New Potential Targets for Treating Myeloma Bone Disease

G. David Roodman

Abstract

Purpose: Myeloma bone disease results in severe pain and pathologic fractures in >80% of patients. Myeloma bone disease is characterized by both increased osteoclast activity and suppressed new bone formation. The basis for both the increased bone destruction and decreased bone formation has been a topic of extensive investigation during the last several years.

Experimental Design: Marrow samples from patients with myeloma were screened by both molecular biological and gene expression profiling techniques to identify factors that may be responsible for the enhanced bone destruction and suppressed bone formation in patients with the disease.

Results: Several novel factors have been identified that directly stimulate osteoclastic bone destruction in myeloma. These include receptor activator of NF-κB ligand, macrophage inflammatory peptide 1α, and interleukin (IL)-3. All of these factors are increased in most patients with myeloma. Furthermore, osteoprotegerin levels are markedly suppressed, further driving osteoclast formation. In addition, four novel inhibitors of osteoblast differentiation or activity have been identified. These include two inhibitors of the Wnt signaling pathway, DKK1 and soluble frizzled protein 2. The Wnt signaling pathway is critical for osteoblast differentiation. Two cytokines, IL-3 and IL-7, have also been reported that directly or indirectly inhibit osteoblast differentiation in patients with myeloma. Interestingly, increased macrophage inflammatory peptide 1α, IL-3, and IL-7 result from abnormal transcriptional regulation of these genes by increased levels of acute myelogenous leukemia-1 to acute myelogenous leukemia-1B transcription factors.

Conclusions: The recent identification of novel stimulators of osteoclast activity and inhibitors of osteoblast differentiation provide new therapeutic targets for treating this devastating bone disease in patients with myeloma.

Materials and Methods

Marrow samples from patients with MM were screened by both molecular biological and gene expression profiling techniques to identify factors that may be responsible for the enhanced bone destruction and suppressed bone formation in patients with MM. The methods used have been previously reported in detail (4–8).

Results

Stimulators of osteoclast formation in myeloma

RANKL. RANKL is a major osteoclastogenic factor involved in myeloma bone disease. Giuliani et al. (9) showed that there is an imbalance between osteoprotegerin, a decoy receptor for RANKL, and RANKL levels in the bone marrow environment of patients with MM. They found that myeloma cells did not express RANKL and produced low amounts of osteoprotegerin. However, in cocultures of human myeloma cells with marrow stromal cells, RANKL expression was up-regulated and osteoprotegerin production strongly down-regulated at both the protein and mRNA levels. In addition, Pearse et al. (10) examined marrow biopsy specimens from patients with MM and found that RANKL expression was markedly up-regulated in bone marrow biopsy specimens from patients with MM, whereas osteoprotegerin was expressed at very low levels.

Inhibitors of the Wnt signaling pathway, including DKK1 and soluble frizzled protein 2.

Multiple myeloma (MM) is a severely debilitating, incurable, and uniformly fatal neoplastic disease of B cell origin (1). The major source of morbidity and possible mortality associated with MM is osteolytic lesions throughout the axial skeleton (2). The increase in osteoclast bone resorption in myeloma is usually associated with a marked impairment in osteoblast function (3). The basis for both the increased bone destruction and decreased bone formation has been a topic of extensive investigation during the last several years. Investigations have identified several potential novel targets for treating myeloma bone disease, including macrophage inflammatory peptide 1α (MIP-1α), interleukin (IL)-3, receptor activator of NF-κB ligand (RANKL), IL-7, and inhibitors of the Wnt signaling pathway, including DKK1 and soluble frizzled protein 2.
compared with normal bone marrow biopsy specimens. Taken together, these data and those of other investigators (11) suggest that there is a marked imbalance between RANKL expression and osteoprotegerin levels that favor osteoclastogenesis and osteoclast activation.

Croucher et al. (12) showed that osteoprotegerin also inhibited the development of osteolytic bone disease in a murine model of myeloma, and Yaccoby et al. (13) reported that when primary myeloma cells are injected into a human fetal bone rudiment implanted into mice with severe combined immunodeficiency, an inhibitor, RANK, decreased bone resorption and tumor burden. Taken together, these studies suggest that blocking bone resorption induced by RANKL may decrease tumor burden and bone destruction in patients with MM. Osteoprotegerin and a neutralizing antibody to RANKL (14, 15) have been used in phase I trials for patients with myeloma or breast cancer. Both of these agents suppress bone resorption markers and anti-RANKL is now being tested extensively in phase II trials.

