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Advances in the understanding of myeloma bone disease and tumour growth

Shmuel Yaccoby
Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, 4301 West Markham, Mail Slot 776, Little Rock, AR, USA

Summary

Advances in multiple myeloma support the notion that the associated bone disease, characterized by increased osteoclastogenesis and suppressed osteoblastogenesis, is both a consequence and necessity of tumour progression. Osteoblastogenesis is suppressed by secreted inhibitors and dysregulation of cell-surface “coupling” factors on osteogenic cells. Osteoclastogenesis is increased as a consequence of osteoblast deactivation and of production of osteoclast-activating factors. Osteoclasts express soluble and cell-surface factors that stimulate myeloma growth, while osteoblasts produce bone-building factors that restrain growth of myeloma cells that are dependent on the microenvironment; detailed molecular mechanisms are discussed. Experimental and clinical findings indicate that pharmacological and experimental osteoblast-activating agents that effectively promote bone formation also reduce growth of myeloma cells within bone, seemingly by simultaneously stimulating osteoblastogenesis and restraining osteoclastogenesis. Unravelling mechanisms of myeloma bone disease expands horizons for developing novel interventions and also facilitates better understanding of the association between induction of osteolysis and disease progression.

Keywords

myeloma; osteoblasts; osteoclasts; Wnt; proteasome

Introduction

During the recent decade, an explosion of studies investigated the role of the bone marrow (BM) microenvironment in the pathogenesis of multiple myeloma (MM). These studies revealed that, while cells of hematopoietic lineage seem to support MM progression (Yaccoby *et al*, 2004; Kukreja *et al*, 2006; Chauhan *et al*, 2009; Zheng *et al*, 2009; Nakayama *et al*, 2004), certain cells of mesenchymal origin may restrain myeloma cell growth (Yaccoby *et al*, 2006; Li *et al*, 2008). Exceptional are neovascular endothelial cells, which are increased in number in myelomatous BM (Vacca *et al*, 1994), but their role in disease progression is not well delineated. Due to its unique growth pattern and the defined form of benign disease, MM is an excellent model for studying tumour–microenvironment interactions.

Correspondence and reprint requests: Shmuel Yaccoby, PhD, Myeloma Institute for Research and Therapy, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, 4301 W. Markham, Mail Slot 776, Little Rock, AR 72205, USA, yaccobyshmuel@uams.edu, Tel: (501) 296-1503, ext. 1424, Fax: (501) 686-6442.

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MM is unique among most hematologic malignancies because of its induction of bone disease. In addition, while in many other bone-associated malignancies (e.g. prostate and breast cancer) tumor growth is associated with increased numbers of osteoclasts and osteoclasts, osteolytic bone resorption in MM is caused by stimulation of osteoclastogenesis and suppression of osteoblastogenesis in areas adjacent to tumour foci (Bataille *et al*, 1991; Barille-Nion & Bataille, 2003; Roodman, 2009; Sezer, 2009). This phenomenon supports the notion that bone disease is both a consequence and necessity of MM progression and metastasis, which is frequently described as a “vicious cycle” between tumour cells and their surrounding cellular environment.

Factual analysis of focal and bone lesions in patients with active MM is achieved by combining computed tomography (CT) with novel, sensitive imaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) (Walker *et al*, 2007; Bartel *et al*, 2009). These imaging techniques and histomorphometric analyses revealed that changes in bone microstructures are typical characteristics of focal lesions and also are evident in patients with benign disease and in those with early-stage MM (Drake M, 2009). Interestingly, novel drugs that are exceptionally effective at clinically controlling MM, such as the immunomodulatory agents (IMiDs) thalidomide and lenalidomide and the proteasome inhibitor bortezomib, seem to affect osteoclast and osteoblast activity in myelomatous bones (Zangari *et al*, 2005; Terpos *et al*, 2007; Sezer, 2009). Moreover, molecular classification of patients’ plasma cells, based on global gene expression profiling, identified a unique group of patients characterized by low level of bone disease and revealed that these patients enjoyed favourable event-free survival (Zhan *et al*, 2006). These clinical findings suggest that continued dissemination of myeloma cells is facilitated by myeloma cell interactions with bone cells within focal lesions.

This review focuses on the effects of bone cells—bone-destroying osteoclasts and bone-building osteoblasts—on MM progression. While the reciprocal interactions between myeloma-induced osteoclastogenesis and osteoclast-induced myeloma growth have been extensively studied, emerging data are revealing the molecular mechanisms of myeloma-induced suppression of osteoblastogenesis and of osteoblasts’ regulation of myeloma cell growth.

