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Tumor-host cell interactions in the bone disease of myeloma

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Abstract

Multiple myeloma is a hematological malignancy that is associated with the development of a destructive osteolytic bone disease, which is a major cause of morbidity for patients with myeloma. Interactions between myeloma cells and cells of the bone marrow microenvironment promote both tumor growth and survival and bone destruction, and the osteolytic bone disease is now recognized as a contributing component to tumor progression. Since myeloma bone disease is associated with both an increase in osteoclastic bone resorption and a suppression of osteoblastic bone formation, research to date has largely focused upon the role of the osteoclast and osteoblast. However, it is now clear that other cell types within the bone marrow, including cells of the immune system, mesenchymal stem cells and bone marrow stromal cells, can contribute to the development of myeloma bone disease. This review discusses the cellular mechanisms and potential therapeutic targets that have been implicated in myeloma bone disease.

Keywords

Multiple myeloma; osteolytic bone disease; osteoclast; osteoblast; bone marrow microenvironment

Introduction

Multiple myeloma is a fatal hematological malignancy that develops within the bone marrow microenvironment. Multiple myeloma is the second most common hematological malignancy and the American Cancer Society estimated that approximately 20,000 new multiple myeloma diagnoses and 10,800 myeloma deaths occurred in 2007 in the United States alone [1]. Myeloma is characterized by the uncontrolled clonal proliferation of malignant plasma cells within the bone marrow. A unique feature of multiple myeloma, in contrast to other hematological malignancies, is the development of a destructive bone disease, resulting in osteolytic bone lesions, bone pain, and pathological fractures. The majority of patients present with, or will develop, bone disease during the course of their myeloma. The bone disease is now recognized as an integral component of the myeloma disease and a contributing factor in tumor progression.

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Multiple myeloma, and other cancers that metastasize to the bone marrow, create an interdependent relationship between tumor cells and cells of the bone marrow microenvironment, which promotes both tumor growth and bone destruction [2]. This was initially described as a reciprocal relationship between tumor growth and osteoclastic bone resorption, whereby myeloma cells release “osteoclast activating factors” and in turn, resorbing bone promotes tumor growth and survival (Figure 1A)[3,4]. It is now clear that myeloma cells interact with numerous cell types with the bone marrow microenvironment and all these interactions have the potential to contribute to the associated bone disease (Figure 1B). In the normal bone marrow microenvironment, bone is constantly undergoing remodeling with a delicate balance between osteoclastic bone resorption and osteoblastic bone formation. Within the myeloma microenvironment, there is a dysregulation in normal remodeling that results in enhanced osteoclastic bone resorption and suppressed bone formation. Myeloma cells play an active role in altering the balance in this process. Importantly, recent research has shown that other cell types within the microenvironment may also contribute to the bone disease. This review considers our current understanding of the mechanism of development of osteolytic bone disease in multiple myeloma

Increased Osteoclastic Bone Resorption

It was early histomorphometric studies that showed that bone resorption was increased in patients with myeloma [5]. These studies demonstrated that the surface undergoing resorption is increased. Furthermore, studies have also suggested that the depth of individual remodeling sites may be increased. This is consistent with both increased numbers of osteoclasts, increased resorptive activity by individual osteoclasts, or both mechanisms occurring simultaneously [6]. The demonstration that osteoclastic resorption was increased resulted in efforts to identify the molecular mediators responsible.

Greg Mundy was the first to demonstrate that myeloma cells produced an ‘osteoclast activating factor’ [3,4]. The identity of this factor(s), for many years, remained elusive. Studies implicated a number of cytokines, including lymphotoxin, interleukin-1 and tumor necrosis factor- α . These studies were limited to providing evidence of expression of molecules in what were often cell lines, or isolated, cultured myeloma cells and there was little evidence of functional data to support a causal role. However, in the last decade, two pathways have been shown to play a fundamentally important role.

