Pathophysiology of myeloma bone disease

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A B S T R A C T

A key clinical feature of multiple myeloma is the development of a devastating osteolytic bone disease, which is characterized by considerable bone pain and increased risk of pathological fracture. This is mediated by both an increase in osteoclastic bone resorption and a sustained inhibition of bone formation. In recent years, new molecular pathways have been identified that mediate these two contributing components to myeloma bone disease. The ligand for receptor activator of NFκB is a critical mediator of osteoclast formation, activity and survival and mediates increased osteoclastic resorption in myeloma. Macrophage inflammatory protein-1α, parathyroid hormone-related protein, interleukin-6, annexin II, ephrin, along with other cytokines have also been implicated in regulating osteoclasts in myeloma, although they generally do so by up-regulating RANKL. A number of molecules, including the soluble Wnt antagonists, dickkopf-1, soluble frizzled related protein (sFRP)-2 and s-FRP-3, activin A, interleukin-3 and -7, hepatocyte growth factor and adiponectin have been shown to suppress osteoblast-mediated bone formation in myeloma, although their relative importance is unclear. In many cases, approaches to targeting these pathways are in advanced clinical development. Finally, inhibitors of bone resorption also decrease myeloma burden suggesting these agents may offer additional benefits beyond treating myeloma bone disease.

Learning goals

At the conclusion of this activity, participants should be able to:
- describe the cellular mechanisms responsible for the development of myeloma bone disease;
- describe the molecular mechanisms responsible for osteoclast stimulation in myeloma;
- describe the molecular mechanisms responsible for osteoblast inhibition in myeloma;
- discuss potential new approaches to treat myeloma bone disease;
- discuss the potential impact of the bone microenvironment on the growth of myeloma.

Introduction

Multiple myeloma is characterized by the growth of malignant plasma cells in the bone marrow. A defining feature is the development of a devastating bone disease characterized by osteolytic bone lesions, bone pain and an increased risk of pathological fracture. These fractures are typically associated with the osteolytic bone lesions; however, there is now increasing evidence that patients with myeloma also have generalized bone loss, or osteoporosis, and have an increased risk of osteoporotic fracture.[1] Furthermore, in patients with monoclonal gammopathy of underdetermined significance, which can progress to myeloma, individuals have a greater than 2-fold increase in fractures of the axial skeleton.[2] Thus, there is now increasing awareness of not only the osteolytic bone disease in myeloma, but also the associated osteoporosis and the impact this may have on fracture risk.

Importantly, in recent years we have seen enormous progress in understanding the cellular and molecular mechanisms responsible for the development of myeloma bone disease. Studies have identified critical regulators responsible for promoting osteoclast formation and function, as well as identified inhibitors of osteoblastic bone formation (Figure 1). This new knowledge has led to the development of new agents that have moved from pre-clinical studies into advanced clinical development. Furthermore, this understanding has also revealed new information about the complex relationship between myeloma cells and the cells of bone, which in turn may lead to new ways of using bone-targeted therapies to modify myeloma development in bone. This review discusses the interactions that occur between myeloma cells and the cells of bone, some of the critical molecular pathways involved, and considers the impact that this has on the development of bone disease.

Cellular mechanisms of bone loss in multiple myeloma

Skeletal integrity is maintained by the coordinated activity of three main cell types in bone. Osteoclasts are the cells responsible for the resorption of bone, osteoblasts are responsible for synthesizing new bone matrix and osteocytes, which are derived from terminally differentiated osteoblasts embedded in the bone matrix, respond to mechanical load and regulate bone turnover. In the adult skeleton,

the amount of bone present and its integrity is maintained through the coordinated activity of these cells in the process of bone remodeling. Osteoclasts resorb bone at discrete sites on the bone surface and osteoblasts then replace this bone by synthesizing new bone matrix, which is subsequently mineralized, returning the bone surface to a state of quiescence.