Activation of osteoclastogenesis in myeloma

The molecular mechanisms by which myeloma cells stimulate osteoclast activity are multifactorial and involve osteoclast-differentiation and -survival factors that are produced by microenvironmental cells and myeloma cells (Figure 1). Several osteoclastogenic factors that are highly produced in myelomatous bone have been shown *in vitro* and/or *in vivo* to be directly involved in MM-induced osteoclast activity: receptor activator of NF-κB ligand (RANKL) (Pearse *et al*, 2001), inflammatory protein-1 alpha (MIP1α) (Choi *et al*, 2000;Han et al, 2001), interleukin (IL)-3 (Lee *et al*, 2004), and stroma-derived factor (SDF)-1 (Zannettino *et al*, 2005). Other factors, including thrombospondin-1 (TSP-1) (Kukreja *et al*, 2009), IL-17 (Dhodapkar *et al*, 2008), and inflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNFα) (for review see Ehrlich & Roodman, 2005), that are associated with bone remodeling have increased levels in BM and/or blood of patients with myeloma, but their roles in this process require further validation.

Roles of osteoblasts

Myeloma cells’ cultivation of BM stromal cells or mesenchymal stem cells (MSCs) is a central mechanism associated with increased osteoclastogenesis in MM. Pearse *et al* (Pearse *et al*, 2001), followed by others (Giuliani *et al*, 2001; Sezer *et al*, 2003), demonstrated that myeloma cells induce stromal cells to upregulate osteoclastogenic factor RANKL and to

downregulate RANKL-decoy receptor osteoprotegerin (OPG). Further detailed studies revealed that the ratio of RANKL-to-OPG expressed by osteogenic cells reflects osteoblast differentiation: as osteoblasts mature, their production of RANKL is reduced and OPG is increased (Glass *et al*, 2005; Spencer *et al*, 2006;Qiang *et al*, 2008b). Recently, Qiang *et al* (Qiang *et al*, 2008a) demonstrated that myeloma cell production of Wnt antagonist dickkopf 1 (DKK1) abrogates the ability of the canonical Wnt ligand, Wnt3a, to stabilize β-catenin and commit immature cells to osteoblastogenesis. As a result, osteoprogenitor cells increased expression of RANKL and reduced expression of OPG, ultimately increasing RANKL/OPG ratios. Immature osteogenic cells and other mesenchymal elements in BM are also significant sources of IL-6 (Gunn *et al*, 2006) and SDF-1 (Kortesidis *et al*, 2005), which may be indirectly involved in inducing local bone resorption in osteolytic lesions (Sandhu *et al*, 1999; Zannettino *et al*, 2005). These studies suggest that suppression of osteoblastogenesis contributes to increased osteoclast activity in MM lytic lesions.

Roles of hematopoietic cells

Hematopoietic cells of various lineages are expanded in or recruited to BM focal lesions and contribute to osteoclastogenesis in MM. Myeloma cells attract osteoclast precursors and macrophages (Yaccoby *et al*, 2004; Zheng *et al*, 2009), which produce high levels of IL-8, a chemokine that directly promotes osteoclast formation (Bendre *et al*, 2003). RANKL and IL-3 are produced by activated T lymphocytes in BM of patients with MM (Giuliani *et al*, 2002; Giuliani *et al*, 2006). IL-3 is also expressed by myeloma cells, which enhances the effects of RANKL and MIP1α on stimulating osteoclast formation (Lee *et al*, 2004).

Recent studies suggest that myeloma cells are infiltrated by dendritic cells (DCs) and that DCs contribute to increased osteoclastogenesis and myeloma cell growth (Kukreja *et al*, 2006; Kukreja *et al*, 2009; Chauhan *et al*, 2009). DCs produce RANKL (Wong *et al*, 1999), and plasmacytoid DC interactions with myeloma cells results in upregulation of other osteoclastogenic factors, such as IL-3, SDF-1, CD40L, and TNFα (Chauhan *et al*, 2009). Interestingly, upon engagement with myeloma cells, DCs produce increased levels of TSP-1, which mediates DC transdifferentiation into bone-resorbing osteoclasts (Kukreja *et al*, 2009). DCs also induce expansion of IL-17-producing T lymphocytes (Dhodapkar *et al*, 2008), which may promote osteoclast formation via their release of IL-17 (Sato *et al*, 2006). Expanded megakaryocytes are also detected in the vascular niche of BM of patients with MM, probably due to high production of cytokines such as IL-6, IL-3, and IL-11. Megakaryocytes produce various bone-associated factors, including RANKL, OPG, and M-CSF, and megakaryocyte RANKL/OPG ratios increase as a result of their interactions with myeloma cells (Yaccoby *et al*, 2005).

