Blood Reviews 41 (2020) 100646

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/blre

Review

Why do myeloma patients have bone disease? A historical perspective

Magne Børset[a,b,*], Anders Sundan[a], Anders Waage[a,c], Therese Standal[a]

[a] Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[b] Department of Immunology and Transfusion Medicine, St. Olavs Hospital, Trondheim University Hospital, Norway
[c] Department of Hematology, St. Olavs Hospital, Trondheim University Hospital, Norway

ARTICLE INFO

Keywords:
Multiple myeloma
Cancer
Bone disease
DKK1
FRZB
SFRP-3
HGF
MIP-1α
CCL3
MIP-3α
RANKL
c-Met
OPG
SOST
Sclerostin
Activin A
BDNF
GDF-15
IL-1β
IL-6

ABSTRACT

The question of how myeloma cells cause destruction of skeletal tissue has interested scientists for many years, and knowledge in this field has developed in parallel with the understanding of physiological bone remodeling. The identification of bioactive proteins of the cytokine class during the last decades of the previous century and mapping of their role in the regulation of anabolic and catabolic processes in bone, led to a sequence of hypotheses about how the same peptides also could be involved in myeloma-driven bone destruction. Although bone remodeling is now understood in detail, there is still no clear unified theory of how myeloma cells degrade bone. The reason for this could be that there is no single mechanism that is active in every patient. The common trait is possibly that myeloma cells benefit from bone destruction *per se*, and the strategy they use to accomplish this vary between patients.

1. Introduction

Multiple myeloma (MM), the cancer of plasma cells, is distinct from other hematological cancers in its strong propensity to degrade bone near the cancer cells. Due to this trait, multiple myeloma can be diagnosed with some certainty even in archeological finds [1]. The question of how myeloma cells are able to cause destruction of bone has interested scientists for several decades. However, lack of knowledge of key aspects of bone metabolism severely hampered research on this matter until at least the mid-nineties, and is possibly still an obstacle to a full explanation. In this review article, we will describe how the knowledge of myeloma bone disease (MBD) has evolved in parallel with the increased knowledge of physiological bone development and remodeling. We also bring an updated overview of the current treatment for MBD.

2. Theories of how MBD develop

It was only in 1974 that the first two papers addressing pathophysiological mechanisms leading to bone destruction were published. Gregory Mundy and colleagues authored both these papers, published in the New England Journal of Medicine. Here they reported the finding of a factor stimulating the bone-resorbing cell, the osteoclast, in MM [2,3]. They found that supernatants from cultured bone marrow cells of patients with MM caused release of calcium from cultures of fetal rat bone. At the time, this “activity” could not be linked to specific molecules, but was found to be distinct from parathyroid hormone and vitamin-D. No cytokines with the ability to cause osteoclast activation had yet been characterized.

* Corresponding author at: Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Postboks 8905, 7491 Trondheim, Norway.
E-mail addresses: magne.borset@ntnu.nu (M. Børset), anders.sundan@ntnu.nu (A. Sundan), anders.waage@ntnu.nu (A. Waage), therese.standal@ntnu.nu (T. Standal).

https://doi.org/10.1016/j.blre.2019.100646

2.1. The cytokine era

After this, no substantial progress was made until the advent of the cytokine era. The next significant paper came from San Antonio, Texas, in 1987, now with Gregory Mundy as senior author and Ross Garrett as first author [4]. By this time, a number of bone-resorbing leukocyte cytokines had been identified, including interleukin-1 (IL-1)β, tumor necrosis factor (TNF) and lymphotoxin (alias TNFβ). The experiments were similar to those in the papers from 1974, but now they could identify lymphotoxin as the possible culprit. However, two years later, a Japanese group led by Atsushi Kuramoto in Hiroshima, made a case for another cytokine, claiming that "IL-1β rather than lymphotoxin" was the bone-resorbing protein produced by myeloma cells [5]. While Garrett had used supernatants from allegedly myeloma cell lines, which are immortalized myeloma cells, the Japanese group had studied supernatant from primary myeloma cells, *i.e.* cells taken fresh from patients. Other groups confirmed these observations, largely with the finding that cell lines made lymphotoxin, whereas supernatant from fresh cells was dominated by IL-1β [6–8].

2.2. Importance of efficient cell sorting and cell authenticity

As it turned out, there were problems with both these approaches. Some of the cell lines used at the time were in fact not genuine myeloma cells lines, but B cell lines immortalized by Epstein Barr virus (EBV) [9,10]. In addition, cultures of primary myeloma cells were generally not separated to purity higher than 90%. Even small numbers of metabolically active cells, like monocytes, can produce large amounts of cytokines, so cytokine production by contaminating cells cannot be ruled out unless the culture purity is very high.

When we came into the field in the early nineties, we realized that we needed to purify myeloma cells better than in previous studies. By picking out myeloma cells with magnetic beads coated with a recently commercially available antibody, termed B-B4, with claimed specificity for myeloma cells, we were able to achieve 98–99% purity [11]. The antigen recognized by this antibody was later found to be syndecan-1 (CD138) [12], and has since become a well-known marker for myeloma cells. Using this method, we found that purified myeloma cells produced neither IL-1, nor IL-6, whereas the bone marrow cells that were sorted away made both these cytokines [11]. IL-6 came into the limelight in MM research in 1988 when the journal Nature published a paper by the Hiroshima group claiming that IL-6 was an autocrine growth factor for myeloma cells [13]. They were right in the observation that IL-6 is a strong myeloma growth factor, but already in 1989 a paper in Blood by Bernard Klein and colleagues from France, disputed the origin of IL-6 in MM [14]. They found that IL-6 did not originate from the myeloma cells, but from other cells in the microenvironment, which was what we could confirm in our work [11]. The IL-1β question remained controversial for a number of years, but now it is generally believed that IL-1β is normally not produced to any large degree by myeloma cells and is unlikely to be the prime cause of the bone disease seen in MM ([15] and Fig. 1).

2.3. Not only activation of osteoclasts

In 1989, an important paper by Regis Bataille et al. appeared in the Journal of Clinical Oncology [16]. Bataille studied the bone disease in MM and found that not only did the cancer destruct bone; there was also diminished bone formation. Healthy bone is constantly being remodeled by a balanced activity of bone-forming osteoblasts and bone-degrading osteoclasts. Bataille saw increased osteoclast activity in virtually all MM patients, including patients with relatively little MBD. The defining trait for patients with excessive MBD was not increased resorption of bone, but bone formation that did not match the increase in degradation. These patients had lost the balance in the activity of the two key cell types remodeling bone.

2.4. HGF

Inspired by Bataille's paper, we started to look for factors that could act as inhibitors of osteoblasts. At the time, one realized that there existed molecules that acted as coupling factors between osteoclasts and osteoblasts. When osteoclasts increased their activity, coupling factors would be activated and signal to osteoblasts that they had to keep pace with the osteoclast in order to preserve bone density. A possible coupling factor candidate was the cytokine transforming growth factor β (TGFβ), which was known to be embedded in the bone matrix and to be released for activity when bone was resorbed [17–19]. We established a biological assay for TGFβ and screened media in which we had grown myeloma cells, for "activity" that inhibited TGFβ [20,21]. As it turned out, some myeloma cell lines produced something that totally abrogated the biological effect of TGFβ in our assay. From the chemical properties of this "something", we realized that it had to be a protein. We purified the protein and had it sequenced. We were disappointed that it was not an unknown protein, but hepatocyte growth factor (HGF) [20], a cytokine that had been purified and cloned a few years before by a Japanese group [22]. However, we soon realized that HGF could still be important in MM [23]. We found that the average level of HGF was higher in serum from patients with MM than in healthy controls [20]. Later, we published that increased HGF levels were associated with an unfavorable prognosis for MM patients [24]. We also found that purified primary myeloma cells tended to express HGF and at the same time to express its receptor, a tyrosine kinase protooncoprotein called c-Met [20]. We demonstrated that c-Met could be activated in an autocrine fashion in myeloma cells, leading to increased proliferation or protection against apoptosis [20,25]. HGF could also potentiate the effects of IL-6 on myeloma cell proliferation [26].

