Dynamic interplay between bone and multiple myeloma: Emerging roles of the osteoblast

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A B S T R A C T

Multiple myeloma is a B-cell malignancy characterized by the unrelenting proliferation of plasma cells. Multiple myeloma causes osteolytic lesions and fractures that do not heal due to decreased osteoblastic and increased osteoclastic activity. However, the exact relationship between osteoblasts and myeloma cells remains elusive. Understanding the interactions between these dynamic bone-forming cells and myeloma cells is crucial to understanding how osteolytic lesions form and persist and how tumors grow within the bone marrow. This review provides a comprehensive overview of basic and translational research focused on the role of osteoblasts in multiple myeloma progression and their relationship to osteolytic lesions. Importantly, current challenges for in vitro studies exploring direct osteoblastic effects on myeloma cells, and gaps in understanding the role of the osteoblast in myeloma progression are delineated. Finally, successes and challenges in myeloma treatment with osteoanabolic therapy (i.e., any treatment that induces increased osteoblastic number or activity) are enumerated. Our goal is to illuminate novel mechanisms by which osteoblasts may contribute to multiple myeloma disease progression and osteolysis to better direct research efforts. Ultimately, we hope this may provide a roadmap for new approaches to the pathogenesis and treatment of multiple myeloma with a particular focus on the osteoblast.

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Multiple myeloma is an incurable plasma cell dyscrasia, a type of B-cell cancer that progresses through stages from monoclonal gammapathy of undetermined significance (MGUS), to asymptomatic smoldering myeloma, and lastly, to overt, symptomatic myeloma. This last stage is associated with significant morbidity, particularly in the form of fractures. Recent reports show that MGUS is also correlated with enhanced skeletal risks and osteopenia at this early stage of plasma cell transformation [1]. During multiple myeloma progression, osteolytic lesions are found throughout the skeleton with multiple tumors or “omas” packing the bone marrow. Osteosclerosis, a hallmark of multiple myeloma-induced bone disease, results from decreased osteoclastic activity and increased osteoclastic activity, releasing growth factors and cytokines embedded in bone matrix to form a “vicious cycle” [2-5]. The degree of osteolysis is an important parameter in the assessment of multiple myeloma patients. While the numbers of osteoblasts and bone formation rates are often increased in the early stages of tumor burden, due to increased osteoclast activity (which feeds back to activate increased osteoblast activity), these numbers become significantly lower when plasma cell infiltration occupies more than 50% of bone marrow [6]. Although bone-building (bone anabolic) treatments are currently being explored in early clinical trials to delay the time to first skeletal-related events (SREs) [7,8], much work remains to be done to validate if these are truly anti-myeloma strategies with long-term clinical benefits.

Preliminary research has demonstrated that osteoblast numbers can be decreased in hematologic malignancies, even in non-osteolytic tumors (a decrease of 55% was found in myelodysplasia and acute myeloid leukemia patients) and that osteoblasts can have an anti-tumor effect in blood cancers [9]. In support of this concept, the genetic depletion of osteoblasts in mouse models of acute leukemia led to increased circulating tumor cells and tumor marrow and spleen engraftment, higher tumor burden, and shorter survival [9]. Myelopoiesis increased and was coupled with a reduction in B-lymphopoiesis and compromised erythropoiesis, suggesting alterations in hematopoietic differentiation. When mice with acute myeloid or lymphoblastic leukemia were treated with a pharmacological inhibitor of duodenal serotonin, a hormone that suppresses osteoblast numbers, osteoblast numbers were increased, as expected. Remarkably, this treatment and subsequent maintenance of the osteoblast pool restored normal marrow function, reduced tumor burden and prolonged survival [9]. Therefore, osteoblasts may play a fundamental role in propagating leukemia in the marrow: pathways mediating this regulation still need identification.

