PROSPECT

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Pathogenesis of Myeloma Bone Disease

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ABSTRACT

Multiple myeloma (MM) is the most common cancer to involve bone with up to 90% of patients developing bone lesions. The bone lesions are purely osteolytic in nature and do not heal in the vast majority of patients. Up to 60% of patients develop pathologic fractures over the course of their disease. Bone disease is a hallmark of MM, and myeloma bone disease differs from bone metastasis caused by other tumors. Although myeloma and other osteolytic metastases induce increased osteoclastic bone destruction, in contrast to other tumors, once myeloma tumor burden exceeds 50% in a local area, osteoblast activity is either severely depressed or absent. The basis for this severe imbalance between increased osteoclastic bone resorption and decreased bone formation has been the topic of intensive investigation over the last several years. These studies have helped to identify novel targets for treating myeloma bone disease and will be discussed in this chapter. J. Cell. Biochem. 109: 283–291, 2010. © 2009 Wiley-Liss, Inc.

KEY WORDS: MYELOMA; BONE DISEASE; METASTASIS

Multiple myeloma (MM) is the most common cancer to involve bone with up to 90% of patients developing bone lesions [Roodman, 2004]. The bone lesions are purely osteolytic in nature and do not heal in the vast majority of patients. Up to 60% of patients develop pathologic fractures over the course of their disease [Melton et al., 2005]. Bone disease is a hallmark of MM, and myeloma bone disease differs from bone metastasis caused by other tumors. Although myeloma and other osteolytic metastases induce increased osteoclastic bone destruction, in contrast to other tumors, once myeloma tumor burden exceeds 50% in a local area, osteoblast activity is either severely depressed or absent [Taube et al., 1992]. The basis for this severe imbalance between increased osteoclastic bone resorption and decreased bone formation has been the topic of intensive investigation over the last several years. These studies have helped to identify novel targets for treating myeloma bone disease and will be discussed subsequently in this chapter.

The clinical and economic impact of myeloma bone disease in patients with myeloma can be catastrophic. Saad et al. [2007] retrospectively evaluated data from patients on the control arms of randomized trials of zoledronic acid to assess the impact of pathologic fractures on survival of patients with malignant disease. A total of 3,049 patients with metastatic bone disease were included in this study, of which 513 had myeloma. Patients with myeloma had the highest incidence of fracture (43%) over the 21 months of the study compared to breast cancer, prostate cancer and lung cancer patients respectively. Myeloma patients who experienced pathologic fractures had at least a 20% increased risk of death compared to myeloma patients without pathologic fractures. Further, patients who had a prior skeletal related event, which included pathologic fracture, spinal cord compression syndrome, surgery to bone or radiation therapy to bone, were more likely to develop new pathologic fractures as compared to patients who did not have a prior skeletal related event. Patients with a skeletal related event prior to entering the studies had a much poorer outcome, including a 40% increase in the risk of sustaining a skeletal related event compared to patients without a prior history of skeletal related events.

In addition to the severe clinical consequences of myeloma bone disease, myeloma bone disease has a tremendous economic burden as well. In a recent analysis of the economic burden of metastatic bone disease in the United States, Schulman and Kohles [2007] compared patients with metastastic bone disease to patients without metastastic bone disease as controls. In their analysis, they found the rate of metastatic bone disease in patients with myeloma was the highest of any cancer during the study period (2000–2004). The incremental cost of having bone disease in patients with myeloma was $57,720 per patient compared to patients without metastatic bone disease. Furthermore the total cost in 2004 dollars for patients

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with myeloma bone disease was estimated at $950,113,852. Thus, myeloma bone disease places a tremendous burden, both economically and clinically, on patients with myeloma.

PATHOGENESIS OF MYELOMA BONE DISEASE

The pathogenesis of myeloma bone disease involves both factors intrinsic to the tumor cells themselves as well as factors in the bone microenvironment. For example, MM has the propensity to home to bone and the bone marrow microenvironment to support the growth of the tumor. MM bone disease induces osteoclastic bone destruction, which enhances both the growth of the tumor, due to the release of activated growth factors from bone during the bone resorptive process, and the production by bone marrow stromal cells and osteoblasts of growth factors and cytokines, which enhance the survival and growth of the tumors. Many of these stromal cell-derived growth factors and cytokines are upregulated due to adhesive interactions between MM cells and bone marrow stromal cells (Fig. 1, Roodman, 2003). In addition, soluble factors produced by tumors themselves, such as TNF-α, further enhance the production of cytokines by bone marrow stromal cells, which also increase tumor survival and growth.