When myeloma develops in the skeleton, the tumor cells are uniquely placed to influence this process of bone remodeling. Histomorphometric studies have demonstrated that osteoclastic bone resorption is increased in patients with myeloma.[3,4] In the early stages of disease development, osteoblastic bone formation may be increased, which likely reflects the tight coupling that normally occurs between resorption and formation. However, as myeloma burden increases, osteoblast driven bone formation is suppressed, which results in an uncoupling of bone remodeling, rapid bone loss and the development of osteolytic lesions. Indeed, myeloma is unusual in that within individual lytic bone lesions there is no bone formation response to the increased resorption, which makes patients bone-scan negative. Furthermore, osteoblast suppression is maintained even in patients in long-term remission making repair of osteolytic bone lesions a particular challenge. The mechanism responsible for the osteoporosis that occurs alongside the focal osteolytic disease is poorly

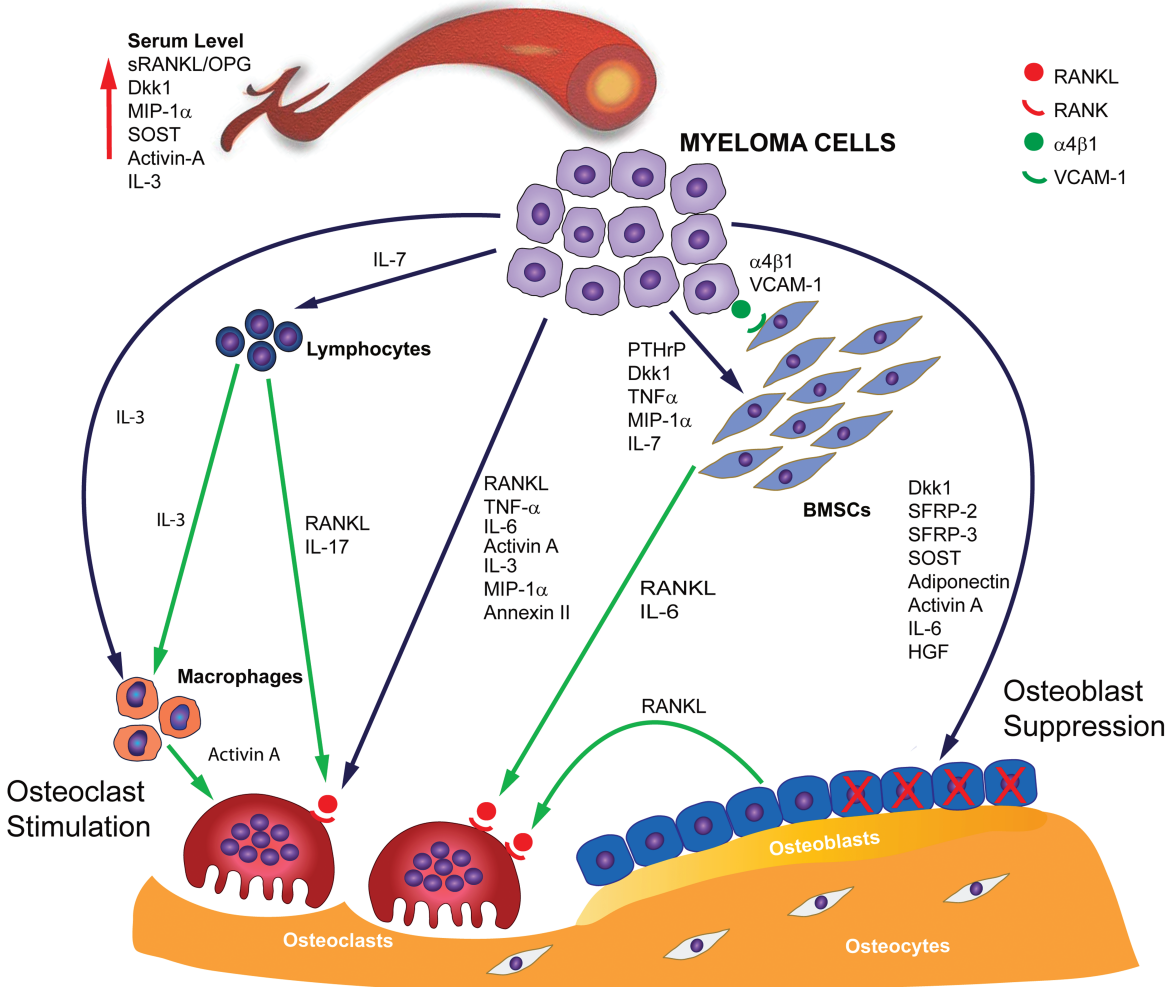

Figure 1. Molecular mechanisms regulating bone resorption and bone formation in multiple myeloma. Osteoclast stimulation and osteoblast suppression in response to myeloma cells in the bone marrow is driven by both direct (blue arrows) and indirect (green arrows) mechanisms. Myeloma cells produce a number of pro-osteoclast factors including RANKL, TNFα, IL-6, Activin A, IL-3, MIP-1α and Annexin II, directly driving osteoclast differentiation, survival and activation. Osteoblast differentiation and survival is also directly inhibited by myeloma cells through the production of factors including, Dkk1, SFRP-2, SFRP-3, SOST, Activin A and IL-6. Stromal cells of the bone marrow environment respond to myeloma cells through soluble factors such as PTHrP, Dkk1, TNFα, MIP-1α and IL-7 and direct contact through α4β1/VCAM-1 interaction. These signals lead to an increase in production of local RANKL and IL-6 by stromal cells to indirectly stimulate osteoclast differentiation, survival and activation. Further, immune cells such as T lymphocytes and macrophages respond to myeloma-produced cytokines (IL-7, IL-3) to indirectly enhance osteoclast formation and function. Serum levels of soluble RANKL, Dkk1, MIP-1α, Activin-A, IL-3 and SOST are all up-regulated in response to the bone marrow infiltration by myeloma cells. It is suggested that this systemic shift may be responsible for the widespread osteoporosis and increased fractures documented in myeloma patients.

