Published in final edited form as:
Semin Hematol. 2012 July ; 49(3): 243–248. doi:10.1053/j.seminhematol.2012.04.011.

Proteasome inhibitors and bone disease

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Abstract

Bone disease in patients with multiple myeloma (MM) is characterized by increase in the numbers and activity of bone-resorpting osteoclasts and decrease in the number and function of bone-formation osteoblasts. MM-triggered inhibition of bone formation may stem from suppression of Wnt/β-catenin signaling, a pivotal pathway in the differentiation of mesenchymal stem cells (MSC) into osteoblasts, and regulating production of receptor activator of nuclear factor-kB ligand (RANKL)/osteoprotegerin (OPG) axis by osteoblasts. Proteasome inhibitors (PIs), such as bortezomib (Bz) induce activation of Wnt/β-catenin pathway and MSC differentiation toward osteoblasts. PIs also suppress osteoclastogenesis, possibly through regulating multiple pathways including NF-κB, Bim and ratio of RANKL/OPG. The critical role of PI in increasing osteoblast function and suppression of osteoclast activity is highlighted by clinical evidence of increases in bone formation and decreases in bone resorption makers. This review will discuss the function of PIs in stimulating bone formation and suppression of bone resorption, and the mechanism underlying this process that leads to inhibition bone disease in MM patients.

Introduction

Bone disease occurs in up to 80% of patients with multiple myeloma (MM).[1] MM-associated osteolytic bone destruction is characterized by imbalanced bone turnover, with increased bone resorption and decreased bone formation. Augmentation of bone resorption results from interaction of MM cells with osteoclasts, leading to stimulation of osteoclast formation and function. Several factors produced directly by MM cells, bone marrow stromal cells, or as a consequence of osteoblasts interaction with MM cells regulate osteoclast activity. [2,3] Prominent among these is the RANKL/OPG axis, which plays a key role in osteoclast formation and activity and is regulated by the Wnt/β-catenin signaling pathway in osteoblast. In contrast to enhanced bone resorption, reduced bone formation in MM patients is caused by impaired osteoblast differentiation.[1,4] Current evidence suggests that MM cells interrupt several important signaling pathways, including the Wnt/β-catenin pathway and Runx2 activity, which are required for osteoblast differentiation and bone formation.

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Conflict-of-interest disclosure: Ya-Wei Qiang declares no conflicting financial interests.

Besides its effect on myeloma cells, [5] inhibition of the ubiquitin-proteasome pathway by PIs has anabolic effect on bone formation. [6,7] The ubiquitin proteasome pathway is responsible for the breakdown of a large variety of cell proteins, including β-catenin, a key protein for osteoblast development and NF-κB pathway activation by RANKL, essential for osteoclast development. Given the importance of proteasome-mediated β-catenin degradation in osteoblast and osteoclast development, inhibition of the ubiquitin proteasome pathway contributes to combating MM-associated bone disease by regulating bone formation and bone resorption.

Studies using an in vitro mouse bone organ culture and an in vivo mouse model have identified the potential pivotal role of PIs in regulating osteoblast differentiation and bone formation under physiological conditions. [8] Chemical compounds, such as PS1, that bind to the catalytic β-subunits of 20S proteasome and suppress proteasome activity stimulated bone formation in bone organ culture. These findings have been corroborated by in vivo studies, illustrating that systemic administration of PS1 to mice for 5 days resulted in significant increase in bone volume and over 70% increase in bone formation rate. [8]

Several independent in vitro cell culture studies reported that Bz induces osteoblast differentiation from MSC isolated from bone marrows of either normal donors or from MM patients. [9–11] In the presence of low concentration (2nM) of Bz in the culture media for 48 hours, a significant increase in the number of pre-osteoblasts was seen, along with increased expression of the bone formation makers osteocalcin and collagen I mRNA. [9] Bz treatment also induced matrix mineralization in human MSC cells during differentiation. [11] The beneficial effect of Bz on bone formation was confirmed in a mouse bone organ culture system [12] and in an in vivo mouse model. [10] Moreover, in the SCID-rab myeloma model, treatment with Bz led to an increase in bone mineral density (BMD). [13]

Several independent clinical studies [6,14-17] reported significant increases in serum levels of the bone formation makers alkaline phosphatase (ALP) [7] and osteocalcin in MM patients responding to Bz treatment, thus validating the findings from in vitro studies and animal models. A recent clinical study in patients with relapsed and refractory myeloma demonstrated that carfilzomib, a novel PI that selectively inhibits the N-terminal threonine protease activity of the proteasome has anabolic effect on bone formation similar to that of Bz.[18]