The ligand for receptor activator of NF κ B (RANKL)

The discovery that RANKL plays a critical role in normal osteoclast formation and function lead to studies investigating its potential importance in the development of myeloma bone disease. Early studies demonstrated that RANKL expression was increased in the bone marrow of patients with myeloma [7,8]. Furthermore, myeloma cells decrease expression of osteoprotegerin (OPG), the decoy receptor, by stromal cells and osteoblasts [9]. Other studies demonstrated that myeloma cells themselves were able to express RANKL directly, suggesting that these cells have the ability to bypass the normal osteoblast dependent induction of osteoclastogenesis [10] [11]. Despite this, not all studies have observed expression of RANKL by myeloma cells. However, more recently, CD38+++ / CD45+ and CD138+ have been shown to express RANKL and induce osteoclast formation directly [12]. Furthermore, other cells within the bone microenvironment may also contribute to increased RANKL expression. For example, myeloma cells induce expression of RANKL in T-cells, a process mediated by interleukin-7 production [13]. Irrespective of the cellular source of RANKL, serum levels are elevated and OPG levels decreased in patients with myeloma and this is associated with the development of bone disease [14].

The demonstration of increased RANKL expression in myeloma has resulted in studies investigating the effect of blocking RANKL in experimental models of myeloma. Recombinant OPG has been shown to inhibit osteoclastogenesis, prevent myeloma induced bone loss and the formation of bone lesions in the 5T2MM murine model of myeloma [11]. OPG peptidomimetics also reduce osteoclast numbers and prevent the development of bone lesions in the same model [15]. Furthermore, lentiviral overexpression of OPG in ARH-77 myeloma cells or in stromal cells has also been shown to prevent myeloma-induced bone loss [16,17]. Similarly, soluble RANKL constructs have been shown to prevent myeloma induced bone destruction in both the 5TGM1, SCID/hu and ARH77 experimental systems [8,18]. Interestingly, in those models in which the myeloma cells only grow in the skeleton, e.g. 5T2MM and SCID/Hu, where the myeloma cells may be considered to be microenvironment dependent, RANKL antagonism was associated with evidence of anti-myeloma effects. This was not seen in the other model systems, e.g. ARH77. This is consistent with the anti-myeloma effect being mediated via the inhibition of bone resorption rather than any direct effect on tumor growth.

Macrophage inflammatory protein-1 α (MIP-1 α)

The second system shown to play a key role is MIP-1 α . MIP-1 α was first implicated in mediating the development of myeloma bone disease by Choi et al [19]. Subsequently, anti-sense inhibition of MIP-1 α and blocking antibody strategies were shown to prevent myeloma bone disease in a number of experimental model systems. MIP-1 α is also elevated in the serum of patients with myeloma and associated with the development of bone disease [20]. Whether MIP-1 α mediates its osteoclastogenic effect via induction of RANKL is unclear. MIP-1 α has been reported to function in a RANKL-independent manner [21]; however, other reports demonstrate that RANKL is required for the actions of MIP-1 α [22,23]. More recently approaches to targeting CCR1, a receptor for MIP-1 α , have been developed and shown to be effective in preventing bone disease [24]. MIP-1 α has also been shown to be a downstream target of FGFR3 [25], which is expressed in a proportion of patients with myeloma. Taken together these studies have demonstrated the importance of this system in contributing to the development of myeloma bone disease.

Other molecules have also been implicated in mediating the increase in osteoclastic bone resorption in myeloma, including IL-3, IL-7, stromal cell-derived factor 1 (SDF1) and hepatocyte growth factor (HGF) [26-29]. Whether these molecules induce osteoclastic resorption via induction of RANKL is unclear. Equally, whether different subgroups of patients aberrantly express these different molecules and utilize them to promote RANKL is also unknown. There is also limited evidence in experimental model systems *in vivo* to support a causal role. Thus, the current data strongly suggest that RANKL and MIP- α are important players in the mediation of osteoclast formation in myeloma.

Osteoblast Suppression and Decreased Bone Formation

The uncoupling of normal bone remodeling not only involves enhanced osteoclastic bone resorption but also the suppression of new bone formation. Despite the effectiveness of bisphosphonates, patients with myeloma still develop skeletal-related events [30] and the existing damage to the bone remains unrepaired. This has led to research focused upon preventing the suppression of bone formation and stimulating repair, which is the subject of a detailed review by David Roodman in this issue of *BONE*.