Recently, the bone marrow has also been shown to play a major role in maintaining MM cells in a dormant state, so they can be reactivated at later states. MM ''stem cells'' home to the bone marrow via cytokines and chemokines, in particular, SDF-1 expressed by osteoblasts and stromal cells in the marrow microenvironment, and its cognate receptor, CXCR4, on MM cells [Alsayed et al., 2007]. SDF-1 is chemotactic for MM cells and directs these cells toward the marrow. The MM cells appear to ''hijack'' the normal marrow mechanism that supports HSCs. Specifically, the marrow microenvironment maintains normal hematopoiesis through production of cytokines and chemokines that enhance the growth and differentiation of hematopoietic stem cells (HSCs) and directs their homing to the marrow. HSCs are not distributed uniformly throughout the bone marrow, but are highly localized in ''niches'' that allow the HSCs to remain in a dormant state. Upon release from the ''niche,'' stem cells enter the cell cycle and proliferate and differentiate to the formed elements of blood [Raaijmakers and Scadden, 2008].

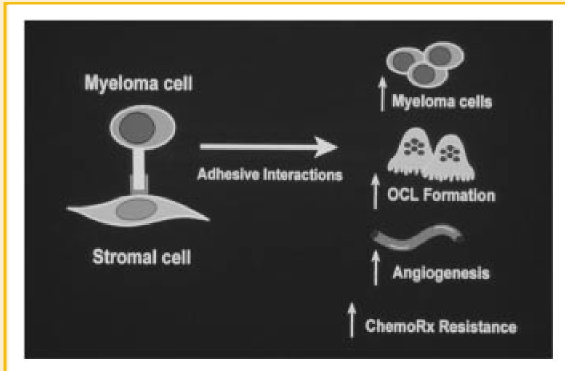

Fig. 1. Adhesive interactions between myeloma cells and stromal cells increase production of cytokines that enhance tumor growth, osteoclast formation and angiogenesis. In addition these adhesive interactions result in the tumor being more resistant to chemotherapy (Chemo Rx).

Similarly, cancers that home to bone, such as MM, appear to use a similar mechanism to either usurp the HSC niche or use a similar type of niche to grow within the marrow and to maintain ''cancer stem cells'' in a dormant state that is resistant to chemotherapeutic agents. Like normal HSCs, cancer stem cells can lay dormant for long periods of time until they are activated to differentiate and proliferate into malignant cells [Vessella et al., 2007].

The cellular composition of the stem cell niche is an area of active investigation, and its components are just beginning to be identified. Osteoblasts are an important component of the stem cell niche, and form the ''endosteal niche'' [Taichman, 2005]. Cytokines and chemokines produced by osteoblasts as well as adhesive interactions between osteoblasts and HSCs maintain HSCs in G_0 and provide a signal for homing of HSCs to the bone marrow. Shiozawa et al. [2008] have reported that prostate cancer cells like HSCs utilize a similar mechanism to home to the bone marrow and lodge there. SDF-1, which is expressed by osteoblasts and endothelial cells, also acts as a chemoattractant for both HSCs and MM cells to home to the bone marrow [Alsayed et al., 2007]. Recently, annexin II (AXII) has been identified as important in HSC and cancer cell lodgment in the bone marrow and the mobilization of HSCs and cancer cells to the peripheral blood [Shiozawa et al., 2008]. We have identified a similar role for annexin II in MM [D'Souza et al., 2009b].

Further, Kollet et al. have showed that OCLs may also participate in HSC mobilization. They showed that OCLs secrete MMP-9 and cathepsin K, which cleave SDF-1, OPN and stem cell factor in the surrounding extracellular matrix [Kollet et al., 2007]. This in turn weakens HSC anchorage provided by the endosteal niche. These results show that OCLs are involved in stem cell mobilization. Whether OCLs are also involved in MM stem cell mobilization remains to be determined, but in preliminary experiments, we have shown that cathepsin K can degrade annexin II, which may be involved in MM cell lodgment (Roodman, unpublished results).