Osteoblast Inhibition in MM

MM-induced suppression of bone formation is characterized by suppression of osteoblast differentiation from MSC.[19,20] Under the regulation of signaling pathways and transcriptional factors, MSC differentiate into osteoblasts, adipocytes, muscle cells, or chondrocytes.[21] Interaction of MSCs with myeloma cells diminishes MSC differentiation into osteoblasts that secret collagen and cause its mineralization with calcium salts and phosphorus to form bone tissue. Specifically, in cocultures of myeloma cells with osteoblast precursors such as the cell line MG63 or MSC from bone marrow of MM patients, a reduction in osteoblastic makers such as ALP, osteocalcin and collagen I were observed.[19,22,23] Interaction with myeloma cells also suppresses osteoblast proliferation,[24] and induces osteoblast apoptosis.[20] Recent studies provided insight into molecular mechanisms responsible for inhibition of osteoblast differentiation and bone formation in MM; [25,26] most prominent were MM-suppression of the Wnt/β-catenin signaling pathway and of Runx2 /Cbfa1 activity.

Suppression of Wnt/β-catenin Pathway Impairs Osteoblasts in MM

Many Wnt effects are mediated through β-catenin, which plays a critical role in regulating differentiation of osteoblast from MSC, [25] and in myeloma pathogenesis.[27,28] β-catenin is a short-lived protein, and intracellular and nuclear levels of this protein are mainly regulated by the ubiquitin-proteasome pathway. [29]

Upon interaction of Wnt proteins with frizzled (Fz) receptors and the low-density lipoprotein-receptor-related protein 5/6 (LRP5/6) co-receptors, β-catenin protein is stabilized, accumulates in the cytoplasm, and translocates to the nucleus. Nuclear localization and association of β-catenin with T-cell factors (TCF)-1, -3, and -4 and lymphoid enhancer-binding factor1 (LEF1) leads to transcriptional activation of target genes that regulate many cellular processes. [29] In the absence of Wnt ligand, β-catenin in the cytoplasm is phosphorylated by GSK3β in a complex that includes axin, the adenomatous polyposis coli (APC) protein, and casein kinase I alpha (CKIα). Phosphorylated β-catenin subsequently is ubiquitinated by ubiquitin ligase and targeted for degradation by the 26S proteosome.[30]

Studies from our laboratory and others' revealed that activation of Wnt/β-catenin signaling acts as a major pathway for guiding MSC differentiation osteoblasts. [31,32] Elevated serum levels of the Wnt antagonist Dkk1 are associated with bone lesion in MM patients. [33–37] Blocking Wnt/β-catenin pathway in MSC by rDKK1 or with serum from MM patients with high concentrations of DKK1, inhibit MSC differentiation into osteoblasts.[37,38] In contrast, increasing Wnt signaling by inhibition of DKK1 with neutralizing anti-DKK1 antibody, by administration of Wnt3a protein, or by injecting Wnt3a-expressing MM cells, increased osteoblast numbers and bone mineral density (BMD) in the SCID-hu myeloma model. [39,4041,42] In addition to myeloma cells secreted DKK1, soluble frizzle-related protein-2 (sFRP2), may also be involved in MM-disruption of Wnt/β-catenin-mediated bone formation. [43]

Activation of Wnt/catenin Signaling by PIs in MM

PI induced activation of Wnt/β-catenin signaling independent Wnt ligands is one of critical mechanisms for their anabolic effect on osteoblast differentiation and bone formation. An E-cadherin pull-down assay, which selectively retrieves the transcriptionally active, free β-catenin, [38,44-] was used to demonstrate that Bz significantly increases the active form of β-catenin in the cytoplasm and nuclei of MSC, leading to enhanced TCF activity. This Bz-induced TCF activity was further validated by co-expressing dominant TCF1 and TCF4.[11] Although multiple Wnts are expressed in human MSC (Qiang unpublished observations), Bz did not increase expression of Wnt ligands. These findings suggest that Bz activates β̃catenin/TCF signaling independently of Wnt ligands. Early studies using immunoblotting, suggested that Bz does not stabilize β̃catenin. [9] It is possible that while E-cadherin pull-down assays retrieve only the active form of β̃catenin, immunoblotting analysis identifies also the inactive forms. A critical role of Bz activated β-catenin/TCF signaling in inducing osteoblast differentiation has been corroborated by evidence that blockage of TCF activity by expressing dominates negative TCF1 and TCF4 abrogates Bz induced increase in matrix mineralization, a prominent maker for bone formation. [11] In addition to direct stabilization of β-catenin, Oyajobi et al reported that Bz also suppresses DKK1 mRNA expression in murine calvaria and bone marrow-derived stromal cells. [12] Clinical studies demonstrated that Bz treatment of patients with MM is associated with reduced levels of circulating DKK1.[45]