Taken together, these studies indicate that hematopoietic cells play critical roles in increasing the gradient of osteoclastogenic factors in focal lesions. The precise involvement of specific hematopoietic elements, particularly certain immune cells, in myeloma bone disease requires further investigation because induction of severe bone resorption occurs in immunodeficient mice after engraftment of MM.

Roles of myeloma cells

In the early 1970s, Mundy and colleagues proposed that myeloma cells produce osteoclast-activating factors (Mundy *et al*, 1974). Osteoclastogenic factors potentially produced by myeloma cells, particularly in response to interactions with BM microenvironmental cells, include MIP1α, IL-3, IL-7, IL-8, and RANKL. Expression of RANKL by myeloma cells is controversial (Farrugia *et al*, 2003; Giuliani *et al*, 2005a; Heider *et al*, 2003), and the roles of IL-7 and IL-8 in forming osteoclasts within the myelomatous bone await validation; however, MIP1α has been extensively studied by Roodman and colleagues. They

demonstrated high levels of this chemokine in BM plasma of patients with MM, and they showed MIP1α promotes osteoclastogenesis directly and indirectly by increasing production of RANKL and IL-6 (Choi *et al*, 2000;Han *et al*, 2001). This group also showed that IL-3 is similarly involved in myeloma-induced osteoclastogenesis (Lee *et al*, 2004). Syndecan-1 (CD138), a heparan sulfate proteoglycan highly produced by myeloma cells, along with heparanase, an enzyme involved in syndecan-1 shedding from surfaces of myeloma cells, regulates osteoclastogenesis by sequestering OPG (Standal *et al*, 2002) and by concentrating factors (*e.g.*, HGF, IL-6, IL-8, SDF-1) that promote osteoclastogenesis, angiogenesis, and myeloma growth (Sanderson *et al*, 2004).

In addition to osteoclastogenic factors, growth factors produced by myeloma cells may increase the life span of monocytes and osteoclast precursors, enhancing their potential to form osteoclasts. Also noteworthy, studies suggest that myeloma cells actively participate in formation of osteoclasts by fusing into osteoclasts (Andersen *et al*, 2007) or by forming themselves into multinucleated cells capable of bone resorption (Silvestris *et al*, 2009).

In summary, levels of multiple key osteoclastogenic factors are increased in the BM milieu of patients with MM. Myeloma cells secrete some of the factors, but the main mechanisms that result in increased levels of these factors and subsequent osteolysis result from myeloma-cell–induced suppression of differentiation and recruitment of osteoblasts and from expansion and cultivation of various hematopoietic and immune elements (Figure 1).

Inactivation of osteoblastogenesis in myeloma

In 1991, Bataille *et al* (Bataille *et al*, 1991) originally reported that increased osteoblast activity coupled with increased bone resorption rate synchronically occur early in conversion from the benign precursor condition (monoclonal gammopathy of undetermined significance, MGUS) to overt MM and that patients who maintained high osteoblast activity did not develop osteolytic disease. MSCs isolated from BM of patients with MM are genetically and phenotypically abnormal and have impaired osteogenic potential (Corre *et al*, 2007; Garayoa *et al*, 2009; Wallace *et al*, 2001). In addition, myeloma cells actively suppress osteoblastogenesis through direct cell–cell contact and production of soluble factors. Co-culturing myeloma cells with osteoblasts resulted in downregulation of the osteoblast marker, osteocalcin (Barille *et al*, 1995), and direct contact between myeloma cells and MSCs, mediated by cell-surface molecules very late antigen 4 (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1), resulted in MSCs downregulating expression of the critical osteoblast transcription factor Runt-related transcription factor 2 (RUNX2). These reports are in accord with histopathological analyses indicating that osteolytic lesions in MM often occur adjacent to the tumour area (Roodman, 2004).

