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Tumor cell p38 MAPK: A trigger of cancer bone osteolysis

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Abstract

Osteolytic bone destruction is a hallmark of bone-metastatic cancers. Current therapy is unable to completely cure or prevent this disease in patients. The p38 mitogen-activated protein kinase (MAPK) affects a diverse range of intracellular responses with well-known roles in development, cell-cycle and differentiation, inflammation, apoptosis, senescence, and tumorigenesis. This article is an overview of the contribution of tumor cell-expressed p38 MAPK to the regulation of osteoclastogenesis, osteoblastogenesis, and osteolytic bone lesions.

Keywords

p38 MAPK; bone destruction; multiple myeloma; breast cancer; cytokines

The mammalian skeleton is continuously remodeling throughout its lifetime. Remodeling of the bone is a carefully choreographed interaction between bone-resorbing osteoclasts and bone-forming osteoblasts, which involves a three-phase cycle: osteoclasts resorb old bones, then osteoblasts deposit type I collagen into resorption lacunae, and subsequently collagen is mineralized to form new bone. This process is essential for maintaining the integrity of the skeleton structure as well as the storage of calcium and phosphorus in bone ^[1].

Osteoclasts arise from hematopoietic monocytic precursors. Osteoclastogenic cytokines can regulate osteoclast maturation. Receptor activator of NF- κ B ligand (RANKL) induces the fusion of mononuclear precursors of monocyte/macrophages to form mature osteoclasts. Macrophage colony-stimulating factor (M-CSF) promotes the growth and survival of monocyte/macrophage precursors ^[2]. Bone marrow stromal cells, osteoblasts, and immune cells (such as activated T cells) primarily produce RANKL and M-CSF ^[3]. On the other hand, osteoblasts originate from mesenchymal stem cells (MSCs) ^[4]. Transcriptional factor Runx2/Cbfa1 and the Wnt signaling pathway promote mature osteoblast formation ^[5].

Osteolysis is found most often in patients with inflammatory bone diseases such as rheumatoid arthritis ^[6]. Especially, osteolytic metastasis is the hallmark of some malignant

tumors, such as multiple myeloma and bone-metastatic breast cancer [7-9]. Bone destruction leads to intractable bone pain, pathological fractures, and hypercalcemia [7]. It is usually associated with a poor prognosis in patients and can severely affect patients' quality of life.

Recent studies have demonstrated that tumor cells impair the balance of the bone formation which is induced by osteoblasts and the bone resorption which is induced by osteoclasts. Several mechanisms by which tumor cells affect osteoclast and osteoblast differentiation and function have been elucidated [10-12]. We found the contribution of tumor cell-expressed p38 mitogen-activated protein kinase (MAPK) to osteolytic bone destruction in multiple myeloma and bone metastatic breast cancer. Combined reports from our and other researchers' laboratories, this review focuses on the functional role of p38 MAPK in the pathogenesis of tumor-induced osteolytic bone destruction.

p38 MAPK signaling pathway

p38 MAPK is an important member of the evolutionarily conserved family of serine/threonine MAPKs. Its main function is to transfer extracellular signals into the intracellular machinery [13]. Along with c-Jun N-terminal kinase, p38 MAPK also acts as stress-activated protein kinases, which can be phosphorylated by a wide range of environmental stresses such as inflammatory cytokines. The phosphorylated p38 MAPK then activates its substrates that ranges from protein kinases, transcription factors, as well as other cytosolic and nuclear proteins, triggering an array of downstream activities, including production of cytokines, inflammatory reactions, cell cycle and differentiation, apoptosis, and etc [14-16].

p38 MAPK in non-malignant bone diseases

The activity of p38 MAPK is elevated in benign bone diseases, such as rheumatoid arthritis and periodontal disease [6, 17-19]. In these diseases, osteolysis occurs frequently due to increased osteoclast bone resorption activities in the bone of the patients under the influences of inflammatory cytokines [6]. Osteoclasts, which are exclusively responsible for bone resorption, are central to the pathogenesis of inflammatory osteolysis [6]. Previous studies have shown that p38 α , a main isoenzyme of p38 MAPK, is highly expressed in mature types of osteoclasts and in osteoclast precursors [18]. Cytokines such as RANKL can upregulate the phosphorylation of p38 α as well as its downstream substrates in the progenitors of osteoclasts. Furthermore, p38 MAPK occupies a central role in the signaling network of interleukin 1 (IL-1) and tumor necrosis factor- α (TNF- α), in which IL-1 participates in the pathophysiology of inflammatory arthritis [19-22] and TNF- α is a dominant cytokine for the induction of inflammatory osteolysis [3, 23-26]. In addition, p38 MAPK signaling enhances the effects of RANKL on the induction of osteoclast differentiation and osteoclast-mediated bone resorption [27, 28]. To date, numerous inhibitors specific for p38 MAPK have been characterized, and several of which have been moved into clinical trials [27]. By inhibiting the production of these proinflammatory cytokines and preventing osteoclast formation, p38 MAPK inhibitors prevent inflammatory osteolytic bone loss in patients.

