Myeloma Bone Disease: Recent Advances in Biology, Diagnosis, and Treatment

ORHAN SEZER

Department of Hematology and Oncology, Charité – Universitätsmedizin Berlin, Berlin, Germany

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ABSTRACT

Bone disease is a hallmark of multiple myeloma (MM). Occurring in the majority of MM patients, it is associated with bone pain, fractures, and hypercalcemia and has major impacts on quality of life. Furthermore, bone resorption activity has been shown to be an independent risk factor for overall survival in patients with symptomatic MM. Myeloma is characterized by a unique form of bone disease with lytic bone destruction that is not followed by reactive bone formation (uncoupling).

This review focuses on recent advances in our understanding of the biology of osteoclast activation and osteoblast inhibition in MM, diagnostic standards, and recent progress in treatment options for myeloma bone disease. Translational research has enabled a rapid transfer of mechanistic insights from the bench to the bedside and will hopefully result in better treatment options and outcome for patients in near future. The Oncologist 2009;14:276–283

Editor’s note: This review focuses on recent advances in our understanding of the biology of osteoclast activation and osteoblast inhibition in multiple myeloma, diagnostic standards, and recent progress in treatment options for myeloma bone disease. See also the article by Silvestris et al. in this issue of the Journal.

BACKGROUND

Multiple myeloma (MM) is characterized by clonal expansion of plasma cells resulting in elevated immunoglobulin levels, immunodeficiency, anemia, renal insufficiency, hypercalcemia, and lytic bone disease [1–4]. MM is the disease with the highest incidence of bone involvement among the malignant diseases. Abnormalities in conventional radiography were found in about 80% of patients with newly diagnosed MM [5]. Bone destruction in MM can result in skeletal complications such as bone pain, pathological fractures requiring surgery and/or radiation to bone, spinal cord compression, hypercalcemia, and deterioration in quality of life [6]. Furthermore, bone resorption activity was shown to be an independent risk factor for overall survival in patients with symptomatic MM [7].

Correspondence: Orhan Sezer, M.D., Ph.D., Department of Hematology and Oncology, Charité – Universitätsmedizin Berlin, 10117 Berlin, Germany. Telephone: 49-30-450-613105; Fax: 49-30-450-527907; e-mail: sezer@charite.de Received January 7, 2009; accepted for publication February 16, 2009; first published online in The Oncologist Express on March 13, 2009. ©AlphaMed Press 1083-7159/2009/$30.00/0 doi: 10.1634/theoncologist.2009-0003

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In a population-based retrospective cohort study, 16 times more fractures were observed than expected in the year before diagnosis, mostly pathologic fractures of the vertebrae and ribs [8]. After follow-up, 58% of these patients experienced at least one new fracture. Patients with a pathological fracture had a worse survival expectation [8]. Thus, skeletal complications are associated with significant morbidity and pain. They compromise mobility and day-to-day independence and negatively impact survival. Moreover, skeletal events increase treatment costs [9].

**Pathogenesis of Myeloma Bone Disease**

The basic principle of increased bone resorption in MM is an uncoupling of normal bone remodeling with enhanced bone resorption and decreased bone formation [10]. By mechanisms discussed in the following, myeloma cells stimulate osteoclast activity and suppress osteoblast differentiation and function. An increase in the number and activity of osteoclasts further promotes myeloma progression directly by cell–cell interactions and indirectly by cytokines released from the bone marrow microenvironment, thus maintaining a vicious circle between bone destruction and tumor cell survival [11].

**Myeloma Cells Increase Osteoclast Activity**

A consistent histological finding in myeloma bone disease is enhanced osteoclast accumulation and bone resorption adjacent to myeloma cells, whereas osteoclasts are not increased in bone not invaded by myeloma [12]. In vitro cocultures of purified preosteoclasts and primary human myeloma cells show that myeloma cells induce the differentiation of progenitors into mature osteoclasts, which in turn support the survival of myeloma cells [11, 13]. It was already suggested, several decades ago, that osteoclasts are stimulated by local osteoclast activating factors that are produced by myeloma cells or cells of the bone microenvironment [14]. Recently, three major groups of factors were identified as main osteoclast inducers in MM: the receptor activator of nuclear factor (NF)-κB (RANK) ligand (RANKL), the chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β, and stromal derived factor-1α (SDF-1α).