New bone formation is inhibited in two ways in myeloma. Firstly, the activity of already existing osteoblasts is suppressed [31-33]. Secondly, differentiation of mesenchymal stem cells (MSCs) into mature osteoblasts is impaired [31,34,35]. In addition to overall suppression of bone formation, this block in differentiation exacerbates the osteolytic bone

disease experienced in these patients as immature osteoblasts provide a rich source of RANKL ligand [36], a critical factor for osteoclastogenesis. The molecular mechanisms responsible for the inhibition of osteoblast differentiation are only now becoming clear.

Wnt Signaling Pathway

Investigations of myeloma-induced osteoblast suppression have largely focused on the Wnt/ β -catenin signaling pathways because of its critical role in normal bone physiology. The first evidence for a role for the Wnt signaling pathway, and specifically the Wnt-signaling antagonist Dickkopf-related protein 1 (Dkk1), in myeloma bone disease came from a study by Tian and colleagues, who demonstrated that patients with multiple myeloma had increased expression of Dkk1, which correlated with the extent of the osteolytic bone disease [37]. Subsequent studies have also observed a significant increase in Dkk1 expression in patients with myeloma, a correlation between Dkk1 expression and osteolytic bone lesions, and a reduction in serum Dkk1 concentrations following anti-myeloma treatment [38-40]. In addition to these findings, Oshima and colleagues demonstrated a role for myeloma cell-derived soluble frizzled related protein-2 (sFRP-2), another antagonist of Wnt signaling, in the suppression of bone formation [41].

In vitro investigations demonstrated that osteoblast differentiation was blocked by bone marrow serum from patients with myeloma, and the inhibitory effect was found to be due to the presence of Dkk1 [37]. Dkk1 was found to inhibit Wnt-3A-induced β -catenin accumulation and BMP-2 mediated osteoblast differentiation. In contrast to these studies, Giuliani and colleagues found that although myeloma cells or bone marrow plasma from myeloma patients could inhibit canonical Wnt signaling in murine osteoprogenitor cells, and express high concentrations of soluble Wnt antagonists, they did not block canonical Wnt signaling in human mesenchymal stem cells or osteoprogenitor cells [42]. In addition to direct effects on myeloma bone disease, Gunn *et al.* have reported that conditioned media from mesenchymal stem cells can promote myeloma cell proliferation and increase expression of Dkk1 by myeloma cells. Dkk1 then acts back on the mesenchymal stem cells to prevent their osteoblastic differentiation and maintain them in an immature state, where they express higher levels of IL-6 and therefore have greater potential to stimulate myeloma cell proliferation. Potentially, this creates a dependency between mesenchymal stem cells and myeloma cells resulting in an increase in myeloma proliferation and a decrease in osteoblastogenesis [43].

Until recently, the major focus has been on Dkk1 derived from myeloma cells; however, there is increasing evidence to suggest that myeloma cells may not be the sole source for Dkk1 within the myeloma bone marrow microenvironment. Several studies have identified an increase in Dkk1 in mesenchymal stem cells isolated from patients with multiple myeloma [44,45]. In support of a role for bone marrow stromal cell derived Dkk1 in myeloma bone disease, Fowler *et al.* have recently demonstrated that myeloma-associated fibroblasts, which are capable of promoting myeloma growth *in vivo*, can induce osteoblast suppression *in vivo* with no requirement for the presence of myeloma cells, and that this effect may be mediated, at least in part, via secretion of Dkk1 [46].

Preclinical studies using murine models of myeloma strongly support targeting the Wnt signaling pathway for the treatment of myeloma bone disease. Inhibition of Dkk1, using neutralizing antibodies, has proven to be effective in several murine models of myeloma, with a significant reduction in myeloma bone disease and tumor burden [47-49]. In addition to directly targeting Dkk1, several studies have investigated targeting other components of the Wnt signaling pathway. Sukhedo *et al.* used a novel small molecule inhibitor, which acts to disrupt the interaction between β -catenin and TCF and so inhibit Wnt signaling [50]. Inhibition of Wnt signaling was found to inhibit tumor growth and prolong survival in a