So far the exact mechanism of the suppression of Dkk1 mRNA expression by Bz is still unknown. Inhibition of proteasome ubiquitin pathway may promote activation of the β-

catenin/TCF pathway, thus overcoming the negative effect of DKK1 on osteoblastogenesis, and resulting in increased osteoblast differentiation and inhibition of myeloma-associated bone resorption.

Enhancement of Runx2/Cbaf Activity by PIs in MM

Suppression of activity of Runx2/Cbfa1 by MM cells has been implicated in MM-triggered inhibition of osteoblast differentiation and bone formation[23]. Runx2/Cbfa is an osteoblast-specific transcription factor, determining MSC differentiation toward early stage osteoblasts in a murine model. [26] In response to factors such as BMP-2, expression of Runx2 mRNA and protein is upregulated.[46] BMP-2 also promotes osteoblast differentiation through phosphorylation of a family of Smad proteins, which in turn activate Runx2 /C-bafa1 transcriptional activity. [47] In mouse osteoblasts, increases in BMP-2 mRNA can be induced by PIs such as PS1[8] and Bz [12].

In addition to transcriptional regulation, Runx2/Cbfa1 is regulated at the post-translational level; Runx2 protein is degraded by smurf1, an E3 ubiquitin ligase, through the ubiquitin-proteasome pathway. [47] Thus, by increasing BMP-2 transcription, which subsequently leads to Runx2 activation, and simultaneously inhibiting Runx2 degradation, PIs should increase Runx-2 activity. Indeed, Mukherjee et al reported that Runx-2 protein increased in response to Bz in mouse MSC. [10] Unlike in the murine system, where dynamic alteration of Runx2/Cbaf1 at both transcriptional and protein level is regulated during osteoblast differentiation, in human, regulation of Runx2/CBfa1 activity, rather than of its mRNA and protein seems to be critical for controlling human osteoblast differentiation.[23,48] Consistent with findings that Runx2/Cbfa1 activity is required for human MSC differentiation to osteoblasts, decreased Runx2/Cbfa1 activity was observed in osteoblast progenitors co-cultured with either MM cell lines or primary plasma cells from MM pateints.[23] This MM-induced suppression of Runx2/Cbfa1 activity seems to reflect, at least partially, interactions between MM cells and osteoblast progenitors via the adhesion molecule VLA4. [23] The downstream signals activated by MM cells adhesion to osteoblast progenitors via VLA4 that are responsible for reduced Runx2/Cbfa1 DNA binding activity are still unknown.

It should be noted that Bz could also induce osteoblast differentiation in *Runx2* null-mice. [10] Thus, Bz may also influence other alternative pathway(s) to regulate MSC differentiation.

Additional mechanisms may contribute to osteoblast suppression in MM. For example, IL3 and IL7 have been reported to suppress osteoblast function. [49] However, the mechanism by which they suppress osteoblasts is unclear.

Enhanced Osteoclast Activity in MM

In addition to suppressing osteoblast development, MM cells stimulate osteoclast differentiation and activity. [2,3,50] Myeloma cells stimulate osteoclast formation and activity directly, by producing various cytokines, also known as osteoclast–activating factor (OAFs), and indirectly by inducing bone marrow stromal cells or osteoblasts to produce OFAs. [2,3,50] The common OFAs include interleukin-3 (IL3), IL6, macrophage inflammatory protein (MIP-1α) and RANKL. RANKL binds to its receptor RANK and is required for osteoclast differentiation and function. This binding is inhibited by OPG, [51] a naturally occurring decoy receptor that competes with RANK for binding of RANKL.[52] The balance of RANKL/OPG plays a critical role in controlling osteoclastogenesis. MM cells stimulate expression of RANKL and suppress expression of OPG by osteoblasts or their progenitors.[53]

