-activated protein kinase (MAPK), blocks cell-cycle progression and induces cell death, while H-Ras^{V12Y}40S, activating phosphoinositide 3-kinase (PI 3-K), lacks this effect. Thus, the H-Ras/Raf/MAPK signaling pathway is thought to be involved in regulating the cell-cycle in ICAM-1-positive osteoblasts. In T-cells, activation of H-Ras/PI 3-K induces integrin-mediated adhesion, suggesting that the systems regulating cell function may differ depending on differences in the second messengers (Tanaka et al., 1999, 2002; Weinstein and Manolagas, 2000) (Fig. 3).

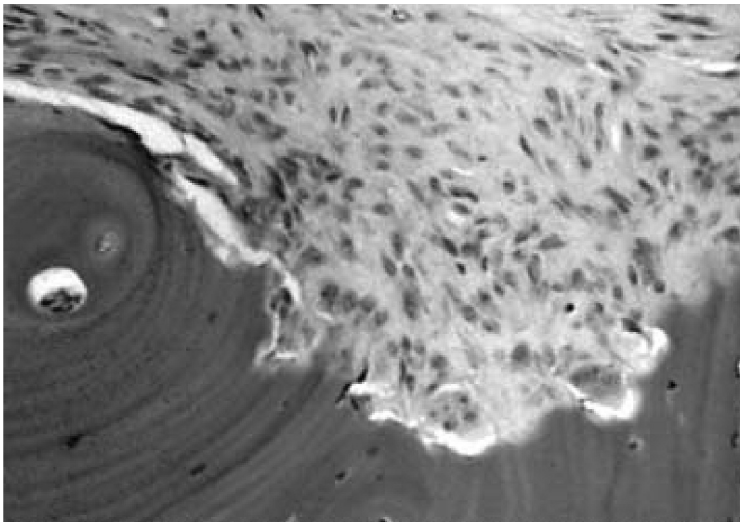

Fig. 4. The interface of synovial membrane and bone in RA. Osteoclasts are activated and resolve bone tissues, where the immune system represented by rheumatoid inflammatory synovium merges the bone system. x 400

Conclusions

In the immune system, cell-cell signaling is achieved via soluble factors such as cytokines as well as through direct contact with immunocompetent cells or matrices, while in the bone system the transmission of signals is often mediated directly or indirectly by osteoblasts. Osteoclast maturation mediated by the adhesion with osteoblasts is coordinated with immune signaling, and thereby, is relevant to pathological events such as secondary osteoporosis observed in RA. During the processes, pro-inflammatory cytokines cause an imbalance in bone metabolism by favouring bone resorption via the expression of RANKL and ICAM-1 and apoptosis of osteoblasts, which are differentially regulated by intracellular signals via H-Ras. Such phenomena play a central role in the pathogenesis of secondary osteoporosis that occurs at the interface between proliferating synovium and bone tissue (Fig. 4). It has been reported that an anti-TNF-α antibody and an IL-1 receptor antagonist, effective for treating RA disease activity, also reduce secondary osteoporosis as well as joint destruction (Wiland et al., 2002; Paleolog, 2003; Taylor, 2003). In future, based on an improved understanding of immune signaling, investigations of the suppression of cell functions may lead to improved understanding and better treatment of diseases of bone metabolism and osteoporosis.

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