One of the most pressing gaps in multiple myeloma biology is a basic biological understanding of the role of osteoblasts in disease progression (see Fig. 1). Recently, bone microstructural changes have been identified, along with elevated DKK1 and MIP-1α levels, in patients with MGUS [10]. Moreover, epidemiological data have demonstrated that low bone mineral density, increased fracture rate, and osteoporosis correlate with MGUS [1]. This provides more evidence that decreased osteoblast number/function, or weaker bones, could not only result from, but also cause or accelerate multiple myeloma [11]. As reviewed here, in vitro and in vivo studies to interrogate this hypothesis are crucial to elevate these correlations to mechanistically de- •

Osteoblasts are highly specific bone cells lining and formulating the mineralized matrix of the skeleton. They result from the osteogenic differentiation of mesenchymal stem cells (MSCs) and pass through a series of pre-osteoblastic stages as osteoprogenitor cells [12], until they become fully functional osteoblasts. When making bone, osteoblasts first deposit a dense organic extracellular matrix, primarily collagen I, and then harden this matrix by producing an inorganic calcium and phosphate-based mineral, hydroxyapatite. Different types of bone are formed by osteoblasts throughout the skeleton during skeletogenesis, remodeling, and fracture healing, including lamellar bone and woven bone [13]. During embryonic development, bone forms predominantly through a complex process termed endochondral ossification, a process including an intermediate cartilage stage [14]. A smaller fraction of human bones, such as the plates of the skull, are formed by intramembranous ossification, a process of direct differentiation of MSCs into mineralizing osteoblasts.

Osteoblasts in distinct anatomical locations respond uniquely to different stimuli and would likely respond differently to tumor cells, complicating studies aimed at using osteoblasts to inhibit multiple myeloma and other osteolytic cancers. What governs osteoblast phenotype and bone turnover in different bone compartments is largely unknown, but much work has been done to unravel the signaling mechanisms, pathways and relationships governing osteogenesis [15,16]. In 2009, Colnot [17] provided direct evidence that the major sources for skeletal stem cells are the periosteum, endosteum, and bone marrow and that while each give rise to osteoblasts, only the periosteum gives rise to chondrocytes, implicating different cellular populations within each distinct microenvironment. The periosteum also contributes to the growth and healing of long bones, demonstrating important differences in cell populations within various anatomical locations [18]. Recent evidence demonstrates that Wnt16 knockout mice have lower cortical bone mass, but no changes to their trabecular bone mass [19], whereas prior reports provide evidence that Wnt10a is necessary for trabecular bone formation, but not for cortical bone formation or maintenance [20,21]. These studies, and others using Klotho, Src, and Sfrp4 null mice [22], demonstrate that osteoblasts and osteoclasts from different anatomical locations respond differently to ligands, trauma/disease, and treatments. This is also found clinically, where some therapeutics show different effects on long bones compared to vertebrae, or cortex versus trabeculae [23]. In sum, these studies suggest that osteoblast progenitors derived from these different locations may have disparate effects on bone remodeling and possibly cancer growth. This is a key nu- ance that may be understood before effective bone anabolic agents can be designed and targeted successfully.

Effects of osteoblasts on multiple myeloma

Unlike bone marrow MSCs, which support myeloma disease progression [24-26], evidence suggests that osteoblasts may suppress myeloma [27]. Osteoblast-derived growth factors play a large role in stimulating the growth of prostate cancers within the bone [28], raising the question of why this does not occur in myeloma. It is not clear if myeloma cells respond differently to these same osteoblast-derived factors, or if myeloma cells, like prostate cancer cells, actually benefit from osteoblasts, but proliferate even more strongly when they activate osteoclastic activity rather than osteoblastic activity. It is interesting that, although very rare, myeloma can also cause osteosclerotic lesions, without other symptoms of POEMS syndrome, suggesting again the possibility that osteoblastic activity may not necessarily be detrimental to plasma-cell-proliferative disorders [29-33].

As recently reviewed by Olechnowicz and Edwards [34], there are numerous other components of the host bone marrow that contribute to the pathogenesis of multiple myeloma, including fibroblasts, immune cells, adipocytes, endothelial cells, and osteoclasts. Contributions from
the sympathetic nervous system and abnormalities in the myeloma-associated extracellular matrix also can support multiple myeloma progression. Perturbation of the osteoblast can lead directly, and spontaneously, to myelodysplasia or AML [35,36], demonstrating the critical influence of the bone microenvironment on hematological malignancies.