It is now evident also that osteoblast differentiation is inhibited by factors secreted by myeloma cells (*e.g.*, Wnt-signaling inhibitors DKK1 [Tian *et al*, 2003] and secreted frizzled-related protein-2 [Oshima *et al*, 2005], IL-7 [Giuliani *et al*, 2005b], and hepatocyte growth factor (HGF) [Standal *et al*, 2007]) and by microenvironmental cells within myelomatous bone (*e.g.*, IL-3 [Lee *et al*, 2004; Ehrlich *et al*, 2005]). Importantly, expression or circulating levels of the osteoblast-inactivating factors often varied among patients, indicating multiple mechanisms by which myeloma cells suppress osteoblastogenesis.

Recently, Pennisi *et al* demonstrated that cell-surface “coupling” factors ephrinB2 and EphB4, which mediate communication between osteoblasts and osteoclasts by bidirectional signaling (Zhao *et al*, 2006), are underexpressed in MSCs from patients with myeloma and that myeloma cells induce downregulation of these genes in healthy MSCs (Pennisi *et al*, 2009c). Osteoclast precursors mainly express ephrinB2, whereas osteoblasts and their precursors, MSCs, express ephrinB2 and EphB4. Forward signaling in MSCs promotes their

osteogenic differentiation, and reverse signaling in osteoclast precursors inhibits their differentiation into multinucleated bone-resorbing osteoclasts (Zhao *et al*, 2006; Pennisi *et al*, 2009c). Activating ephrinB2–EphB4 bidirectional signaling by using chimeric proteins ephrinB2-Fc and EphB4-Fc in the SCID-hu model for MM resulted in increased osteoblast activity and bone mass of the myelomatous bone (Pennisi *et al*, 2009c). Inhibition of osteoclastogenesis, angiogenesis, and myeloma growth also were associated with treating mice with clustered EphB4-Fc (which stimulates reverse signaling) but not with clustered ephrinB2-Fc (which stimulates forward signaling). These studies indicate that cell-surface factors for coupling bone remodeling, such as ephrinB2 and particularly EphB4, are potential therapeutic targets for MM bone disease (Figure 1).

Role of bone disease in myeloma progression: *In vitro* studies

Involvement of osteoclasts

The absolute numbers of osteoclasts in healthy bone and in MM focal lesions are low compared to other cellular compartments; nevertheless, the impact of osteoclasts on myeloma cell survival and growth is significant. Osteoclasts influence myeloma cells directly, by physical cell–cell contact and production of growth factors, and indirectly, by effects on the extracellular compartment (*e.g.*, increased levels of bone-resorption products) and other cellular elements (*e.g.*, angiogenesis stimulation) (Tanaka *et al*, 2007). For instance, we showed that co-culturing osteoclasts and primary myeloma plasma cells in a 1:1,000 ratio was sufficient to effectively support survival of myeloma cells and protect them from drug-induced apoptosis (Yaccoby *et al*, 2004; Yaccoby, 2005); cell contact seems to be essential for these effects to occur (Abe *et al*, 2004; Yaccoby et al, 2004), but it is not yet clear whether osteoclasts significantly affect survival of distant myeloma cells in lytic lesions. In patients with MM, some osteoclasts may be formed by fused myeloma cells (Andersen *et al*, 2007; Silvestris *et al*, 2009) or transdifferentiation of DCs (Kukreja *et al*, 2009), but little is known about the properties, structure, and functional activities of osteoclasts from myeloma patients.

An intriguing observation indicated that after *in vitro* interactions with osteoclasts (Yaccoby, 2005) or stromal cells (Dezorella *et al*, 2009), myeloma cells acquire an immature phenotype. This suggests that, through an adhesion mechanism, certain microenvironmental elements can revert or induce de-differentiation of the recognizable myeloma plasma cells into an immature phenotype that protects these cells from spontaneous and drug-induced apoptosis. This notion is in line with *in vivo* studies that demonstrated high aggressiveness of murine myeloma cells due to alteration of bone turnover by external factors (Libouban *et al*, 2003; Libouban *et al*, 2004). Recently, imaging techniques such as MRI and PET-microCT revealed that plasma cells can persist in focal lesions of patients who achieve clinical complete remission (defined by detection of monoclonal protein and random BM examination [Walker *et al*, 2007; Bartel *et al*, 2009]), suggesting the presence of immature, resilient, apoptosis-resistant malignant plasma cells in clinical myeloma.