Myeloma cells lead to an imbalance in the RANKL–osteoprotegerin (OPG) system in the tumor microenvironment. RANKL has been characterized as the key mediator of osteoclast differentiation and activation. RANKL is a member of the tumor necrosis factor superfamily [15] and is produced mainly by osteoblastic lineage cells and stromal cells. The cellular receptor for RANKL, RANK, is expressed on the surface of osteoclast precursors and mature osteoclasts. RANKL induces differentiation, formation, fusion, and survival of preosteoclasts [16]. Moreover, it has direct effects on mature osteoclasts, causing actin ring formation and cytoskeletal rearrangements that precede bone resorption, and activating mature osteoclasts to resorb bone.

OPG acts as a decoy receptor antagonist for RANKL [17]. It is secreted mainly by osteoblastic lineage and stromal cells. A balanced RANKL/OPG ratio is essential for normal bone turnover. In animal models, unbalanced expression of these cytokines led to extreme skeletal phenotypes, for example, severe osteopetrosis in RANKL knockout mice [18] or osteopenia in OPG-deficient mice [19]. In humans, an abnormal RANKL/OPG ratio is found in both benign and malignant bone diseases [20]. Several studies investigated the role of the RANKL–RANK–OPG system in myeloma bone disease [21–23]. It could be shown that myeloma cells induce RANKL expression by stromal cells within the bone microenvironment through direct cell-to-cell contact. Moreover, animal models [24] as well as studies on human primary cells could show direct RANKL expression of myeloma cells [25, 26].

In addition to the effects on RANKL expression, myeloma cells decrease OPG availability within the bone microenvironment. They lead to reduced OPG secretion by osteoblasts and stromal cells [21, 22, 27]. Moreover, myeloma cells produce and shed syndecan-1 (CD 138), a transmembrane proteoglycan that binds to the heparin-binding domain of OPG and mediates its internalization, and consecutively, lysosomal degradation by myeloma cells [28]. The combination of these effects results in an increased RANKL/OPG ratio in the bone marrow microenvironment that favors the formation and activation of osteoclasts. Furthermore, the ratio of the serum level of soluble RANKL to OPG was found to be a prognostic factor for survival in newly diagnosed myeloma patients [29].

MIP-1α and MIP-1β are other cytokines that are important for myeloma bone disease. MIP-1α belongs to the RANTES (regulated on activation normal T cell expressed and secreted) family of chemokines. MIP-1α is chemotactic for osteoclast precursors, induces late-stage differentiation of human osteoclast progenitors, and promotes osteoclast formation in bone marrow cultures [30–33]. Both MIP-1α and MIP-1β are produced and secreted by myeloma cells. In preclinical experiments, antibodies against MIP-1α and MIP-1β or their receptor, CCR5 [32], as well as transfection of myeloma cells with an antisense construct to MIP-1α, could block enhanced bone resorption [31]. Studies on the action of MIP-1α and MIP-1β suggested that their effects on osteoclasts are dependent on the RANKL pathway [33]. MIP-1α and MIP-1β enhance RANKL expression in stromal cells. In a murine model of
myeloma, injection of recombinant MIP-1α produced a strong increase in osteoclast formation in normal mice, but not in RANK−/− animals [33]. In addition to these effects, MIP-1α can directly act on myeloma cells, because they express the receptor CCR5. Studies showed that MIP-1α promotes growth, survival, and migration of myeloma cells [34]. It could be shown that MIP-1α-induced signaling involved activation of the phosphatidylinositol 3-kinase /Akt and mitogen-activated protein kinase (MAPK) signaling pathways in myeloma cells, leading to increased proliferation and protection against apoptosis [34].