Direct effects of osteoblasts on myeloma growth

Biology of direct effects of osteoblasts on myeloma cells

Osteoblasts have been reported to directly inhibit multiple myeloma cells in vitro. One group demonstrated that some osteoblastic cells (MC3T3-E1 pre-osteoblastic cells and bone marrow-derived stromal cells), when differentiated into mineralized osteoblasts, induce apoptosis and cell cycle arrest in myeloma cells (i.e., cells such as RPMI8226, U266, KMS-12, INA6, 5TGM1, and primary patient samples) [27]. Decorin, the main small leucine-rich proteoglycan produced by osteoblasts, has also been identified as an endogenous, osteoblast-derived factor that suppresses multiple myeloma cell growth and survival [37].

In general, however, there is controversy about the net effect of osteoblasts on myeloma cells, as osteoblasts also produce factors that could support myeloma growth, such as osteocalcin, osteopontin, fibroblast growth factors, and transforming growth factor beta family members, although direct studies on this are lacking. One study demonstrated that quiescent myeloma cells prefer to reside in the endosteal/osteoblastic regions of the bone marrow compared with the vascular regions or spleen, indicating that osteoblasts may play a unique role in maintaining myeloma cells within a specific niche [38]. Another study showed that osteoblasts may be either supportive or inhibitory of multiple myeloma cells, and interestingly, these effects were dependent on the patient source of myeloma cells [39,40]. A better understanding of the direct anti-myeloma effects of osteoblasts is mandatory before bone anabolic treatments can be used successfully to inhibit multiple myeloma progression.

