REVIEW

Regulators of Osteoclast Differentiation and Cell–Cell Fusion

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Osteoclasts are multinuclear giant cells derived from osteoclast/macrophage/dendritic cell common progenitor cells. The most characteristic feature of osteoclasts is multinucleation resulting from cell–cell fusion of mononuclear osteoclasts. Osteoclast cell–cell fusion is considered essential for re-organization of the cytoskeleton, such as the actin-ring and ruffled boarder to seal the resorbing area and to secret protons, respectively, to resorb bone; the fusion process is thus critical for osteoclast function. Various molecules, such as E-cadherin and macrophage fusion receptor (MFR), have been identified as regulators of osteoclast or macrophage cell–cell fusion. Laboratory production of osteoclasts used to be performed in a co-culture of osteoclast progenitors with osteoblastic cells, but recent advances in the identification of nuclear factor of kappa B ligand (RANKL) enabled the isolation of osteoclast-specific molecules involving osteoclast cell–cell fusion and differentiation regulators from purified osteoclast mRNA, since osteoclasts can be formed without osteoblasts. The essential cell–cell fusion regulator, dendritic cell-specific transmembrane protein (DC-STAMP), was isolated by a cDNA subtractive screen between mononuclear macrophages and RANKL-induced multinuclear osteoclasts. The cell–cell fusion of osteoclasts and foreign body giant cells (FBGCs) was completely abrogated in DC-STAMP-deficient mice *in vivo* and *in vitro*. Bone resorbing activity was significantly reduced but was still detected in DC-STAMP-deficient osteoclasts. DC-STAMP expression is positively regulated by two transcriptional factors: nuclear factor of activated T cells 1 (NFATc1) and c-Fos, both of which are essential for osteoclast differentiation. Furthermore, a novel osteoclastogenesis-regulating pathway involving two transcriptional repressors [B cell lymphoma 6 (Bcl6) and B lymphocyte-induced maturation protein 1 (Blimp1)] under RANKL stimulation has been discovered. The expression of osteoclastic genes such as *DC-STAMP*, *NFATc1*, and *Cathepsin K*, as well as osteoclast differentiation, was inhibited by Bcl6. Bcl6-deficient mice showed enhanced osteoclastogenesis and reduced bone mass, whereas osteoclast-specific Blimp1-conditional knockout mice showed elevated Bcl6 expression, osteoclastic gene expression, and osteoclast differentiation and increased bone mass. In this review, recent advances in our understanding of the regulators of osteoclast differentiation and cell–cell fusion are discussed. (Keio J Med 60 (4) : 101–105, December 2011)

***Keywords*: osteoclasts, DC-STAMP, Blimp1, Bcl6**

Introduction

Osteoclasts are unique bone-resorbing cells, and the loss of osteoclast differentiation or function seen in osteopetrotic mice (such as *op/op* mice), c-Fos-deficient mice, and receptor activator of nuclear factor kappa B ligand

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(RANKL)-deficient mice results in the abrogation of bone resorption, which leads to lack of bone marrow and the absence of tooth eruption.[1–3] [RANKL is also known as the osteoclast differentiation factor (ODF), osteoprotegerin ligand (OPGL), and tumor necrosis factor [TNF]-related activation-induced cytokine (TRANCE).[6–9]] In contrast, elevated-osteoclast differentiation or function seen in osteoporosis and metastatic bone cancer patients results in reduced bone mass and bone destruction, respectively.[4,5] Thus, it is necessary to understand the mechanisms of osteoclastogenesis to control bone mass in osteoporosis and metastatic bone cancer patients.

Until the identification of RANKL, osteoclastogenesis had been induced in the laboratory in a co-culture system with osteoclast progenitor cells and osteoblastic cells under the stimulation of osteotropic factors, such as vitamin D3.[10] The identification of RANKL enabled the induction of osteoclastogenesis without osteoblastic cells in the presence of RANKL and M-CSF. An osteoclast culture system was established by isolating osteoclast progenitor cells using flow cytometric sorting and cultivation in the presence of RANKL and M-CSF[11,12]; in this way, various osteoclastic factors were isolated (**Fig. 1**).