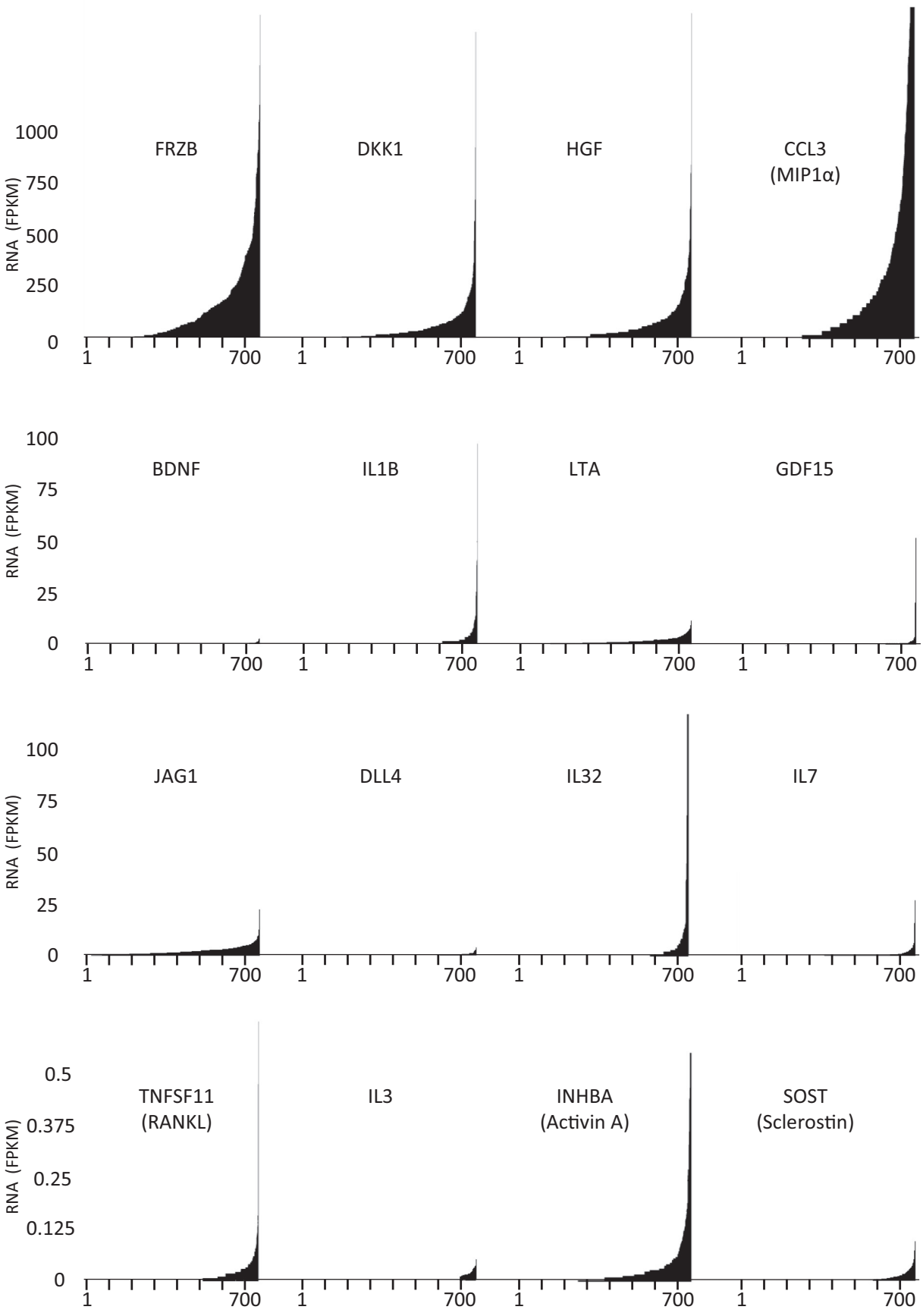
HGF is a heparin-binding growth factor and we realized that it would bind syndecan-1, the myeloma marker molecule, since syndecan-1 is a proteoglycan with heparan sulfate side chains. We hypothesized that syndecan-1, by presenting HGF to its high-affinity receptor c-Met, would potentiate the effect of HGF, and showed this in a paper in 2000 [27]. Patrick Derksen in Steven Pals' research group in Amsterdam later showed that such presentation of HGF to myeloma cells promoted proliferation [28,29]. Although there were reports that HGF promoted osteoclast formation and activity [30–32], the question remained whether HGF really was the factor causing bone resorption in MM.

2.5. MIP-1α

In the meantime, other cytokines were shown to have bone-resorbing properties and were detected as produced by myeloma cells or by other cells in the bone marrow of patients with MM. Around the turn of the century, work from *G. David* Roodman's lab demonstrated increased levels of macrophage inflammatory protein (MIP)-1α in MM bone marrow, whereas previous candidate molecules, IL-6, IL-1β and lymphotoxin, were undetectable [33]. Antibodies against MIP-1α could block bone resorption caused by MM bone marrow plasma in *in vitro* experiments [33] and antisense RNA against MIP-1α blocked bone resorption *in vivo* [34]. The latter experiment was done with ARH-77 cells, which are no longer considered real myeloma cells, but EBV-transformed cells [9]. However, the expression of MIP-1α from a large proportion of genuine myeloma cells is firmly documented (Fig. 1). The same group later identified IL-3 as another possible mediator of myeloma-induced osteoclast stimulation [35].

2.6. RANKL and OPG

In 1997–98, the long-sought coupling factors between osteoblast- and osteoclast activity were found. Receptor activator of nuclear factor-kappa B ligand (RANKL) emerged as the prime osteoclast-activating factor and osteoprotegerin (OPG) as a decoy receptor that binds and



(caption on next page)

Fig. 1. RNA-seq data showing myeloma cell expression of a selection of genes that have been linked to myeloma bone disease.
Histograms of mRNA levels as quantified by RNA-seq of samples purified by positive selection of CD138+ myeloma cells from 766 patients (CoMMpass IA12 database). Individual samples are ordered by expression level. mRNA levels are shown in fragments per kilobase of exon per million reads mapped (FPKM). Note the large differences in y-axis scale between plots. There is a skewed expression pattern of all genes, which makes it unlikely that any single gene expressed by myeloma cells, is responsible for myeloma bone disease in all afflicted patients. *FRZB, DKK1, HGF* and *CCL3* are expressed at a high level in a substantial number of patients, which could indicate that each of these genes is of biological relevance in a subgroup of patients. *JAG1* and *DLL4* were the two Notch ligands with the highest expression.

inactivates RANKL, thereby favoring bone formation [36,37]. Interestingly, both seem to be produced mainly by pre-osteoblasts, osteoblasts [38] and by osteocytes [38,39], although during inflammation certain T cells can also make RANKL [40]. When the ratio between RANKL and OPG is high, bone resorption predominates, and bone formation dominates when the ratio is low. It was soon established that there is an imbalance in MM between these two proteins in favor of RANKL [41–44]. Some studies indicated production of RANKL by myeloma cells [45], whereas others failed to do so [42]. Recently, available large datasets from high-capacity sequencing of mRNA from purified patient myeloma cells show lack of *RANKL* expression in the large majority of patients (CoMMpass database IA12, Fig. 1), a finding that should rule out direct production of RANKL by myeloma cells as the prime reason for MBD. OPG is a heparan sulfate-binding protein and thus binds to the heparan sulfate side chains of syndecan-1. We demonstrated that this binding leads to internalization and degradation of OPG by myeloma cells, a factor that could contribute to the altered RANKL/OPG balance in patients with myeloma [46].

2.7. DKK1 and SFRP-3

The next major finding in the quest for factors that contribute to bone degradation in MM came after the introduction of global gene expression profiling. John D. Shaughnessy's lab in Bart Barlogie's group in Arkansas were pioneers in the use of this technology and were able to correlate gene expression in purified myeloma cells to the degree of MBD in patients. The only genes encoding secreted factors within the top 50 upregulated genes in myeloma cells from patients with a high level of MBD, were genes coding for dickkopf 1 (*DKK1*) and secreted frizzled-related protein (x)-3 (*FRZB*), both proteins from the Wnt family [47,48]. DKK1 is an inhibitor of Wnt signaling, and in bone, it blocks differentiation of osteoblasts, as seen by reduced production of alkaline phosphatase (ALP) [48]. Antibodies against DDK-1 increased the number of osteoblasts and suppressed the bone disease in MM mouse models [49,50], and blocking SFRP-2, another inhibitor of Wnt signaling, increased mineralization in an *in vitro* bone formation assay [51].

