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Hematopoietic Cell Regulation of Osteoblast Proliferation and Differentiation

Monique Bethel,

Department of Orthopaedic Surgery, Indiana University School of Medicine, 1120 South Drive, FH 115, Indianapolis, IN 46202, USA

Edward F. Srou, and

Departments of Medicine, Pediatrics, and Microbiology and Immunology, Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, 980 West Walnut Street, R3-C312, Indianapolis, IN 46202, USA

Melissa A. Kacena

Department of Orthopaedic Surgery, Indiana University School of Medicine, 1120 South Drive, FH 115, Indianapolis, IN 46202, USA

Monique Bethel: mobethel@iupui.edu; Edward F. Srou: esrou@iupui.edu; Melissa A. Kacena: mkacena@iupui.edu

Abstract

The last several decades have revealed numerous interactions between cells of the hematopoietic lineage and osteoblasts (OBs) of the mesenchymal lineage. For example, OBs are important players in the hematopoietic stem cell (HSC) niche and OBs are known to impact osteoclast (OC) development. Thus, although much is known regarding the impact OBs have on hematopoietic cells, less is known about the impact of hematopoietic cells on OBs. Here we will review this reciprocal relationship: the effects of hematopoietic cells on OBs. Specifically, we will examine the impact of hematopoietic cells such as HSCs, lymphocytes, and megakaryocytes, as well as the hematopoietic cell-derived OCs on OB proliferation, differentiation, and function.

Keywords

Hematopoiesis; Hematopoietic stem cells; Osteoclasts; B cells; T cells; Megakaryocytes; Osteoblasts; Proliferation; Differentiation

Introduction

Although hematopoietic cells in adults reside within bone cavities, until recently the hematopoietic and bone systems were studied in isolation. Importantly, a growing body of evidence shows numerous interactions between cells of the hematopoietic lineage and the bone-forming osteoblast (OB). One of the most commonly studied interactions is that between OBs and hematopoietic stem cells (HSCs) in the hematopoietic niche. Much is also known about the regulation of osteoclast (OC) formation and differentiation by OBs. However, although the crosstalk between OBs and OCs and the influence of OBs on HSCs are relatively well established, it is less clear how these cells regulate OB proliferation and

differentiation in normal physiologic and in disease states. The regulation of OBs by hematopoietic lineage cells is the focus of this review.

OBs are of the mesenchymal lineage and their main function is to secrete a collagen-based matrix that is then calcified to form bone. It is important to understand the development process of the OB, as the disruption of this process is believed to contribute to the disordered bone turnover of several disease states. Briefly, mesenchymal stem cells are induced to develop into pre-OBs via several cytokines. Once at the pre-OB stage, Runx2 is an essential transcription factor that allows for further OB differentiation and prevents progenitor cells from differentiating into adipocytes or chondrocytes. Finally, osterix is another transcription factor, downstream of Runx2, that directs the maturation of pre-OBs into immature OBs [1]. Interruptions in this process may lead to abnormal or diseased bone, and are associated with several malignancies [2, 3]. How hematopoietic cells or their derivatives impact these differentiation and specification pathways will be discussed below.

Hematopoietic Stem Cell—Osteoblast Interactions

As mentioned above, it has been known for some time that HSCs reside in a “niche,” or a specialized microenvironment that prevents the uncontrolled proliferation of these cells while preserving their ability to renew [4•]. Although the details of the inner workings of the niche are still being elucidated, mounting evidence is indicating that OBs are key components of the niche, directly involved in determining the ultimate fate of HSCs.

Although Schofield [5] first proposed the concept of the HSC niche in the 1970s, the importance of OBs in the niche has been recognized just this past decade [6, 7]. There are a number of studies that have documented the effects of altering OB number or function on the number of HSCs. As an example, Calvi et al. [6] examined the Col1-*ca*PTH1R mouse, in which the parathyroid hormone receptor (PTH1R) was constitutively active. They determined that these mice had elevated numbers of OBs as well as elevated numbers of HSCs. In studies by Zhang et al. [7], inactivation of the bone morphogenetic protein (BMP) 1A receptor (BMPRI1A) in mice resulted in the development of increased numbers of spindle-shaped N-cadherin⁺CD45⁺ OBs (SNO cells) along with a parallel increase in HSCs. Interestingly, these HSCs were seen to be attached to the SNO cells [7]. In both of these models, HSCs also lacked either the PTH or BMP receptors; therefore, the increases could not be attributed to elevation in PTH or blocking of BMP alone, thus implicating OBs in this process. Since then, the mechanisms by which OBs regulate HSCs and the possible roles of endosteal cells in the creation of hematopoietic niches have been the subject of intense investigation and debate (reviews of different points of view are presented by Kiel and Morrison [8•], Porter and Calvi [9•], Purton and Scadden [10], and Lymperi et al. [4•]). Although these examples clearly illustrate the effects of OBs on HSCs, here we will review the limited data available regarding the reciprocal relationship: the effects of HSCs on OBs.