understood. Although it is generally regarded that myeloma cells are able to interact directly with osteoclasts, and osteoblasts, and their precursors, to alter bone remodeling, there is evidence that they may also mediate these effects indirectly via other cells in bone. Cells of the immune system that reside in the bone marrow have been implicated in the development of bone disease in myeloma. For example, regulatory T cells are increased and associated with disease progression[5] and in the presence of myeloma cells, increased numbers of T lymphocytes in the bone marrow up-regulate pro-osteoclastic molecules.[6] Furthermore, T-helper cells are abundant in the bone marrow of patients and their production of interleukin-17 promotes osteoclast formation.[7] Hence myeloma cells drive changes in the immune system, which are associated with production of pro-osteoclastic factors, which indirectly accelerate bone disease.

Molecular mechanisms regulating osteoclast formation and function in myeloma

Early studies, led by Mundy and colleagues, focused on identifying the molecules that promoted bone resorption and demonstrated that human myeloma cell lines and primary cells isolated from patients with myeloma produced an osteoclast activating factor.[8,9] However, the identity of the molecules that comprise this activity was, for many years, unclear. Interleukin-1β, lymphotoxin and tumor necrosis factor-α were all implicated, but little functional data were available to support a causal role.[10-13] More recently new pathways, including the ligand for receptor activator of NFκB (RANKL) and macrophage inflammatory protein (MIP)-1α, have been identified, shown to be functionally important and are likely the major regulators of osteoclast formation in myeloma. The other pathways implicated, and there are a number, are likely to do so via regulation of RANKL in the bone microenvironment. Importantly, there is increasing recognition that molecules that regulate bone resorption in myeloma may also directly effect bone formation and conversely pathways that have a key role in suppressing bone formation may also regulate expression of critical osteoclastogenic factors.