Other mediators involved in osteoclast activation in MM include interleukin (IL)-6 and IL-11, which are predominantly produced by stromal cells [35]. IL-3 and hematocyte growth factor (HGF) are factors mainly produced by myeloma cells. SDF-1α is another chemokine expressed by marrow stromal cells and myeloma cells [36]. SDF-1α binds to its receptor, CXCR4, which is expressed on osteoclast precursors, thereby inducing chemotaxis and matrix metalloproteinase (MMP)-9 activity. In vitro, SDF-1α increased osteoclast motility and bone-resorbing activity. In this model, osteoclast activation mediated by myeloma cells could be reduced using a CXCR4-specific inhibitor [36].

In this issue of The Oncologist, Silvestris et al. [37] advocate that malignant plasma cells can transdifferentiate to functional osteoclast-like cells in the marrow microenvironment and directly participate in bone resorption. This is an interesting hypothesis, but clearly needs confirmatory data before it can be generally accepted. The osteoclast-like activity of all or a subset of myeloma cells needs confirmation in myeloma patients. Furthermore, the authors claim that patients with advanced MM are usually pancytopenic as the result of repeated chemotherapy and have a small marrow reserve of hematopoietic progenitors able to differentiate into osteoclasts; the remaining monocytes would not be able to produce adequate amounts of osteoclasts necessary to produce extensive bone resorption. As summarized above, the bone marrow microenvironment is heavily altered in MM due to cell-to-cell interactions and paracrine factors, all favoring both osteoclast differentiation from progenitors and an increase in the activity of mature osteoclasts. These factors can well counterbalance a reduction in the number of osteoclast progenitors. At present, osteoclasts are considered to represent the major cells resorbing bone, in myeloma and also in other cancer-induced bone diseases [38]. Thus, although the hypothesis from Silvestris et al. [37] must be taken into account, it is unclear, at this time, to which extent myeloma cells directly contribute to bone resorption in MM patients, and further studies are needed to clarify this issue. Furthermore, osteoblast differentiation and activity are substantially impaired in MM, resulting in unbalanced bone resorption.

**MYELOMA CELLS SUPPRESS OSTEOCLAST DIFFERENTIATION AND FUNCTION**

Whereas most studies on myeloma bone disease initially focused on osteoclast activation, the influence of myeloma cells on osteoclasts has been characterized more recently. In contrast to bone metastases in other malignancies, MM causes bone destruction without a sufficient osteoblastic reaction. Histomorphometric analysis of bone biopsies from patients with overt myeloma showed a reduced number and activity of osteoclasts on bone surfaces adjacent to myeloma cells [10]. Moreover, in vitro studies revealed that osteoblast growth and function are inhibited when cocultured with myeloma cells or in medium conditioned by myeloma cells, suggesting that this effect is a result of soluble osteoblast inhibiting factors [39].

The canonical Wingless-type (Wnt) pathway was demonstrated to be a major signaling pathway in osteoclasts. Wnt glycoproteins bind to the Wnt receptor and its coreceptors low-density lipoprotein receptor-related protein (LRP)5/LRP6 and lead to a stabilization of β-catenin. This results in its cytoplasmatic accumulation, translocation into the nucleus, and stimulation of expression of osteoblastic target genes [40, 41]. In the absence of a Wnt signal, β-catenin is phosphorylated and degraded by the proteasome. Extracellular Wnt antagonists prevent the binding of Wnt glycoproteins to their receptors and can be divided into two functional classes [42]. Members of the Dickkopf (DKK) family bind to the LRP5/LRP6 component of the Wnt receptor complex, whereas secreted frizzled-related proteins (sFRP), for example, sFRP-2 and sFRP-3 (synonym, FrzB), bind to Wnt proteins. Both result in suppression of Wnt signaling and reduced osteoblast function.

Using gene-expression profiles of myeloma patients, Tian et al. [43] found an overexpression of the DKK-1 gene in MM patients with focal bone lesions. Moreover, DKK-1 protein could be detected in myeloma cells, and elevated levels of DKK-1 were detected in peripheral blood and bone marrow plasma from patients with osteolytic lesions. In vitro, recombinant human DKK-1 or bone marrow plasma with high DKK-1 levels inhibited osteoblast function. This effect was neutralized by a polyclonal anti-DKK-1 antibody.

