

## **PERSPECTIVES**

### **Myeloma-Bone Interaction: A Vicious Cycle**

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#### **Abstract**

Multiple myeloma is a disease that results in devastating bone destruction. Myeloma cells stimulate bone resorption through the enhancement of osteoclast formation and activation. These effects are mediated through the impact of macrophage inflammatory protein (MIP)-1 on RANKL-RANK signaling. Bone marrow stromal cells, as well as the interaction between myeloma cells and osteoclasts, contribute to the growth and survival of myeloma cells. B-cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) play important roles in the interaction between myeloma cells and osteoclasts. Myeloma cells also suppress bone formation through the secretion of Wnt antagonists, including secreted Frizzled-related protein (sFRP)-2. An understanding of the molecular mechanism of bone destruction and tumor progression in multiple myeloma is necessary to develop effective therapies for myeloma patients. *BoneKEy-Osteovision*. 2006 March;3(3):8-14.

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Multiple myeloma is characterized by the accumulation of monoclonal plasma cells in the bone marrow and destructive bone lesions. Multiple myeloma originates in lymphoid tissues, but develops and expands almost exclusively in the bone marrow. Myeloma cells enhance bone resorption, and cause devastating bone destruction by osteoclasts. The bone destruction generates multiple disabling fractures with debilitating clinical symptoms including intractable bone pain, compression myelopathy and hypercalcemia. Severity of bone lesions correlates with the tumor burden and clinical stage of the disease. Furthermore, the aggressive features of myeloma bone lesions contribute significantly to its poor prognosis despite the recent development of intensive chemotherapeutic regimens with or without stem cell transplantation (1;2). Elucidation of the molecular mechanism of bone destruction and tumor progression is essential for the development of effective therapies to improve survival as well as quality of life for myeloma patients. We will summarize the current understanding of myeloma cell and bone marrow cell

interactions, focusing on the vicious cycle of interactions that leads to multiple myeloma.

#### **Enhancement of Bone Resorption**

Myeloma cells reside in close contact with stromal cells and enhance the formation and activation of osteoclasts in bone destructive lesions. Interactions between receptor activator of nuclear factor- $\kappa$ B (RANK) on the surface of osteoclast lineage cells and RANK ligand (RANKL) on stromal cells play a key role in the formation and activation of osteoclasts. Several studies have demonstrated that myeloma cells enhance RANKL expression by bone marrow stromal cells via cell-to-cell contact (3;4). RANKL-RANK signaling is counteracted by osteoprotegerin (OPG), a decoy receptor for RANKL secreted from stromal and other types of cells. Myeloma cells stimulate osteoclast formation by triggering a coordinated increase in RANKL and decrease in OPG in the bone marrow (4-6). In addition to the stromal cell-mediated enhancement of RANKL-RANK signaling, a majority of myeloma cells secrete vascular

endothelial growth factor (VEGF) and a subset of myeloma cells also express RANKL (7;8). Because VEGF can substitute for M-CSF in the induction of osteoclastogenesis by RANKL-RANK interaction, there may be a direct interaction of myeloma cells with osteoclasts and their precursors to enhance osteoclastogenesis and bone resorption.

An important question is what stimulates RANKL-RANK signaling. Choi *et al.* first reported that an osteoclastogenic C-C chemokine, macrophage inflammatory protein (MIP)-1 $\alpha$  is a candidate for osteoclast stimulatory factor in multiple myeloma (9). They later showed that MIP-1 $\alpha$  is responsible for the enhanced bone resorption in myeloma by demonstrating blockade of bone destruction in an animal model using an antisense strategy against MIP-1 $\alpha$  (10). We have reported that MIP-1 $\beta$  is also secreted by most primary myeloma cells from patients with multiple osteolytic lesions (11). *In vitro* experiments demonstrated that myeloma cells potently enhance both the formation and function of osteoclasts, and these effects were mostly abrogated by neutralizing antibodies against MIP-1 $\alpha$  and  $\beta$  or their cognate receptor, CCR5. These results suggest critical roles for MIP-1 $\alpha$  and  $\beta$  in the enhancement of bone resorption by myeloma cells. The ability of myeloma cells to secrete these chemokines correlates well with the extent of bone lesions as well as with levels of bone resorption markers in patients with myeloma, suggesting a causal role for these chemokines in the development of lytic bone lesions (12). Because the osteoclastogenic activity of MIP-1 $\alpha$  and  $\beta$  is blocked by OPG, and because these chemokines induce RANKL expression by stromal cells, the effects of myeloma cells on bone resorption appear to be mostly mediated via the stimulation of RANKL expression in stromal cells by MIP-1 (11). Oyajobi *et al.* demonstrated that athymic mice that were either inoculated with CHO cells secreting human MIP-1 $\alpha$  or injected with recombinant MIP-1 $\alpha$  developed lytic bone lesions. The bone resorptive effect of MIP-1 $\alpha$  was dependent upon RANKL/RANK signaling

because MIP-1 $\alpha$  had no effect in *RANK(-/-)* mice (13). They further demonstrated that in murine 5TGM1 myeloma-bearing mice, inhibition of MIP-1 $\alpha$  action by neutralizing anti-MIP-1 $\alpha$  antibodies, but not blockade of osteoclastic bone resorption, suppressed tumor growth and reduced tumor burden. They suggest that MIP-1 $\alpha$  stimulates not only bone resorption but also the survival and growth of myeloma cells (13).

