Osteoclasts play critical roles in bone resorption at the site of inflammatory joints, and receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) are required for osteoclastogenesis. RANKL, a member of tumor necrosis factor (TNF) family cytokine, is critically involved in the differentiation and fusion of precursors into mature osteoclasts. Binding of RANKL to its receptor RANK activates TNF receptor-associated factor 6 (TRAF6), which is linked to the nuclear factor-κB (NF-κB) and/or mitogen-activated protein kinases (MAPKs). Among these signaling molecules, much attention has been raised to MAPKs as the therapeutic targets for bone resorptive diseases. In this review, we summarized the involvement of MAPKs and the studies using the specific inhibitors of MAPKs in osteoclastogenesis. The inhibitor of tumor progression locus 2 (Tpl2) effectively suppressed osteoclastogenesis, suggesting that the blockade of the particular MAPK pathway could be of clinical importance as the treatment option for bone destructive diseases including rheumatoid arthritis.


*Correspondence should be addressed to:
Takahisa Sugita, Ph.D., Pharmacology Research Laboratories I, Research Division, Mitsubishi Tanabe Pharma Corporation, 1000, Kamoshida-cho, Aoba-ku, Yokohama, Kanagawa 227-0033, Japan. Phone: +81-45-963-4370, Fax: +81-45-963-3326, E-mail: sugita.takahisa@mp.mt-pharma.co.jp

**Key words** receptor activator of nuclear factor-κB ligand, mitogen-activated protein kinase, osteoclastogenesis, tumor progression locus 2, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the presence of inflammatory synovitis accompanied by the destruction of joint cartilage and bone. An increasing body of evidence has demonstrated that osteoclasts are the principal cell type responsible for the bone resorption in inflammatory joint diseases. Multi-nucleated giant cells with the phenotypic features of osteoclasts are present at erosion sites in RA and collagen-induced arthritis animal models. Furthermore, it has been reported that mice lacking osteoclasts were resistant to arthritic bone resorption. Thus, chemical compounds that could inhibit the generation of osteoclasts at inflammatory sites would be useful for the treatment of RA.

Macrophage colony-stimulating factor (M-CSF) and receptor for activation of nuclear factor-κB ligand (RANKL) are required for osteoclastogenesis from monocytes. The binding of RANKL to its receptor RANK recruits TRAF6 followed by the sequential downstream events such as up-regulation of mitogen-activated protein kinases (MAPKs), nuclear factor-κB (NF-κB), AP-1, nuclear factor of activated T cells (NFAT) c1, and results
in the differentiation of monocytes into osteoclasts\textsuperscript{(11)}. In mammalian cells, three major subfamilies of MAPKs; extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, have been identified\textsuperscript{(20)}. p38 and JNK, which belong to the stress-activated protein kinases (SAPKs), are activated in response to inflammatory cytokines, ultraviolet irradiation, heat shock or osmotic shock, whereas ERK are mainly activated by mitogenic stimuli\textsuperscript{(15)}. These MAPKs are also activated by RANKL and other osteoclastogenic stimuli. In this review, we summarized the involvement of MAPK pathways in RANK signaling pathways and the attempts to inhibit osteoclastogenesis by MAPKs inhibitors.