In vitro challenges of studying osteoblasts and myeloma interaction

There are several challenges that must be overcome to understand the role of osteoblasts in impeding myeloma growth. First, in vitro coculture studies with plasma cells and osteoblasts are limited by the lack of relevant osteoblast cell lines. The human bone marrow stroma cell lines HS-5 and HS-27 do not differentiate into osteoblasts, and other cell lines that do mineralize, such as Saos2 [41] and MG-63 [42], are actually osteosarcoma rather than osteoblast cell lines. Certain cell lines, such as the human fetal osteoblastic cell line hFOB1.19 [43], which proliferate at 33.4 °C and differentiate at 39.4 °C or in osteogenic...
maintained. Most
This cycle is essential for maintaining bone mass and strength[53].
Increased osteoblastic activity leads to increased osteoclastic activity,
Osteoclasts
Indirect effects of osteoblasts on myeloma cells through interaction with
model the interactions between myeloma cells and any other cells, in
arti
to model the process of differentiation of MSCs into a mineralized, porous
are now become more established[24,51,52]. Tissue-engineered 3D
fl
oma cell growth relates to the
patient-derived osteoblasts are utilized.
changes that occur in patients, which are between myeloma cells and
myeloma-associated osteoblasts. Future studies may more accurately
understanding the relationships between these cells types if myeloma-
patient-derived osteoblasts are utilized.
A third challenge to studying the direct effects of osteoblasts on multi-
type myeloma is that, as with myeloma-derived MSCs [49], myeloma-
derived osteoblasts differ substantially from their healthy-donor coun-
terparts [50]. Specifically, their proliferation and osteogenic potential
are significantly inhibited and their expression of the CCL3 receptor
(CCR1) is signi
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are signi
creases that occur in patients, which are between myeloma cells and
myeloma-associated osteoblasts. Future studies may more accurately
understand the relationships between these cells types if myeloma-
Osteoclasts
The most well-documented osteoblast relationship in the bone is the
forward-feedback mechanism with osteoclasts known as remodeling.
Increased osteoblastic activity leads to increased osteoclastic activity,
which can then trigger recruitment of more osteoclasts and vice versa.
This cycle is essential for maintaining bone mass and strength [53].
The pathophysiology of myeloma-induced bone disease progressing
through the “vicious cycle” occurs when myeloma cells hijack the
normal bone remodeling process and skew the balance towards increased
osteolytic processes. This state of inhibited osteoblastic activity and in-
creased osteoclastic activity, stimulated through molecules such as
RANKL from myeloma cells and osteoblasts [54], leads to osteolytic le-
sions, weakened bone, pathological fracture, and a release of bone-
embedded growth factors that further promote tumor cell growth [2,
45].
Osteoclasts not only degrade bone matrix to release tumorigenic fac-
tors, but also directly promote the survival and proliferation of myeloma
activities through agents such as bisphosphonates may have the oppo-
site effect, due to the subsequent suppression of osteoblast function
in vivo, hence diminishing any potential osteoblastic anti-multiple my-
eloma action. Therefore, understanding the regulation of the timing, lo-
cation, and responses of osteoclasts to osteoblasts, and the reverse, is
Adipocytes
There is growing interest in understanding interactions between
bone and fat cells in normal physiology and disease, and the dynamic rel-
relationships between osteoblasts and marrow adipocytes are likely to af-
fect multiple myeloma within the microenvironment in numerous
ways. Previously, it was thought that obesity was associated with stron-
gier bones, but more evidence has surfaced that obesity and osteoporosis
share common genetic and environmental factors and that excessive fat
and obesity may not protect against osteoporosis but could, in fact, ac-
the osteogenic pathway, it may still appear as a supportive stromal
and could be exploited further. The two best options for osteoblast
models may be primary human osteoblasts [45], or primary bone
marrow-derived MSCs, which can be expanded and then induced to dif-
fentiate into mineralizing osteoblasts [24,46]. The challenge with
using differentiated MSCs to model osteoblasts is delineating the mo-
ment when an MSC becomes a “pre-osteoblast,” or has matured into a
fully differentiated osteoblast, or has overshoot the osteoblast stage to
become an osteocyte. If the MSC is not differentiated far enough down
the osteogenic pathway, it may still appear as a supportive stromal
cell, accelerating the growth of myeloma cells rather than inhibiting
them, as it is believed that osteoblasts may do in vivo. The mouse cell
line MC3T3-E1 [47] is a well-accepted albeit unique pre-osteoblast cell
line that undergoes linear osteogenic differentiation. Primary mouse
calvarial osteoblasts are also widely used [48], but studies with mouse
osteoblasts add some risk of missing human cell-specific signaling.
A second challenge for studying direct effects of osteoblasts on multi-
type myeloma is that, as with myeloma-derived MSCs [49], myeloma-
derived osteoblasts differ substantially from their healthy-donor coun-
terparts [50]. Specifically, their proliferation and osteogenic potential
are significantly inhibited and their expression of the CCL3 receptor
(CCR1) is significantly increased, which is one pathway contributing
to their decreased osteogenic capacity [50]. By studying interactions be-
tween normal osteoblasts and myeloma cells, we may not observe the
changes that occur in patients, which are between myeloma cells and
myeloma-associated osteoblasts. Future studies may more accurately
understand the relationships between these cells types if myeloma-
Hematopoietic niche and immune cell interaction with myeloma cells
Because the osteoblastic niche is also a site for hematopoietic stem
cell (HSC) and immune cell homing and homeostasis [70], osteoblasts
may inhibit multiple myeloma growth partially by supporting anti-
myeloma immune cell homing to the bone marrow. However, specific
types of osteoblasts may play different immune supportive roles, as it
appears that only a subtype of osteoblasts, those termed spindle-
shaped N-cadherin+/CD45- Osteoblasts (SNOs), located next to the
endosteal surface of bone, function to retain the so called Long-Term
(LT)-HSCs in a quiescent status [12]. The relationship between osteo-
blasts and immune cells is complex. For example, although sclerostin
null mice have high bone mineral density, they have increased B-cell
apoptosis due to decreased osteoblast-derived CXCL12, resulting from
increased Wnt signaling [71]. Moreover, although the immune system
in general suppresses multiple myeloma [72], not all immune cells
mediate this role. Regulatory T-cells and immunosuppressive myeloid-derived suppressor cells [73] are now being identified as important new targets that inhibit the immune response in multiple myeloma [74]. Interestingly, cellular immunity was found to be decreased in myeloma patients, including decreased ratio of CD4⁺/CD8⁺, DC1/DC2, and Th1/Th2 cells, as well as an increased ratio of regulatory T cells, and some of these metrics of immune function (CD4⁺/CD8⁺ ratio and CD4⁺/CD25⁺/CD3⁺T ratio) were significantly positively correlated with the quantity of osteoblasts [75]. Hence, the potential effects of osteoblast loss on multiple myeloma via inhibition of the immune system require further investigation.