Recently, our laboratory began to examine the effects of hematopoietic progenitors on OBs. To do this we have cultured OBs for 1 week with bone marrow (BM)-derived HSCs selected for Lin-Sca1+cKit⁺ (LSK), and then examined OB morphology, cell density, and gene expression. Our studies demonstrated that OBs cultured with LSK cells have a similar morphology and cellular density as that observed in cultures of OBs alone [11]. In more recent studies, we demonstrated that mRNA expression of a number of genes that are important in OB differentiation (eg, Runx2, alkaline phosphatase, osteopontin, osteocalcin, and type I collagen) were unaltered in OBs co-cultured with LSK cells compared with those cultured alone [11, 12]. Further, as Notch signaling has been shown by our group [13] as well as others [14] as being important in OB-mediated hematopoietic enhancing activity, we examined mRNA expression of Jagged 1 and Jagged 2, which are both Notch ligands that

are highly expressed on OBs. Jagged 1 and Jagged 2 expression was unaltered when OBs were cultured in the presence or absence of LSK cells [11, 12]. Although more studies are required to conclusively demonstrate whether HSCs impact OBs, these limited observations suggest that primitive HSCs have a negligible or not yet determined OB-modulating capacity as with 1 week or less of co-culture, LSK cells do not impact OBs in terms of morphology, cell number, or mRNA expression. However, these data must be interpreted cautiously as limited numbers of outcomes and only one concentration of LSK cells was examined. Thus, at this time, it is not clear whether HSCs impact OBs; although the reciprocal regulation is evident.

B and T Cell—Osteoblast Interactions

As in the case of HSCs, our understanding of how normal lymphocytes including B and T cells impact OB biology is limited at best. However, outcomes of lymphocyte-OB interactions have been characterized in several disease states. Evidence indicates that B cells and T cells interact with OBs in certain pathologic states such as rheumatoid arthritis and T-cell leukemia/lymphoma [15, 16]. Although many cancers are associated with metastatic osteolytic lesions, multiple myeloma (MM) is different. In most malignancies where bone lesions develop, it is more often attributed to increased osteoclastic bone destruction. However, in MM, in addition to overstimulation of bone resorption by OCs, there is a significant reduction in new bone formation by OBs in the area of the lesions [2]. There is evidence that the malignant B cells of MM can inhibit OB proliferation and differentiation via mechanisms involving direct cell-to-cell contact as well as secretion of soluble factors. Guiliani et al. [2] showed that MM cells grown in culture with osteoprogenitor cells decreased both the numbers of OB precursors that developed and hindered further maturation of these cells by decreasing Runx2 activity in the OBs. The inhibition was much stronger when the cells were allowed direct contact; however, interleukin-7, a soluble factor elevated in MM, inhibited OB differentiation via a decrease in Runx2 activity [2]. Similarly, another cytokine that is elevated in MM, interleukin-3, has been shown to inhibit OB activity, although its inhibitory action is indirect and involves macrophages/monocytes [3]. In another study, Silvestris et al. [17] found that co-culture with MM cells activated apoptotic pathways in OBs.

Adult T-cell leukemia/lymphoma (ATLL) is another malignancy in which abnormal OB function may contribute to disordered bone remodeling. Approximately 80% of patients with ATLL develop humoral hypercalcemia of malignancy associated with osteolytic lesions of the bone [16]. Shu et al. [16] conducted an experiment where malignant T cells infected with the human T lymphotropic virus (the cause of ATLL) were grown with pre-osteoblastic cells. Real-time polymerase chain reaction data from pre-OBs co-cultured with malignant T cells showed increased expression of receptor activator of nuclear factor- κ B ligand (RANKL) with a concomitant decrease in osteoprotegerin (OPG) and Runx2 expression. The reduced expression of OPG along with the elevated RANKL expression in OBs is thought to be in part responsible for the upregulation in osteoclastogenesis observed in ATLL.