2.8. More inhibitors of osteoblasts

The concept of MM-induced loss of bone formation was a confirmation of Regis Bataille's observations from the late 80s and was also supported by subsequent work of Nicola Giuliani and colleagues who saw reduced activity of the osteoblast transcription factor RUNX2/CBFA1 in co-cultures of bone marrow cells and myeloma cells. Myeloma cells had an inhibitory effect on osteocalcin, ALP and collagen I mRNA levels in human preosteoblastic cells [52]. They suggested that IL-7 could be a factor that mediated the observed osteoblast inhibition [52]. The same group had earlier found IL-7 to be overexpressed in the bone marrow of patients with MM and that it induced RANKL production in T cells and thus promoted osteoclast activity [53]. They also added IL-3 to the list of osteoblast-inhibiting molecules, although its effect was indirect *via* CD45+ cells in the microenvironment [54]. Sonia Vallet and colleagues from Harvard Medical School found that MIP-1α also had a direct inhibitory effect on osteoblast activity [55].

We studied the effects of HGF on bone cells *in vitro* and found that it was inhibitory to osteoblast differentiation, much in the same way as had been described for DKK1 [56]. Osteoblast development is promoted by bone morphogenetic proteins (BMPs), leading to production of RUNX2/CBFA1, ALP and osteocalcin. HGF prevented these signs of BMP-induced osteoblast differentiation, and reduced formation of bone nodules *in vitro*. We also found an inverse correlation between the levels of HGF and markers of bone formation in serum from patients with MM [56]. Myeloma cells were found to be the likely source of the elevated level of this cytokine in serum [57]. Increased serum levels in MM patients of the enzyme HGF activator, which converts pro-HGF to its active form, suggests that HGF is not only present, but also bioactive in the patients [58]. Positive immunohistochemical staining of phosphorylated c-Met in myeloma biopsies further supports an active HGF/c-Met signaling axis in MM bone marrow [59].

Lately, yet another osteoblast-inhibiting molecule of the Wnt family, sclerostin, has been implicated in MBD. Targeting sclerostin or its gene *SOST*, prevented bone loss, reduced the number of osteolytic lesions and increased bone formation in different preclinical MM models; and inhibitors of sclerostin are emerging as promising drug candidates against myeloma-induced bone disease [60–62]. While a few early reports suggested that sclerostin was expressed by myeloma cells, it now seems evident that osteocytes and to some extent other cells of the osteoblast lineage, are the main producers of sclerostin in MM [60–62]. Since inhibiting sclerostin did not reduce tumor burden, treatment directed against sclerostin will probably have to be combined with other anti-myeloma drugs. Interestingly, DKK1 secretion from myeloma cells may be key to enhance *SOST* expression by osteocytes/osteoblasts [60]. Evangelos Terpos and colleagues found significantly elevated levels of sclerostin in the serum of patients who presented with bone fractures at diagnosis, but the difference in serum levels between patients with advanced MBD (> 3 lytic lesions and/or fractures) and other patients with myeloma did not meet statistical significance [63].

2.9. Other suspects

A series of additional molecules have been proposed as candidates for mediating the bone disease. Some studies have focused on Notch signaling. Inhibitors of Notch receptor signaling block MM-induced osteoclast activation *in vitro* [64], as well as *in vivo* in a mouse model of MM [65]. Previous reports of high expression of Notch ligands [66] does not find support in expression data from purified myeloma cells in the CoMMpass data base (Fig. 1), but Notch ligand expression may be induced of by cell-to-cell contact in the microenvironment.

A study by Jesus Delgado-Calle and coworkers in Teresita Bellido's lab in Indianapolis focused on direct physical interaction between myeloma cells and osteocytes [65]. Osteocytes have been difficult to study due to their relative inaccessibility as they are embedded in solid bone. However, it is now realized that they are important in orchestrating bone remodeling by balancing the expression of sclerostin, RANKL, OPG and other molecules [67]. In patients with myeloma there is increased osteocyte death, a situation that favors osteoclast activation, and osteocytes produce increased amounts of the osteoclast-activating cytokine IL-11 [68]. We found that HGF from myeloma cells were able to induce IL-11 production in osteoblast-like cells [69].

Activin A is a cytokine that antagonizes BMP-6 and -9 [70] and possibly inhibits osteoblast activity. In a panel of 18 detectable cytokines in bone marrow plasma from patients with MM, activin A was the only one that was significantly elevated in the subgroup of patients with

MBD [71]. SDF-1α [72] and VEGF [73], both factors that have been suggested to promote bone disease in MM, showed *lower* levels in patients with MBD, but these differences did not reach statistical significance [71]. Other relevant cytokines were either undetectable (IL-1β, IL-17, IL-32 and MIP-1α) or were not represented in the cytokine array (IL-3, IL-7, IL-11, Lymphotoxin, DKK1, SFRP-3, HGF, GDF-15, MIP-3α, RANKL and OPG). The main source of activin A was bone marrow stromal cells. Myeloma cells secreted little or no activin A [71], suggesting that myeloma cells instruct cells in their neighborhood to produce this cytokine. Additional support for activin A as an important mediator in MBD came from an MM mouse model where inhibition of activin A by a decoy receptor stimulated bone formation and prevented bone destruction [74].

Growth differentiation factor (GDF)-15 is another cytokine that has been found in increased amount in serum from MM patients [75], and which stimulates osteoclasts [76] and inhibits osteoblasts [75].

Brain-derived neurotrophic factor (BDNF) is yet another cytokine with bone-destructive properties that has been implicated as a candidate for causing MBD [77,78]. BDNF induced RANKL production from bone marrow stromal cells, and knockdown of ***BDNF*** in ARH-77 cells rescued mice from the bone disease seen in mice grafted with wild-type cells [77]. Again, it can be argued that ARH-77 cells are not genuine myeloma cells. However, the same group, led by Yu Hu in Wuhan, China, also demonstrated increased levels of BDNF in bone marrow plasma from MM patients and saw positive correlation between BDNF and MBD [79]. Similarly to many of the proposed culprits, ***BDNF*** shows low or no expression in the majority of purified myeloma samples in the CoMMpass data base (Fig. 1).

After it was found that Th17 cells could produce RANKL during inflammation and activate osteoclasts [40], several reports demonstrated a skewed balance of the T cell repertoire in MM bone marrow towards a Th17 profile and production of IL-17 [80], possibly mediated by dendritic cells [81] or by the action of another overexpressed cytokine, MIP-3α (CCL20) [82]. Both IL-17 and MIP-3α can lead to osteoclast activation, but are themselves not myeloma cell products [80,82]. In a recent study of correlations between MBD and levels of selected cytokines in bone marrow, MIP-1α, MIP-3α, Activin-A and DKK1 were significantly higher in patients with high bone disease than in patients with low MBD [83]. Levels of IL-3 and RANKL did not come out as significant in this study.

2.10. Lessons from expression studies

Ida Christensen in Niels Abildgaard's lab in Denmark examined snap-frozen crude bone marrow samples from patients with MM with quantitative PCR and looked for correlations with level of MBD. She found significantly higher ***DKK1***, ***FRZB***, ***HGF*** and ***MET*** (c-Met gene) expression in samples from patients with lytic bone disease, whereas a series of other genes, including the genes encoding MIP-1α, RANK, RANKL, OPG, syndecan-1 and sclerostin did not correlate significantly with bone disease. In accordance with this, she found higher protein levels of DKK1, SFRP-3 and HGF in bone marrow plasma from MM patients with extensive bone disease than in similar samples from patients with low bone disease [84,85]. In other studies, positive correlation with the extent of bone disease has also been found for serum levels of MIP-1α [86,87]. In the study by Tsirakis and colleagues, the extent of MBD correlated more strongly with HGF than with MIP-1α [87].