xenograft model of myeloma, however the effects of this small molecule have not been evaluated in models of myeloma bone disease. Edwards *et al.* used a systemic pharmacological approach, by treatment with lithium chloride, which acts to inhibit glycogen synthase kinase 3 β (GSK-3 β) and so activate β -catenin[51]. Lithium chloride was found to significantly prevent myeloma bone disease and reduce tumor burden within bone in the 5TGM1 murine model of myeloma. In support of this, a small molecule inhibitor of GSK-3 has been shown to prevent myeloma bone disease in the 5T2MM model [52]. Qiang *et al.* have also demonstrated that systemic Wnt3A treatment could prevent the development of myeloma bone disease and reduce tumor burden in a SCID mouse model of myeloma [53].

Although there is compelling evidence that targeting Dkk1 and Wnt signaling prevents myeloma bone disease in experimental models, concern has been raised over the implications for tumor growth. Activation of the Wnt signaling pathway through β -catenin plays a critical oncogenic role in many human malignancies and expression of β -catenin has been demonstrated in myeloma cell lines and in malignant plasma cells from patients with multiple myeloma [42,54]. Currently, published data are conflicting as to the role of Wnt signaling in myeloma cells [51,53-55]. Importantly, in all studies *in vivo*, when the tumor cells were present within the bone marrow microenvironment, activation of Wnt signaling resulted in a reduction in tumor burden and prevention of myeloma bone disease. [51,53]. These data highlight the importance of interactions in the local microenvironment and demonstrate that, despite potential direct effects to increase tumor growth at extraosseous sites, increasing Wnt signaling in the bone marrow microenvironment can prevent the development of myeloma bone disease. Overall, targeting the Wnt signaling pathway represents an attractive therapeutic approach for the treatment of myeloma bone disease. However, further work needs to be undertaken to establish the effects of blocking Dkk1 and promoting Wnt signaling on myeloma growth and survival in both intra-osseous and extramedullary sites.

Runx2 and E4 promoter binding protein 4 (E4BP4)

Several groups have begun to elucidate the molecular mechanisms by which myeloma cells suppress osteoblasts. Myeloma cells have negative effects on osteoblast differentiation by both cell-cell contact dependent and by secreted factor dependent mechanisms. Not only is differentiation altered, but also osteoblasts are functionally inhibited as demonstrated by extensive histomorphometric analysis [31,56]. Giuliani *et al.* found that myeloma suppression of osteoblast activity was mediated by the reduction of osteoblast-specific Runx2 transcriptional activity [27]. This effect was specific to myeloma cells and upon screening various myeloma cell lines and primary cells from patients with myeloma, they identified Dkk1, IL-7, and sFRP-3 as likely mediators of the osteoblast suppression. These authors primarily focus on IL-7 being responsible for the majority of suppression through decreased Runx2 activity. Currently, there are no strong correlative studies providing association between IL-7 levels and the extent of bone disease and functional studies to support a causal role are required. Suppression of Runx 2 transcriptional activity in myeloma may be mediated via activation of the repressor gene E4BP4. These studies suggested that E4BP4 acts as an osteoblast transcriptional repressor by inhibiting Runx2 and Osterix in patients with myeloma bone disease [57].

Activin A

Recent studies have also shown that myeloma cells promote release of activin A, a member of the transforming growth factor- β (TGF- β) superfamily, from bone marrow stromal cells, and activin A inhibits osteoblast differentiation and bone formation. Bone marrow plasma levels of activin A are also increased in patients with multiple myeloma with bone lesions,

compared to those with out bone lesions or those without myeloma [58]. A soluble form of the activin receptor, the murine ActRIIA receptor fused to a murine Fc construct (ActRIIA.muFc or RAP011), which binds activin and prevent signaling, stimulates bone formation *in vitro* and *in vivo*. Furthermore, treatment of 5T2MM bearing mice with ActRIIA.muFc, prevents myeloma-induced suppression of bone formation, loss of cancellous bone and the development of bone lesions [59]. Interestingly, treatment had no effect on osteoclastic resorption suggesting that osteoblast suppression plays a key role in determining lesion development. Similar effects on osteoblast suppression and bone disease have also been observed when INA6 myeloma cells are maintained in the SCID/hu system [58].