As a result of the influences of osteoclasts, myeloma cells decrease JNK activation (Colla *et al*, 2007) and activate survival signaling pathways that include p44/p42 MAPK, STAT3, and PI3K/Akt pathways (Hecht *et al*, 2008). Soluble growth factors and cytokines in the MM microenvironment, such as IL-6, osteopontin (Abe *et al*, 2004; Yaccoby *et al*, 2004), B cell activating factor of the TNF family (BAFF), and a proliferation-inducing ligand (APRIL) (Yaccoby *et al*, 2008), have been implicated in osteoclast-induced myeloma cell survival. Global gene expression profiling in osteoclasts cultured alone or co-cultured with primary myeloma cells identified few genes not expressed by myeloma cells but commonly upregulated in osteoclasts after co-culture with myeloma cells (Ge *et al*, 2006); notable among these was fibroblast activation protein (FAP), a type-II integral-membrane

glycoprotein that belongs to the serine protease family known as DASH (dipeptidyl peptidase-IV activity and/or structure homologs). Further studies revealed that it is also upregulated in MSCs after co-culture with myeloma cells and that inhibiting FAP (with siRNA or specific inhibitor) reduced the stimulatory effects of osteoclasts and MSCs on myeloma cell growth in co-cultures (Pennisi *et al*, 2009a) and *in vivo* in SCID-hu mice (Pennisi *et al*, 2009a). The study using FAP inhibitor suggests that this serine protease is involved in regulating adhesion molecules in osteoclasts, some of which are implicated in tumourigenesis and osteoclastogenesis (Pennisi *et al*, 2009a). Together, the studies of interactions between myeloma cells and osteoclasts shed light on critical cell-surface and soluble factors associated with myeloma cell survival in bone, which are potentially important for innovative targeted therapies (Figure 2).

Involvement of osteoblasts

Studies of the roles of osteoblasts on myeloma cell growth have been inconclusive, partially due to heterogeneous characteristics of MM and to the use of ill-defined populations of osteoblasts (*e.g.*, using differentiating rather than terminally differentiated osteoblasts). In addition to their production of bone-building products, mature osteoblasts differ from MSCs and immature osteoblasts by their expression of cytokines, osteoclastogenic factors, and "coupling factors." It is now recognized that bone-building osteoblasts produce high levels of OPG and reduced levels of RANKL (Glass *et al*, 2005; Spencer *et al*, 2006; Qiang *et al*, 2008b). Expression of myeloma growth factor IL-6 is reduced as MSCs differentiate into osteoblasts (Gunn *et al*, 2006). It is proposed, therefore, that inhibition of osteogenic differentiation in MM creates favourable conditions for propagating myeloma cells and inducing osteolytic lesions (Gunn *et al*, 2006; Corre *et al*, 2007; Stewart & Shaughnessy, Jr., 2006).

Although BM stromal cells and MSCs often protect myeloma cells from spontaneous and drug-induced apoptosis (Mitsiades *et al*, 2006), recent findings indicate that mature osteoblasts inhibit survival of myeloma cells taken from a large subset of patients, and they interfere with the stimulatory effects of osteoclasts on myeloma cell survival and growth (Yaccoby *et al*, 2006). Li *et al* (Li *et al*, 2008) demonstrated that decorin, a small leucine-rich proteoglycan that is highly produced by bone-building osteoblasts, inhibited survival of myeloma cells and attenuated the stimulatory effects of osteoclasts on myeloma cells. They also demonstrated that blocking activity or expression of decorin reduced osteoblasts' inhibitory effects on myeloma cell growth and survival, but overexpression of decorin in MSCs lessened the ability of these cells to support myeloma cell survival (Li *et al*, 2008). Mechanistically, decorin may directly induce tumour cell apoptosis by activating caspase 3 and upregulating $p21^{WAF}$, and it may indirectly inhibit tumour growth by degrading critical cell-surface growth-factor receptors such as MET and epidermal growth factor receptor (EGFR) (for review, see Goldoni & Iozzo, 2008) and by suppressing myeloma-induced osteoclastogenesis and angiogenesis (Li *et al*, 2008). It is likely that bone-building osteoblasts produce other factors that negate myeloma cell growth. Collectively, these findings suggest that increasing numbers of terminally differentiated osteoblasts may help control MM by producing bone-building factors that directly and indirectly restrain myeloma growth by inhibiting osteoclastogenesis and angiogenesis and by altering the net balance between osteoclastogenic and antiosteoclastogenic factors in myelomatous bone (Figure 2).