Large-scale gene array studies revealed that expression of mRNA for HGF was one of the traits that most significantly distinguished myeloma cells from healthy plasma cells [88]. Interestingly, ***HGF*** and ***MET***, were ranked first and third on the list of angiogenesis-related genes that were overexpressed in myeloma cells as compared to normal bone marrow plasma cells [89]. Moreover, ***HGF*** was ranked number 21 on the list of genes whose expression distinguished malignant plasma cells from cancer cells purified from patients with chronic lymphocytic leukemia and Waldenström's macroglobulinemia, two hematological malignancies that do not cause bone destruction, but which otherwise are closely related to MM [90]. It seems reasonable to presume that genes whose expression is responsible for the perturbed bone remodeling in patients with MM should appear on this list. Indeed, ***DKK1*** and ***FRZB*** were ranked number seven and nine, respectively. Other candidate molecules mentioned in this review were absent among the > 500 genes on this list, with the exception of the Notch ligand ***JAG1***, which was ranked number 519. Of course, even if the absence of a specific mRNA on this list reduces the likelihood of that gene being highly expressed by myeloma cells, it does not exclude the corresponding protein from being an important mediator of the bone disease. It might well be that other cells in the bone marrow are instructed by myeloma cells to express a given bone-destructive protein.

2.11. Role of exosomes

In recent years, it has become evident that communication between myeloma cells and other cells in the bone marrow is not only mediated by soluble factors and direct cell-cell contact but also by extracellular vesicles (EVs) or exosomes. The cargo of EVs/exosomes is dependent on microenvironmental cues. In our lab, we found that upon hypoxia both primary myeloma cells and cell lines express the pro-inflammatory cytokine IL-32 and that IL-32 is exported from the cells in EVs. Such EVs promoted osteoclast activation both ***in vitro*** and ***in vivo*** [91]. Importantly, silencing ***IL-32*** in JJN3 myeloma cells significantly reduced the osteolytic capacity of this cell line, demonstrating the potent effect of this particular cytokine on osteoclast activation. Patients with MBD have higher expression of IL-32 in the plasma cells than patients without bone disease, supporting that IL-32 may play a role for the development of bone disease [91]. A recent study also nicely demonstrated that MM-cell-derived EVs in addition to enhancing osteoclast differentiation also potently inhibited osteoblast differentiation in a MM mouse model [92]. Whether exosome secretion is a useful target in the treatment of myeloma bone disease remains to be investigated.

2.12. Role of non-coding RNAs

Exosomes carry not only protein cargo, but also non-coding RNAs. And recent research has revealed that bone remodeling is heavily influenced by RNAs that do not code for proteins but have various regulatory roles [93,94]. In a paper by Bingzong Li and colleagues from China they demonstrated that exosomes from MM cell lines carried a long non-coding RNA called lncRUNX2-AS1 [95]. When delivered to mesenchymal stromal cells (MSCs), this RNA targeted mRNA coding for the transcription factor RUNX2 and thereby inhibited differentiation of the stem cells to osteogenic cells. Small non-coding RNAs may also play a role by regulating translation of mRNAs. A recent paper from Irene Ghobrial's lab at Harvard, written together with colleagues from Denmark, focused on microRNA-138 (miR-138), which is one of several miRNAs that target mRNAs coding for proteins that are important for osteogenic differentiation of MSCs [96]. They showed that miR-138 is overexpressed by myeloma cells and by MSCs from myeloma patients. Targeting miR-138 by an anti-miR-138 oligonucleotide increased osteogenic differentiation of MSCs and increased the number of osteoblastic linage cells in a multiple myeloma mouse model.

3. Current diagnostics of MBD

MBD is a serious clinical manifestation of MM and may have a major negative impact on quality of life. Active bone disease can be detected in > 80% of patients with MM [97]. It causes fractures leading to pain and often changes in body shape, including sometimes 10–15 cm shortening of height. MBD constitutes one of the so-called CRAB criteria [hypercalcemia (C), renal impairment (R), anemia (A), bone destruction (B)] that distinguishes MM from smoldering myeloma and from the

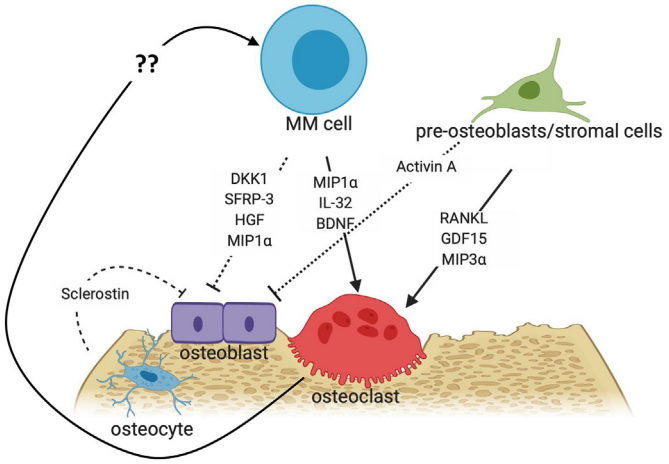


Fig. 2. Proteins proposed to be involved in myeloma bone disease.
The figure depicts proteins that directly affect either osteoblast or osteoclast activity and are found in a substantial fraction of myeloma cases at a level that correlates with extent of bone disease.

premalignant condition monoclonal gammopathy of undetermined significance (MGUS). This means that start of MBD heralds that an active cancer has developed from a slowly developing plasma cell disorder and that the patient now will benefit from treatment.

We can distinguish two types of bone destructions. Osteolytic lesions with destruction of cortical bone is the most obvious and can be detected by conventional skeletal survey (X-ray) and low-dose whole-body CT. CT is the more sensitive and will pick up approximately 25% more lesions in the axial skeleton and flat bones, but not in the long bones [98].

Low-dose whole body CT is therefore recommended as the standard investigation of MBD. A second, more subtle type of bone disease destructs trabecular bone in the bone marrow and appears as focal lesions by magnetic resonance imaging (MRI) or PET-CT. These lesions have prognostic significance and indicate that treatment should be started. The recommended diagnostic set up is therefore screening for MBD by low-dose whole body CT. If this demonstrates bone disease, no further examinations are required. If no bone disease is detected, the examinations should be supplemented by MRI (whole body or spine/pelvis) or PET-CT in patients with seemingly smoldering myeloma. This extended examination is not required in patients with MGUS without pain, who rarely have these lesions. However, not all modalities for bone examination are available in all hospitals and the diagnostic procedures must be adjusted accordingly. More details can be found in a recent update [99].

4. Current treatment of MBD

Anti-myeloma treatment is also the best treatment against the bone disease. Treatment directed more specifically against MBD can be divided in two: prophylactic treatment and treatment against osteolytic lesions and their consequences. The latter includes radiation against painful lesions and surgery and will not be discussed further.

Prophylactic treatment with bisphosphonates has been standard care since the 1990s. Several bisphosphonates such as zoledronic acid, pamidronate and clodronate, have been used, today most commonly zoledronic acid. Bisphosphonates are pyrophosphate analogues that bind to hydroxyapatite and are incorporated into areas of active bone remodeling [100]. All bisphosphonates share a core phosphate-carbon-phosphate backbone, but their potency varies dependent on the composition of the two side chains coupled to the central carbon atom.

Bisphosphonate given to MM patients reduces time to first skeletal-related events. Zoledronic acid and pamidronate were compared directly and had an equal effect [101].

Interestingly, a study comparing clodronate and zoledronic acid demonstrated that zoledronic acid extended overall survival by 5.5 months [102]. These results confirm preclinical studies indicating a direct anti-myeloma effect of zoledronic acid. Pamidronate may also have a similar effect as indicated in a Cochrane network meta-analysis [103].

The only approved treatment specifically targeting one of the molecules that have been discussed in this review as mediators of MBD, is denosumab, a monoclonal antibody against RANKL. In a phase 3 clinical trial, the effect of denosumab was found to be equipotent to that of zoledronic acid [104]. Due to its higher cost, denosumab is seldom used except in myeloma patients with renal failure, for whom bisphosphonates may have harmful side effects.

