The Role of Jak/STAT Pathways in Osteoclast Differentiation

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Abstract

Osteoclasts are bone-resorbing cells of monocyte/macrophage origin and are culprits of bone destruction associated with osteoporosis, rheumatoid arthritis, and cancer bone metastasis. Recent advances in osteoclast biology revealed central roles of various cytokines in regulating osteoclastogenesis both in vitro and in vivo. However, exact underlying mechanisms including signaling pathways downstream of receptor ligation are still under pursuit. In the present review, the role of Jak/STAT proteins and their regulators will be discussed in connection with osteoclastogenesis, since growing evidence indicates that a number of cytokines and growth factors utilizing Jak/STAT signaling pathways affect osteoclastogenesis. A better understanding on the role of Jak/STAT pathways in osteoclast differentiation will not only strengthen our knowledge on osteoclast biology but also provide invaluable insights into the development of anti-resorptive strategies for treating bone-lytic diseases.

Key Words: Jak, STAT, Osteoclast, Differentiation

OSTEOCLAST

Osteoclasts are the bone-resorbing cells of monocyte/macrophage origin (Boyle et al., 2003). In mice, CD45R⁻CD11b⁻bone marrow cells were identified as osteoclast precursors at least in vitro (Jacquin et al., 2006). Mizoguchi et al. established that cell cycle arrest in osteoclast precursors is prerequisite for osteoclast differentiation (Mizoguchi et al., 2009). These cells were identified as c-Fms⁺ (M-CSF receptor) RANKL⁺ cells at the site of osteoclastogenesis in vivo. Upon stimulation of these osteoclast precursors with macrophage colony-stimulation factor (M-CSF) and receptor activator of nuclear factor kappa B ligand (RANKL), these cells differentiate and finally fuse to form multinuclear functional osteoclasts through a multi-step process. M-CSF supports the survival and proliferation of osteoclast precursors. The critical role of M-CSF in osteoclastogenesis was revealed by the osteopetrotic bone phenotype of op/op mice, which lack functional M-CSF leading to the absence of osteoclasts (Yoshida et al., 1990). Genetic ablation of RANKL also induced osteopetrosis accompanied by complete loss of osteoclasts (Kong et al., 1999). Ligation of RANKL receptor (RANK) by RANKL recruits TNF receptor associated factors (TRAFs) and stimulates NF-κB, c-Fos, and NFATc1-mediated gene transcription by activating multiple signaling cascades including mitogen-activated protein kinases such as ERK, JNK, and p38 as well as phosphatidylinositol 3-kinase (PI3K)/Akt pathways (Lee and Kim, 2003). The major osteoclast signaling pathway is depicted in Fig. 1. Transcription factors playing critical roles also have been identified by loss of function mutation studies in mice. One of these transcription factors involved in osteoclastogenesis is hematopoietic transcription factor PU.1. Mice deficient in PU.1 developed osteopetrosis due to the loss of osteoclasts (Tondravi et al., 1997). Another family of transcription factors affecting osteoclastogenesis is microphthalmia-associated transcription factor (MITF). Mice having mutations in this transcription factor have long been known for their osteopetrotic bone phenotype (Walker, 1975). The importance of NFκB in osteoclastogenesis was suggested by the study by Iotsova et al., which reported severe osteopetrosis in NFκB1 (p50) and NFκB2 (p52) double knockout mice (Iotsova et al., 1997). Mice deficient in c-Fos also developed osteopetrosis due to a lineage shift in myeloid precursors that blocked osteoclastogenesis while stimulating macrophage differentiation (Grigoriadis et al., 1994). In addition to these transcription factors, nuclear factor of activated T cells cytoplasmic 1 (NFATc1) is considered as a master transcription factor for osteoclast terminal differentiation and function (Takayanagi et al., 2002a). Not only NFATc1-deficient cells did not differentiate into osteoclasts in response to RANKL stimulation, overexpression of NFATc1...
Osteoclast signaling pathway. Upon RANK ligation by RANKL, TRAF is recruited followed by activation of MAPKs such as ERK, JNK, and P38. Consequently, c-Fos expression and activity is increased resulting in the induction of NFATc1. Alternatively, increased in the intracellular Ca\(^{2+}\) concentration upon RANKL stimulation activates Ca\(^{2+}\)-dependent phosphatases calcineurin. Dephosphorylation by calcineurin promotes nuclear translocation of NFATc1, supporting the transcription of osteoclastogenic genes.