PATHOGENESIS OF THE INCREASED OSTEOCLAST ACTIVITY IN MYELOMA

OCL activity is greatly increased in MM. MM patients lose bone much more rapidly than age-matched controls. Diamond et al. [1997] reported that patients with MM who received glucocorticoid containing treatment lost approximately 6% of their bone mineral density at the lumbar spine and almost 10% of their bone mineral density at the femoral neck over a 12-month period compared to age-matched controls who were not on steroid therapy. The controls did not lose bone at the lumbar spine and lost approximately 1% of their bone mineral density at the femoral neck over the same time period. Further, the frequency of skeletal-related events is very high in patients with MM, with more than 30% of the patients having a fracture or requiring radiation therapy for bone pain on the placebo arm of a 21-month clinical trial [Berenson et al., 1998]. These data clearly show that OCL activity is markedly increased in MM. Further, histologic studies of bone biopsies from patients with MM show that

increased OCL activity occurs mainly adjacent to MM cells. This suggests that local cytokines produced or induced by MM cells are responsible for the increased bone resorptive activity and OCL formation in MM. Growth factors released by the increased bone resorptive process also increase the growth of MM cells [Edwards et al., 2008]. Increased OCL activity appears to contribute both to the increased angiogenesis and tumor growth in MM. Further, recent studies have shown that OCL themselves can support the growth of MM cells through cell-to-cell contact, which results from production of interleukin (IL)-6 and osteopontin [Yaccoby et al., 2002; Abe et al., 2004] by the OCL. Similarly, Tanaka and coworkers reported that OCL enhance angiogenesis in MM through production of factors such as osteopontin, which together with VEGF produced by MM cells, increase angiogenesis [Tanaka et al., 2007]. We have recently reported that OCL themselves produce angiogenic factors, which further contribute to this process in particular MMP9 [Cackowski et al., in press].

Adhesive interaction between marrow stromal cells and MM cells play a critical role in the bone destructive process [Nefedova et al., 2004; Nimmanapalli et al., 2008]. When MM cells home to the marrow, they adhere to bone marrow stromal cells through binding of vascular cell adhesion molecule-1 (VCAM-1) on stromal cells and $\alpha_4\beta_1$ integrin on MM cells. These adhesive interactions increase production of a multiplicity of factors in the bone microenvironment, which enhance OCL formation. Among the important factors that have been identified that are involved in the increased OCL formation in MM include RANKL, osteoprotegerin (OPG), MIP-1α, IL-6 and IL-3.

The RANK/RANKL signaling pathway is a critical component of the normal and malignant bone remodeling process. RANK is a transmembrane signaling receptor, which is a member of the tumor necrosis receptor superfamily. It is found on the surface of OCL precursors [Nakagawa et al., 1998; Hsu et al., 1999]. RANK ligand (RANKL) is expressed as a membrane-bound protein on marrow stromal cells and osteoblasts, and secreted by activated lymphocytes. Its expression is induced by cytokines that stimulate bone resorption [Boyle et al., 2003] such as PTH, 1,25 OH Vitamin D_3 and prostaglandins [Hofbauer and Heufelder, 1998; Yasuda et al., 1998]. RANKL binds to RANK receptor on OCL precursors and induces OCL formation. RANK signals through the NF-κB and JunN terminal kinase pathways and induces increased osteoclastic bone resorption and enhanced OCL survival [Roodman, 2007]. The important role of RANKL in normal osteoclastogenesis has been clearly demonstrated in RANKL or RANK gene knockout mice. These animals lack OCLs and develop severe osteopetrosis [Tsukii et al., 1998; Dougall et al., 1999; Kong et al., 1999].

OPG is a soluble decoy receptor for RANKL and is a member of the TNF receptor superfamily [Lacey et al., 1998]. It is produced by osteoblasts as well as other cell types including B cells [Weitzmann et al., 2002]. Cells and blocks the interactions of RANKL with RANK, thereby limiting osteoclastogenesis. In normal subjects, the RANKL/OPG ratio is very low. Studies using knockout mice for the OPG gene have shown the importance of OPG. OPG deficient mice develop severe osteopenia and osteoporosis [Simonet et al., 1997; Bucay et al., 1998; Lacey et al., 1998; Dougall et al., 1999; Li et al., 2000]. Pearse et al. [2001] were the first to demonstrate that RANKL expression was upregulated in bone marrow biopsies of MM patients, while OPG expression was decreased. Giuliani and coworkers showed that circulating levels of OPG and RANKL correlated with both clinical activity of MM, severity of bone disease and poor prognosis [Terpos et al., 2003b]. Furthermore, murine MM models demonstrated that inhibition of RANKL can prevent bone destruction in either the SCID-hu model or the T2 MM syngeneic model of MM [Yaccoby et al., 2002; Terpos et al., 2003b]. These studies confirmed that blocking RANKL, either with a soluble form of the RANK receptor or by OPG, inhibited bone destruction and decreased tumor burden. However, RANKL is not the only OCL stimulating factor involved in MM.