Growth Factors; HGF, TGF- β

There are many growth factors that are either produced by myeloma cells or increased within the myeloma bone marrow microenvironment, all of which have the potential to contribute to the suppression of bone formation in myeloma. HGF is not only implicated in regulating osteoclastic resorption but may also be involved in osteoblast suppression. HGF is produced by myeloma cells, and is increased in the serum of patients with myeloma, where levels can correlate with and predict response to anti-myeloma therapies [60-63]. In relation to myeloma bone disease, serum concentrations of HGF negatively correlate with markers of bone formation, and HGF inhibits BMP-2-induced osteoblastogenesis *in vitro* [64,65]. TGF- β , a ubiquitous, multi-functional growth factor is released from the bone matrix during osteoclastic bone resorption and acts to inhibit osteoblast differentiation. TGF- β signaling has been shown to block the ability of myeloma cells to inhibit osteoblast differentiation *in vitro* and inhibition of the TGF- β type I receptor kinase in experimental models resulted in a decrease in tumor burden and a reduction in bone destruction [66].

Therapeutic Approaches; Proteasome inhibition, Dkk1 and Activin A

The recent preclinical studies in experimental murine models of myeloma bone disease suggest that targeting osteoblastic bone formation in myeloma may be an effective therapeutic approach. Certainly, targeting the Wnt signaling pathway is effective in promoting bone formation and preventing myeloma bone disease. A fully human anti-Dkk1 neutralizing antibody (BHQ880) is now in phase I/II clinical trials in patients with multiple myeloma. Furthermore, a human ActRIIA.muFc construct (ACE011) has been shown promote bone formation in cynomolgus monkeys and is now in clinical development for patients with myeloma [67].

Proteasome inhibitors are already in use in the treatment of patients with multiple myeloma. These agents have had a significant impact in reducing myeloma burden in some patients with myeloma. However, there is now accumulating evidence that proteasome inhibition also prevents myeloma bone disease. Initial studies from Garrett *et al.* demonstrated that proteasome inhibitors could act directly on osteoblasts to stimulate osteoblast differentiation and bone formation *in vitro* and *in vivo* [68]. Subsequent studies have suggested that the bone anabolic effect of proteasome inhibition can be inhibited by Dkk1 [69], and that proteasome inhibitors also act on mesenchymal stem cells to promote osteoblast differentiation [70-72]. It has also been reported that proteasome inhibitors may inhibit osteoclastic bone resorption directly [73-75]. Furthermore, treatment of myeloma-bearing SCID-rab, or mice bearing 5T2MM myeloma cells, with Bortezomib results in a reduction in both tumor burden and osteolytic bone disease [76,77]. In the studies in the SCID-rab model the effect of bortezomib treatment was compared with that of melphalan, which has direct anti-myeloma effects but no direct effect on bone. Only bortezomib prevented myeloma bone disease suggesting that these effects of bortezomib were not a consequence of reduced tumor burden but a direct effect on bone cells. Bortezomib was found to have a

similar effect, to promote osteoblastogenesis and inhibit osteoclastogenesis in normal non-myeloma bearing mice. Using the well-characterized 5TGM1 murine model of myeloma, Edwards *et al.* have recently demonstrated that myeloma cells exhibit a greater response to proteasome inhibition when located within the bone microenvironment, as compared with extra-osseous sites, suggesting that myeloma cells may be more sensitive to proteasome inhibitors when located within the bone marrow [78]. These data are consistent with reports in patients with myeloma that bortezomib treatment is associated with increases in biochemical markers of bone formation [79-84]. Although, to date, there are no studies investigating the effect of bortezomib on skeletal related events, in patients with myeloma the evidence suggests these agents have the capacity to prevent myeloma bone disease. Since tumor burden and bone disease are inextricably linked in multiple myeloma, agents that target both the tumor and the environment, such as proteasome inhibitors, remain important therapeutic approaches.