Tumor necrosis factor superfamily members – the ligand for receptor activator of NFkB and tumor necrosis factor

The ligand for receptor activator of NFκB (RANKL) plays a central role in regulating skeletal homeostasis through promoting osteoclastic bone resorption. RANKL is a member of the tumor necrosis factor (TNF) family and is expressed by a range of cell types including cells of the osteoblast lineage and activated T cells.[14] RANKL binds its receptor, receptor activator of NFκB (RANK) a member of TNF receptor family, expressed by osteoclasts and their precursors,[15] to stimulate osteoclast differentiation, formation and survival. The pro-resorptive activity of the RANKL/RANK interaction is regulated by osteoprotegerin, which functions as a decoy receptor by binding RANKL, preventing its interaction with RANK and inhibiting osteoclastogenesis and bone resorption.[16] Myeloma cells have been shown to express RANKL and to stimulate osteoclastogenesis directly.[17-20] Myeloma cells also up-regulate expression of RANKL in human BMSCs and osteoblasts, and down-regulate production of OPG, altering the balance in favor of increased osteoclastogenesis.[21-23] This may be mediated by interactions between $\alpha_4\beta_1$ on myeloma cells and vascular cell adhesion molecule-1 (VCAM1) on bone marrow stromal cells in a contact dependent mechanism,[24] or via production of local factors such as dickkopf-1 (Dkk1) by myeloma cells, and may be dependent upon signaling through the p62 adapter protein.[25,26] Myeloma cells also produce interleukin-7 (IL-7) which has been shown to induce RANKL in T cells, indirectly contributing to osteoclast formation.[27] Finally, myeloma cells also remove local OPG through an endocytic pathway via an ability to bind syndecan-1 on the cell surface.[28] Thus, multiple interactions and mechanisms can contribute to increased expression of RANKL relative to OPG in the local environment, although the relative contribution of the different cell types and mechanisms has yet to be defined.

The changes in the balance between RANKL and OPG are also reflected in serum concentrations of soluble RANKL and OPG in patients with myeloma. For example, OPG has been reported to be decreased in the serum of patients with myeloma and associated with the development of lytic lesions,[29] whereas the ratio of serum soluble RANKL to OPG is increased and this is associated with poor survival.[30] In individuals with MGUS the RANKL/OPG is also increased when compared to control subjects but remains significantly lower than patients with myeloma.[31] Interestingly, in a murine model of myeloma sRANKL has been shown to be released by myeloma cells and contribute to bone loss at distant sites, raising the possibility that sRANKL may also contribute to the development of osteoporosis in myeloma.[32]

Critically, targeting RANKL with either recombinant OPG constructs, soluble RANK or OPG peptidomimetics has been shown to prevent osteoclast formation and the development of osteolytic bone lesions in a number of murine models of myeloma.[17,21,33,34] These studies have paved the way for studies in patients, initially with OPG, which reduces biochemical markers of bone resorption in patients with myeloma[35] and more recently the anti-RANKL antibody, denosumab, which is now in clinical development (see below).[36-38]

In addition to RANKL, other TNF family members also play a role in promoting osteoclastic resorption in myeloma. TNFα is increased in the bone marrow of patients with myeloma and can be produced by myeloma cells.[13] TNFα is a major regulator of osteoclastogenesis and can induce osteoclast formation directly, independently of RANKL. However, TNFα also induces expression of X-box binding protein 1 spliced (XBP1s), a transcription factor that promotes expression of RANKL and IL-6 in bone marrow stromal cells.[39] XBP1s also increases VCAM-1 expression in stromal cells, which may contribute further to RANKL expression via interactions with $\alpha_4\beta_1$ on myeloma cells.[24] Thus, in addition to potential direct osteoclastogenic effects, TNFα may play a critical role in regulating bone resorption in myeloma by inducing expression of RANKL in the local environment.