Other cells in the osteoprogenitor lineage

MSCs, the osteoblast progenitors, and myeloma progression. Bone marrow-derived MSCs are osteoprogenitor cells capable of differentiating into osteoblasts, adipocytes, and chondrocytes, among other cells, and much research has demonstrated their support of multiple myeloma adhesion, growth, and drug-resistance in vitro [24–26,76]. The expression of signaling cytokines, extracellular matrix factors, and adhesion molecules is the basis for their important role in myelomagenesis, bone marrow homing, and proliferation [24,77–80]. MSCs from myeloma patients are abnormal in terms of osteogenic differentiation, proliferation, gene expression, and other functions [24,49,81]. By inhibiting osteogenic differentiation of MSCs, multiple myeloma cells may be cleverly retaining a population of cells known to support their survival while inhibiting the maturation of osteoblasts, which generate bone matrix and have suppressive effects on myeloma cells.

In vitro, both osteoblasts and osteocytes can support MSC osteogenesis, in part due to soluble osteogenic cytokines [82]. However, osteoblasts seem to support an initial proliferation of MSCs and a delayed differentiation, while osteocytes promote an initial osteogenic differentiation [82]. However, contrasting the HSC niche roles of osteoblasts, it is less well understood how osteoblasts affect MSC homing, quiescence, and differentiation in vivo in healthy bone marrow, and even less so in myeloma-infiltrated bone marrow.

One study found that human placenta-derived adherent cells (PDACs), a type of MSC, inhibit H929 myeloma cell growth in a subcutaneous tumor model (tumor cells grown subcutaneously, later injected with PDACs). However, when the tumor was grown instead in a rabbit bone that was implanted subcutaneously into a SCID mouse (the SCID-rab model), the injected PDACs inhibited growth of H929 myeloma cells [82]. This may indicate that MSCs in multiple myeloma are dependent on the presence of the bone microenvironment to show anti-myeloma effects. Hence, osteoblasts may be essential regulators of the osteoprogenitor phenotype, and they may support a more anti-myeloma phenotype in MSCs.

Osteocytes, the osteoblast descendant, and myeloma progression. Upon becoming encased in osteon, osteoblasts become osteocytes and play a key regulatory role in bone homeostasis, osteoclast activity, and osteoblast regulation. Osteocytes are the mechanosensing cells that reside in lacuna and connect with each other through dendritic processes extending through lacunar-canaliculuar networks. They have been considered switchboard operators, as they direct a number of different signals that control cells behavior. For example, they extend processes into the vasculature within the bone, and out into the osteoblast-lined surfaces of the marrow and peristeum. With age, these lacunar-canaliculuar networks become compromised with large sections of bone lacking live osteocytes, suggesting one mechanism whereby diseases that have increasing incidence with age, such as myeloma, may have enhanced growth potential and progression. In several cancers, it has been shown that osteocytes affect tumor evolution through a number of local signaling and endocrine mechanisms [12,83,84].

The relationship between osteoblasts and osteocytes is complicated by the addition of myeloma cells. Since osteoblasts give rise to osteocytes as they become encased in bone matrix, myeloma inhibition of osteoblasts and osteoblastic activity may be a major cause of the decreased osteocytes observed in clinical samples [85]. However, since osteocytes are one of the major producers of sclerostin, a Wnt antagonist, a decrease in osteocytes for any reason typically decreases sclerostin levels, which then stimulates osteoblastic activity to produce a stable bone equilibrium. Unfortunately, the net balance in multiple myeloma patients is osteolysis and loss of osteoblasts/osteocytes; the attempt by the bone to normalize itself is futile and eventually toppled by the burden of osteoclastic activity. For more on the roles of osteocytes in multiple myeloma, refer to the elegant review by Roodman et al. [86].