MIP-1α is a chemokine that is produced by MM cells in 70% of MM patients, and is a potent inducer of human OCL formation. MIP-1α can increase OCL formation independently of RANKL and can potentiate both RANKL and IL-6 stimulated OCL formation [Han et al., 2001]. Magrangeas et al. [2003] reported that gene expression profiling demonstrated that MIP-1α is the gene most highly correlated with bone destruction in MM. Further, Abe and coworkers reported that elevated levels of MIP-1α portended an extremely poor prognosis in MM [Hashimoto et al., 2004]. In vivo models of MM further demonstrated that MIP-1α can induce OCL formation and bone destruction. Blocking MIP-1α expression in MM cells injected into immunodeficient mice or treating the animals with a neutralizing antibody to MIP-1α decreased tumor burden and bone destruction [Alsina et al., 1996; Choi et al., 2001]. MIP-1α also plays an important role in lodgment of MM cells in the bone marrow. MIP-1α increases adhesive interactions between MM cells and marrow stromal cells by increasing expression of β_1 integrins on MM cells. This results in increased production of RANKL, IL-6, VEGF and TNFα by marrow stromal cells, which further enhances MM cell growth, angiogenesis and bone destruction. Masih-Khan et al. [2006] reported that the t4:14 translocation results in a constitutive expression of the FGFR3 receptor, and in high levels of MIP-1α. Patients with the t4:14 translocation have a very poor prognosis, which may reflect the increased MIP-1α production in this patient population. Consistent with this notion, Terpos et al. [2003a] showed that serum levels of MIP-1α correlated with the extent of bone disease in MM patients and predicted survival for patients with MM. Patients with high MIP-1α levels had a significantly shorter survival.

IL-3, in addition to RANKL and MIP-1α, is also significantly elevated in bone marrow plasma of MM patients as compared to normal controls [Lee et al., 2004]. IL-3 can induce OCL formation in human bone marrow cultures at levels similar to those measured in MM patients and OCL formation induced by marrow plasma from MM patients can be inhibited using a blocking antibody to IL-3 [Lee et al., 2004]. IL-3 also can enhance the effects of RANKL and MIP-1α on the growth and development of OCLs as well as stimulates MM cell growth [Lee et al., 2004].

We and others have shown that adhesive interactions between MM and stromal cells play a significant role both in homing of MM cells in the bone marrow and augmenting the bone destructive process. These adhesive interactions result in increased activation of NF-κB and p38 MAP-kinase. The latter is involved in induction of RANKL expression by osteoblasts. Blocking p38MAP-kinase results

in a potent inhibition of IL-6 and VEGF production as well as decreased adhesion of MM cells to marrow stromal cells [Nguyen et al., 2006]. Similarly, Vanderkerken et al. [2006] reported that inhibition of p38 MAP kinase in the 5T2 MM murine model of MM decreased tumor cell burden, prevented development of bone disease and increased overall survival of mice injected with 5T2 cells. Therefore, blocking p38MAPK signaling may be a potential therapeutic target for novel therapies to ameliorate MM disease.

We recently reported that p62, an adapter protein, appears to play an important role in the intrinsic changes in myeloma. p62 is an adapter protein that has no intrinsic enzymatic activity but plays an important role in the NF-κB signaling pathway and serves as a platform for formation of multiple signaling complexes that can affect MM cell growth and OCL formation (Fig. 2A,B). The p62 protein contains a number of motifs that serve as a focal point for protein-protein interactions involved in cell signaling [Geetha and Wooten, 2002] (Fig. 2C), including an atypical PKC-interacting domain (PB1) that also is a p62 dimerization domain, a Zn-finger domain that mediates binding to RIP1 (ZZ), a p38 and LIM interaction domain, a TRAF6-interacting domain, and a UBA domain that mediates non-covalent interaction with ubiquitin.