Several other drugs targeting molecular mediators of the bone disease are being explored, but have not yet reached full documentation for clinical use.

5. Future considerations

Can we arrive at a conclusion about the question of how myeloma causes bone degradation? When we started looking for myeloma-produced molecules that could explain this important manifestation of the disease, we presumed that there would be a single protein or a unifying molecular program that was responsible in all MM patients with MBD. Many hypotheses have been put forward over the years and most of them have focused on a specific extracellular protein with signaling properties. However, today, almost thirty years later, nobody has come up with a mechanism that can explain the disease in every patient. If we look at the expression in primary myeloma cells of genes coding for proteins that have been implicated in MBD, we have difficulty finding a candidate molecule that can be involved in every patient (Fig. 1, Fig. 2 and Table 1). The majority of genes are expressed at such low levels that it is difficult to imagine that their gene product could be responsible for anything unless maybe in a small fraction of patients. The genes encoding RANKL, IL-1β, lymphotoxin, IL-3, IL-7, sclerostin, activin-A, Notch ligands, BDNF and GDF-15 fall within this group. The expression patterns of other candidate molecules are also conspicuously skewed across a population of patients (Fig. 1). Genes coding for MIP-1α, DKK1, HGF and SFRP-3 are active in a substantial number of patients, but each of them relatively silent in a higher proportion of patients than the percentage who do not suffer from bone disease.

So then, what is the unifying pattern we are unable to see? The presence of monoclonal immunoglobulin in the circulation is a unifying trait of most MM patients. We recently demonstrated that serum proteins are differently glycosylated in MM patients compared with healthy individuals [105]. In rheumatoid arthritis, altered N-glycosylation of autoantibodies promotes bone loss [106], and it will be interesting to explore if immunoglobulins play a similar role in MBD.

Alternatively, maybe the unifying pattern is benefit from bone destruction to myeloma cells no matter how the destruction is accomplished. Could it be that myeloma cells are dependent on some product from active osteoclasts, and have a series of strategies to promote the production of this substance (Fig. 2)? In support of this notion, in an elegant study using two-photon intravital microscopy, Michelle Lawson and colleagues in Peter Croucher's lab demonstrated that osteoclast activation promoted reactivation of dormant myeloma cells [107]. Thus, our quest should perhaps focus more on the interaction between myeloma cells and osteoclasts and particularly on osteoclast products.

Practice points

- The bone disease is a particular trait of multiple myeloma, affecting over 80% of patients.
- MBD causes pain and pathological fractures, leading to serious suffering for many patients.
- MBD is far from fully understood. To form a basis for new treatment

Table 1
Overview of proteins proposed to be involved in myeloma bone disease. BM = bone marrow, MBD = myeloma bone disease.

	Main mode of action		Dysregulated in myeloma?	Gene expressed by myeloma cells?	Expression in myeloma patients correlates with MBD?
	Promoting osteoclasts	Inhibiting osteoblasts			
Lymphotoxin	Yes, directly [4,108]			No [5]	
IL-1β	Yes, directly [108]		Yes [82]	No, or low by subgroup. Fig. 1, [11,15,82]	BM plasma levels did not correlate significantly [82]
HGF	Yes [30,32]	Yes [56]	Yes [23,24]	Yes, by subgroup. Fig. 1, [23]	Yes, mRNA expression, BM plasma levels [84] and blood serum levels [87]
MIP-1α	Yes, directly and *via* RANKL [33,34]	Yes [55]	Yes [33,87]	Yes, by subgroup. Fig. 1, [33,34]	Yes [serum levels: [86,87] and BM levels [83]]
IL-3	Stimulates OC precursors [35], but several studies indicate inhibition of osteoclasts by IL-3 [109]	Yes, indirectly *via* CD45+ cells [54]	Yes [35]	No. IL-3 is produced by osteoblasts. Fig. 1, [109]	BM levels did not correlate significantly [83]
RANKL	Yes, directly [37]		Yes [41,42,45]	No Fig. 1 [42]	Serum RANKL/OPG ratio correlated with MBD [44], but BM RANKL expression did not [85]
DKK1		Yes [50]	Yes [47,48]	Yes, by subgroup. Fig. 1, [47,48]	Yes (mRNA expression and BM plasma levels) [85]
SFRP-3		Yes? [51] (Shown for SFRP-2)	Yes [47,48]	Yes, by subgroup. Fig. 1, [47,48]	Yes (mRNA expression and BM plasma levels) [85]
IL-7	Yes, *via* RANKL [53,110]	Yes [52]	Yes [53]	No, or low by subgroup. Fig. 1	
Sclerostin		Yes [111]	Yes [60–63]	No. Fig. 1	Correlation between serum sclerostin and MBD with borderline significance (p = .08) [63]
GDF15	Yes [76]	Yes [75]	Yes [75]	No, or low by subgroup. Fig. 1	Yes, blood serum levels [75]
IL-32	Yes, directly [91,112]		Yes [91]	Yes, by small subgroup. Fig. 1	Yes, IL32 gene expression [91]
Activin A	Yes [113]	Yes [70]	Yes [71]	No, Fig. 1, [71]	Yes, BM plasma levels [71]
IL-17	Yes, *via* T cells [80]		Yes [80]	No, Fig. 1	Yes, BM plasma levels [80]
MIP-3α	Yes [114]		Yes [82]	No, Fig. 1	Yes, BM levels [82,83]
BDNF	Yes [79]		Yes [77,78]	No, or low by subgroup, Fig. 1	Yes, BM plasma levels [79]

it is important to understand better how MBD develops.
- Many bioactive proteins have been implicated as mediators of MBD, but only a few of them firmly documented as myeloma cell products in a substantial proportion of patients: SFRP-3, DKK1, HGF and MIP-1α.
- Clinical studies targeting the identified cytokines or their downstream signaling pathways should be conducted with level of MBD as a clinical endpoint.

Research agenda

- There is likely to be a vicious circle between myeloma cells and osteoclasts where they stimulate each other.

- The components of this circle can best be identified through research focused on the interplay between myeloma cells and other cells in the microenvironment: osteoclasts, osteoblast, osteocytes and immune cells.
- The effect of immunoglobulin glycosylation of bone metabolism should be examined.

Funding

The authors work on MBD has been supported by the Norwegian Cancer Society; the Liaison Committee for education, research and innovation in Central Norway; the Norwegian Research Council and the Cancer Fund, St. Olavs Hospital.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Reference

[1] Zink A, Rohrbach H, Szeimies U, Hagedorn HG, Haas CJ, Weyss C, et al. Malignant tumors in an ancient Egyptian population. Anticancer Res 1999;19:4273–7.
[2] Mundy GR, Raisz LG, Cooper RA, Schechter GP, Salmon SE. Evidence for the secretion of an osteoclast stimulating factor in myeloma. N Engl J Med 1974;291:1041–6.
[3] Mundy GR, Luben RA, Raisz LG, Oppenheim JJ, Buell DN. Bone-resorbing activity in supernatants from lymphoid cell lines. N Engl J Med 1974;290:867–71.
[4] Garrett IR, Durie BG, Nedwin GE, Gillespie A, Bringman T, Sabatini M, et al. Production of lymphotoxin, a bone-resorbing cytokine, by cultured human myeloma cells. N Engl J Med 1987;317:526–32.
[5] Kawano M, Yamamoto I, Iwato K, Tanaka H, Asaoku H, Tanabe O, et al. Interleukin-1 beta rather than lymphotoxin as the major bone resorbing activity in human multiple myeloma. Blood 1989;73:1646–9.
[6] Bataille R, Klein B, Jourdan M, Rossi JF, Durie BG. Spontaneous secretion of tumor necrosis factor-beta by human myeloma cell lines. Cancer 1989;63:877–80.
[7] Cozzolino F, Torcia M, Aldinucci D, Rubartelli A, Miliani A, Shaw AR, et al. Production of interleukin-1 by bone marrow myeloma cells. Blood 1989;74:380–7.
[8] Yamamoto I, Kawano M, Sone T, Iwato K, Tanaka H, Ishikawa H, et al. Production of interleukin 1 beta, a potent bone resorbing cytokine by cultured human myeloma cells. Cancer Res 1989;49:4242–6.
[9] Drexler HG, Dirks WG, MacLeod RA. False human hematopoietic cell lines: cross-contaminations and misinterpretations. Leukemia 1999;13:1601–7.
[10] Drexler HG, Matsuo Y, MacLeod RA. Persistent use of false myeloma cell lines. Hum Cell 2003;16:101–5.
[11] Børset M, Helseth E, Naume B, Waage A. Lack of IL-1 secretion from human myeloma cells highly purified by immunomagnetic separation. Br J Haematol 1993;85:446–51.
[12] Wijdenes J, Vooijs WC, Clement C, Post J, Morard F, Vita N, et al. A plasmocyte selective monoclonal antibody (B-B4) recognizes syndecan-1. Br J Haematol 1996;94:318–23.
[13] Kawano M, Hirano T, Matsuda T, Taga T, Horii Y, Iwato K, et al. Autocrine

generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 1988;332:83–5.
[14] Klein B, Zhang XG, Jourdan M, Content J, Houssiau F, Aarden L, et al. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. Blood 1989;73:517–26.
[15] Sati HI, Greaves M, Apperley JF, Russell RG, Croucher PI. Expression of interleukin-1beta and tumour necrosis factor-alpha in plasma cells from patients with multiple myeloma. Br J Haematol 1999;104:350–7.
[16] Bataille R, Chappard D, Marcelli C, Dessauw P, Sany J, Baldet P, et al. Mechanisms of bone destruction in multiple myeloma: the importance of an unbalanced process in determining the severity of lytic bone disease. J Clin Oncol 1989;7:1909–14.
[17] Chenu C, Pfeilschifter J, Mundy GR, Roodman GD. Transforming growth factor beta inhibits formation of osteoclast- like cells in long-term human marrow cultures. Proc Natl Acad Sci U S A 1988;85:5683–7.
[18] Mundy GR, Bonewald LF. Role of TGF beta in bone remodeling. Ann N Y Acad Sci 1990;593:91–7.
[19] Noda M, Camilliere JJ. In vivo stimulation of bone formation by transforming growth factor-beta. Endocrinology 1989;124:2991–4.
[20] Børset M, Lien E, Espevik T, Helseth E, Waage A, Sundan A. Concomitant expression of hepatocyte growth factor/scatter factor and the receptor c-MET in human myeloma cell lines. J Biol Chem 1996;271:24655–61.
[21] Børset M, Waage A, Sundan A. Hepatocyte growth factor reverses the TGF-beta-induced growth inhibition of CCL-64 cells. A novel bioassay for HGF and implications for the TGF-beta bioassay. J Immunol Methods 1996;189:59–64.
[22] Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, et al. Molecular cloning and expression of human hepatocyte growth factor. Nature 1989;342:440–3.
[23] Børset M, Hjorth-Hansen H, Seidel C, Sundan A, Waage A. Hepatocyte growth factor and its receptor c-met in multiple myeloma. Blood 1996;88:3998–4004.
[24] Seidel C, Børset M, Turesson I, Abildgaard N, Sundan A, Waage A. Elevated serum concentrations of hepatocyte growth factor in patients with multiple myeloma. The Nordic Myeloma Study Group. Blood 1998;91:806–12.
[25] Hov H, Holt RU, Ro TB, Fagerli UM, Hjorth-Hansen H, Baykov V, et al. A selective c-met inhibitor blocks an autocrine hepatocyte growth factor growth loop in ANBL-6 cells and prevents migration and adhesion of myeloma cells. Clin Cancer Res 2004;10:6686–94.
[26] Hov H, Tian E, Holien T, Holt RU, Vatsveen TK, Fagerli UM, et al. c-Met signaling promotes IL-6-induced myeloma cell proliferation. Eur J Haematol 2009;82:277–87.
[27] Seidel C, Børset M, Hjertner O, Cao D, Abildgaard N, Hjorth-Hansen H, et al. High levels of soluble syndecan-1 in myeloma-derived bone marrow: modulation of hepatocyte growth factor activity. Blood 2000;96:3139–46.
[28] Derksen PW, Keehnen RM, Evers LM, van Oers MH, Spaargaren M, Pals ST. Cell surface proteoglycan syndecan-1 mediates hepatocyte growth factor binding and promotes Met signaling in multiple myeloma. Blood 2002;99:1405–10.
[29] Derksen PW, de Gorter DJ, Meijer HP, Bende RJ, van DM, Lokhorst HM, et al. The hepatocyte growth factor/Met pathway controls proliferation and apoptosis in multiple myeloma. Leukemia 2003;17:764–74.
[30] Adamopoulos IE, Xia Z, Lau YS, Athanasou NA. Hepatocyte growth factor can substitute for M-CSF to support osteoclastogenesis. Biochem Biophys Res Commun 2006;350:478–83.
[31] Fuller K, Owens J, Chambers TJ. The effect of hepatocyte growth factor on the behaviour of osteoclasts. Biochem Biophys Res Commun 1995;212:334–40.
[32] Grano M, Galimi F, Zambonin G, Colucci S, Cottone E, Zambonin Zallone A, et al. Hepatocyte growth factor is a coupling factor for osteoclasts and osteoblasts in vitro. Proc Natl Acad Sci U S A 1996;93:7644–8.
[33] Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD, et al. Macrophage inflammatory protein 1-alpha is a potential osteoclast stimulatory factor in multiple myeloma. Blood 2000;96:671–5.
[34] Choi SJ, Oba Y, Gazitt Y, Alsina M, Cruz J, Anderson J, et al. Antisense inhibition of macrophage inflammatory protein 1-alpha blocks bone destruction in a model of myeloma bone disease. J Clin Invest 2001;108:1833–41.
[35] Lee JW, Chung HY, Ehrlich LA, Jelinek DF, Callander NS, Roodman GD, et al. IL-3 expression by myeloma cells increases both osteoclast formation and growth of myeloma cells. Blood 2004;103:2308–15.
[36] Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.
[37] Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci U S A 1998;95:3597–602.
[38] Han Y, You X, Xing W, Zhang Z, Zou W. Paracrine and endocrine actions of bone-the functions of secretory proteins from osteoblasts, osteocytes, and osteoclasts. Bone Res 2018;6:16.
[39] Nakashima T, Hayashi M, Fukunaga T, Kurata K, Oh-Hora M, Feng JQ, et al. Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med 2011;17:1231–4.
[40] Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 2006;203:2673–82.
[41] Giuliani N, Bataille R, Mancini C, Lazzaretti M, Barille S. Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. Blood 2001;98:3527–33.
[42] Pearse RN, Sordillo EM, Yaccoby S, Wong BR, Liau DF, Colman N, et al. Multiple myeloma disrupts the TRANCE/ osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc Natl Acad Sci U S A 2001;98:11581–6.