Inhibition of Osteoclast Activity by PIs in MM

Zavrski et al demonstrated that proteasome inhibition by the PIs MG132 and MG-262 suppresses RANKL-mediated human osteoclast precursor differentiation and the bone resorption ability of mature osteoclasts, and suggested inhibition of RANKL induced activation of NF-κB via the increased stabilization of the NF-B inhibitor, IκB, as a possible mechanism. [54] This was also supported by studies demonstrating that treatment of the osteoclast-like cell line RAW264.7 with MG132 attenuated RANKL-mediated NF-κB activity by stabilizing p62, CYLD, and IκBα, all negative regulators of RANKL-mediated NF-kB activation. [55] However, recent studies addressing this question reported that Bz had no inhibitory effect on NF-κB activity despite its significantly increasing IκBα protein levels in preosteoclasts.[56]

Metzler reported that Bz abrogates human osteoclast precursor differentiation into tartrate-resistant acid phosphatase (TRAP) expressing mature osteoclasts, and decreases osteoclast activity. [56] Breitkreautz et al also reported that Bz inhibits osteoclastogenesis, as evidenced by decrease in αVβ3-integrin/TRAP positive osteoclasts and reduced bone resorption. [57]

In agreement with suppression of osteoclast activity by PI in vitro and in myelomatous mice, clinical studies provide the evidence that Bz may directly suppress osteoclastogenesis and bone resorption.[9,45,58,59] Terpos and colleagues reported a significant decrease in serum levels of the osteoclast marker tartrate-resistant acid phosphatase isoform-5 (TRACP-5b), and carboxy-terminal telopeptide of Type-I collegen crosslinks (CTX), a specific marker of bone resorption, after treatment of MM patients with Bortezomib for 3 months.[59] In agreement with these finding, Uy et al demonstrated a reduction in the urinary excretion of amino-terminal collagen crosslinks (NTX) in thirty-nine patients with MM following administration of Bz for longer than 6 months. [60] It should be noted that such inhibitory effect on osteoclastogenesis was observed after long term (3 to 6 moths) Bz therapy, where Bz also diminishes myeloma burden, which may be responsible for the decreases in osteoclast activity and function. Thus, these studies could not distinguish whether Bz's effect on osteoclasts was direct, or indirect through reduction of MM. Boissy et al offer an argument for the direct influence on osteoclast activity, as they reported significantly decreased levels of serum CTX and urine NTX in MM patients within 24 hours following Bz administration.[58]

Suppression of β-catenin /TCF Signaling causes an imbalance in RANKL/ OPG in MM

Recent studies identified OPG and RANKL as target genes of TCF, responding to activation of Wnt/β-catenin pathway.[61–63] In embryonic carcinoma cells, increased canonical Wnt signaling activity upregulates OPG mRNA expression.[64] Studies using *in vivo* murine models demonstrated that enhanced Wnt signaling, either by over expression of β-catenin [61] or deleting APC, a component of the complex leading to phosphorylation of β-catenin, resulted in reduced osteoclastogenesis.[62] In these mice, osteoblasts with increased Wnt signaling were found to express reduced levels of RANKL and high levels of OPG, whereas osteoblasts with reduced Wnt signaling had a reduced expression of OPG. Recent studies demonstrated that overexpression of Dkk1 or a dominant negative TCF4 in osteoblasts leads to increased RANKL expression and decreased OPG production. [32,44,65] Conversely, increasing Wnt signaling in the bone marrow microenvironment by administration of neutralizing DKK1 antibody [33,40,42] or increased Wnt3a expression in either bone marrow or in myeloma cells reduced osteoclast numbers and attenuated MM-induced bone resorption.[39] It is interesting to note that, unlike Wnt3a-activated β-catenin/TCF signaling that promotes OPG expression in osteoblasts, [32,44,65] activation of β-catenin/TCF signaling

by Bz does not lead to increased OPG secretion by osteoblasts. It is possible that Bz may also stabilize negative regulators for βcatenin- mediated regulation of OPG/RANKL secretion.