p62 plays a critical role in PKCζ-mediated NF-κB activation. p62 recruits PKCζ to the signaling complex upon ligation of TNF-α, interleukin-1, or nerve growth factor [Sanz et al., 1999, 2000; Wooten et al., 2001] without affecting PKCζ catalytic activity [Wooten et al., 2001]. Ligation of the TNF receptor increases the interaction of p62 with receptor interacting protein (RIP), bringing PKCζ to the TNF receptor-signaling complex where PKCζ phosphorylates IKKβ [Muller et al., 1995; Sanchez et al., 1998]. Interestingly, the p62 gene can be induced by various extracellular signals activating NF-κB, including treatment of cells with PMA, serum, PDGF, or oxidative stress [Lee et al., 1998].

In support of the importance of p62 in MM, we recently reported that knockdown of p62 in 1° stromal cells from MM patients decreased both IL-6 production and support of MM cell growth by stromal cells [Hiruma et al., 2009]. Further, RANKL expression in response to TNF-α and IL-6, in both p62 siRNA transduced human stromal cells and $p62^{-/-}$ murine marrow stromal cells, was significantly decreased. Importantly, co-culture of normal OCL precursors with $p62^{-/-}$ stromal cells resulted in impaired osteoclastogenesis in response to TNF-α or IL-6, factors implicated in MM bone disease. Taken together, these results suggest that p62 plays an important role in tumor progression and bone destruction in MM through it capacity to form signaling complexes in stromal cells. Thus, targeting p62 may provide a mechanism for blocking the enhanced NF-κB, p38, and AKT signaling, which is upregulated and appears to be intrinsic to the marrow microenvironment exposed to myeloma cells.

OSTEOBLAST DYSFUNCTION IN MYELOMA

In addition to increased OCL activity, osteoblast activity is markedly suppressed in MM. Histomorphometric studies have shown that bone remodeling is uncoupled in MM with increased bone resorption and decreased or absent bone formation. Further, osteoblast apoptosis is increased due to high levels of soluble cytokines such as TNF-α and physical interaction between osteoblasts and MM cells [Silvestris et al., 2004]. MM patients have low levels of bone formation markers, such as alkaline phosphatase and osteocalcin [Hjorth-Hansen et al., 1999], and

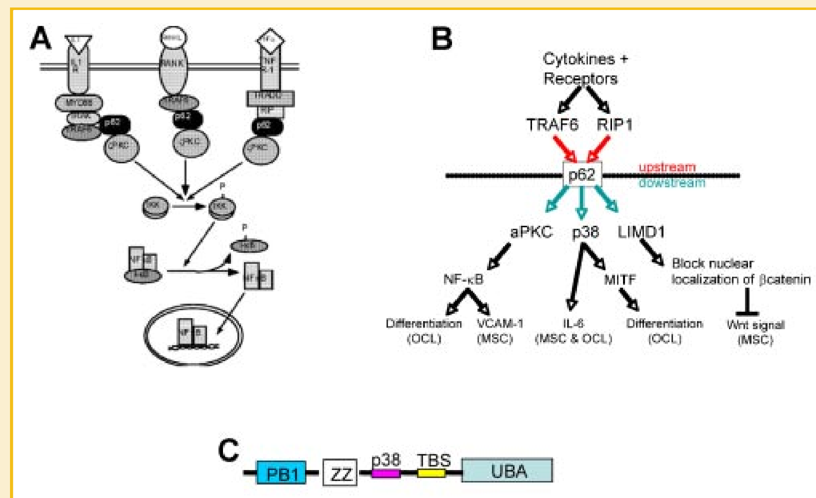

Fig. 2. **A:** The role of p62 and PKCζ in IL-1, TNF, and RANKL signaling. p62 sits at the crossroads for the cytokines that activate NF-κB. **B:** p62 mediates the effects of inflammatory cytokines on marrow stromal cells (MSC) and OCL precursors through its involvement in the formation of multiple signaling pathways in addition to NF-κB, including p38 activation and inhibition of β-catenin nuclear translocation. **C:** Structure of the p62 protein. aPKC and ERK interact with the PB1 (SH2 + AID) domain which is also responsible for p62 homodimerization, whereas RIP1 binds to the ZZ domain, and TRAF6 interacts with the domain indicated. The UBA domain binds to polyubiquitin chains. Also denoted is a p38 interaction domain corresponding to amino acids (aa) 173–182 that overlaps the LIM-domain protein binding (LB) region within aa 170–220. Not show is the LC3-interacting region (LIR) within aa 321–342. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

marrow stromal cells from MM patients have an impaired capacity to differentiate to osteoblasts. This explains why bone scans underestimate the extent of MM bone disease since bone scans reflect new bone formation.