Alternative Contributions of the Bone Marrow Microenvironment to the Development of Myeloma Bone Disease

Osteoblasts and osteoclasts are critical cells in the development of myeloma bone disease, but there is increasing evidence to suggest that the other cells present within the bone marrow also participate in the increasingly complex relationship that promotes myeloma bone disease. To date, the study of the role of the host microenvironment in myeloma has been limited, due to the lack of appropriate mouse models of myeloma bone disease. However, Fowler *et al.* have recently developed a mouse model of myeloma that utilizes mice deficient in the recombinae activating gene-2 (RAG-2) [85]. These mice host the 5T murine myeloma cells, are easy to breed with other genetically modified mice and so permit examination of the host microenvironment in myeloma *in vivo*. The ability to target specific factors and cell types within the bone marrow microenvironment will both enhance our understanding of the cellular and molecular mechanisms that contribute to myeloma bone disease and identify and validate novel therapeutic approaches.

Bone marrow mesenchymal stem cells/Bone marrow stromal cells/fibroblasts

Recently Toderto et al, performed gene expression analysis of MSCs and osteoblasts from normal donors, and patients with monoclonal gammopathy of undetermined significance (MGUS) or myeloma. For those patients with myeloma they also compared those with or without osteolytic bone lesions [86]. There were no differences in the phenotype of MSCs or osteoblasts from any of the groups examined. However, these studies demonstrated that Dkk1 expression was higher in MSCs from patients with myeloma with osteolytic bone disease than without bone disease. Prior to the gene expression studies performed by Toderto *et al.*, gene expression studies by Bourin and colleagues examined bone marrow mesenchymal stem cells in normal, MGUS, and myeloma patients [45]. In contrast to the more recent study, the investigation by this group used bone marrow cells that were in culture for an extended period of time. Therefore the expression profiles in this population of cells could be altered due to the plasticity of these cells in culture. Among some of the genes that were identified as differentially expressed in myeloma BM MSCs compared to normal MSCs were Dkk1, IL-6, and IGF-1. These factors are known to play an important role in osteoblast differentiation [37], osteoclastogenesis [87], and support of myeloma growth and survival [88,89]. Additionally, the authors found that MSCs from myeloma patients were less capable of forming mineralized nodules, indicative of mature osteoblasts, during *in vitro* differentiation studies [45]. Even though these studies focused on the MSC populations specifically, they raise the question of the extent to which other cell types contribute to disease progression and development of osteolytic bone disease, and whether these changes are a cause or consequence of the presence of tumor and bone disease.

Dendritic cells

Myeloma cells can affect other resident cells within the bone marrow microenvironment in favor of myeloma progression and further propagate the associated bone disease. Dhodapkar and colleagues found that myeloma cells can stimulate dendritic cells to differentiate into osteoclast-like cells [90]. Co-culture of immature DCs with myeloma cells led to formation of functional multinucleated bone-resorbing giant cells. This effect was specific to the interactions between DCs and myeloma cells, both primary cells and cell lines. Additionally, no other monocyte population or cancer cell type had the ability to stimulate dendritic cell differentiation into these cells. The formation of functional osteoclast-like cells was dependent on cell-cell contact, local cytokines and the interaction between thrombospondin-1 and CD47. Whether these cells form *in vivo* and have the capacity to resorb bone in this setting is unclear. Of note the formation of these cells was inhibited by OPG suggesting this is a RANKL dependent process and raising the possibility that RANKL may be produced by dendritic cells in this system.

T cells

RANKL was first identified in T cells following antigen stimulation [91,92] that can occur from exposure to infectious agents. Any activation of T cells under inflammatory or even pathologic conditions could potentially augment osteoclast formation and activity [93-95]. Recent studies have suggested that myeloma cells can influence T cells, specifically increasing expression of RANKL, and that these T cells can subsequently promote osteoclastogenesis [13]. Colucci *et al.* demonstrated that T cells from multiple myeloma patients with osteolysis have the ability to promote osteoclastogenesis [96]. The authors found that osteoclasts derived from PBMC cultures taken from multiple myeloma patients had prolonged survival by disrupting the induction of apoptosis via downregulation of the death receptor. Additionally, osteoclasts in the presence of T cells upregulated the expression of the anti-apoptotic protein, Bcl-2. These data further support the concept of the surrounding bone marrow microenvironment becoming altered in order to promote myeloma bone disease.