Canopy-lining cells, the osteoblast cousin, and myeloma progression. Although similar in lineage to active, bone-matrix-secreting osteoblasts, canopy lining cells are quiescent, bone marrow protecting cells. These cells isolate areas of turnover to create a tightly connected, single-cell wide physical barrier to seal-off the osteoclast/osteoblast resorption pit from the marrow [87]. The relatively flat, elongated cells immunostain for osteoblast markers osteocalcin, osteonectin, pro-collagen type I (PINP), pro-collagen type III (PICP) and NCAM (CD56), demonstrating that the cell originates from the osteoblast lineage [88]. Importantly, they are Ki-67 negative (hence, non-proliferative) and negative for lymphocytic and monocytic markers. How these cells differ, if at all, from the more classically described quiescent bone lining cells remains to be delineated.

Canopy lining cells may play an important role in the dysregulation of bone remodeling in general [89] and could be a novel target cell type in multiple myeloma. Osteoblasts seem to require these cells to properly lay down matrix; as multiple myeloma biopsies analyzed for the presence of these canopies over the bone remodeling compartment (BRC) demonstrated frequent disruptions in 66% of the biopsies. Importantly, frequent disruption (holes) in the canopies correlated with extensive resorption without matrix reconstruction, not observed in biopsies with normal, intact canopies over the BRCs [83,88,90]. Only in multiple myeloma bone surfaces with disrupted canopies did the researchers observe an absence of coupling between bone formation and resorption in patient biopsies [90]. It remains to be determined if BRC canopy destruction in multiple myeloma is a cause or result of deficient bone formation. The microanatomical structures may function through multiple unclear mechanisms (e.g., exerting physical constraints for cells or chemooattractants or acting as anchorage points for certain progenitors), but it is evident that their disruption results in direct physical contact between myeloma cells, osteoclasts, and osteoblasts, and coincides with the occurrence of osteolytic lesions.

Systemic effects

Osteoblasts, osteoclasts, and osteocytes contribute not only to local modifications of bone but also to systemic changes in whole body homeostasis through secretion of specific peptides and growth factors. Traditionally, the action of these cells define the bone as an endocrine organ, responding to hormones and soluble signaling molecules such as estrogen via estrogen receptor α (ERα) [91], calcium, PTH, 1, 25-Dihydroxycholecalciferol, and vitamin D, which communicate with other endocrine organs throughout the body, such as the thyroid, parathyroid, pituitary glands, adrenal glands, and pancreas, as reviewed elsewhere [92]. More recently, the secretion of metabolically active peptides such as osteocalcin has been shown to regulate insulin sensitivity and secretion. During states of high bone turnover, the release of matrix and cell-derived undercarboxylated osteocalcin impact adipose tissue sensitivity to insulin which in turn could release adipokines that further modulate myeloma progression [93]. On the other hand, bone metabolism may appear normal, as judged by biochemical measurements such as urinary excretion of calcium, hydroxyproline, and n-telopeptide, but significant bone destruction may be present. Hence, experiments to alter the local bone milieu, to dissect the roles of osteoblasts on...
myeloma growth, must be performed in conjunction with monitoring other systemic changes resulting from alterations of osteoblasts. Measuring bioactive factors that are liberated during bone destruction may help quantify bone turnover, but cannot be used as a definitive readout, due to a variety of confounding systemic effects resulting from osteoblast stimulation or inhibition [6].