In the last few years, signaling pathways involved in osteoblastic differentiation have been identified, which provide a better understanding of how osteoblast activity is inhibited in MM. In addition, these studies have identified several potential therapeutic targets for treating MM bone disease.

The formation and differentiation of osteoblasts from mesenchymal cells require the activity and function of the transcription factor Runx2/Cbfal. Runx2/Cbfal deficient mice (Runx2) completely lack osteoblasts and bone formation [Kobayashi and Kronenberg, 2005]. Human osteoblast differentiation is associated with increased Runx2/Cbfal activity without a change in Runx2 protein levels, although Runx2/Cbfal overexpression can also impair bone formation [Franceschi and Xiao, 2003]. These results indicate that a time dependent expression of Runx2 drives osteoblast differentiation and plays a critical role in this process.

Inhibition of Runx2/Cbfal activity in MM bone disease has been demonstrated. When MM cells were co-cultured with osteoprogenitor cells, the MM cells inhibited osteoblast differentiation and reduced numbers of both early osteoblast precursors, as well as the more differentiated precursors [Giuliani et al., 2005]. This effect was mediated by blocking Runx2/Cbfal activity in osteoprogenitor cells. In addition, since Runx2/Cbfal stimulates secretion of the RANKL decoy receptor, OPG, in osteoprogenitor cells [Thirunavukkarasu et al., 2000], it is possible that inhibition of Runx2/Cbfal activity also increases osteoclastogenesis. The interaction between Runx2/Cbfal and MM cells is mediated both by cell-to-cell interaction between MM cells and osteoprogenitors and soluble factors produced by MM cells [D'Souza et al., 2009a]. This cell-to-cell interaction is dependent on $\alpha_4\beta_1$ on MM cells and VCAM-1 on osteoblast precursors, since neutralizing anti-$\alpha_4\beta_1$ antibodies reduce the inhibitory effect of MM cells on Runx2/Cbfal activity [Giuliani et al., 2005].

IL-3 appears to play a dual role in the bone destructive process in MM. As noted above, it can stimulate OCL formation and bone resorption but in addition indirectly inhibit osteoblast formation. Treatment of primary mouse or human marrow stromal cells with marrow plasma from MM patients that expressed high IL-3 levels inhibited osteoblast differentiation, which was reversed by an anti-IL-3 antibody [Ehrlich et al., 2005].

IL-7 also can inhibit osteoblasts in MM. IL-7 levels are increased in marrow plasma samples from patients with MM compared to normal controls [Giuliani et al., 2005]. IL-7 is a very potent inhibitor of osteoblast differentiation and can affect osteoblast formation in several ways including interfering with Runx2 activity [Toraldo et al., 2003; Giuliani et al., 2005; Lee et al., 2006].

TNF-α, an inflammatory cytokine, is also increased in the MM microenvironment [Li et al., 2007] and is known to stimulate apoptotic pathways [Tan et al., 2006]. TNF-α also can block OB differentiation by inhibiting precursor recruitment from progenitor cells, inhibiting their expression of matrix protein genes and stimulating gene expression that enhances osteoclastogenesis, such as RANKL [Mukai et al., 2007]. TNF-α has also been reported to suppress the expression of the critical OB transcription factors Runx2 [Gilbert et al., 2002], TAZ (a Runx2 coactivator) [Li et al., 2007], and Osx [Lu et al., 2006] in OB progenitors. Further, TNF-α neutralizing antibody can partially prevent MM cells from suppressing osteogenesis [Li et al., 2007].

The MM-induced inhibition of MSC differentiation into osteoblasts may also further contribute to tumor growth. MSC exposed to MM cells produce increased levels of MM supportive cytokines such as IL-6 and TNF-α (see above). Thus, even in remission the bone microenvironment is locked into a MM supportive phenotype that is primed to protect and promote the growth of any MM cells that escaped eradication. While MM-exposed MSC support MM cell growth, Yaccoby et al. [2007] reported that osteoblasts can suppress the growth of MM cells in vivo, suggesting that reversing osteoblast suppression in MM can have additional anti-tumor effects.