Serum DKK-1 levels are higher in newly diagnosed MM patients than in patients with monoclonal gammopathy of undetermined significance or controls [44]. Kaiser et al. [45] quantified DKK-1 serum levels in 184 previously untreated MM patients. Importantly, myeloma patients without lytic lesions on conventional radiography had
significantly lower DKK-1 levels than patients with lytic bone disease. Furthermore, serum DKK-1 correlated with the number of bone lesions [45]. In a recent study, DKK-1 serum levels were examined in MM patients receiving different treatment regimens, also including novel agents [46]. Serum DKK-1 decreased in myeloma patients responding to therapy, irrespective of the regimen chosen, but not in nonresponders. This finding suggests that myeloma cells are the main source of the circulating DKK-1 protein.

In vitro and in vivo experiments showed a reduction in osteoprogenitor cell differentiation by DKK-1 via inhibition of Wnt signaling [47] that could be antagonized by an anti–DKK-1 antibody [48]. In addition to its role in osteoblast inhibition, recent results by Qiang et al. [27] suggested that DKK-1 also stimulates osteoclastogenesis by modulating RANKL and OPG production in osteoblasts and thereby increasing the RANKL/OPG ratio. A study by Yacoby et al. [48] tested the effect of anti-DKK-1 therapy on bone metabolism and tumor growth in a severe combined immunodeficient (SCID)-rab system. SCID-rab mice were engrafted with primary myeloma cells and treated with control and DKK-1–neutralizing antibodies for 4–6 weeks. The bone mineral density of the implanted myelomatous bone was reduced in control mice but increased in mice treated with anti–DKK-1 antibody. Histological examination revealed that myelomatous bones of anti-DKK-1–treated mice had higher numbers of osteocalcin-expressing osteoblasts and a lower number of multinucleated tartrate-resistant acid phosphatase (TRAP)-expressing osteoclasts [48]. Thus, DKK-1 is a potential therapeutic target in MM, and the effects of DKK-1–neutralizing antibodies remain to be evaluated in MM patients.

Furthermore, myeloma cell lines and primary myeloma cells from patients with bone lesions have been shown to produce the soluble Wnt inhibitor sFRP-2 and thereby suppress mineralization and alkaline phosphatase activity in osteoblasts. Immunodepletion of sFRP-2 significantly restored mineralized nodule formation in vitro [49].

In addition, osteoblasts induce expression of MMP-1 and upregulate expression of MMP-2, urokinase plasminogen activator, and HGF in myeloma cells [50]. In turn, interaction with myeloma cells leads to abundant MMP-1 expression in osteoblasts. The mechanisms responsible for MMP-1 upregulation are mediated by both membrane-bound and soluble factors, and involve the p38 MAPK pathway. Interaction with osteoblasts enhances the capability of myeloma cells to transmigrate and invade through Matrigel or type I collagen [50].

Other mechanisms may add to the effect of myeloma cells on osteoblasts and include an upregulation of IL-6 secretion through cell-to-cell contact [51] and a down-regulation of OPG mRNA by osteoblastic lineage cells [21]. IL-3, a factor produced by myeloma cells, has also been shown to inhibit osteoblast differentiation [52]. Myeloma cells inhibit osteoblast formation via cell-to-cell contact by suppressing the activity of Runx2/Cbfa1, another critical osteoblast transcription factor in preosteoblastic cells [53].

Further research on the interaction between myeloma cells and osteoblasts is needed in order to understand the mechanisms of osteoblast inhibition and to identify possible therapeutic targets in the treatment of myeloma bone disease [54–56].