Role of bone disease in myeloma progression: Clinical and *in vivo* experimental studies

Osteoclast inhibitors

Until recently, *in vivo* animal studies to unravel the roles of bone disease in myeloma tumour growth focused on the use of osteoclast inhibitors. Currently, MM bone disease is treated mainly by controlling MM tumour burden and inhibiting osteoclast activity with bisphosphonates. Osteoclasts have heterogeneous enzymatic repertoires and bisphosphonate sensitivities (Andersen *et al*, 2004;Everts *et al*, 2006). In fact, the report that trabecular osteoclasts are more responsive to bisphosphonates than are cortical osteoclasts (Chappard *et al*, 1991) may explain why bisphosphonates are better for reducing fractures of the appendicular skeleton, which is mainly composed of trabecular bone, than fractures of long bones, which are made of compact Haversian bone (Kanis & McCloskey, 2000). Experimental studies using animal models, such as the 5T or SCID-hu mouse models, have shown that inhibition of osteoclast activity by bisphosphonates or inhibitors of RANKL (*e.g.*, RANK-Fc or OPG) effectively prevented MM-induced osteolysis (Yaccoby *et al*, 2002; Pearse *et al*, 2001; Croucher *et al*, 2003; Vanderkerken *et al*, 2003; Croucher *et al*, 2001). In many of these experiments, inhibition of osteoclast activity reduced growth of medullary but not extramedullary myeloma. These findings, however, are inconsistent with clinical observations: bisphosphonates, mainly pamidronate and zoledronate, reduce skeletal complications in patients with MM, but the beneficial effects on myeloma progression are inconclusive (Berenson *et al*, 1996; Roodman, 2008). In a phase II clinical trial with patients with relapsed or plateau-phase MM, some patients who were treated with RANKL-neutralizing antibody denosumab experienced MM disease stabilization (Vij *et al*, 2009).

The discrepancies in anti-myeloma efficacy of osteoclast inhibitors seen in experimental and clinical studies could be due to several factors. Clinically, bisphosphonates do not completely block bone resorption, and it often progresses (Roodman, 2008). This point could be significant in light of recent clinical observations suggesting that certain bisphosphonates may induce formation of viable, detached giant osteoclasts in BM of patients with osteoporosis (Weinstein *et al*, 2009). In experimental systems, however, significant inhibition of bone disease serves as an indicator of drug efficacy that allows investigation of the association between inhibition of bone disease and tumour progression. Conversely, many experimental animal studies initiated treatment when tumour burden was low. These studies also utilized young animals or bones capable of compensating for bone resorption by increasing osteoblast activity; thus, the experimental conditions may not reflect the bone biology of elderly MM patients. For instance, Croucher *et al* (Croucher *et al*, 2001) showed that, in the 5T mouse model, treatment with OPG prevented development of osteolytic bone disease and preserved bone mineral density and bone volume to levels similar to or higher than nonmyelomatous mice, suggesting restoration of normal osteoblastogenesis or perhaps a concomitant increase in osteoblast activity. Overall, these studies suggest that, by effectively inhibiting osteoclast activity and retaining normal levels of osteoblast activity, osteoclast inhibitors may create an inhospitable BM microenvironment and restrain growth of medullary myeloma cells, which otherwise induce lytic lesions that facilitate their growth within the bone.

Wnt activation

Recent *in vivo* studies dealt with the effects of using osteoblast-activating agents to inhibit MM bone disease and tumour progression. Studies demonstrating the critical role of Wnt signaling in bone hemostasis (Clevers, 2006), along with the discovery that myeloma cells produce Wnt inhibitors, prompted the examination of agents that promote Wnt signaling in bone indirectly (*e.g.*, neutralizing activity of MM-produced Wnt antagonist DKK1 [Yaccoby

et al, 2007]) or directly (*e.g.*, Wnt3a [Qiang *et al*, 2008c], lithium chloride [Edwards *et al*, 2008]). The effects of DKK1-neutralizing antibody on MM bone disease and tumour growth were investigated in SCID-rab mice that were each engrafted with myeloma cells from one of 11 patients (Yaccoby *et al*, 2007). In this animal model, rabbit bone is implanted in a SCID mouse, and primary human myeloma cells are engrafted in the implanted bone; typical MM disease manifestations occur, including induction of severe osteolytic bone disease. In this animal study, as in the clinical setting, anti-DKK1 heterogeneously affected bone disease and tumour burden, but the overall effects were significantly increased osteoblast numbers and bone mass and reduced osteoclast numbers and myeloma burden in myelomatous bones (Yaccoby *et al*, 2007). A humanized DKK1-neutralizing antibody, BHQ880, which is currently being evaluated in clinical trials, has shown similar effects in SCID-hu mice engrafted with IL6-dependent myeloma cell line INA6 (Fulciniti *et al*, 2009). In the 5T2MM murine model of MM, BHQ880 promoted osteoblastogenesis but had no effect on osteoclastogenesis or tumour growth (Heath *et al*, 2009), suggesting that inducing a BM microenvironment that is inhospitable for myeloma cells by such intervention requires simultaneous stimulatory effects on osteoblasts and inhibitory effects on osteoclasts.