AGENTS THAT TARGET FACTORS INVOLVED IN MYELOMA BONE DISEASE

As noted above there is a multiplicity of factors, both osteolytic and inhibitors of osteoblast differentiation that contribute to myeloma bone disease, one of the principles among these is RANKL. Recently a fully humanized monoclonal antibody to RANKL has been developed, which has high specificity for RANKL and mimics the endogenous effects of osteoprotegrin (OPG), a soluble to RANKL. Phase 1 trials in patients with myeloma or breast cancer have shown that single and multiple subcutaneous injections of denosumab caused rapid and sustained suppression of osteoclastic bone resorption markers with an excellent safety profile [Body et al., 2006]. A recent phase II study of denosumab as a single agent in patients with either progressive or plateau phase myeloma showed that it could significantly suppress bone resorption markers, although had no effect on tumor burden [Vij et al., 2007]. Phase III trials are currently underway with denosumab for myeloma and other tumors metastatic to bone that are not breast cancer or prostate cancer and preliminary reports suggest that denosumab appears to be equivalent to bisphosphonates, which are the standard treatment for myeloma bone disease. In addition, small molecule antagonist of the CCR1 receptor, which is the principle receptor for MIP-1α which is increased in myeloma, have been developed and have been in clinical trials for other diseases such as multiple sclerosis and are being considered for development to treat osteolytic bone disease in myeloma.

Immunomodulatory drugs such as lenalidomide and pomalidomide have also shown anti-osteoclastic activities in myeloma. Anderson et al. reported that CC-4047 (Actimid), which is a derivative of thalidomide and has similar actions as lenalidomide [Anderson et al., 2006], also inhibited OCL development by affecting the lineage commitment of OCL precursors. CC-4047 down-regulated the expression of PU.1, a critical transcription factor for the development of OCLs. The down-regulation of PU.1 in hematopoietic progenitor cells resulted in a complete shift of lineage development towards granulocytes and away from OCL. This inhibited OCL formation with a concomitant accumulation of immature granulocytes. Similarly, Breitkreutz et al. [2008] demon-

strated that lenalidomide inhibited OCL formation by targeting PU.1 and down-regulating cathepsin K.

In addition to therapies targeting stimulators of osteoclastic bone destruction other novel therapies are being targeted to factors, which inhibit osteoblast activity in myeloma. These include anti-DKK1, which inhibits the Wnt signaling pathway in myeloma. As noted above, anti-DKK1 has been tried in preclinical models and appears promising and is being developed for myeloma bone disease. There is already an ongoing trial with anti-DKK1 and osteoporosis. Further bone anabolic agents are also being developed to stimulate osteoblast differentiation in myeloma. These include an antagonist to the Activin receptor.

In addition to Wnt signaling antagonists in myeloma, Activin A recently has been shown to be elevated in patients with myeloma. Activin A is produced by osteoclasts and bone marrow stromal cells in myeloma patients and can increase osteoclast formation and block osteoblast differentiation [Vallet et al., 2008]. Raje et al. have shown that Activin A stimulates production of IL-6 by bone marrow stromal cells and that myeloma cells induce Activin A expression by bone marrow stromal cells. More importantly, Croucher and coworkers, at the 11th Annual International Myeloma Workshop in 2007, reported that an Activin A inhibitor prevented development of myeloma bone disease in the 5T2 murine model of myeloma. Clinical trials of the human antagonist to the Activin A receptor are now ongoing in patients with myeloma.

Finally a new treatment for myeloma, the proteosome antagonist, bortezomib, also appears to have bone anabolic effects and possibly osteoclast inhibitory effects in myeloma (Fig. 3). Clinical trials with bortezomib have indicated that may also increase osteoblast activity, induce new bone formation and potentially repair lytic bone lesions. Degradation of proteins by the ubiquitin-proteasome pathway plays a critical role in MM cell survival, and clinical trials of proteasome inhibition with bortezomib have shown it to be an effective anti-MM agent [Kropff et al., 2006]. The proteasome pathway is also an important regulator of osteoblast differentiation [Garrett et al., 2003] and proteasome inhibition can induce new bone formation in rodents.