Other Molecular Mechanisms Underlying Action of PIs in Osteoclasts

Recent studies suggest that Bz suppresses p38 MAKP activity, which is critical for osteoclast development, [56,57] and its upstream factor TRAF6. [66] Additionally, PI inhibition of degradation of the pro apoptotic protein Bim by the ubiquitin-proteasome pathway, thus causing osteoclast apoptosis, has also been suggested as a mechanism of Bz anti-osteoclast effect. [67,68] In addition to their direct suppressing effect on osteoclast formation via activation of Bim and IkB in osteoclasts, PIs have also been implicated in regulating factors associated with activation of osteoclasts. Bz diminishes secretion of IL6, an important osteoclastogenic factor, by MM-derived endothelial cells. [1,69]

Conclusion and Perspectives

Bone disease in patients with MM is characterized by suppressed bone formation and enhanced bone resorption. Myeloma cells inhibit bone formation and enhance bone resorption by producing or inducing cells in their microenvironment to secrete factors that suppress bone formation and stimulate bone resorption. These factors deregulate several signaling pathways that control osteoblast differentiation from MSC or osteoclast formation and function. Among these factors, suppression of the Wnt/β-catenin pathway by MM-derived Dkk1 and sFRP2 affect both decrease in bone formation and increases of bone resorption. Activation of Wnt/β-catenin pathway is required not only for osteoblast differentiation and bone formation, but also for regulating osteoclast formation and bone resorption through manipulating osteoblast production of OPG/RANKL. β-Catenin, a key factor in the Wnt signaling pathway, is degraded by the ubiquitin-proteasome pathway when Wnt signaling is absent or blocked. Inhibition of the ubiquitin proteasome pathway by PIs results in activation of the β̃-catenin/TCF pathway by blocking β̃-catenin degradation, thus overcoming Wnt suppression. Additionally, PI-mediated increased *Runx2* activity may contribute to inducing osteoblast differentiation and bone formation. Clinical data support the critical role of Bz as potential anabolic effector on bone formation in MM patients who respond to Bz. In contrast to their anabolic effect on bone formation, PIs suppress bone resorption through direct and indirect inhibition of osteoblast formation and function. PIs directly suppress osteoclast formation and function by inhibiting RANKL activated NF-κB. Inhibition of Bim by PIs is also responsible for inducing apoptosis in osteoclast. Bz directly suppresses osteoclast activity, although the decrease in bone resorption in MM patients is only transient, rather than permanent. Bz abolishes Dkk1 expression, but does not promote OPG production in osteoblasts, as does Wnt3a. It remains to be determined what causes the different effects of Wnt3a and Bz, given they both activate β-catenin/TCF signaling and have a similar role in regulating bone formation.

Acknowledgments

This research was supported by MMRF (YWQ)

References

1. Roodman GD. Pathogenesis of myeloma bone disease. Blood Cells Mol Dis. 2004; 32:290–292. [PubMed: 15003820]
2. 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; 98:3527–3533. [PubMed: 11739153]