[43] Seidel C, Hjertner O, Abildgaard N, Heickendorff L, Hjorth M, Westin J, et al. Serum osteoprotegerin levels are reduced in patients with multiple myeloma with lytic bone disease. Blood 2001;98:2269–71.
[44] Terpos E, Szydlo R, Apperley JF, Hatjiharissi E, Politou M, Meletis J, et al. Soluble receptor activator of nuclear factor kappaB ligand-osteoprotegerin ratio predicts survival in multiple myeloma: proposal for a novel prognostic index. Blood 2003;102:1064–9.
[45] Farrugia AN, Atkins GJ, To LB, Pan B, Horvath N, Kostakis P, et al. Receptor activator of nuclear factor-kappaB ligand expression by human myeloma cells mediates osteoclast formation in vitro and correlates with bone destruction in vivo. Cancer Res 2003;63:5438–45.
[46] Standal T, Seidel C, Hjertner O, Plesner T, Sanderson RD, Waage A, et al. Osteoprotegerin is bound, internalized, and degraded by multiple myeloma cells. Blood 2002;100:3002–7.
[47] Shaughnessy Jr. JD, Barlogie B. Interpreting the molecular biology and clinical behavior of multiple myeloma in the context of global gene expression profiling. Immunol Rev 2003;194:140–63.
[48] Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med 2003;349:2483–94.
[49] Heath DJ, Chantry AD, Buckle CH, Coulton L, Shaughnessy Jr. JD, Evans HR, et al. Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. J Bone Miner Res 2009;24:425–36.
[50] Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy Jr. JD. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. Blood 2007;109:2106–11.
[51] Oshima T, Abe M, Asano J, Hara T, Kitazoe K, Sekimoto E, et al. Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. Blood 2005;106:3160–5.
[52] Giuliani N, Colla S, Morandi F, Lazzaretti M, Sala R, Bonomini S, et al. Myeloma cells block RUNX2/CBFA1 activity in human bone marrow osteoblast progenitors and inhibit osteoblast formation and differentiation. Blood 2005;106:2472–83.
[53] Giuliani N, Colla S, Sala R, Moroni M, Lazzaretti M, La MS, et al. Human myeloma cells stimulate the receptor activator of nuclear factor-kappa B ligand (RANKL) in T lymphocytes: a potential role in multiple myeloma bone disease. Blood 2002;100:4615–21.
[54] Ehrlich LA, Chung HY, Ghobrial I, Choi SJ, Morandi F, Colla S, et al. IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. Blood 2005;106:1407–14.
[55] Vallet S, Pozzi S, Patel K, Vaghela N, Fulciniti MT, Veiby P, et al. A novel role for CCL3 (MIP-1alpha) in myeloma-induced bone disease via osteocalcin down-regulation and inhibition of osteoblast function. Leukemia 2011;25:1174–81.
[56] Standal T, Abildgaard N, Fagerli UM, Stordal B, Hjertner O, Børset M, et al. HGF inhibits BMP-induced osteoblastogenesis: possible implications for the bone disease of multiple myeloma. Blood 2007;109:3024–30.
[57] Rampa C, Tian E, Vatsveen TK, Buene G, Slordahl TS, Børset M, et al. Identification of the source of elevated hepatocyte growth factor levels in multiple myeloma patients. Biomark Res 2014;2:8.
[58] Wader KF, Fagerli UM, Holt RU, Stordal B, Børset M, Sundan A, et al. Elevated serum concentrations of activated hepatocyte growth factor activator in patients with multiple myeloma. Eur J Haematol 2008;81:380–3.
[59] Wader KF, Fagerli UM, Børset M, Lydersen S, Hov H, Sundan A, et al. Immunohistochemical analysis of hepatocyte growth factor and c-Met in plasma cell disease. Histopathology 2012;60:443–51.
[60] Eda H, Santo L, Wein MN, Hu DZ, Cirstea DD, Nemani N, et al. Regulation of sclerostin expression in multiple myeloma by Dkk-1: a potential therapeutic strategy for myeloma bone disease. J Bone Miner Res 2016;31:1225–34.
[61] McDonald MM, Reagan MR, Youlten SE, Mohanty ST, Seckinger A, Terry RL, et al. Inhibiting the osteocyte-specific protein sclerostin increases bone mass and fracture resistance in multiple myeloma. Blood 2017;129:3452–64.
[62] Delgado-Calle J, Anderson J, Cregor MD, Condon KW, Kuhstoss SA, Plotkin LI, et al. Genetic deletion of Sost or pharmacological inhibition of sclerostin prevent multiple myeloma-induced bone disease without affecting tumor growth. Leukemia 2017;31:2686–94.
[63] Terpos E, Christoulas D, Katodritou E, Bratengeier C, Gkotzamanidou M, Michalis E, et al. Elevated circulating sclerostin correlates with advanced disease features and abnormal bone remodeling in symptomatic myeloma: reduction post-bortezomib monotherapy. Int J Cancer 2012;131:1466–71.
[64] Schwarzer R, Kaiser M, Acikgoez O, Heider U, Mathas S, Preissner R, et al. Notch inhibition blocks multiple myeloma cell-induced osteoclast activation. Leukemia 2008;22:2273–7.
[65] Delgado-Calle J, Anderson J, Cregor MD, Hiasa M, Chirgwin JM, Carlesso N, et al. Bidirectional Notch Signaling and osteocyte-derived factors in the bone marrow microenvironment promote tumor cell proliferation and bone destruction in multiple myeloma. Cancer Res 2016;76:1089–100.
[66] Houde C, Li Y, Song L, Barton K, Zhang Q, Godwin J, et al. Overexpression of the NOTCH ligand JAG2 in malignant plasma cells from multiple myeloma patients and cell lines. Blood 2004;104:3697–704.
[67] Bellido T. Osteocyte-driven bone remodeling. Calcif Tissue Int 2014;94:25–34.
[68] Giuliani N, Ferretti M, Bolzoni M, Storti P, Lazzaretti M, Dalla PB, et al. Increased osteocyte death in multiple myeloma patients: role in myeloma-induced osteoclast formation. Leukemia 2012;26:1391–401.
[69] Hjertner O, Torgersen ML, Seidel C, Hjorth-Hansen H, Waage A, Børset M, et al.