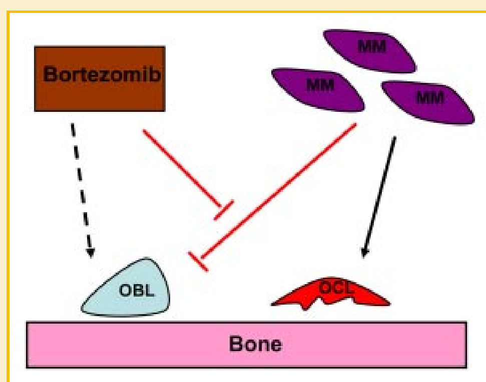

Fig. 3. Myeloma cells (MM) produce osteoclast (OCL) activating factors (see text for details), which increase osteoclast formation (black arrow) as well as produce osteoblast (OBL) inhibiting factor (red inhibitor). Bortezomib can enhance bone formation by increasing several osteoblast transcription factors (see text for details) (black arrow). In addition, bortezomib can inhibit osteoclast formation in addition to blocking growth of myeloma cells (red inhibitor). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Zangari et al. [2005] conducted a retrospective analysis of three trials of bortezomib in patients with relapsed MM. In all three trials, patients who had a partial response to bortezomib therapy had a transient increased alkaline phosphatase level compared to non-responders. Additionally, patients who responded to bortezomib treatment were compared with those who responded to dexamethasone. The bortezomib-treated group had higher serum levels of alkaline phosphatase than the dexamethasone responders, suggesting that the increase in the osteoblast marker is not merely a result of reduced tumor burden.

A prospective study by Heider et al. [2006] analyzed relapsed MM patients enrolled in clinical trials using bortezomib (alone or with dexamethasone) compared to other agents. Patients treated with bortezomib had increased levels of bone-specific alkaline phosphatase (BAP) and osteocalcin, both markers of increased osteoblast activity. The increase in BAP and osteocalcin was observed in patients whose MM responded to bortezomib treatment as well as non-responders, but was not seen in the other treatment groups regardless of response [Barille-Nion and Bataille, 2003].

Giuliani et al. [2007] investigated the in vitro and in vivo effects of bortezomib in patients with MM. In human osteoblast precursor cultures, bortezomib increased markers of osteoblast differentiation and osteoblast-specific transcription factors and enhanced bone nodule formation. Bone marrow samples of patients responding to bortezomib had a significantly increased number of osteoblastic cells compared to non-responders. These studies suggest that bortezomib can stimulate osteoblasts in patients whose MM responded to bortezomib. Oyajobi et al. [2007] have also reported that bortezomib can increase bone formation in murine models of MM. Although, the mechanism of action for bortezomib's effect on osteoblast stimulation in MM has not been fully determined, Terpos et al. [2006] reported that bortezomib decreased DKK1 and RANKL serum levels in MM patients suggesting bortezomib affects DKK1 production.

There currently is an ongoing trial to examine prospectively the effects of bortezomib on bone formation using bone formation markers, DEXA, as well as high resolution CT to detect new bone formation in response to bortezomib.

SUMMARY

The identification and characterization of the pathophysiologic mechanisms underlying myeloma bone disease have provided important new therapeutic targets for treating these patients, who are currently incurable. Myeloma bone disease takes a tremendous toll on patients both physically, financially and impacts their survival. Current therapies using intravenous bisphosphonates have greatly improved the outlook of the patients with myeloma bone disease, but only slow the progression of the disease rather than completely eradicating it [Coleman et al., 2005]. The identification of RANKL as a major mediator of the bone destructive process in myeloma bone disease has led to phase III clinical trials of a human

antibody to RANKL, Denosumab. Further, antagonists to CCR1, which blocks the effects of MIP-1α, antibodies to DKK1, and Activin receptor antagonists are all in trial for myeloma bone disease and may show promise in the future. It is unclear if any of these therapies will heal bone lesions in patients with myeloma if the bone marrow microenvironment in myeloma is so adulterated that new bone formation cannot occur. However, preclinical studies in rodent models of myeloma suggest that anti-DKK1 or other anabolic agents, including the activin receptor antagonist, can prevent bone lesions. These new therapies will become increasingly important as the survival of patients with myeloma continues to improve with novel therapies directed at the tumor. Ameliorating the bone disease will have even greater importance to these patients so that they can have an improved quality of life.

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