3. Pearse RN, Sordillo EM, Yaccoby S, 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; 98:11581–11586. [PubMed: 11562486]
4. Bataille R, Chappard D, Marcelli C, 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; 7:1909–1914. [PubMed: 2585025]
5. Richardson PG, Mitsiades C, Hideshima T, Anderson KC. Bortezomib: proteasome inhibition as an effective anticancer therapy. Annu Rev Med. 2006; 57:33–47. [PubMed: 16409135]
6. Terpos E, Sezer O, Croucher P, Dimopoulos MA. Myeloma bone disease and proteasome inhibition therapies. Blood. 2007; 110:1098–1104. [PubMed: 17494860]
7. Zangari M, Esseltine D, Lee CK, et al. Response to bortezomib is associated to osteoblastic activation in patients with multiple myeloma. Br J Haematol. 2005; 131:71–73. [PubMed: 16173965]
8. Garrett IR, Chen D, Gutierrez G, et al. Selective inhibitors of the osteoblast proteasome stimulate bone formation in vivo and in vitro. J Clin Invest. 2003; 111:1771–1782. [PubMed: 12782679]
9. Giuliani N, Morandi F, Tagliaferri S, et al. The proteasome inhibitor bortezomib affects osteoblast differentiation in vitro and in vivo in multiple myeloma patients. Blood. 2007; 110:334–338. [PubMed: 17371942]
10. Mukherjee S, Raje N, Schoonmaker JA, et al. Pharmacologic targeting of a stem/progenitor population in vivo is associated with enhanced bone regeneration in mice. J Clin Invest. 2008; 118:491–504. [PubMed: 18219387]
11. Qiang YW, Hu B, Chen Y, et al. Bortezomib induces osteoblast differentiation via Wnt-independent activation of beta-catenin/TCF signaling. Blood. 2009; 113:4319–4330. [PubMed: 19196662]
12. Oyajobi BO, Garrett IR, Gupta A, et al. Stimulation of new bone formation by the proteasome inhibitor, bortezomib: implications for myeloma bone disease. Br J Haematol. 2007; 139:434–438. [PubMed: 17910634]
13. Pennisi A, Li X, Ling W, Khan S, Zangari M, Yaccoby S. The proteasome inhibitor, bortezomib suppresses primary myeloma and stimulates bone formation in myelomatous and nonmyelomatous bones in vivo. Am J Hematol. 2009; 84:6–14. [PubMed: 18980173]
14. Heider U, Kaiser M, Muller C, et al. Bortezomib increases osteoblast activity in myeloma patients irrespective of response to treatment. Eur J Haematol. 2006; 77:233–238. [PubMed: 16923110]
15. Shimazaki C, Uchida R, Nakano S, et al. High serum bone-specific alkaline phosphatase level after bortezomib-combined therapy in refractory multiple myeloma: possible role of bortezomib on osteoblast differentiation. Leukemia. 2005; 19:1102–1103. [PubMed: 15830008]
16. Zangari M, Yaccoby S, Cavallo F, Esseltine D, Tricot G. Response to bortezomib and activation of osteoblasts in multiple myeloma. Clin Lymphoma Myeloma. 2006; 7:109–114. [PubMed: 17026821]
17. Lund T, Soe K, Abildgaard N, et al. First-line treatment with bortezomib rapidly stimulates both osteoblast activity and bone matrix deposition in patients with multiple myeloma, and stimulates osteoblast proliferation and differentiation in vitro. Eur J Haematol. 2010; 85:290–299. [PubMed: 20528908]
18. Zangari M, Aujay M, Zhan F, et al. Alkaline phosphatase variation during carfilzomib treatment is associated with best response in multiple myeloma patients. Eur J Haematol. 2011; 86:484–487. [PubMed: 21477075]
19. Silvestris F, Cafforio P, Calvani N, Dammacco F. Impaired osteoblastogenesis in myeloma bone disease: role of upregulated apoptosis by cytokines and malignant plasma cells. Br J Haematol. 2004; 126:475–486. [PubMed: 15287939]
20. Silvestris F, Cafforio P, Tucci M, Grinello D, Dammacco F. Upregulation of osteoblast apoptosis by malignant plasma cells: a role in myeloma bone disease. Br J Haematol. 2003; 122:39–52. [PubMed: 12823344]
21. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science. 1997; 276:71–74. [PubMed: 9082988]

22. Barille S, Collette M, Bataille R, Amiot M. Myeloma cells upregulate interleukin-6 secretion in osteoblastic cells through cell-to-cell contact but downregulate osteocalcin. Blood. 1995; 86:3151–3159. [PubMed: 7579410]

23. Giuliani N, Colla S, Morandi F, et al. Myeloma cells block RUNX2/CBFA1 activity in human bone marrow osteoblast progenitors and inhibit osteoblast formation and differentiation. Blood. 2005; 106:2472–2483. [PubMed: 15933061]

24. Evans CE, Ward C, Rathour L, Galasko CB. Myeloma affects both the growth and function of human osteoblast-like cells. Clin Exp Metastasis. 1992; 10:33–38. [PubMed: 1733645]

25. Krishnan V, Bryant HU, Macdougald OA. Regulation of bone mass by Wnt signaling. J Clin Invest. 2006; 116:1202–1209. [PubMed: 16670761]

26. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell. 1997; 89:747–754. [PubMed: 9182762]

27. Qiang YW, Endo Y, Rubin JS, Rudikoff S. Wnt signaling in B-cell neoplasia. Oncogene. 2003; 22:1536–1545. [PubMed: 12629517]

28. Qiang YW, Walsh K, Yao L, et al. Wnts induce migration and invasion of myeloma plasma cells. Blood. 2005; 106:1786–1793. [PubMed: 15886323]

29. Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. beta-catenin is a target for the ubiquitin-proteasome pathway. Embo J. 1997; 16:3797–3804. [PubMed: 9233789]

30. Kisselev AF, Goldberg AL. Proteasome inhibitors: from research tools to drug candidates. Chem Biol. 2001; 8:739–758. [PubMed: 11514224]

31. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005; 8:739–750. [PubMed: 15866164]

32. Qiang, YW.; Rudikoff, S. Wnt Signaling Pathways in Multiple Myeloma. In: Georgiev, M.; Bachev, E., editors. Multiple Myeloma: Symptoms, Diagnosis and Treatment. Vol. Chapter 2. New York: Nova Science Publishers; 2010. p. 51-75.