Macrophage inflammatory protein-1α

MIP-1α, also known as chemokine C-C motif ligand, is produced by multiple myeloma cells, elevated in the bone marrow of patients with myeloma and able to promote osteoclastogenesis.[40-42] MIP-1α signals through a number

of receptors including CCR1 and CCR5, which are expressed on osteoclast precursors, and CCR9, regulating downstream signaling pathways associated with a range of functional processes including proliferation, survival and migration. MIP-1α may regulate effects on osteoclastic bone resorption in a RANKL independent manner; however, recombinant MIP-1α has been reported to be unable to induce osteoclastogenesis in RANK deficient mice, supporting the notion that it does so via regulation of RANKL expression.[43] Certainly, MIP-1α can stimulate expression of RANKL in stromal cells indirectly stimulating osteoclast differentiation.[42] Serum concentrations of MIP-1α are also elevated in patients with myeloma and associated with the presence of osteolytic lesions.[44,45] Inhibition of MIP-1α using antisense technology or antibodies to MIP-1α prevents the development of myeloma bone disease in a number of model systems.[40,46] Furthermore, antagonism of CCR1 also prevents development of osteolytic bone disease in models of myeloma.[47,48]

Interleukin-6

Interleukin (IL)-6 is able to promote myeloma cell survival and growth as well as modify bone remodeling. IL-6 is found in the local bone microenvironment and can be expressed by myeloma cells themselves.[49] IL-6 is a potent inducer of osteoclast formation via induction of IL-1 in the local environment.[50] The IL-6 receptor is expressed on osteoclasts and their progenitors, which is consistent with a direct effect on osteoclastogenesis.[51] However, functional studies also demonstrate that IL-6 can bind its receptor on cells of the osteoblast lineage to stimulate RANKL expression and hence also regulate osteoclastogenesis indirectly.[52]

Additional regulators - parathyroid hormone related protein, Annexin II and Ephrin

Other molecules have also been implicated in regulating osteoclast formation in myeloma often by up-regulating RANKL expression either in myeloma cells or in bone marrow stromal cells. For example, parathyroid hormone related protein (PTHrP) is produced by a number of tumors that grow in bone and plays a critical role in the development of bone metastasis, particularly breast cancer metastasis to bone.[53,54] PTHrP is a potent stimulator of bone resorption and mediates its effect by up-regulating RANKL in osteoblasts via the PTH-R1.[55] PTHrP has been shown to be expressed by myeloma cells and PTHrP signaling, via the PTH-RI, increases expression of RANKL in myeloma cells.[56,57] Annexin II is also expressed by myeloma cells.[58] Annexin II is important in osteoclast formation, promotes RANKL expression in bone marrow cells[59] and regulates the growth of myeloma cells.[60] The ephrinB2/EphB4 axis is involved in the coupling of resorption and formation and levels are reduced in stromal cells from patients with myeloma. A soluble EphB4 construct has been shown to reduce osteoclast formation and myeloma growth in tumor-bearing mice, suggesting this system may regulate bone resorption in myeloma.[61]

Molecular mechanisms regulating osteoblast suppression in myeloma

In addition to progress in understanding the molecular mechanisms responsible for promoting osteoclastic resorption, major advances have been made in defining the pathways responsible for osteoblast suppression in myeloma. Osteoblast suppression remains a major determinant of osteolytic disease, as despite success in the clinic with inhibitors of bone resorption, osteolytic lesions do not heal. Indeed, it has been argued that stromal cells isolated from patients with myeloma retain an aberrant phenotype *in vitro* for sustained periods, suggesting there are permanent changes in resident stromal cell populations.[62] This may pose particular challenges for development of new treatments. However, despite these challenges a number of pathways, including inhibitors of the Wnt pathway, the activin-A signaling system and hepatocyte growth factor have all been implicated in regulating osteoblast suppression in myeloma.

Soluble Wnt antagonists – Dickkopf-1, soluble frizzled related proteins and sclerostin