Identification of DC-STAMP, which is essential for cell–cell fusion in osteoclasts and FBGCs

Osteoclasts and macrophages are derived from common progenitors in the presence of M-CSF plus RANKL and M-CSF alone, respectively.[13] M-CSF-induced macrophages are mononuclear cells, whereas M-CSF plus RANKL-induced osteoclasts are multinuclear cells. A cell–cell fusion regulator, DC-STAMP, was isolated by a cDNA subtractive screen between osteoclasts and macrophages.[14] DC-STAMP is a seven-transmembrane protein and has been isolated from dendritic cells, IL-4-induced macrophages, and osteoclasts.[15–17] Interestingly, although the expression of osteoclast differentiation markers was normal, multinucleation of osteoclasts was completely abrogated in DC-STAMP-deficient mice both *in vivo* and *in vitro*, indicating that DC-STAMP is specifically required for osteoclast cell–cell fusion rather than differentiation.[14] Similarly, the cell–cell fusion of FBGCs, which are also derived from common progenitor cells of osteoclasts and macrophages, was completely abrogated in DC-STAMP-deficient mice.[14,18] Thus, DC-STAMP is essential for cell–cell fusion of both osteoclasts and FBGCs.

Role of osteoclast cell–cell fusion in bone resorption and bone homeostasis

The absence of cell–cell fusion in osteoclasts resulted in the severe reduction of bone-resorbing activity, which in turn increased bone mass in DC-STAMP-deficient mice.[14] Thus, DC-STAMP plays a role in regulating bone resorbing efficiency and physiological bone mass. Indeed, DC-STAMP-overexpressing transgenic (DC-STAMP Tg) mice showed accelerated osteoclast bone-resorbing activity and reduced bone mass.[19] Interestingly, although DC-STAMP is not expressed in osteoblasts, osteoblastic activity is upregulated in DC-STAMP-deficient mice, but it is downregulated in DC-STAMP Tg mice,[19] suggesting that DC-STAMP likely regulates osteoblastic activity through osteoclast cell–cell fusion. Thus, DC-STAMP is a bone-remodeling factor regulating both osteoclasts and osteoblasts. A similar phenomenon was reported in v-ATPase V0 subunit d2 (ATP6v0d2)-deficient mice; in addition, severe inhibition of osteoclast cell–cell fusion, as well as increased osteoblastic activity, was detected in ATP6v0d2-deficient mice.[20] Thus, osteoclast cell–cell fusion might be critically involved in bone homeostasis.

Regulation of cell–cell fusion in osteoclasts

Identification of RANKL further supports the isolation of the master transcriptional regulator of osteoclastogenesis, nuclear factor of activated T cells 1 (NFATc1), since osteoclasts could be formed without osteoblastic cells, and osteoclast-specific molecules have been isolated.[21] Osteoclastogenesis is reportedly regulated by NFATc1 and AP1 cooperatively under RANKL stimulation. Since the expression of DC-STAMP and ATPv0d2 was regulated by NFATc1,[18,22] osteoclast cell–cell fusion was induced along with the differentiation of osteoclasts.

Identification of a novel osteoclast differentiation pathway

As described above, NFATc1 is required for osteoclastogenesis and, interestingly, auto-amplification of NFATc1, i.e., NFATc1-dependent NFATc1 induction, has been induced in osteoclasts.[23] Thus, inhibiting NFATc1 was predicted to result in increased bone mass by suppressing osteoclast formation. NFAT inhibitor FK506 has been utilized clinically as an immune-suppressor for organ transplantation; however, reduced bone mass in FK506-treated patients as well as in mice has been observed[24]; this was due to the inhibition of bone formation by FK506.[24] FK506 inhibits NFAT-family molecules, and NFATs are critically involved in regulating osteoblastogenesis and, although osteoclastogenesis is strongly inhibited by FK506, osteoblastogenesis is more strongly suppressed by FK506, which results in reduced bone mass.[24] Thus, although NFATc1-activating pathways have been extensively identified,[25] other osteoclastogenesis-regulating pathways are sought as they might be potential therapeutic targets because inhibiting NFATc1 resulted in decreased bone mass.