In an attempt to directly increase canonical Wnt signaling, Qiang *et al* (Qiang *et al*, 2008c) demonstrated that the canonical Wnt ligand, Wnt3a, similarly reduced bone disease and growth of myeloma cell line H929 and primary myeloma cells that were engrafted in the human bone in SCID-hu mice, but Wnt3a had no effect when H929 myeloma cells were grown subcutaneously in SCID mice. In a different approach to directly stimulate Wnt signaling, Edwards *et al* (Edwards *et al*, 2008) used lithium chloride, a GSK-3β inhibitor, in the 5TGM1 myeloma mouse model. Treatment with lithium chloride resulted in stabilization of β-catenin, prevention of MM-induced bone disease, and reduction in tumour burden in bone; however, lithium chloride treatment stimulated tumour growth when 5TGM1 cells were inoculated subcutaneously (Edwards *et al*, 2008). Because growth of most patients' MM cells is restricted to bone and requires a specialized BM microenvironment, the animal studies investigating the consequences of increased Wnt signaling on medullary myeloma (Qiang *et al*, 2008c) are clinically relevant and suggest that increased Wnt signaling may restrain myeloma growth by increasing bone formation and preventing bone resorption, which result from stimulation of osteoblastogenesis and suppression of osteoclastogenesis.

Proteasome inhibitors

Incorporating proteasome inhibitor bortezomib into clinical treatment of myeloma uncovered its unique effects on bone remodeling (Zangari *et al*, 2005; Zangari *et al*, 2007; Zangari *et al*, 2008). Proteasome inhibitors stimulate osteoblastogenesis and bone formation (Garrett *et al*, 2003; Mukherjee *et al*, 2008), which supports the notion that the ubiquitin–proteasome pathway is a promising therapeutic target for inhibiting myeloma cell growth and bone disease. In successive clinical observations, Zangari *et al* (Zangari *et al*, 2007; Zangari *et al*, 2008) demonstrated that the anti-myeloma response of bortezomib is associated with increases in both bone mass and circulating levels of bone alkaline phosphatase (Zangari *et al*, 2008) and that increased levels of alkaline phosphatase predict treatment response (Zangari *et al*, 2007). Clinical observations by others support the perception that bortezomib is a bone-anabolic agent in MM (Terpos *et al*, 2006; Heider *et al*, 2006). In addition to their clinical value, these observations demonstrate that osteoblast activation is feasible in patients with MM, despite reports indicating that patients' MSCs are genetically, phenotypically, and functionally abnormal (Corre *et al*, 2007; Garayoa *et al*, 2009; Wallace *et al*, 2001;Todoerti *et al*, 2009).

The clinical association between increased osteoblast activity and response to bortezomib therapy is supported by experimental data. Edwards *et al* (Edwards *et al*, 2009) reported that, in the 5TGM1 model, the effect of bortezomib on tumour reduction was higher when

myeloma cells were grown within the BM than in extra-osseous sites (Edwards *et al*, 2009). In the SCID-rab model for primary myeloma, the anti-myeloma effects of bortezomib, but not melphalan, were associated with increased bone mass in responding hosts (Pennisi *et al*, 2009b), suggesting that bortezomib's effects on skeletal homeostasis are not a consequence of reduced tumour burden but, rather, of direct effects on bone cells (Pennisi *et al*, 2009b).