B cells have several well-established roles in the pathophysiology of rheumatoid arthritis: B cells attract T cells to the sites of inflammation and then act as antigen-presenting cells for T cells, secrete autoantigens that activate the complement cascade and induce angiogenesis and granulation tissue formation, and form memory B cells to autoantigens [15]. In contrast to these activities that promote destruction in rheumatoid arthritis, recent data suggest that B cells may also play a role in protecting bone from destruction via an interaction with OBs. In 2008, Hayer et al. [18•] created a new mouse model, combining a mouse strain that constitutively expresses human tumor necrosis factor (henceforth called hTNFtg) and

develops a rheumatoid arthritis-like condition, with another strain, $Btk^{-/-}$, that forms decreased numbers of B cells with defective immunoglobulin production and response to B-cell activators (progeny of this cross are referred to as $hTNFtg/Btk^{-/-}$). This new mouse strain developed arthritis similar to the $hTNFtg$ mouse; however, there were decreased numbers of OBs, decreased endosteal bone formation, and decreased expression of bone matrix proteins osteocalcin and osteopontin compared with the $hTNFtg$ mouse [18•]. Levels of BMP-6 and BMP-7, important mediators of new bone formation, were also decreased in the $hTNFtg/Btk^{-/-}$ [18•]. In this model, B cells may influence OBs and thus bone formation through the production of these proteins. Of note, there is also some evidence that differentiation of OBs is inhibited in rheumatoid arthritis. Mohanty et al. [19] found decreased numbers of OBs at the endocortical surface in the tibia of mice in a model of rheumatoid arthritis. It is not yet known if B cells function similarly in human rheumatoid arthritis. T cells, conversely, likely act primarily to stimulate OCs in rheumatoid arthritis via their production of RANKL and macrophage colony-stimulating factor (M-CSF) and have little documented interaction with OBs in rheumatoid arthritis [20].

Osteoclast-Osteoblast Interactions

To maintain skeletal homeostasis, the process of bone resorption and bone formation must be tightly coupled. Many skeletal diseases, such as osteoporosis and metastatic bone disease, are the result of an imbalance in bone resorption and bone formation. In the physiologic state, it has been proposed that small microcracks or other defects in bone are detected by osteocytes that recruit OC precursors to the site, which then differentiate into mature OCs [21]. Cells of the OB lineage are critical in this process, as part of their function includes regulation of OC function, via their expression of M-CSF, RANKL, and OPG. RANK is a transcription factor expressed on cells of the OC lineage that is essential for OC maturation. OPG is a decoy receptor for RANKL; its binding to RANKL prevents binding of RANKL to RANK. Variations in the RANKL/OPG ratio by OBs can therefore regulate OC function [21]. Importantly, in addition to the role of OBs in OC formation and differentiation, it is becoming clear that OCs also influence OBs.

In 2008, Sanchez-Fernandez et al. [22] demonstrated that platelet-derived growth factor (PDGF-bb) released by mature OCs was a chemoattractant for immature and mature OBs. Specifically, they showed that conditioned medium from the OC-like RAW264.7 cell line was able to attract immature and mature OBs to a greater extent than conditioned medium derived from RAW264.7 cells in which PDGF-bb expression was markedly reduced using small interfering RNA. This is just one example of how OCs can affect OBs.

Another example of OCs affecting OBs comes from data examining the bidirectional interaction between an EphrinB2 ligand on OC progenitors and the EphB4 receptor on pre-OBs [23, 24]. The forward signaling effect of EphrinB2 binding to EphB4 led to increased differentiation of murine calvarial OB, whereas the reverse signaling of EphB4 binding to EphB2 in osteoclastic cultures inhibited osteoclastogenesis [24]. Of note, disorders in the EphrinB2-EphB4 signaling pathway have also been implicated in the osteolytic lesions of MM. In a study comparing MM patients to healthy controls, Pennisi et al. [25] found that both EphrinB2 and EphB4 were expressed at lower levels in MM patients. Furthermore, mice engrafted with tumor cells from patients with active disease showed an increase in bone mineral density after treatment with phosphorylated EphrinB2 and EphB4 by $16\% \pm 6\%$ and $9\% \pm 7\%$, respectively [25].

In addition to OC-OB interactions, studies have demonstrated a role for OC progenitors in OB regulation. Specifically, it has been shown that unsorted calvarial-derived OBs (which are heterogeneous and contain a population of macrophages termed OsteoMacs) express

higher levels of osteocalcin and increased mineralization as determined by von Kossa staining compared with enriched OB populations from which hematopoietic cells have been removed [26•]. Similarly, when enriched OBs were co-cultured with OsteoMacs, mineralization was enhanced compared with enriched OBs cultured alone. These in vitro studies were then confirmed in vivo where depletion of OsteoMacs resulted in the loss of mature cuboidal OBs on the bone surface. Taken together, these data demonstrate that both macrophages as well as mature OCs can regulate OBs.