alone was sufficient to induce osteoclastogenesis in the absence of RANKL, suggesting that NFATc1 is both necessary and sufficient for osteoclast differentiation. Genes induced by these osteoclastogenic transcription factors include proteases, ion pumps, and membrane proteins involved in osteoclast fusion and function. For example, protease cathepsin K, produced upon MITF transactivation (Hu et al., 2007), catalyzes collagen matrices to degrade bone and cartilage. A proton pump vacuolar ATPase (V-ATPase) showed significantly higher expression in mature osteoclasts than in their precursors. Interestingly, deletion of d2 subunit of V-ATPase during early stages of osteoclastogenesis resulted in defective osteoclasts. Furthermore, deletion of d2 subunit of V-ATPase during early stages of osteoclastogenesis resulted in defective osteoclasts and osteoclast fusion (Lee et al., 2006). However, when depleted during later stages of osteoclast differentiation, it was evident that V-ATPase functions as proton pump that acidifies extracellular matrices during bone resorption (Wu et al., 2009). Dendritic cell-specific transmembrane protein (DC-STAMP) was identified as a protein highly expressed in osteoclast compared with its precursor (Yagi et al., 2005) and its expression was greatly induced by NFATc1 (Kim et al., 2008). In DC-STAMP-deficient mice, osteoclast fusion was completely impaired although expression of osteoclast differentiation markers was normal, suggesting that the function of DC-STAMP is cell fusion-specific (Yagi et al., 2005). Upon these concerted actions of multiple genes, mature multinucleated osteoclasts are formed. These osteoclasts undergo structural changes that allow the formation of sealing zone between bone surface and osteoclast basal membrane, and the secretion of acids as well as lytic enzymes into the lacunae leading to the bone resorption (Boyle et al., 2003).

**BONE-DESTRUCTIVE DISEASES AND ANTI-OSTEOCLASTOGENESIS DRUGS**

Since bone homeostasis is maintained by bone-formation by osteoblasts and bone-degradation by osteoclasts, many of the skeletal diseases involve unregulated osteoclastogenesis that leads to excessive bone resorption (Arai et al., 1999; Teitelbaum, 2000; Boyle et al., 2003). These include osteoporosis, rheumatoid arthritis, periodontal diseases, cancer metastasis, and multiple myeloma bone diseases. Thus the inhibition of osteoclast differentiation and/or activity is expected to alleviate bone destruction associated with these conditions. Most widely used anti-osteoclastogenic drugs are bisphosphonates (Favus, 2010). This class of drugs has high affinity to bones and is ingested by bone-resorbing osteoclasts (Sato et al., 1991; Masarachia et al., 1996). Once inside the cells, bisphosphonate induces apoptosis of osteoclasts either by acting as ATP analogue (non-nitrogen containing bisphosphonates), or by inhibiting protein prenylation that is important for the survival and activity of osteoclasts (nitrogen containing bisphosphonates) (Frith et al., 1997; van Beek et al., 2003).

Another promising class of drugs targets RANKL-RANK axis. Among them is a humanized anti-RANKL antibody denosumab developed by Amgen, which is approved by FDA in 2010 for use in post-menopausal osteoporosis. This drug mimics endogenous osteoprotegerin (OPG), which binds to RANKL and blocks RANKL-dependent osteoclastogenesis.

In the last decade extensive studies on osteoclast biology as well as on the regulation of osteoclastogenesis by immune and endocrine system greatly advanced our understanding on the process of osteoclastogenesis. Upon this understanding, several drug pipelines are under clinical trials (Yasothan and Kar, 2008), and still more possible target molecules are being suggested through basic and clinical studies. Among the wide variety of mechanisms reported to govern osteoclastogenesis, the present review will focus on the Janus kinase/signal transducers and activators of transcription (Jak/STAT) signaling pathways.

**THE JAK/STAT PATHWAY**

Jak/STAT signaling pathway is one of the most extensively studied signal transduction cascade in mammals. More than 30 cytokines and receptor ligands have been shown to utilize Jak/STAT pathway to integrate extracellular signal to modulate gene expression and cellular functions (Rawlings et al., 2004; Murray, 2007). These include type I and type II interferons, GP130 family cytokines, interleukins, and growth hormones. There are four Jak family members (Jak1, Jak2, Jak3, and Tyk2) (Stark et al., 1998) and seven STAT family members (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) (Darnell, 1997). The importance of Jak/STAT signaling has been underscored by embryonic or perinatal lethality or severe defects in immune system or growth hormone pathways in knockout mice (Shuai and Liu, 2003). Upon ligation, the Jak-binding receptors multimerize allowing trans-phosphorylation of Jaks (Murray, 2007). Subsequently, phosphory-