In vivo models of osteoanabolism as a therapeutic approach to multiple myeloma

Osteoanabolic treatment is defined as any treatment that stimulates osteoblastic activity and bone formation. As a therapy for multiple myeloma, this strategy has yielded conflicting conclusions, with in vivo efficacy depending on the model system and treatments used. Some mouse models, such as the patient xenograft SCID-hu model, demonstrated that osteoanabolic treatments hold promise for inhibiting multiple myeloma, although results were highly variable and patient-specific [40]. There is mounting evidence that the anti-myeloma proteasome inhibitors carfilzomib [94] and bortezomib [95] have bone anabolic effects on bone and induce osteogenic differentiation of MSCs, which may contribute to their anti-myeloma effects [96], but concrete evidence remains elusive to demonstrate that these agents can produce anti-myeloma effects via changes in the bone microenvironment. The use of anti-resorptive agents, such as bisphosphonates, cathepsin K inhibitors, or RANKL inhibitors [54], in combination with osteoanabolic agents, may maximize the use of the bone microenvironment to inhibit myeloma. Collectively, these results highlight the need for better in vivo models and deeper understanding of exactly how we predict osteoanabolic treatments may function to inhibit multiple myeloma.

Anti-sclerostin and anti-DKK1 antibodies also have osteoanabolic effects in preclinical models and in clinical trials and are currently under investigation for the treatment of osteoporosis and osteolytic disease [97,98]. Anti-sclerostin treatments may prove useful for osteolytic cancers in general, but especially for myeloma, since myeloma cells secrete sclerostin that inhibits osteoblast activity [99]. Anti-DKK1 treatments may also be viable mechanisms for inhibiting myeloma bone disease, as DKK1, a canonical Wnt pathway inhibitor, is overexpressed in myeloma cells and patient serum [100], and DKK1 levels correlate with the extent of lytic bone disease [101]. Anti-DKK1 antibody therapy has also been shown to significantly increase osteoblast bone formation and bone mineral density in both murine and human healthy and multiple myeloma models [97,102,103]. Anti-DKK1 therapy in myeloma also inhibits osteolysis in multiple myeloma SCID-rab models (SCID mouse with rabbit bone subcutaneous implantation) [104]. However, the rates of success at lowering IgG levels or decreasing tumor growth rate, measured by tumor size, were only 36% (4/11) and were patient specific, suggesting that bone anabolic treatments may work only for a subset of myeloma patients [104]. A different myeloma model used to test anti-DKK1 antibodies is the SCID-hu model with fetal bone chips in a SCID mouse, injected with INA-6 myeloma cells. In this model, treatment with the Novartis antibody BHQ880, which neutralizes both human and murine DKK1, promoted osteoblastogenesis and decreased tumor burden, as measured by in vivo IL6 levels [102].

Other bone anabolic agents, including dasatinib, a multitargeted tyrosine kinase inhibitor [105], and soluble decoy receptors of activin A, a known osteoclast activating factor [106], also inhibit multiple myeloma, suggesting their clinical utility and supporting the hypothesis that increasing bone volume and osteoblast number is a practical method for inhibiting multiple myeloma [107,108]. Similarly, TGF-β3, a potent inhibitor of terminal osteoblast differentiation abundant in the bone matrix, has also been identified as a novel target. Anti-TGF-β3 therapies are able to restore osteoblast differentiation suppressed in MM conditions in vitro and suppress myeloma cell growth within the bone marrow (using the SCID-rab/INA6 myeloma model) while preventing bone destruction in myeloma-bearing animal models [27]. This study demonstrated that osteoblasts, defined as mineralized MC3T3-E1 cells, were able to induce apoptosis and G1 cell cycle arrest in 5TGM1 myeloma cells, although the exact mechanisms by which osteoblasts potentiated these effects were not explored [27].

In vivo studies using daily administered parathyroid hormone (PTH) in SCID-rab and SCID-hu mouse models demonstrated that PTH treatment increased bone mineral density and reduced tumor burden [109]. PTH also increased the number of osteoblasts and other bone formation parameters and pre-treatment with PTH before injecting tumor cells also increased bone mineral density and delayed tumor progression. This research supports the hypothesis that an increase in bone mineral density and osteoblast number may provide a net anti-myeloma effect. Importantly, PTH can clinically lead to increased bone formation and osteoblast activity within the first 6 months of treatment. However, with longer-term PTH administration, osteoblast activation slows, and importantly, bone resorption increases significantly. Theoretically, this could compromise any positive effects of this approach for slowing myeloma progression [110]. Thus, osteoanabolic therapies for bone utilize a range of different approaches and target pathways, nicely summarized in a recent review [111]. However, it still remains controversial whether reported anti-tumor effects of bone-modulating therapies are clinically significant [112]. The current challenge in myeloma therapeutics thus becomes not only to develop biologic agents that have the desired effect of killing cancer cells but also to prevent any rebound or compensation that could make the skeletal changes worse.