Megakaryocyte-Osteoblast Interactions

Perhaps the most characterized interaction whereby hematopoietic cells modulate OB function is that which takes place between OBs and megakaryocytes (MKs). The influence of MKs on OBs was recognized within the past decade, and this interaction has been the focus of our laboratory. The discovery of an interaction between MKs and OBs in the GATA-1 and NF-E2 deficient mouse models was important in demonstrating a physiologic role for MKs in regulating both OBs and bone formation. GATA-1 or NF-E2 are transcription factors essential for the normal maturation of MKs, and eventual release of platelets. In both of these mouse models, in which GATA-1 or NF-E2 are markedly reduced or knocked out, respectively, the mice were observed to have an abundance of immature MKs both in the BM cavity and the spleen, few mature MKs and platelets, but a striking two- to threefold increase in bone volume [27]. In addition, histomorphometry demonstrated that in vivo OB number was significantly elevated, as were bone formation parameters. Furthermore, co-culture of OBs with MKs resulted in a three- to sixfold increase in OB proliferation [27–30]. Using two distinct experimental models (incubating OBs in MK-conditioned medium or placing a cell-impermeable membrane between MKs and OBs) it was determined that direct MK-to-OB contact was required to increase OB number in vitro [27].

Two other mouse models have also shown an increased bone mass phenotype in association with MK-platelet dysregulation. Mice that overexpress the principle MK growth factor, thrombopoietin, have a fourfold increase in BM MK number, and develop osteosclerosis [31–33]. Another mouse model, discovered in 2008, also has a high bone mass phenotype and a dysregulation of platelets involving the glycoprotein Ib-IX (GP Ib-IX) receptor for von Willebrand factor. These mice have a genetic mutation in the GP Ib-IX receptor that impairs platelet adhesion to damaged endothelial surfaces and includes elevated splenic levels of MKs with significantly decreased numbers of OCs, but no effect on OB number or function [34]. This is in contrast to the other three mouse models where increased levels of MKs were observed in the BM. Therefore, it appears that the mechanism causing increased bone mass is not the same in this model as the other three previously mentioned mouse models. It appears that the splenic MKs may secrete a factor(s) that can enter the circulation and inhibit OC formation and differentiation. The notion that MKs can secrete a factor(s) that inhibits OC development has been described by two separate groups using in vitro methodologies [30, 35, 36]. Therefore, in contrast to the mice bearing a mutation in the GP Ib-IX receptor, when BM MK number is elevated, anabolic bone formation is increased, leading to a high bone mass phenotype [27]. Thus, these mouse models suggest that during anabolic bone formation, MKs must be present in the BM where they can be in direct contact with OBs.

Additional in vivo evidence of the MK-OB connection comes from recent studies by Dominici et al. [37•]. In their studies, in mice treated with lethal total body irradiation, surviving MKs were shown to migrate to endosteal bone surfaces (in close contact with OBs) and stimulate a twofold increase in OB number. It is thought that MKs migrated to these areas to re-establish the HSC niche and begin to repopulate BM cells.

The ability of MKs to stimulate bone formation in vivo was also shown in adoptive transfer models. In these studies, spleen cells from NF-E2^{-/-} mice were transplanted into lethally irradiated control recipient mice (spleen cells contain very high numbers of immature MKs and HSCs). At 4 weeks, the irradiated mice receiving spleen cells from the NF-E2^{-/-} had developed a bone phenotype indistinguishable from that of the NF-E2^{-/-} mouse [38].