**DIAGNOSIS OF MYELOMA BONE DISEASE**

The majority of MM patients have skeletal involvement with bone pain, lytic lesions, diffuse osteoporosis, or pathologic fractures at the time of diagnosis, and almost all patients with symptomatic MM develop bone manifestations in the later clinical course [8]. The most common osteolytic lesions include the vertebrae, ribs, scull, femur, hip, and humerus, whereas in approximately 15% of patients diffuse osteopenia is the only bone manifestation. The standard diagnostic procedure for the detection of skeletal affections is conventional radiography, although this technique is relatively insensitive and requires a loss of 30%–50% of the trabecular bone to have a detectable lytic lesion. Bone scans are obsolete in MM, because they primarily reflect osteoblast activity and underestimate myeloma bone disease. Because histomorphometric studies have shown that abnormal bone degradation can exist in the absence of osteolytic lesions on skeletal radiography, the diagnostic sensitivity of conventional radiography appears to be low in early myeloma. Low-dose whole-body multidetector computed tomography was developed as an alternative to conventional x-ray imaging [57].

Magnetic resonance imaging (MRI) was established as a noninvasive technique that can recognize bone marrow abnormalities rather than myeloma bone disease [58]. For suspected cord compression, MRI is the technique of choice. MRI can be helpful in the distinction between benign and malignant compression fractures [59]. Abnormal MRI is a negative prognostic factor in asymptomatic MM [60]. In patients with symptomatic MM, the number of focal lesions detected by MRI was shown to have independent prognostic value for survival [61].

In addition to imaging techniques, new biochemical markers have been evaluated for monitoring the present bone metabolism in MM, but are not yet in routine use [62].
TREATMENT OF MYELOMA BONE DISEASE

Bisphosphonates

Bisphosphonates are the mainstay of the treatment of myeloma bone disease. These drugs induce osteoclast apoptosis or inhibit osteoclast activity [63]. The aim is the reduction of skeletal-related events in patients with myeloma bone disease, that is, MM patients with one or more lytic lesions or diffuse osteopenia as a result of myeloma-induced increased bone resorption. Large, randomized, placebo-controlled clinical trials have proven the efficacy of i.v. pamidronate or oral clodronate in the therapy of myeloma bone disease [64–67]. Zoledronic acid has an effect that is superior to that of pamidronate in the treatment of hypercalcemia of malignancy [68], but a clinical efficacy comparable with that of pamidronate in MM in the prevention of skeletal-related events [69]. The shorter infusion duration over 15 minutes is a possible advantage. Intravenous bisphosphonates have the potential to cause acute or chronic renal dysfunction. Osteonecrosis of the jaw (ONJ) is rather uncommon, but potentially serious, complication of i.v. bisphosphonates, which is characterized by the presence of exposed bone in the mouth [70]. Invasive dental procedures are a major risk factor for the development of ONJ [71]. Zoledronic acid was associated with a higher incidence of ONJ in retrospective evaluations. Guidelines concerning bisphosphonate treatment in MM were recently published elsewhere [72, 73]. Before the start of bisphosphonates and during this treatment, dental status should be monitored at least on an annual basis and good oral hygiene should be maintained. Dental conditions should be treated before initiating bisphosphonate therapy. After therapy initiation, unnecessary invasive dental procedures should be avoided. When dental procedures are required, patients should be treated conservatively, minimizing invasive procedures. Temporary suspension of bisphosphonate treatment should be considered if invasive dental procedures are necessary. Bisphosphonates should be given for 2 years, and thereafter physicians should seriously consider discontinuing bisphosphonates in patients with responsive or stable disease [72, 73]. Two recent studies of patients with MM or solid tumors showed that preventive measures, such as a detailed assessment of dental status and avoidance of invasive dental procedures during bisphosphonate treatment, had the potential to reduce the incidence of ONJ by about 75% [74, 75]. The treatment of established ONJ is conservative in general. Initial therapy of ONJ should include discontinuation of bisphosphonates until healing occurs.