In contrast to the positive regulators of osteoclastogen-

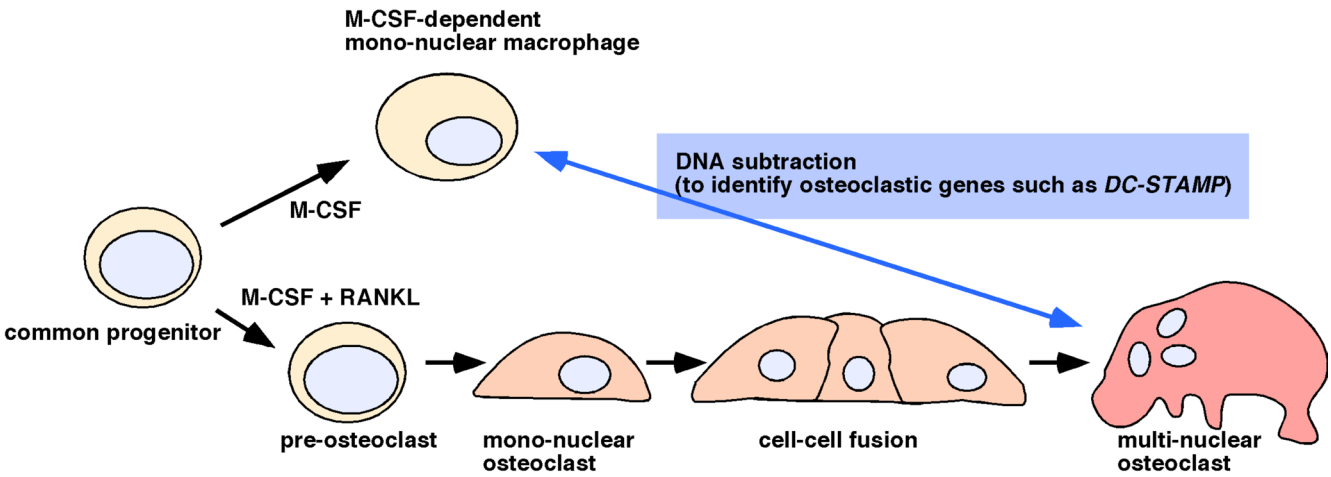

Fig. 1 Strategy for isolating RANKL target genes, including cell–cell fusion regulators, by a DNA subtractive screen between multinuclear osteoclasts induced by M-CSF plus RANKL and mononuclear macrophages induced by M-CSF alone.

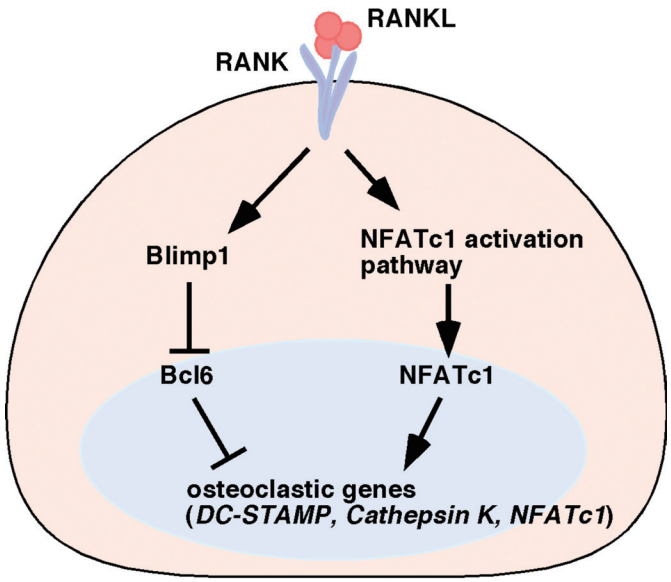

Fig. 2 The Blimp1-Bcl6-osteoclastic gene axis is critical for regulating osteoclastogenesis and bone homeostasis.
NFATc1 is crucial as a positive regulator for osteoclastogenesis under RANKL stimulation. In contrast, Blimp1 and Bcl6 are both transcriptional repressors and are also critical regulators for osteoclast differentiation. Blimp1 inhibits the expression of Bcl6, which inhibits osteoclastic genes, such as *DC-STAMP*, *Cathepsin K*, and *NFATc1*.

esis such as AP1 and NFATc1, the negative regulators of osteoclast differentiation have not been fully characterized. B cell lymphoma 6 (Bcl6) is a transcriptional repressor that was identified as being highly expressed in osteoclast progenitors but was significantly down-regulated during osteoclastogenesis by RANKL.[26] Bcl6 was shown to directly bind to *DC-STAMP* promoter and became dissociated upon RANKL stimulation.[26] Thus DC-STAMP expression is a good indicator for analyzing regulators of osteoclast differentiation. Similarly, Bcl6 bound to *Cathepsin K* promoter and *NFATc1* promoter in the absence of RANKL and became dissociated in the presence of RANKL; thus, suppression of Bcl6 during differentiation is required for appropriate osteoclastogenesis and regulation of bone homeostasis. Lack of Bcl6 results in hyper-osteoclast differentiation, which in turn leads to loss of bone mass.[26] Thus, osteoclastogenesis and bone homeostasis are regulated in a delicate balance