MIP-1 $\alpha$  and  $\beta$  bind to their cognate receptors, CCR1 and 5, to exert their diverse effects. Myeloma cells express both CCR1 and 5, and MIP-1 acts on myeloma cells in an autocrine/paracrine fashion to activate integrins including VLA-4. Stromal cells express VCAM-1 as a ligand for VLA-4, and MIP-1 stimulates adhesion of myeloma cells to stromal cells by enhancing binding of VLA-4 with VCAM-1. Myeloma cell adhesion through VLA-4/VCAM-1 interaction is reported to enhance production of osteoclast-stimulating activity (14). In addition, VLA-4/VCAM-1 interaction enhances the secretion of MIP-1 by myeloma cells (Abe *et al.* unpublished data). Taken together, these observations are consistent with the notion that close cell-to-cell interaction between myeloma cells and bone marrow stromal cells creates a microenvironment in which a vicious cycle is established: integrin-mediated cell-to-cell contact enhances MIP-1 production and MIP-1 enhances integrin-mediated cell adhesion as well as osteoclastic bone resorption.

### **Myeloma Cell Growth and Survival**

The fact that myeloma cells grow and expand almost exclusively in the bone marrow suggests the importance of the bone marrow microenvironment in supporting myeloma cell growth and survival. By analogy to cancer metastasis in which a pre-metastatic niche is formed by migration and seeding of hemopoietic progenitor cells to metastatic sites (15), bone marrow cells appear to provide myeloma cells with a microenvironment suitable for their growth and survival. Such a microenvironment may be called a "myeloma niche". Stromal cells are a component of this microenvironment

that have been extensively studied. Damiano *et al.* reported that cell adhesion-mediated drug resistance (CAM-DR) is generated by adhesion of myeloma cells to stromal cells via an interaction between VLA-4 or -5 and fibronectin (16). Their results demonstrated that fibronectin-mediated adhesion confers a survival advantage for myeloma cells exposed to cytotoxic drugs, by inhibiting drug-induced apoptosis. The interaction between VLA-4 and fibronectin is similar to what was observed in a "pre-metastatic niche" formed by VEGFR1 and VLA-4-positive bone marrow-derived hematopoietic progenitor cells (15), and may play an important role in the formation of the "myeloma niche" in the bone marrow microenvironment.

Myeloma cells also adhere to osteoclasts, and their interaction contributes to disease. Administration of bisphosphonates or inhibitors of RANK ligand such as RANK-Fc and osteoprotegerin not only prevents myeloma cell-induced bone destruction but also interferes with tumor progression in animal models of multiple myeloma (3;17). Yaccoby *et al.* previously reported that in an animal model of human myeloma using a SCID-human host system, myeloma cell growth in the bone marrow microenvironment was suppressed by inhibition of osteoclast activity by bisphosphonates, and suggested that myeloma growth in the bone marrow is dependent upon osteoclast activity (18). We have developed a culture system to examine interactions between primary myeloma cells and autologous or allogenic human osteoclasts developed from peripheral blood mononuclear cells *in vitro* (19). Growth of myeloma cells was potently enhanced by cell-to-cell interaction with osteoclasts, and the effect of osteoclasts was only partially inhibited by an anti-human interleukin (IL)-6 neutralizing antibody, despite an increased production of IL-6 by osteoclasts via the interaction with myeloma cells. In addition, although mouse and rabbit IL-6 cannot act on human cells bearing human IL-6 receptors, growth of human myeloma cells was enhanced even under interactions with mouse or rabbit osteoclasts (19). These results demonstrated that osteoclasts

stimulate myeloma cell growth independent of the enhancement of IL-6 secretion. Furthermore, prevention of cellular contact between myeloma cells and osteoclasts abolished the effect of osteoclasts on myeloma cell growth, suggesting that close cell-to-cell interaction is required for osteoclast-mediated stimulation of myeloma cell growth.