Myeloma cell p38 MAPK in osteolytic bone destruction

Multiple myeloma is a plasma cell malignancy that exploits the bone marrow microenvironment for myeloma survival and propagation. Osteolytic bone lesions are observed in more than 80% of myeloma patients and cause great suffering in myeloma patients [29]. Previous studies have demonstrated that myeloma cells are responsible for bone destruction. Clinically, osteolytic lesions are detected only in the area of bones where myeloma cells infiltrate [29]. Histomorphometric studies have shown that myeloma patients with osteolytic bone lesions exhibit less bone formation and more bone resorption, which results from the decreased osteoblastogenesis and increased osteoclastogenesis that occur proximately to the infiltrated myeloma cells. We and other groups found that p38 MAPK, which is constitutively activated in myeloma cells, triggers osteolytic bone destruction [30-33]. Vanderkerken *et al.* [34] reported that treatment of murine myeloma (5T2MM and 5T33MM)-bearing mice with the p38 MAPK inhibitor SCIO-469 decreases tumor burden and bone lesions, and prolongs mouse survival. In line with these findings, we observed that inhibition of p38 MAPK by the inhibitor SD-169 significantly reduced myeloma-induced osteolytic bone lesions by reducing osteoclastogenesis and enhancing osteoblastogenesis in xenografted myeloma mouse models. One potential problem with those p38 MAPK inhibitors is lack of specific toxicity towards myeloma cells. When administrated, blockage of p38 MAPK activity occurs indiscriminately in both myeloma cells and the surrounding normal bone marrow cells, which include stromal cells, the progenitors of osteoclasts and osteoblasts.

It is still unclear what role p38 MAPK in myeloma cells plays in bone destruction. We specifically selected myeloma cells with different levels of phosphorylated p38 MAPK and injected these cells into mice. Severe bone resorption was observed in mice injected with myeloma cells with high or detectable p38 MAPK activity, whereas no or less visible bone resorption was seen in mice injected with myeloma cells with low or undetectable p38 MAPK activity. Furthermore, when p38 MAPK protein expression was knocked down in myeloma cells using specific shRNAs, less bone resorption was seen in mice injected with myeloma cells with knocked down p38 MAPK than in mice injected with control cells. Thus, myeloma cell-expressed p38 MAPK contributes to osteolytic bone lesions [12].

Myeloma cell p38 MAPK in osteoclastogenesis activation

Increased osteoclast differentiation and osteoclast-mediated bone resorption is an important mechanism in the pathogenesis of myeloma-associated osteolytic bone destruction. The p38 MAPK signaling pathway has been shown to participate in the development of the progenitors into mature osteoclasts [10]. This signaling can upregulate the expression and secretion of inflammatory cytokines like TNF- α and RANKL in the progenitors of osteoclasts and, in turn, RANKL upregulates the transcriptional activity of c-Fos and NFATc1 via the p38 MAPK signaling pathway, which is important for the expression of osteoclast differentiation-associated genes. Studies have shown that myeloma cells upregulate the secretion of these osteoclast activators from bone marrow stromal cells via p38 MAPK signaling. Our results showed that knockdown of myeloma cell-expressed p38 MAPK substantially downregulates p38 MAPK phosphorylation in the surrounding normal

bone marrow cells, indicating that myeloma cell-expressed p38 MAPK is responsible for the activation of p38 MAPK signaling in the bone marrow microenvironment.

Dickkopf1 (DKK-1), which is an inhibitor of Wnt/ β -catenin signaling, has been demonstrated to involve in osteoclast differentiation [35]. We found that myeloma cell-expressed p38 MAPK upregulates the secretion of DKK-1, which upregulates RANKL secretion from MSCs and osteoblasts [10]. In addition, monocyte chemotactic protein 1 (MCP-1) is a chemokine that is mainly released from bone marrow stromal cells and recruits immune cells to the infected location or the wounded tissue. The production of MCP-1 is also upregulated during osteoclastogenesis. Previous studies showed that MCP-1 enhanced the fusion of hematopoietic precursors to osteoclast-like cells in vitro and dictated osteoclast behavior in inflammatory osteoporosis via binding to receptor CCR2. We found that myeloma cells also secrete MCP-1 and that its production is stimulated by p38 MAPK. Mechanistic studies have shown that MCP-1 upregulates the expression of the RANKL receptor in both the mature types of osteoclasts and osteoclast precursors. Binding of RANKL to RANK thus activates the NF- κ B, p38 MAPK, and ERK signaling pathways in osteoclasts progenitors, leading to enhanced osteoclast differentiation and bone resorption activity.