Proteasome Inhibition

Although bisphosphonates significantly reduce skeletal-related events in comparison with placebo, about 50% of MM patients who received pamidronate for 21 months still developed skeletal-related events, thus there is room for improvement [65]. Recently, there has been growing evidence that proteasome inhibitors might suppress osteoclast activity. The binding of RANKL to its transmembrane receptor RANK activates signaling cascades, including the NF-κB pathway [13]. Von Metzler et al. [76] first showed that inhibition of proteasome with bortezomib, a drug used in the treatment of MM [77–79], reduced NF-κB activity in osteoclasts and inhibited osteoclast differentiation and the activity of mature osteoclasts [76, 80, 81]. Inhibition of osteoclast activity can be achieved with other NF-κB inhibitors [82], but the effects of bortezomib in osteoclastogenesis also include the modulation of other signaling pathways that are crucial in these cells [76]. Importantly, the favorable potential effects of bortezomib on myeloma bone disease are not limited to osteoclasts. Zangari et al. [83] reported a significant increase in total alkaline phosphatase in myeloma patients receiving bortezomib. In another study, Heider et al. [84] found a significant increase in serum concentrations of two markers of osteoblast activity, bone-specific alkaline phosphatase (BAP) and osteocalcin, in MM patients treated with bortezomib, but not in patients treated with other antimyeloma agents. Of interest, the increase in BAP was significant in both responders and nonresponders to bortezomib, and this finding suggested a direct effect of proteasome inhibition on osteoblastic activity. Bortezomib was found to increase transcription factor Runx2/Cbfa1 activity in human osteoblast progenitors and osteoblasts [85] and induce mesenchymal stem cells to preferentially undergo osteoblastic differentiation [86]. Although the findings of positive effects of bortezomib on both osteoclasts and osteoblasts are highly interesting, there are no data available yet from randomized clinical trials showing patient benefit in regard to myeloma bone disease with the use of bortezomib [87, 88].

DKK-1 Antagonists

As summarized in the section on osteoblasts in MM, a reduction in osteoprogenitor cell differentiation could be antagonized by anti–DKK-1 antibodies. In addition to its role in osteoblast inhibition, DKK-1 also stimulates osteoclastogenesis by modulating RANKL and OPG production in osteoblasts and thereby increasing the RANKL/OPG ratio [27]. DKK-1–neutralizing antibodies restored the bone mineral density of implanted myelomatous bone in mice, increased the number of osteocalcin-expressing osteo-
blasts, and reduced the number of multinucleated TRAP-expressing osteoclasts [48].

Another DKK-1–neutralizing antibody (BHQ880) was able to increase the differentiation of mesenchymal stem cells to osteoblasts and overcome the negative effect of MM cells in a dose-dependent manner [89]. In a SCID-hu murine model, treatment with BHQ880 led to an increase in trabecular bone and the number of osteoblasts 1 month after the initial dose. A higher human osteocalcin level in the serum of BHQ880-treated mice compared with controls was also detected, reflecting an increase in osteoblastic activity. IL-6 levels were also reduced by this treatment. These results support DKK-1 as an important therapeutic target in myeloma and provide the rationale for clinical evaluation of DKK-1 inhibition to improve bone healing in MM.

**RANKL Antagonists**

Denosumab (AMG 162), a human monoclonal antibody to RANKL, was developed to treat patients with skeletal diseases resulting from increased bone resorption. In a randomized study, the safety and efficacy of denosumab were evaluated in patients with MM or breast cancer with radiologically confirmed bone lesions. Patients received a single dose of either denosumab or pamidronate. The treatment with denosumab resulted in a decrease in bone turnover markers that was similar in magnitude to but more sustained than that with i.v. pamidronate [90]. Further studies with denosumab are ongoing.

**CONCLUSIONS**

Increased bone resorption is a hallmark of MM and is caused by osteoclast activation and osteoblast inhibition. Myeloma bone disease significantly deteriorates the quality of life of MM patients. Recently, bone resorption activity was shown to be an independent risk factor for overall survival in patients with symptomatic MM. During the last few years, major advances occurred in our understanding of the mechanisms of myeloma bone disease. These insights are being rapidly transferred from the bench to the bedside and are expected to extend treatment options in myeloma bone disease and hopefully improve outcome for MM patients.

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