The Wnt signaling pathway has long been known to play a critical role in regulating many physiological processes including bone formation. Wnt signaling is regulated by soluble Wnt antagonists, including the dickkopfs, soluble frizzled related proteins and sclerostin, many of which play critical roles in regulating normal bone turnover. Pivotal studies have demonstrated that Dickkopf-1 (DKK1) is expressed by myeloma cells and can decrease bone formation.[63,64] DKK1 concentrations are increased in the serum of a proportion of patients with myeloma and correlate with the presence of osteolytic disease.[64,65] However, it is unclear whether local levels in the bone microenvironment are sufficient to regulate beta-catenin.[64] DKK1 is also able to up-regulate RANKL expression in osteoblasts increasing osteoclast activity, which may indirectly promote development of osteolytic disease.[66] Importantly, studies have shown that blocking DKK1 with anti-DKK1 antibodies can prevent osteoblast suppression in models of myeloma. The retention of osteoblast activity is associated with prevention of myeloma-induced bone loss and the development of osteolytic bone lesions.[67-69] In at least some of the studies, anti-DKK1 treatment had no effect on osteoclastic bone resorption suggesting that retaining the coupling between resorption and formation is sufficient to prevent bone destruction. Interestingly, promoting Wnt signaling by treatment with lithium chloride prevented myeloma induced bone loss.[70] Lithium treatment was also associated with decreased tumor burden in the skeleton; however, burden was increased at extra-skeletal sites.[70] Anti-DKK1 antibodies are now in clinical development for the prevention of bone disease in patients with myeloma (see below).

In addition to DKK1, other soluble Wnt antagonists have also been implicated in osteoblast suppression in myeloma. For example, the RPMI8226 and U266 myeloma cell lines suppress bone formation *in vitro* and express soluble frizzled related protein (sFRP)-2, but not other soluble Wnt antagonists.[71] Soluble-FRP-2 is also found in myeloma cells isolated from the bone marrow of patients with bone disease.[71] In addition, sFRP-3 is produced by some myeloma cells and elevated concentrations of sFRP-3 are present in the bone marrow of patients with myeloma when compared to those with MGUS, and associated with the presence of osteolytic disease.[64] More recently, the discovery of the bone-specific Wnt antagonist protein scle-

rostin (SOST) has resulted in studies examining its role in myeloma. SOST expression by myeloma cells has been reported and serum levels of SOST are increased.[72,73] However, with the exception of DKK1, there are limited functional data to support a causal role in animal models and data are unclear as to the relative importance of these different antagonists and/or whether they are important in different sub-classes of myeloma.

Despite the limited understanding of the role of DKK1, sFRPs and SOST in the pathogenesis of myeloma bone disease, both anti-DKK1 and anti-SOST are being developed as bone anabolic agents for the treatment of osteoporosis[74,75] and may offer potential in patients with myeloma bone disease.

Activin-A

Activin is a member of the TGF-β superfamily with complex effects on bone. Activin A has been shown to inhibit bone formation and in some studies promote osteoclastic bone resorption, although this may prove to be context specific. Activin-A is increased in the bone marrow of patients with myeloma and serum level are increased in patients with newly diagnosed myeloma and associated with elevated bone resorption.[76,77] Activin-A signaling occurs through the Activin A type IIA receptor to inhibit osteoblastic bone formation. Blocking activin-A signaling using a soluble ActRIIA murine Fc fusion protein (ActRIImuRc) has been shown to prevent activin A mediated osteoblast suppression, but have no effect on osteoclast formation *in vitro*.[78] Furthermore this construct has been shown to prevent myeloma-induced osteoblast suppression in murine models of myeloma bone disease. Although treatment had no effect on osteoclastic resorption, treatment prevented the bone loss including the development of osteolytic bone lesions.[76,78] Inhibitors of activin A are currently being evaluated in clinical trials.

Interleukin-3 (IL-3) expression is also increased in myeloma and is able to stimulate bone resorption and inhibit bone formation. Interestingly, IL-3 promotes release of activin-A from bone marrow macrophages and anti-activin A antibodies prevent the effects of IL-3 on bone resorption in models of myeloma.[79] The reason why some studies report positive effects of activin-A on promoting bone resorption, whereas others do not, is unclear but may reflect differences in experimental systems, the temporal nature of changes in osteoclastogenesis *in vivo* and/or the different approaches to blocking activin. However, IL-3 also inhibits osteoblast differentiation,[80] which is consistent with the inhibitory effect of activin-A on osteoblast differentiation, suggesting this axis may play a critical role in promoting myeloma bone disease, particularly through osteoblast suppression. T lymphocytes, as well as myeloma cells, have been suggested to produce IL-3 in myeloma, which is consistent with immune cells playing a role in the development of myeloma bone disease.[81]