33. Heider U, Kaiser M, Mieth M, et al. Serum concentrations of DKK-1 decrease in patients with multiple myeloma responding to anti-myeloma treatment. Eur J Haematol. 2009; 82:31–38. [PubMed: 19067746]

34. Kaiser M, Mieth M, Liebisch P, et al. Serum concentrations of DKK-1 correlate with the extent of bone disease in patients with multiple myeloma. Eur J Haematol. 2008; 80:490–494. [PubMed: 18331598]

35. Pinzone JJ, Hall BM, Thudi NK, et al. The role of Dickkopf-1 in bone development, homeostasis, and disease. Blood. 2009; 113:517–525. [PubMed: 18687985]

36. Politou MC, Heath DJ, Rahemtulla A, et al. Serum concentrations of Dickkopf-1 protein are increased in patients with multiple myeloma and reduced after autologous stem cell transplantation. Int J Cancer. 2006; 119:1728–1731. [PubMed: 16646053]

37. Tian E, Zhan F, Walker R, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med. 2003; 349:2483–2494. [PubMed: 14695408]

38. Qiang YW, Barlogie B, Rudikoff S, Shaughnessy JD Jr. Dkk1-induced inhibition of Wnt signaling in osteoblast differentiation is an underlying mechanism of bone loss in multiple myeloma. Bone. 2008; 42:669–680. [PubMed: 18294945]

39. Qiang YW, Shaughnessy JD Jr, Yaccoby S. Wnt3a signaling within bone inhibits multiple myeloma bone disease and tumor growth. Blood. 2008; 112:374–382. [PubMed: 18344425]

40. Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy JD Jr. Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. Blood. 2007; 109:2106–2111. [PubMed: 17068150]

41. Edwards CM, Edwards JR, Lwin ST, et al. Increasing Wnt signaling in the bone marrow microenvironment inhibits the development of myeloma bone disease and reduces tumor burden in bone in vivo. Blood. 2008; 111:2833–2842. [PubMed: 18094333]

42. Ng AC, Khosla S, Charatcharoenwitthaya N, et al. Bone microstructural changes revealed by HRpQCT imaging and elevated DKK1 and MIP-1alpha levels in patients with monoclonal gammopathy of undetermined significance. Blood. 2011