Clinical studies have shown that the treatment of multiple myeloma patients with bisphosphonates significantly overall survival and progression-free survival [113,114]. Zoledronic acid and bortezomib both have anti-myeloma effects. Zoledronic acid is thought to directly impact myeloma cells, and bortezomib additionally may induce “pro-bone” mechanisms, including increasing osteogenic differentiation and inhibiting osteoclasts [95,115]. In fact, based on these studies, a phase II clinical trial recently completed in smoldering multiple myeloma patients treated with low dose bortezomib had a primary endpoint, “to evaluate the bone anabolic effect of bortezomib in patients with smoldering myeloma” and a secondary endpoint “to evaluate the effect of bortezomib on the natural history of smoldering myeloma” [116]. Another interesting phase II trial in the recruitment stage aims to test Sotatercept, an activin-A antagonist that interferes with the SMAD pathway. This signaling network, when activated, can lead to increased bone formation and anti-tumor activity in multiple myeloma [117] and bone anabolic improvements in bone mineral density and in bone formation [118]. The effects of Sotatercept on patient-specific outcomes such as skeletal-related events (i.e., fractures, impaired healing, bone pain) as well as delayed-progression, or progression-free or overall survival, remain to be elucidated. Results from these ongoing and future trials may open the door for similar treatments to be tested in patients with early stage or even overt myeloma.

Future directions and conclusions

One of the new directions in osteoblast-myeloma research, and in tumor-host interaction studies in general, is the use of CRISPR-Cas9 knockout technologies [119]. With this technology, researchers have already demonstrated an ability to more specifically target genes such as Ikaros family zinc finger proteins 1 and 3 (IKZF1 and IKZF3) in myeloma and hence dissociate the anti-tumor and teratogenic activities of thalidomide-like drugs [120]. The use of CRISPR technologies for modulating host osteoblasts and bone marrow cells would provide abundant information regarding the roles of different genes in bone cells and could suggest novel mechanisms for modulating the bone microenvironment to induce a less hospitable environment for the growth of cancer cells. Also, if investigators can overcome the obstacles and potential off-target effects of microRNA delivery, these may be a potential novel osteoanabolic treatment. “Osteogenic microRNAs” have been identified [121] and are currently under investigation for in vivo efficacy. Interestingly, some of these have been identified as differentially expressed in
multiple myeloma versus healthy MSCs and capable of functionally rescuing MSCs for their ability to produce bone matrix [24].

Multiple myeloma is considered by some a prototype for metastatic bone disease although there are clear phenotypic distinctions from other malignancies such as prostate cancer. Nevertheless, studies of osteoblastic function in myeloma could be extrapolated to other conditions that have classically been considered osteolytic, such as metastatic breast cancer [122–125]. This review described the multitude of ways in which osteoblasts may function to support or inhibit myeloma growth, and discussed new potential targets in the relationship between osteoblasts and myeloma cells to treat or prevent multiple myeloma. Osteoblasts act as an important hub of activity, affecting other cells within the bone marrow niche and mediating both direct and indirect effects on myeloma cells (Fig. 1). The future of bone anabolic treatments for anti-myeloma therapy is bright, but to optimize the use and design of such agents, it will be critical to view the osteoblast within a larger context and to visualize its interactions with other cells in the bone microenvironment and roles in whole body homeostasis.

Author contributions

MRR conceived and wrote the manuscript; LL designed and created the figure; LCJ, CJR, and IMG provided intellectual input and edited the manuscript.

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Conflicts of interest

The authors declare no competing financial interest. Ghbrial: BMS: Advisory Board; Celgene: Advisory Board, Advisory Board Other; Millennium: Advisory Board, Advisory Board Other; Onyx: Advisory Board, Advisory Board Other.

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