Because myeloma cells become refractory to chemotherapeutic agents in advanced stages when systemic bone destruction develops, there is a possibility that the interaction with osteoclasts may have a protective role against cytotoxic effects of anti-cancer agents. In fact, cell-to-cell interaction with osteoclasts not only enhances the growth of myeloma cells but also causes marked resistance to cytotoxic effects of doxorubicin (19). These observations are in agreement with clinical findings that myeloma patients at advanced stages with extensive osteolytic lesions show refractoriness to chemotherapies, and suggest that increased osteoclast number and/or activity contributes to the aggressiveness or drug resistance of adjacent myeloma cells. These findings are another demonstration of the vicious cycle of interactions between myeloma cells and osteoclasts: osteoclasts that are generated and activated by myeloma cells not only resorb and destroy bone but also enhance myeloma cell growth and survival, even during treatment with chemotherapeutic agents, largely via cell-to-cell interaction with myeloma cells.

There are several candidates that may mediate the cellular interaction between myeloma cells and osteoclasts. B-cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL), members of the tumor necrosis factor (TNF) family, have been implicated as growth and survival factors for myeloma cells under cytokine stimulation (20;21). BAFF binds to three TNF receptor family members, B-cell maturation antigen (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and BAFF receptor (BAFF-R), whereas APRIL binds to BCMA, TACI and heparan sulfate

proteoglycans such as syndecan-1 (22). Myeloma cells in primary culture constitutively express BCMA as well as syndecan-1 (20;21), while myeloma cells dependent upon the bone marrow microenvironment express high levels of TACI (23). When TACI-Fc was added in co-cultures with osteoclasts as a decoy receptor for BAFF and APRIL, apoptosis of myeloma cells was substantially enhanced, indicating a supporting role for BAFF and/or APRIL in osteoclast-mediated myeloma cell growth and survival (24). These results are consistent with the hypothesis that BAFF and/or APRIL play important roles in the formation of a vicious cycle between myeloma cells and osteoclasts under close cell-to-cell interactions in the myeloma bone marrow microenvironment. This hypothesis provides a rationale for interference in the interactions between these factors and their cognate receptors by TACI-Fc as a novel therapeutic approach against multiple myeloma.

### Suppression of Bone Formation

In typical destructive bone lesions of myeloma patients, enhanced bone resorption is accompanied by impaired bone formation and mineralization, causing the well-known "punched-out" lesions visible on X-rays. Analyses of bone turnover by biochemical bone markers also suggested an imbalance of bone turnover with enhanced bone resorption and suppressed bone formation (12). However, little is known about the mechanism(s) responsible for the impairment of bone formation. A canonical Wntless-type (Wnt) signaling pathway has recently been shown to play a critical role in osteoblast differentiation (25;26). Wnt proteins are secreted cysteine-rich glycoproteins that regulate differentiation of hematopoietic and mesenchymal cells as well as embryonic development. Because conditioned media from myeloma cells suppressed osteoblast differentiation and mineralization, and because several secreted Frizzled-related protein (sFRP) and Dickkopf (DKK) family members are soluble Wnt antagonists, the expression of sFRP-1, -2 and -3 as well as DKK-1 was examined in myeloma cells. Various myeloma cell lines

including U266, RPMI8226 and ARH77 secreted only sFRP-2 at detectable levels. Although DKK-1, an inhibitor of an LDL receptor-related protein (LRP)5/6 Wnt co-receptor, was reported to play an important role in the impairment of bone formation by myeloma (27), DKK-1 mRNA could be detected only in U266 cells. More importantly, sFRP-2 mRNA and protein was detected in most myeloma cells from patients with advanced or terminal stages of myeloma.

In an effort to clarify the role of sFRP-2 in the suppression of bone formation by myeloma cells, recombinant sFRP-2 was added to MC3T3-E1 osteoblastic cell cultures together with BMP-2. Exogenous sFRP-2 partially suppressed alkaline phosphatase activity and almost completely blocked mineralized nodule formation enhanced by BMP-2 (28). Furthermore, immunodepletion of sFRP-2 restored mineralized nodule formation by MC3T3-E1 cells in the presence of myeloma cell conditioned media. These results demonstrate that myeloma cell-derived sFRP-2 is an important factor in the impairment of bone formation by multiple myeloma. In contrast to sFRP-2, DKK-1 was preferentially expressed in mature types of myeloma cells, but barely expressed in plasmablastic or immature types of myeloma cells (27). Nevertheless, because myeloma cells have been shown to secrete factors other than sFRP-2 that are implicated as inhibitors of osteoblast differentiation, including DKK-1, IL-3 and insulin-like growth factor-binding protein 4, sFRP-2 may act in concert with other factors to cause a potent suppression of bone formation in destructive bone lesions. Further elucidation of the mechanism of the development of destructive bone lesions by myeloma cells should lead to novel therapeutic approaches to prevent devastating bone destruction and myeloma cell expansion.

**Conflict of Interest:** The authors report that no conflicts of interest exist.

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