MIP-1α. We identified MIP-1α as a novel osteoclast actuating factor produced by myeloma cells (4). We found that MIP-1α mRNA and protein levels were elevated in freshly isolated bone marrow from ~70% of patients with MM compared with healthy patients (4). Levels of MIP-1α were mildly increased in 21% of patients with other hematologic diagnoses, and were not elevated in marrow samples from healthy controls. Furthermore, recombinant human MIP-1α induced osteoclast formation in human bone marrow cultures (4), and importantly, the addition of a neutralizing antibody to MIP-1α to human bone marrow cultures treated with freshly isolated marrow plasma from patients with MM blocked the increased osteoclast formation induced by these marrow plasma samples. Anti-MIP-1α had no effect on control levels of osteoclast formation. Marrow plasma samples from healthy controls did not induce osteoclast formation. Thus, high levels of MIP-1α are present in marrow samples from patients with active MM but not in marrow from patients with other hematologic disorders or healthy controls, and support an important role of MIP-1α in patients with active myeloma. Abe et al. (16) also showed that elevated levels of MIP-1α were present in 15 of 20 patients with MM, and that MIP-1α induced rabbit osteoclast formation. Uneda et al. (17) showed that the expression levels of MIP-1α produced by myeloma cells correlated with bone lesions in 16 of 18 patients with MM who expressed elevated MIP-1α levels.

In vivo studies with murine models of myeloma bone disease have also shown an important role of MIP-1α in vivo. When severe combined immunodeficiency mice were implanted with human MM cells that could no longer express MIP-1α because they had been stably transfected with an antisense construct to MIP-1α, both tumor burden and bone destruction were markedly decreased compared with animals implanted with MM cells that expressed high levels of MIP-1α (18). These data showed that blocking MIP-1α could have a profound effect on bone destruction and tumor growth in MM.

Recently, Oyabji et al. (19) showed that murine myeloma cells also produced MIP-1α, and that a neutralizing antibody to MIP-1α blocks the bone destruction and decreases tumor burden in syngeneic animals implanted with this murine myeloma cell line. These findings suggest that MIP-1α is an excellent target for blocking bone destruction in myeloma.

The finding that MIP-1α was overexpressed in myeloma cells and not in other hematologic malignancies suggested that the regulation of expression of MIP-1α in myeloma was abnormal. Choi and coworkers (5) showed an imbalance for the expression of two forms of the acute myelogenous leukemia (AML)-1A transcription factor in MM cells. AML-1B normally drives the transcription of genes regulated by AML-1, and AML-1A is a truncated form of AML-1B that inhibits the binding of AML-1B to AML-1 transcription factor binding sites. In normal cells, AML-1B expression levels are higher or equal to AML-1A. In myeloma cells that express high levels of MIP-1α, the ratio of AML-1A to AML-1B was reversed such that there is much more AML-1A than AML-1B. Furthermore, transfecting myeloma cells with AML-1A enhances the production of MIP-1α, whereas transfecting them with AML-1B markedly suppresses MIP-1α production. More importantly, other genes that are regulated by AML-1 (e.g., IL-3 and IL-7) were also increased in marrow plasma from patients with myeloma. IL-3 and IL-7 have profound effects on both osteoclast formation and inhibition of bone formation in myeloma.

Inhibitors of osteoblast differentiation in myeloma

IL-3. We reported that treatment of primary mouse and human marrow stromal cells with IL-3 inhibited basal and BMP-2-stimulated osteoblast formation in a dose-dependent manner, without affecting cell growth. Importantly, marrow plasma from MM patients with high IL-3 levels inhibited osteoblast differentiation and the osteoblast inhibition could be blocked by anti-IL-3. However, IL-3 did not inhibit osteoblast differentiation of osteoblast-like cell lines. In contrast, IL-3 increased the number of CD45+ hematopoietic cells in primary stromal cell cultures. Depletion of the CD45+ cells abolished the inhibitory effects of IL-3 on osteoblasts, and reconstitution of the cultures with CD45+ cells restored the capacity of IL-3 to inhibit osteoblast differentiation. These data suggest that IL-3 plays a dual role in the bone destructive process in myeloma by both stimulating osteoclasts and indirectly inhibiting osteoblast formation.