Myeloma cell p38 MAPK in the inhibition of osteoblastogenesis

Decreased osteoblast differentiation and osteoblast-mediated bone formation is another important mechanism of myeloma-induced osteolytic bone destruction [10]. Studies from Greenblatt *et al.* [36] showed that the p38 MAPK signaling pathway is essential for normal skeletogenesis in mice. They found that in a mouse model, deletion of p38 MAPK or its downstream genes exhibited significantly lower bone mass and defective osteoblastogenesis. Moreover, bone morphogenetic protein2 or transforming growth factor β 1 enhances osteoblast differentiation through the p38 MAPK and ERK signaling pathways. Tian *et al.* [35] found that myeloma cells inhibit differentiation from MSCs by secreting the Wnt antagonist DKK-1, although there is not a drastic increase in the secretion level of DKK-1 from myeloma cells. Recent studies also found that myeloma cells produce other Wnt antagonists such as secreted frizzled-related proteins. These soluble antagonists disrupt the binding of Wnt ligands to the frizzled receptor, thereby suppressing the Wnt/ β -catenin signaling pathway [37].

In addition, myeloma cells stimulate the secretion of inflammatory cytokine IL-7 from bone marrow stromal cells via the very late antigen 4-vascular cell adhesion molecule signaling pathway. Stromal cell-derived IL-7 reduces the transcriptional activity of Runx2, leading to suppression of the expression of osteoblast differentiation-associated genes [38]. We found that constitutively activated myeloma cell-expressed p38 MAPK upregulates DKK-1 expression and secretion in vitro and in myeloma-bearing mice, whereas inhibition or shRNA knockdown of p38 MAPK activity in myeloma cells significantly downregulates DKK-1 secretion. Analysis of the DKK-1 promoter region showed that several CREB-binding sites localize 1.3kb upstream from the transcriptional start site. CREB is a p38 MAPK-targeted transcriptional factor. The phosphorylation of CREB by p38 MAPK signaling was found in all examined myeloma cells (unpublished data). In our future studies,

we will investigate whether p38 MAPK signaling upregulates the transcriptional expression of DKK-1 in myeloma cells via CREB.

Isoforms of p38 MAPK in bone-metastatic breast cancer

Bone is a common place for metastatic breast cancer cells to settle and grow. Bone metastasis is present in more than 70% of patients with advanced breast cancer and often induces osteolytic bone destruction [39]. Previous studies showed that breast cancer cells within bone marrow enhance mature osteoclast formation and osteoclast-mediated bone resorption [39, 40]. We have found that constitutive activity of p38 MAPK in breast cancer cells plays an important role in osteoclast differentiation and activity.

There are four isoforms of p38 MAPK, which includes MAPK14 (p38 α), MAPK11 (p38 β), MAPK12 (p38 γ) and MAPK13 (p38 δ). Among them, p38 α and p38 β are the predominant forms [11]. Breast cancer cells express p38 α and p38 β but little or none of the other isoforms. It has been reported that breast cancer cells with inhibited p38 α are less likely to metastasize to bone [41, 42]. However, the roles of the three other isoforms in bone are not clear. In our study, we demonstrated the importance of p38 β in osteolytic bone destruction induced by breast cancer cells and elucidated a novel mechanism by which p38 β upregulates the expression and secretion of MCP-1, leading to enhanced osteoclast differentiation and bone resorption [11]. Thus, p38 β is a novel potential target in clinical trials to improve the current treatment efficacy in patients with bone-metastatic breast cancer.

Conclusion

Cancer cells depend on the tumor microenvironment to grow, invade, and metastasize [43]. This complex microenvironment is shaped by cross-talk between varieties of cell types that are capable of secreting many different kinds of inflammatory cytokines. p38 MAPK in tumor cells is activated by those cytokines. In addition, p38 MAPK can further stimulate tumor cells to produce cytokines and chemokines that are involved in osteolytic bone destruction. The combination of tumor growth, invasion, and bone destruction forms a vicious cycle in the bone marrow microenvironment of bone-related tumors. Better understanding of the functional role of tumor cell-expressed p38 MAPK in the pathogenesis of tumor-induced osteogenesis and bone destruction would illuminate the mechanisms by which tumor cells manipulate this microenvironment. While the underlying activation of p38 MAPK in different types of tumors requires further investigation, interruption of p38 MAPK signaling in tumor cells offers a novel and potentially more effective approach in the therapy for tumor-induced osteolytic bone destruction.

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