Additional regulators - hepatocyte growth factor, interleukin-7 and adiponectin

Early studies demonstrated that the JJN-3 myeloma cell line causes profound osteoblast suppression *in vivo* and mice bearing these tumor cells have increased serum concentration of hepatocyte growth factor (HGF).[82] HGF can be increased in serum of patients with myeloma and associated with low levels of the bone formation marker bone-specific alkaline phosphatase.[83] HGF signals through the c-MET tyrosine kinase receptor and can decrease expression of the osteoblast-specific transcription factors *Runx2* and *Osterix* and bone formation possibly by stimulating interleukin-11 production from osteoblasts.[83,84] IL-11 also increases bone resorption. Approaches to targeting c-MET are under development.[85] Interleukin-7 is also increased in the bone marrow of patients with myeloma, able to block Runx2 activity and can suppress bone formation.[86] IL-7 has recently been shown to induce Gfi1 a transcriptional repressor of *Runx2*. Gfi1 is induced in stromal cells, elevated in bone marrow stromal cells isolated from patients with myeloma and able to inhibit bone formation.[87] It has been suggested that Gfi1 plays a role in the long-term suppression of bone formation by regulating recruitment of histo-modifying enzymes to the Runx2 promoter.[62] Finally, studies of genes differentially expressed in the bone marrow from mice that support myeloma development compared to those that do not support myeloma development found adiponectin to be decreased.[88] Restoration of adiponectin expression with the peptidomimetic L-4F increased osteoblast surface and bone formation rates and reduced myeloma-induced bone loss.[88] This was seen in the absence of effects on osteoclasts and raises the possibility of targeting this pathway to prevent osteoblast suppression to stop development of myeloma bone disease.

Implications for myeloma growth in bone

There is increasing evidence to demonstrate that myeloma cells are dependent upon the cells of bone for critical growth and survival signals. This may be most important in the early stages, when myeloma cells first arrive in bone, and are microenvironment dependent and less important when the tumor burden increases and the cells are environment independent. Certainly, osteoblasts produce a range of molecules that can support myeloma cell survival (e.g. IL-6 and OPG) which prevents TNF apoptosis inducing ligand-induced apoptosis of myeloma cells.[23] This may have important implications as agents that target osteoclasts and osteoblasts in efforts to treat myeloma bone disease may indirectly influence tumor burden. In experimental models, bisphosphonates, which inhibit osteoclasts, also reduce tumor burden and clinical studies have reported increases in survival in individuals treated with bisphosphonates.[89-91] OPG, soluble RANKL constructs and OPG peptidomimetics have all been shown to decrease bone resorption and reduce myeloma burden in similar models.[21,33,92] Furthermore, targeting MIP-1α signaling, p38MAP kinase and other pathways that regulate osteoclast formation are also typically associated with reductions in tumor burden.[47,93] The demonstration that targeting multiple pathways, all of which inhibit osteoclastic resorption, decreases myeloma burden supports the notion that these effects are mediated by osteoclasts rather than via direct anti-myeloma effects. In contrast, agents that promote bone formation, although effective in promoting bone formation are not typically associated with alterations in myeloma burden.[68,78,88] When anti-myeloma effects of bone anabolic agents have been reported these are also associated with increases in resorption. These

observations raise the possibility that, in the future, optimizing use of anti-resorptive treatments may offer additional benefits beyond simply preventing bone resorption.