Hepatocyte growth factor (HGF) induces interleukin-11 secretion from osteoblasts: a possible role for HGF in myeloma-associated osteolytic bone disease. Blood 1999;94:3883–8.
[70] Olsen OE, Wader KF, Hella H, Mylin AK, Turesson I, Nesthus I, et al. Activin a inhibits BMP-signaling by binding ACVR2A and ACVR2B. Cell Commun Signal 2015;13:27.
[71] Vallet S, Mukherjee S, Vaghela N, Hideshima T, Fulciniti M, Pozzi S, et al. Activin a promotes multiple myeloma-induced osteolysis and is a promising target for myeloma bone disease. Proc Natl Acad Sci U S A 2010;107:5124–9.
[72] Zannettino AC, Farrugia AN, Kortesidis A, Manavis J, To LB, Martin SK, et al. Elevated serum levels of stromal-derived factor-1alpha are associated with increased osteoclast activity and osteolytic bone disease in multiple myeloma patients. Cancer Res 2005;65:1700–9.
[73] Tanaka Y, Abe M, Hiasa M, Oda A, Amou H, Nakano A, et al. Myeloma cell-osteoclast interaction enhances angiogenesis together with bone resorption: a role for vascular endothelial cell growth factor and osteopontin. Clin Cancer Res 2007;13:816–23.
[74] Chantry AD, Heath D, Mulivor AW, Pearsall S, Baud'huin M, Coulton L, et al. Inhibiting activin-a signaling stimulates bone formation and prevents cancer-induced bone destruction in vivo. J Bone Miner Res 2010;25:2633–46.
[75] Westhrin M, Moen SH, Holien T, Mylin AK, Heickendorff L, Olsen OE, et al. Growth differentiation factor 15 (GDF15) promotes osteoclast differentiation and inhibits osteoblast differentiation and high serum GDF15 levels are associated with multiple myeloma bone disease. Haematologica 2015;100:e511–4.
[76] Hinoi E, Ochi H, Takarada T, Nakatani E, Iezaki T, Nakajima H, et al. Positive regulation of osteoclastic differentiation by growth differentiation factor 15 up-regulated in osteocytic cells under hypoxia. J Bone Miner Res 2012;27:938–49.
[77] Ai LS, Sun CY, Zhang L, Zhou SC, Chu ZB, Qin Y, et al. Inhibition of BDNF in multiple myeloma blocks osteoclastogenesis via down-regulated stroma-derived RANKL expression both in vitro and in vivo. PLoS One 2012;7:e46287.
[78] Pearse RN, Swendeman SL, Li Y, Rafii D, Hempstead BL. A neurotrophin axis in myeloma: TrkB and BDNF promote tumor-cell survival. Blood 2005;105:4429–36.
[79] Sun CY, Chu ZB, She XM, Zhang L, Chen L, Ai LS, et al. Brain-derived neurotrophic factor is a potential osteoclast stimulating factor in multiple myeloma. Int J Cancer 2012;130:827–36.
[80] Noonan K, Marchionni L, Anderson J, Pardoll D, Roodman GD, Borrello I. A novel role of IL-17-producing lymphocytes in mediating lytic bone disease in multiple myeloma. Blood 2010;116:3554–63.
[81] Dhodapkar KM, Barbuto S, Matthews P, Kukreja A, Mazumder A, Vesole D, et al. Dendritic cells mediate the induction of polyfunctional human IL17-producing cells (Th17-1 cells) enriched in the bone marrow of patients with myeloma. Blood 2008;112:2878–85.
[82] Giuliani N, Lisignoli G, Colla S, Lazzaretti M, Storti P, Mancini C, et al. CC-chemokine ligand 20/macrophage inflammatory protein-3alpha and CC-chemokine receptor 6 are overexpressed in myeloma microenvironment related to osteolytic bone lesions. Cancer Res 2008;68:6840–50.
[83] Palma BD, Guasco D, Pedrazzoni M, Bolzoni M, Accardi F, Costa F, et al. Osteolytic lesions, cytogenetic features and bone marrow levels of cytokines and chemokines in multiple myeloma patients: role of chemokine (C-C motif) ligand 20. Leukemia 2016;30:409–16.
[84] Kristensen IB, Christensen JH, Lyng MB, Moller MB, Pedersen L, Rasmussen LM, et al. Hepatocyte growth factor pathway upregulation in the bone marrow microenvironment in multiple myeloma is associated with lytic bone disease. Br J Haematol 2013;161:373–82.
[85] Kristensen IB, Christensen JH, Lyng MB, Moller MB, Pedersen L, Rasmussen LM, et al. Expression of osteoblast and osteoclast regulatory genes in the bone marrow microenvironment in multiple myeloma: only up-regulation of Wnt inhibitors SFRP3 and DKK1 is associated with lytic bone disease. Leuk Lymphoma 2014;55:911–9.
[86] Terpos E, Politou M, Szydlo R, Goldman JM, Apperley JF, Rahemtulla A. Serum levels of macrophage inflammatory protein-1 alpha (MIP-1alpha) correlate with the extent of bone disease and survival in patients with multiple myeloma. Br J Haematol 2003;123:106–9.
[87] Tsirakis G, Roussou P, Pappa CA, Kolovou A, Vasilokonstantaki C, Miminas I, et al. Increased serum levels of MIP-1alpha correlate with bone disease and angiogenic cytokines in patients with multiple myeloma. Med Oncol 2014;31:778.
[88] Zhan F, Hardin J, Kordsmeier B, Bumm K, Zheng M, Tian E, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. Blood 2002;99:1745–57.
[89] Hose D, Moreaux J, Meissner T, Seckinger A, Goldschmidt H, Benner A, et al. Induction of angiogenesis by normal and malignant plasma cells. Blood 2009;114:128–43.
[90] Chng WJ, Schop RF, Price-Troska T, Ghobrial I, Kay N, Jelinek DF, et al. Gene-expression profiling of Waldenstrom macroglobulinemia reveals a phenotype more similar to chronic lymphocytic leukemia than multiple myeloma. Blood 2006;108:2755–63.
[91] Zahoor M, Westhrin M, Aass KR, Moen SH, Misund K, Psonka-Antonczyk KM, et al. Hypoxia promotes IL-32 expression in myeloma cells, and high expression is associated with poor survival and bone loss. Blood Adv 2017;1:2656–66.
[92] Faict S, Muller J, De VK, De BE, Maes K, Vrancken L, et al. Exosomes play a role in multiple myeloma bone disease and tumor development by targeting osteoclasts and osteoblasts. Blood Cancer J 2018;8:105.
[93] Wu QY, Li X, Miao ZN, Ye JX, Wang B, Zhang F, et al. Long non-coding RNAs: a new regulatory code for osteoporosis. Front Endocrinol (Lausanne) 2018;9:587.
[94] Taipaleenmaki H. Regulation of bone metabolism by microRNAs. Curr Osteoporos Rep 2018;16:1–12.
[95] Li B, Xu H, Han H, Song S, Zhang X, Ouyang L, et al. Exosome-mediated transfer of lncRUNX2-AS1 from multiple myeloma cells to MSCs contributes to osteogenesis. Oncogene 2018;37:5508–19.
[96] Tsukamoto S, Lovendorf MB, Park J, Salem KZ, Reagan MR, Manier S, et al. Inhibition of microRNA-138 enhances bone formation in multiple myeloma bone marrow niche. Leukemia 2018;32:1739–50.
[97] Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc 2003;78:21–33.
[98] Hillengass J, Moulopoulos LA, Delorme S, Koutoulidis V, Mosebach J, Hielscher T, et al. Whole-body computed tomography versus conventional skeletal survey in patients with multiple myeloma: a study of the International Myeloma Working Group. Blood Cancer J 2017;7:e599.
[99] Hillengass J, Usmani S, Rajkumar SV, Durie BGM, Mateos MV, Lonial S, et al. International myeloma working group consensus recommendations on imaging in monoclonal plasma cell disorders. Lancet Oncol 2019;20:e302–12.
[100] Rodan GA, Fleisch HA. Bisphosphonates: mechanisms of action. J Clin Invest 1996;97:2692–6.
[101] Rosen LS, Gordon D, Kaminski M, Howell A, Belch A, Mackey J, et al. Zoledronic acid versus pamidronate in the treatment of skeletal metastases in patients with breast cancer or osteolytic lesions of multiple myeloma: a phase III, double-blind comparative trial. Cancer J 2001;7:377–87.
[102] Morgan GJ, Davies FE, Gregory WM, Cocks K, Bell SE, Szubert AJ, et al. First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC myeloma IX): a randomised controlled trial. Lancet 2010;376:1989–99.
[103] Mhaskar R, Redzepovic J, Wheatley K, Clark OA, Miladinovic B, Glasmacher A, et al. Bisphosphonates in multiple myeloma: a network meta-analysis. Cochrane Database Syst Rev 2012:CD003188https://doi.org/10.1002/14651858.
[104] Raje N, Terpos E, Willenbacher W, Shimizu K, Garcia-Sanz R, Durie B, et al. Denosumab versus zoledronic acid in bone disease treatment of newly diagnosed multiple myeloma: an international, double-blind, double-dummy, randomised, controlled, phase 3 study. Lancet Oncol 2018;19:370–81.
[105] Zhang Z, Westhrin M, Bondt A, Wuhrer M, Standal T, Holst S. Serum protein N-glycosylation changes in multiple myeloma. Biochim Biophys Acta Gen Subj 2019;1863:960–70.
[106] Pfeifle R, Rothe T, Ipseiz N, Scherer HU, Culemann S, Harre U, et al. Regulation of autoantibody activity by the IL-23-TH17 axis determines the onset of autoimmune disease. Nat Immunol 2017;18:104–13.
[107] Lawson MA, McDonald MM, Kovacic N, Hua KW, Terry RL, Down J, et al. Osteoclasts control reactivation of dormant myeloma cells by remodelling the endosteal niche. Nat Commun 2015;6:8983.
[108] Feng W, Guo J, Li M. RANKL-independent modulation of osteoclastogenesis. J Oral Biosci 2019;61:16–21.
[109] Soysa NS, Alles N. The role of IL-3 in bone. J Cell Biochem 2018;120:6851–9.
[110] Weitzmann MN, Cenci S, Rifas L, Brown C, Pacifici R. Interleukin-7 stimulates osteoclast formation by up-regulating the T-cell production of soluble osteoclastogenic cytokines. Blood 2000;96:1873–8.
[111] Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J 2003;22:6267–76.
[112] Mabilleau G, Sabokbar A. Interleukin-32 promotes osteoclast differentiation but not osteoclast activation. PLoS One 2009;4:e4173.
[113] Fuller K, Bayley KE, Chambers TJ. Activin A is an essential cofactor for osteoclast induction. Biochem Biophys Res Commun 2000;268:2–7.
[114] Lisignoli G, Piacentini A, Cristino S, Grassi F, Cavallo C, Cattini L, et al. CCL20 chemokine induces both osteoblast proliferation and osteoclast differentiation: increased levels of CCL20 are expressed in subchondral bone tissue of rheumatoid arthritis patients. J Cell Physiol 2007;210:798–806.