IL-7. Giuliani and coworkers (7) reported that IL-7 is increased in marrow plasma samples from patients with myeloma and that IL-7 inhibits osteoblast differentiation of both early human osteoblast precursors (CFU-F) and more differentiated osteoblast precursors (CFU-OB). These workers further showed that anti-IL-7 reversed the inhibition of human osteoblast differentiation by myeloma cell lines and primary myeloma cells, and that IL-7 could inhibit bone nodule formation by osteoblast precursors in vitro. Furthermore, they showed that IL-7 blocked the activity of the cbfa-1 transcription factor, which is a critical transcription factor for osteoblast differentiation. Thus, IL-7 was a potent inhibitor of osteoblast differentiation in myeloma and is a potential target for reversing the severe suppression of the osteoblast activity in myeloma.
**DKK1.** Tian and coworkers (8) reported that DKK1 may be involved in the suppression of osteoblast activity in myeloma. DKK1 is an inhibitor of the Wnt signaling pathway, which is a critical pathway of osteoblast differentiation. These authors showed that DKK1 was secreted by myeloma cells, and marrow plasma from patients with myeloma who had >12 ng/mL of DKK1 inhibited the osteoblast differentiation of a murine mesenchymal stem cell line in vitro. They further showed that DKK1 gene expression levels were highly correlated with the extent of bone disease in patients with myeloma. However, other workers reported that DKK1 is not expressed by MM cells (7, 21), suggesting that DKK1 is not universally expressed by myeloma cells.

Recently, the administration of proteosome antagonists has been reported to increase bone formation in animals by increasing expression of BMP-2, a bone growth factor (22). Zangari and coworkers have reported that bone formation markers are increased in patients with myeloma receiving the proteosome antagonist, bortezomib (23). Their results suggest that these agents may be able to increase bone formation in patients with myeloma in addition to treating underlying malignancy and reverse the inhibitory effects of myeloma on bone formation.

**Discussion**

Several new potential therapeutic targets have been identified for treating myeloma bone disease. These include RANKL, for which a neutralizing antibody to RANKL is currently in clinical trial; MIP-1α, for which receptor antagonists for the MIP-1α receptor, CCR1, are being explored; IL-3 and IL-7; and the Wnt antagonist DKK1. A clinical trial is being initiated using parathyroid hormone–related peptide, a Wnt agonist, to try to stimulate bone formation in MM. Finally, small molecules that could target the expression of AML-1a in MM cells could also be a potential new therapy for MM bone disease and decrease production of MIP-1α, IL-3, and IL-7. In addition, radiopharmaceuticals, such as Quadramet (24), that target bone, are now being tested in combination with autologous stem cell transplantation to treat myeloma. Thus, new therapeutic targets are available for treating myeloma bone disease, which could be used to treat MM and myeloma bone disease.

**Open Discussion**

**Dr. Berenson:** One of the concerns I have with the model described by John Shaughnessy is that as the disease progresses, DKK1 is not there. If you get rid of DKK1, you make myeloma more aggressive and resistant to treatment.

**Dr. Roodman:** In preliminary experiments, we can’t find DKK1 up-regulated in myeloma, but we haven’t looked in as many patients as Dr. Shaughnessy, who looked at hundreds. We’ve looked at 10 or 15, so I don’t know whether our subset doesn’t express DKK1. The bigger problem with all of these inhibitors is the clinical finding that myeloma bone lesions never heal. You don’t make new bone once you get rid of myeloma. These are all soluble inhibitors that are being made by myeloma cells, so there has to be something more profound that happens to the bone marrow marker environment instead of being bathed with a reversible inhibitor, such as a Wnt signaling inhibitor.

**Dr. Berenson:** That’s part of the concern, because there are data showing that the Wnt pathway is activated in myeloma cells.

**Dr. Roodman:** There are 20 different Wnt signaling ligands, some of which stimulate growth of myeloma cells. You have to be careful with those types of studies because you have to pick one that doesn’t affect myeloma but does block DKK1.