Antigen-presenting cells produce various cytokines that are responsible for induction and differentiation of T cells. More recently, studies have implicated the T helper cells known as Th17 cells in myeloma and its bone disease. Th17 cells are defined by the coexpression of IFN- γ and IL17. Studies investigating Th17 cells have demonstrated the ability of DCs to activate Th17 cells [97,98] and myeloma tumors often become infiltrated with DCs. Assessment of peripheral blood mononuclear cells (PBMCs) found that there were only a small proportion of Th17 cells circulating in healthy individuals [99]. Dhodapkar and colleagues showed that inflammatory cytokine-stimulated DCs were capable of activating polyfunctional Th17 cells, compared to immature DCs. The authors demonstrated that matured DCs were more potent antigen-presenting cells, able to induce Th17 expansion, when compared to monocytes. However, the authors provide no strong clinical evidence to demonstrate a direct connection between DCs and increased Th17 cells present in myeloma patients. The high prevalence of these cells could account for the elevated serum concentrations of IL-17 also seen in myeloma patients [100]. IL-17 is a known stimulator of osteoclasts [101,102] and interestingly the proportion of Th17 cells present in the bone marrow of myeloma patients correlates with lytic bone disease [99].

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) have received increasing attention in recent years due to their high prevalence in the peripheral blood of cancer patients [103]. MDSCs are defined by their dual expression of CD11b, a marker for myeloid cells in the macrophage lineage, and Gr-1, a marker for granulocytes. MDSCs were initially

demonstrated as having immunosuppressive capabilities [104]. These immunosuppressive effects are mediated through the production of arginase [105], nitrogen oxide [106], and reactive oxygen species (ROS) [107,108], resulting in impaired B, T, and NK cell function [109]. Additionally, MDSCs inhibit maturation of dendritic cells and promote the formation of type II macrophages, which have been shown to aid tumor progression.

MDSCs produce high concentrations of TGF- β , raising the possibility that release of TGF- β by MDSCs could contribute to the suppression of bone formation in myeloma. Since MDSCs are found within the bone marrow and have a cell surface phenotype that overlaps with that of progenitors in the osteoclast lineage, this raises the possibility that MDSCs may have the capacity to develop into osteoclasts. Zhuang *et al.* have shown that MDSCs are increased in the bone marrow and spleen of myeloma-bearing mice and correlate with disease progression [110]. MDSCs isolated from mice with myeloma also had a greater capacity to form osteoclasts than MDSCs from control mice [110]. Furthermore, when MDSCs isolated from lacZ-positive mice were inoculated into recipient mice, cells positive for both lacZ and TRAP were observed on the bone surface, demonstrating the MDSCs have the capacity to differentiate into osteoclasts *in vivo* [110,111].

Conclusions

Osteolytic bone disease is one of the many devastating features associated with multiple myeloma. Despite the advancement in myeloma research, the contributions of the various cell types found within the bone marrow microenvironment to myeloma bone disease are not fully understood. Our current knowledge is limited due to the difficulty of studying the intact bone marrow microenvironment in its entire complexity. It will be critical in the coming years to begin to understand what components of the bone marrow are important for progression. The relationship between myeloma cells and these cells within the microenvironment contributes to the destructive bone disease, which is one of the defining features of multiple myeloma. Emerging cancer research of recent years not only demonstrates genetic alterations to the cancer cells but also to the surrounding microenvironment. The future of effective cancer therapeutics will have a dual focus; treating both the tumor cells and the altered microenvironment. Fortunately, progress over recent years has provided us with new approaches to targeting both the osteoclast and now the osteoblast. It is likely that these new approaches will have a real impact on both the development of bone disease and also on the progression of myeloma itself.

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Figure 1. Progression of our understanding of the complex cellular relationships in myeloma bone disease

(A) The original studies first described the relationship between myeloma cells and osteoclasts, whereby myeloma cells released "osteoclast activating factors" (OAFs) that stimulated osteoclastic bone resorption which in turn released growth factors which promoted myeloma cell growth and survival. (B) Our current knowledge has identified many more cell types and factors which contribute to disease progression, although the original concepts of tumor cells promoting bone destruction which in turn promote tumor growth remain the fundamental aspects of this increasingly complex network of interactions.