Emerging treatments for myeloma bone disease

Bisphosphonates (BPs) remain the most common treatment for myeloma bone disease. Clodronate was the first BP to be used and was shown to reduce skeletal-related events.[94,95] This was followed by the introduction of pamidronate and, more recently, zoledronic acid, both of which are effective in reducing SREs.[91,96-99] Bisphosphonate treatment has also been reported to be associated with survival benefit,[91,95,96] although in the majority of studies this is seen in subgroup analysis only.[95,96] However, this is consistent with pre-clinical studies[89,90] and a meta-analysis, which suggested that, at least for zoledronic acid, when compared to placebo, treatment is associated with an improvement in overall survival.[100]

However, improvements in understanding of the molecular mechanisms responsible for myeloma bone disease have led to a number of new approaches that are now in clinical development in patients with myeloma. The most advanced is probably the anti-RANKL neutralizing antibody, denosumab, which blocks RANKL-mediated osteoclastic bone resorption. Denosumab reduces biochemical markers of bone resorption in patients with myeloma[36,37,101] even in studies of individuals who previously received bisphosphonate treatment, although in the latter study the majority of patients had solid tumors rather than myeloma.[101] More recently, a randomized double blind phase III study in patients with advanced cancer, which included individuals with myeloma, compared denosumab with zoledronic acid and showed superior reductions in bone turnover markers with denosumab, but no difference in the time to SREs.[38] A phase III trial comparing denosumab with zoledronic acid in patients with myeloma is underway (*clinicaltrials.gov identifier:01345019*).

Agents that promote bone formation are also being explored clinically. These include agents that primarily target the tumor but may have additional bone effects, such as proteasome inhibitors (reviewed by Terpos *et al.*[102]) and novel bone targeted agents. For example, ACE-011, (sotatercept), a soluble human activin type IIA receptor fusion construct, that prevents activin signaling in a similar manner to the mouse construct, RAP-011,[76,78] has been investigated in a phase IIa safety study of patients with myeloma (*clinicaltrials.gov identifier:00747123*) and showed evidence of increased biochemical markers of bone formation.[103] However, patient numbers were low and further studies are required to verify these data. Furthermore, early phase clinical trials of the anti-DKK1 antibody, BHQ88, which is effective in pre-clinical models, are underway in combination with bisphosphonates in relapsed or refractory patients with myeloma (*clinicaltrials.gov identifier:00741377*), in untreated patients with renal insufficiency (*clinicaltrials.gov identifier:01337752*) and patients with high-risk smoldering or untreated MM (*clinicaltrials.gov identifier:01302886*). Finally, agents with efficacy in other disease areas may also offer potential in patients with myeloma. For example, antibodies that neutralize the wnt antagonist sclerostin (e.g. romosozumab) have emerged as potential new treatment strategies in osteoporosis.[104] Pre-clinical investigations of anti-sclerostin agents will be required to provide the rationale for investigating this approach in patients with myeloma. However, these examples illustrate how understanding the molecular mechanisms responsible for bone disease development can lead to new agents to treat this important component of myeloma.

Conclusions

Enormous progress has been made in understanding the pathogenesis of myeloma bone disease. A number of molecules have now been shown to promote osteoclastic bone resorption in patients with myeloma, although it is likely that the majority do so by promoting RANKL expression by myeloma cells or in cells of the local bone marrow microenvironment. It remains to be established whether different classes of myeloma, due to different molecular rearrangements, utilize different molecules to promote RANKL induced-osteoclast formation. However, the demonstration that RANKL is pivotal supports the notion that the approaches to targeting RANKL, with anti-RANKL antibodies, will successfully translate into the clinic for the treatment of all patients with myeloma bone disease. Considerable progress has also been made towards understanding the suppression of bone formation with a number of pathways shown to be functionally important. Approaches to targeting these pathways are under development in the clinic. Evidence also points to the fact that retaining the coupling between resorption and formation is sufficient to prevent myeloma bone disease, suggesting that bone anabolic approaches will be effective in preventing development of new bone lesions and osteoporosis in patients with myeloma. This also provides an opportunity to capitalize on progress in the osteoporosis field in which the development of bone anabolic agents, including the anti-sclerostin antibody, is a major priority. However, a key challenge for the future will be developing strategies to repair existing bone lesions in patients with myeloma. In order to achieve this, a more profound understanding of the sustained suppression of bone formation seen in patients with myeloma is required.

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