**Dr. Berenson:** What about bortezomib?

**Dr. Roodman:** There’s a nice paper by Greg Mundy’s group in the Journal of Clinical Investigation (22) in which they showed that proteosome antagonists induce BMP production, which resulted in new bone formation in animal models. Several groups looked at bone formation markers in patients with myeloma. In several cases, the patients who are treated with bortezomib have elevated alkaline phosphatase levels compared with patients who don’t get it and those who respond have a higher alkaline phosphate level than those who don’t respond.

**Dr. Clohisy:** Why do we have localized punched-out lesions that are 3 inches away in metaphyseal bone?

**Dr. Roodman:** You have a very local production of relatively high concentrations of stimulators of osteoclastogenesis, but if you really measure how much RANK ligand there is, they are not high. In contrast to other papers, we can’t find elevated MIP1-α in the serum in patients with myeloma, even though their marrow plasma has high levels. What you are having is a local high concentration that stimulates increased numbers of osteoclasts around myeloma cells, but if you go down the block, you don’t have increased numbers of osteoclasts. The osteoclasts are destroying bone, not the myeloma cells.

**Dr. Clohisy:** Have those myeloma cells found a place where they can successfully grow?

**Dr. Roodman:** Are there niches in the bone marrow for the growth of myeloma cells like there are niches in the bone marrow for the growth of hemopoietic cells, and myeloma cells express the same homing receptor as hematopoietic cells. They can be mobilized from the bone marrow in a similar fashion using GCSF. When you do a stem cell autotransplantation, you mobilize some myeloma cells. About 2% of your total transplant is myeloma cells on average, so you can mobilize them and they home to these niches. It is interesting that you don’t get hematopoiesis as a diffuse process throughout the bone marrow; it occurs in very discrete sites. It may be that those sites have up-regulated SDF1 by the osteoblasts or stromal cells, which allows the cells to home.

**Dr. Bruland:** I remember recently having treated three patients with osteolytic bone foci of myeloma/plasmocytoma. Two of the lesions healed nicely following fractionated radiotherapy. However, the third patient presented with an eminent fracture. Despite pain relief, the fracture had to be stabilized by an orthopedic surgeon. My experience is that a myeloma lesion per se can be healed by radiotherapy, but you need standard fractionated radiotherapy up to a reasonable dose.

**Dr. Roodman:** What’s been shown in patients who get TBI followed by stem cell rescue with an autotransplantation and myeloma?

**Dr. Bruland:** Then the dose pr. fraction and total radiation dose is very much lower. For total body radiation we now give 1.3 Gy twice daily over 5 days.
Dr. Suva: I don’t think it is at all surprising that there are differences in the type of genes and proteins that are expressed from population to population.

Dr. Roodman: We’ve done cytokine analysis of marrow plasma from 100 patients and haven’t found DKK1. We find IL-8, IL-6, and MIP1-α. Other groups have shown that in myeloma mouse models, DKK1 is up-regulated in the stroma, not in the myeloma cells. Therefore, if it is up-regulated in the microenvironment of myeloma, you’ll pick it up if you have low levels of contamination. It doesn’t matter where it comes from, it’s whether or not it’s up-regulated.

Dr. Weilbaecher: Are there any chromosomal rearrangements in AML-1?

Dr. Roodman: No, there are no chromosomal abnormalities in AML-1. There is a splice variant in myeloma and an AML fusion gene in acute leukemia.

Dr. Weilbaecher: Is the anemia associated with myeloma, for example, related to decreased osteoblasts in stem cells? It sounds like there are osteoblast defects.

Dr. Roodman: A paper from a group in Japan back in the middle 1990s looked at CFUE and BFUE in myeloma marrow samples and reported that MIP1-α was suppressing erythropoiesis and was in part responsible for the anemia in those patients (25). MIP1-α will stimulate CFU-GM, but it will not stimulate CFU-E and BFU-E. Whether that is really the mechanism or not is still unclear.

Dr. Suva: Do you think that giving PTHrP(1-36) to stimulate bone mass is going to have any effect on the local lesions or is it just going to increase bone mass systemically?

Dr. Roodman: It probably will have an effect, because it has a strong anabolic action similar to PTH but more potent. The question is whether you can get any kind of osteoblastic response and whether you do it in the face of a bisphosphonate, because these patients routinely get monthly zoledronic acid or pamidronate, some of them for years.

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**References**

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