43. Oshima T, Abe M, Asano J, et al. Myeloma cells suppress bone formation by secreting a soluble Wnt inhibitor, sFRP-2. Blood. 2005; 106:3160–3165. [PubMed: 16030194]
44. Qiang YW, Chen Y, Stephens O, et al. Myeloma-derived Dickkopf-1 disrupts Wnt-regulated osteoprotegerin and RANKL production by osteoblasts: a potential mechanism underlying osteolytic bone lesions in multiple myeloma. Blood. 2008; 112:196–207. [PubMed: 18305214]
45. Terpos E, Heath DJ, Rahemtulla A, et al. Bortezomib reduces serum dickkopf-1 and receptor activator of nuclear factor-kappaB ligand concentrations and normalises indices of bone remodelling in patients with relapsed multiple myeloma. Br J Haematol. 2006; 135:688–692. [PubMed: 17107351]
46. Komori T. Runx2, a multifunctional transcription factor in skeletal development. J Cell Biochem. 2002; 87:1–8. [PubMed: 12210716]
47. Zhao M, Qiao M, Oyajobi BO, Mundy GR, Chen D. E3 ubiquitin ligase Smurf1 mediates core-binding factor alpha1/Runx2 degradation and plays a specific role in osteoblast differentiation. J Biol Chem. 2003; 278:27939–27944. [PubMed: 12738770]
48. Shui C, Spelsberg TC, Riggs BL, Khosla S. Changes in Runx2/Cbfa1 expression and activity during osteoblastic differentiation of human bone marrow stromal cells. J Bone Miner Res. 2003; 18:213–221. [PubMed: 12568398]
49. Giuliani N, Rizzoli V, Roodman GD. Multiple myeloma bone disease: pathophysiology of osteoblast inhibition. Blood. 2006; 108:3992–3996. [PubMed: 16917004]
50. Sezer O, Heider U, Zavrski I, Kuhne CA, Hofbauer LC. RANK ligand and osteoprotegerin in myeloma bone disease. Blood. 2003; 101:2094–2098. [PubMed: 12424190]
51. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 1997; 89:309–319. [PubMed: 9108485]
52. O'Brien CA. Control of RANKL gene expression. Bone. 2010; 46:911–919. [PubMed: 19716455]
53. Terpos E, Szydlo R, Apperley JF, 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; 102:1064–1069. [PubMed: 12689925]
54. Zavrski I, Krebbel H, Wildemann B, et al. Proteasome inhibitors abrogate osteoclast differentiation and osteoclast function. Biochem Biophys Res Commun. 2005; 333:200–205. [PubMed: 15936724]
55. Ang E, Pavlos NJ, Rea SL, et al. Proteasome inhibitors impair RANKL-induced NF-kappaB activity in osteoclast-like cells via disruption of p62, TRAF6, CYLD, and IkappaBalpha signaling cascades. J Cell Physiol. 2009; 220:450–459. [PubMed: 19365810]
56. von Metzler I, Krebbel H, Hecht M, et al. Bortezomib inhibits human osteoclastogenesis. Leukemia. 2007; 21:2025–2034. [PubMed: 17581612]
57. Breitkreutz I, Raab MS, Vallet S, et al. Lenalidomide inhibits osteoclastogenesis, survival factors and bone-remodeling markers in multiple myeloma. Leukemia. 2008; 22:1925–1932. [PubMed: 18596740]
58. Boissy P, Andersen TL, Lund T, Kupisiewicz K, Plesner T, Delaisse JM. Pulse treatment with the proteasome inhibitor bortezomib inhibits osteoclast resorptive activity in clinically relevant conditions. Leuk Res. 2008; 32:1661–1668. [PubMed: 18394701]
59. Terpos E, Kastritis E, Roussou M, et al. The combination of bortezomib, melphalan, dexamethasone and intermittent thalidomide is an effective regimen for relapsed/refractory myeloma and is associated with improvement of abnormal bone metabolism and angiogenesis. Leukemia. 2008; 22:2247–2256. [PubMed: 18769451]
60. Uy GL, Trivedi R, Peles S, et al. Bortezomib inhibits osteoclast activity in patients with multiple myeloma. Clin Lymphoma Myeloma. 2007; 7:587–589. [PubMed: 18186967]
61. Glass DA 2nd, Bialek P, Ahn JD, et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell. 2005; 8:751–764. [PubMed: 15866165]
62. Holmen SL, Zylstra CR, Mukherjee A, et al. Essential role of beta-catenin in postnatal bone acquisition. J Biol Chem. 2005; 280:21162–21168. [PubMed: 15802266]
63. Spencer GJ, Utting JC, Etheridge SL, Arnett TR, Genever PG. Wnt signalling in osteoblasts regulates expression of the receptor activator of NFkappaB ligand and inhibits osteoclastogenesis in vitro. J Cell Sci. 2006; 119:1283–1296. [PubMed: 16522681]

64. Willert J, Epping M, Pollack JR, Brown PO, Nusse R. A transcriptional response to Wnt protein in human embryonic carcinoma cells. BMC Dev Biol. 2002; 2:8. [PubMed: 12095419]

65. Qiang YW, Chen Y, Brown N, et al. Characterization of Wnt/beta-catenin signalling in osteoclasts in multiple myeloma. Br J Haematol. 2010; 148:726–738. [PubMed: 19961481]

66. Hongming H, Jian H. Bortezomib inhibits maturation and function of osteoclasts from PBMCs of patients with multiple myeloma by downregulating TRAF6. Leuk Res. 2009; 33:115–122. [PubMed: 18778854]

67. Meller R, Cameron JA, Torrey DJ, et al. Rapid degradation of Bim by the ubiquitin–proteasome pathway mediates short-term ischemic tolerance in cultured neurons. J Biol Chem. 2006; 281:7429–7436. [PubMed: 16431916]

68. Akiyama T, Bouillet P, Miyazaki T, et al. Regulation of osteoclast apoptosis by ubiquitylation of proapoptotic BH3-only Bcl-2 family member Bim. EMBO J. 2003; 22:6653–6664. [PubMed: 14657036]

69. Roccaro AM, Hideshima T, Raje N, et al. Bortezomib mediates antiangiogenesis in multiple myeloma via direct and indirect effects on endothelial cells. Cancer Res. 2006; 66:184–191. [PubMed: 16397231]