Indeed, accumulating data shed light on mechanisms by which proteasome inhibitors directly affect the fate of bone cells. Inhibition of the ubiquitin–proteasome pathway induces osteoblast differentiation by increasing expression of bone morphogenetic protein 2 (Garrett *et al*, 2003) and preventing proteolytic degradation of RUNX2 (Bellido *et al*, 2003; Giuliani *et al*, 2007). Bortezomib also promotes bone formation by inhibiting DKK1 expression in osteogenic cells (Oyajobi *et al*, 2007) and by stabilizing β-catenin in osteogenic cells in a mechanism that is independent of canonical Wnt ligands (Qiang *et al*, 2009). Proteasome inhibitors also reduce NF-κB activity in osteoclast precursors, resulting in inhibition of osteoclast formation (Zavrski *et al*, 2005; von Metzler *et al*, 2007). Bortezomib reduces circulating levels of DKK1 and RANKL in patients with relapsed MM (Terpos *et al*, 2006; Kaiser *et al*, 2008), further suggesting that proteasome inhibitors increase bone mass by stimulating osteoblastogenesis and inhibiting osteoclastogenesis. Despite these convincing data, the long-term effects of proteasome inhibition on bone remodeling, particularly with regard to the fate of osteoprogenitor cells, should be carefully investigated because the stimulatory effects of bortezomib on circulating levels of osteoblastic markers, at least in patients with MM, seem to be transient (Zangari *et al*, 2005).

Conclusions

Understanding the roles of bone disease in myeloma tumour growth is challenging due to the heterogeneous characteristics of the disease. Nevertheless, clinical observations reveal a high correlation between numbers of focal and/or lytic lesions and disease stage, as assessed by standard clinical parameters and molecular classification of myeloma cells (Walker *et al*, 2007; Bartel *et al*, 2009; Zhan *et al*, 2006). Because osteoclasts and bone-building osteoblasts appear to induce contrasting effects on myeloma cell survival and proliferation, induction of osteolytic bone destruction in MM is likely to promote disease progression. In animal studies, pretreating with bisphosphonates to inhibit osteoclast activity (Yaccoby *et al*, 2002) or with intermittent parathyroid hormone to increase bone mass (Pennisi A *et al*, 2007) significantly slowed engraftment of MM, suggesting that, in some cases, bone disease drives development of MM. Partially inhibiting osteoclast activity without maintaining normal or increased levels of osteoblast activity may not be sufficient to create an inhospitable BM environment for myeloma cells. Therefore, development of interventions that simultaneously stimulate osteoblastogenesis and restrain osteoclastogenesis may also control myeloma progression while effectively promoting bone formation.

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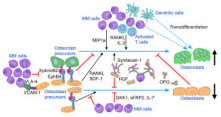

Figure 1.

Main cellular and molecular factors associated with MM-induced bone disease. Osteolytic lesions are induced by uncoupling of bone metabolism, resulting in increased bone resorption by osteoclasts and reduced bone formation by osteoblasts. Interactions between MM cells and various BM cells (*e.g.*, osteoblast and osteoblast precursors, activated T lymphocytes, dendritic cells) result in increased production of osteoclast-activating factors (*e.g.*, RANKL, IL-3, MIP1α) and/or osteoblast-inactivating factors (*e.g.*, DKK1, sFRP2, IL-7). OPG levels in lytic lesions are low as a consequence of reduced osteoblast numbers and of OPG internalization and degradation by MM cells in a mechanism mediated by binding to syndecan-1 on MM cell surfaces. Syndecan-1 molecules shed from MM cells also bind and localize factors such as HGF that act on bone cells to promote osteolysis. Osteoblastogenesis is impaired by cell–cell contact mediated by VCAM-1 on MM cells and VLA-4 on osteoblast precursors and also by dysregulation of cell-surface coupling factors such as ephrinB2 and EphB4 on osteoblast precursors. Osteoclasts may form by MM cells fusing together or by dendritic cells fusing with osteoclast precursors, contributing to increased numbers of osteoclasts. Other cellular elements of BM, such as megakaryocytes, macrophages, and Th17 T lymphocytes, may also be cultivated by MM to promote bone disease (see text for details).

Figure 2.
Roles of bone cells in myeloma cell survival and growth. In focal/lytic lesions, osteoclasts and mesenchymal cells (*e.g.*, MSCs) are cultivated by myeloma cells to express tumour-promoting factors (*e.g.*, FAP) and produce MM growth factors (*e.g.*, osteopontin, IL-6), which induce survival signalling and promote dedifferentiation of myeloma cells into an immature, apoptosis-resistant phenotype. In contrast, bone-building osteoblasts may inhibit myeloma cell growth by producing bone-building products, including some SLRPs. Agents that suppress osteoclast activity and/or stimulate osteoblastogenesis help re-establish balanced bone remodeling and contribute to controlling MM (see text for details); these agents include bortezomib, anti-DKK1, Wnt-activators, bisphosphonates, IMiDs, and anti-RANKL.