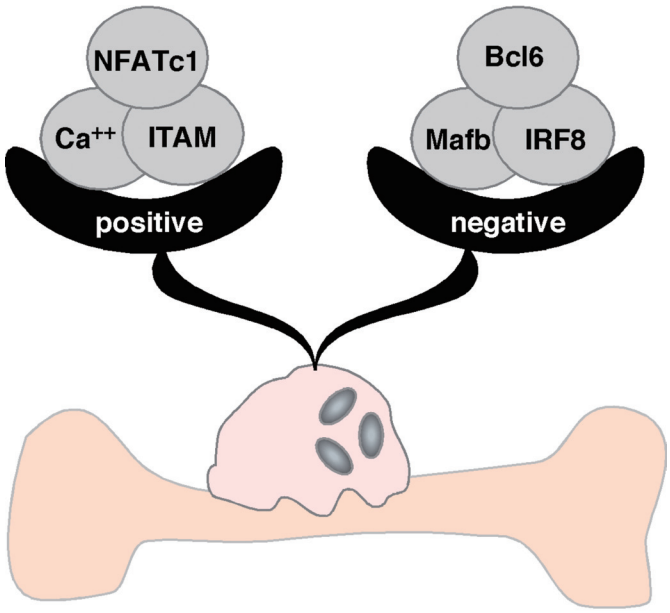

Fig. 3 Osteoclastogenesis and bone homeostasis are regulated by positive and negative regulators.
Positive regulators of osteoclastogenesis are calcium and the immunoreceptor tyrosine-based activation motif (ITAM) signal, which activate NFATc1, a master positive regulator of osteoclastogenesis. Negative regulators of osteoclastogenesis are Bcl6, IRF8, and Mafb.

between NFATc1-induced positive regulation and Bcl6-induced negative regulation of osteoclast differentiation. Moreover, Bcl6 expression in osteoclasts was further negatively regulated by another transcriptional repressor, Blimp1 (**Fig. 2**).[26,27] Lack of Blimp1 in osteoclasts resulted in loss of Bcl6 suppression, which in turn inhibited osteoclast differentiation in the presence of RANKL and increased bone mass.[26] Thus, the negative axis involving Blimp1-Bcl6 is a novel pathway regulating osteoclast differentiation and bone homeostasis.

To date, other negative regulators of osteoclast differentiation, such as interferon regulatory factor 8 (IRF8) and v-maf musculoaponeurotic fibrosarcoma oncogene family protein B (Mafb), have been identified,[28,29] and suppression of these molecules is also required for appropriate osteoclastogenesis. Further studies are needed to clarify the mechanisms of osteoclastogenesis regulation by positive and negative regulators.

Future Directions

Bone remodeling is regulated in a coupled manner by osteoclasts and osteoblasts. Indeed, increased bone resorption by osteoclasts has been observed, but accelerated osteoblastic activity has also been detected in osteoporosis patients, indicating that both osteoclast and osteoblast activities are accelerated. Meanwhile, inhibited osteoclast activity by bisphosphonate also results in inhibited osteoblastic activity, and thus both osteoclast and osteoblast activities are inhibited. This means that osteoclast bone resorption reduces bone mass, but is also required for subsequent bone formation by osteoblasts. Interestingly, bone remodeling in DC-STAMP-deficient and ATP6v0d2-deficient mice is regulated in an uncoupled manner, and inhibited osteoclast activity and elevated osteoblast activity were seen in these mice, which in turn increased bone mass. Thus, osteoclast cell–cell fusion is considered a therapeutic target for bone diseases such as osteoporosis.

In addition, osteoclast-specific Blimp1-conditional knockout mice showed inhibited osteoclastogenesis and increased bone mass.[28,29] Osteoclastogenesis and bone homeostasis are controlled in a delicate balance between osteoclast-positive regulators, such as NAFTc1, and negative regulators, such as Bcl6 (**Fig. 3**). Since inhibition of NFATc1 fails to increase bone mass and rather reduces bone mass, Blimp1 is an interesting therapeutic target for bone diseases. Further studies are required to identify potential therapies that increase bone mass in a more physiological manner in the future.

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