**p38 MAPK**

p38 MAPKs are widely activated by harmful stimuli such as UV radiation, heat shock, osmotic shock, cytotoxic agents and inflammatory cytokines as well as RANKL stimulation\textsuperscript{(14)}. Involvement of MAPK cascades in RANK signaling pathway are illustrated (Fig. 1). The major MAPKKK, TAK1, is activated by RANKL stimulation. TAK1 forms a complex with TRAF6, where TAB2 functions as an adapter molecule\textsuperscript{(15)}. RANKL stimulation facilitates the formation of a RANK-TRAF6-TAB2-TAK1 complex, leading to activation of TAK1. Huang et al. reported that TAK1 phosphorylates MKK3/6 followed by the phosphorylation of p38 MAPKs in RANK signaling\textsuperscript{(16)}. They have also shown that the p38 inhibitor, SB203580 and dominant-negative TAK1 and MKK6 suppressed RANKL-induced NF-κB activation and NFATc1, the essential transcription factor for osteoclastogenesis. Moreover, activated p38 MAPK phosphorylates transcription factor ATF2\textsuperscript{(17)}. Indeed, Lee et al reported that p38 inhibitor suppressed RANKL-induced activation of ATF2\textsuperscript{(18)}. Activation of TAK1 was shown to lead to the activation of IKK\textsuperscript{(19)}. It is indicated that TAK1 is an upstream activator of IKK in the RANK signaling pathway\textsuperscript{(15)}. Therefore, as shown in Fig. 1, the signaling pathways both TAK1-MKK3/6-p38-NF-κB pathway and TAK1-IKK-β-NF-κB pathway are utilized for osteoclastogenesis by RANKL.

The p38 MAPK family is composed of four isoenzymes, p38α, p38β, p38γ, and p38δ, and p38α was shown to be involved in osteoclastogenesis. Matsumoto et al.\textsuperscript{(16)} and Bohm et al.\textsuperscript{(20)} reported that the dominant negative form of p38α or p38α-deficient monocytes caused decreased osteoclasts differentiation in vitro. Kirkwood et al. showed that the specific inhibitor of p38α, SB-282, significantly reduced the number of tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts in lipopoly saccharide (LPS)-induced experimental rat model of osteoclastogenesis\textsuperscript{(20)}.

![Fig. 1 Signaling events involved in RANKL-induced osteoclast differentiation from bone marrow monocyte/macrophage lineage cells](image_url)

These data indicated that the p38α would be one of the therapeutic targets for the treatment of inflammatory bone destructive diseases.

**JNK**

JNKs are also activated in response to inflammatory cytokines, ultraviolet irradiation, heat shock or osmotic shock\textsuperscript{(13)}. JNKs are composed of at least 10 different isoforms encoded by three different genes, *Jnk1*, *Jnk2* and *Jnk3*. Genetically disrupted mouse of each gene is viable and morphologically normal\textsuperscript{(22,24)}. The typical substrate of the JNKs is c-Jun, the components of AP-1. In RANKL stimulation, JNK is activated by TAK1\textsuperscript{(25)}. In addition, MKK7 is also required for the activation of JNK in RANK signaling pathway\textsuperscript{(26)}. It has been reported that RANKL-induced osteoclastogenesis is accompanied by the JNK-induced c-Jun phosphorylation\textsuperscript{(27,28)}. Activation of JNK1 but not JNK2 is required for efficient osteoclastogenesis from bone marrow monocyte demonstrated by using the JNK1 or JNK2 gene deficient.
Conclusion

Inhibition of MAPK cascade in RANK signaling pathway
might be useful for the treatment of bone resorption in a particular disease condition, and investigation to develop pharmaceutical agents focusing on the osteoclastogenesis has been extensively conducted. Denosumab, fully human monoclonal IgG2 antibody that binds RANKL and inhibits its activity, is a powerful resort on osteoporosis\cite{40}. For the application to the inflammatory diseases such as RA, anti-bone resorptive action as well as anti-inflammatory action will be needed to satisfy the unmet medical needs. We found that the Tpl2 inhibitor could be potentially useful to suppress osteoclastogenesis. Recently, we also reported that the inhibitor suppressed the production of TNFα one of the major pro-inflammatory cytokines\cite{41}. Biological therapeutics including soluble TNF receptor and monoclonal antibodies for the neutralization of TNFα such as infliximab, have been developed and are now powerful therapeutic options\cite{42}. The Tpl2 inhibitor could be a new drug to suppress osteoclastogenesis and TNFα production. Further studies will be needed to clarify in vivo efficacy of bone protective effects at inflammatory condition.

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