There is also emerging evidence that MKs play a role in skeletal homeostasis in humans as well. In 2000, Bord et al. [39] compared three sets of women to evaluate the effect of long-term estrogen replacement on bone. Included in the study were 10 postmenopausal women who had received conventional hormone replacement therapy (HRT), 11 women who received estradiol implants on demand following total hysterectomy and salpingo-oophorectomy (high-dose group), and six age-matched controls received no hormonal therapy (4 postmenopausal and 2 premenopausal women) [39]. Biopsies were taken from the iliac crest and the BM was examined histologically for MKs. It was found that women receiving high doses of estrogen had the highest numbers of MKs, followed by women undergoing 2 years of HRT. Of note, BM from women in both groups had significantly higher numbers of MKs than that observed in pretreatment (control) postmenopausal women [39]. Of interest, the two premenopausal women in the control group had the highest MK number in their group (higher than all members of the pretreatment, postmenopausal group) [39]. This was followed up by a 2004 in vitro study by the same group showing that treatment of MK progenitors with estrogen increased megakaryopoiesis, decreased RANKL expression, and increased OPG expression in MKs [40]. As estrogen status has been associated with bone mass [41], these data suggest that MK number may be associated with bone mass. Further, multiple MK-related diseases result in osteosclerosis [42, 43]. Osteosclerosis is observed in several myeloproliferative disorders in which MK number is also increased. As an example, in idiopathic myelofibrosis, megakaryocytosis is common and 80% of patients present with osteosclerosis; the remainder usually have findings of early fibrosis with hypercellularity [42]. Overall, these findings suggest that MKs may play a significant role in bone pathology and may also regulate normal skeletal homeostasis. Taken together, it appears that the interactions seen between MKs and OBs and/or bone mass in mice may persist in humans as well.

Aside from the in vivo studies presented above, more recent investigations have been conducted to begin elucidating the mechanisms by which MKs alter OB proliferation and differentiation. It was shown by two separate groups that stimulation of OB proliferation by MKs requires juxtacrine communication (direct MK-OB contact) between the cells [27–30, 44]. As OBs are known to communicate to each other and other cells via gap junction intercellular communication (GJIC), this juxtacrine mechanism was explored in MK-mediated OB proliferation [28]. Connexin 43 (Cx43) is one of the 20 mammalian connexins, or proteins, that form the channels in the gap junctions [45]. Cx43 expression has been identified in both OBs and MKs [46, 47]. In 2009, Ciovacco et al. [28] confirmed the expression of Cx43 on MKs and demonstrated that GJIC existed between MKs and OBs using a standard parachute assay. Pharmacologically blocking GJIC between MKs and OBs with the GJIC inhibitors α GA or oleamide resulted in the surprising finding that MK-mediated OB proliferation was further increased, suggesting that GJIC between MKs and OBs inhibits the ability of MKs to enhance OB proliferation.

Because GJIC was not responsible for the MK-mediated increase in OB number additional mechanisms were explored, such as integrin signaling [29]. Integrin signaling was examined as previous studies demonstrated that MKs enhance fibroblast proliferation via integrins [48, 49]. To examine integrin involvement in OBs co-cultured with MKs, OBs were cultured alone or in the presence of MKs and the following known inhibitors of integrin binding and/or signaling were titrated into cultures: ethylenediaminetetraacetic acid (EDTA), Arg-gly-

asp-ser (RGDS, a soluble protein), as well as neutralizing antibodies directed against integrin $\alpha 3$, and integrin $\alpha 5$. All of these inhibitors were able to significantly reduce MK-mediated OB proliferation while having little effect on OB monocultures, suggesting that integrin involvement is important in MK-mediated OB proliferation [29]. Thus, although more work is needed to understand precisely how MKs stimulate OB proliferation, these studies are the first steps in that process.

The effects of MKs on OB differentiation are less well understood than the effects of MKs on OB proliferation. Bord et al. [50] examined OB mRNA 1 day following co-culture with MKs for expression of type I collagen and OPG and showed that MKs enhanced expression of both genes. In studies by our group where OBs were co-cultured with MKs for 14 days, we unexpectedly found that MKs inhibited all markers of OB differentiation (mRNA expression of type I collagen, osteocalcin and alkaline phosphatase as well as functional assays examining alkaline phosphatase activity and bound calcium as a measure of mineralization) [28]. These data were unexpected based on the previously published data by Bord et al. [50]. These apparently contradictory findings may be explained by differences in culture duration in both studies. Regardless of the explanation, it is evident that much work is required to understand the role of MKs in regulating OB differentiation.

Conclusions

What is now clear is that interactions between cells of the hematopoietic lineage and bone-forming OBs occur in normal and pathologic conditions. Alterations in these cell-cell communications are factors in many disease states, from malignancy to inflammatory states to osteoporosis. Better understanding the mechanisms by which these cells interact may lead to the development of novel therapeutic targets for multiple and varied disease processes.

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References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
 - Of major importance
1. Marie PJ. Transcription factors controlling osteoblastogenesis. *Arch Biochem Biophys*. 2008; 473:98–105. [PubMed: 18331818]
 2. 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]
 3. Ehrlich LA, Chung HY, Ghobrial I, et al. IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. *Blood*. 2005; 106:1407–1414. [PubMed: 15878977]
 4. Lymperi S, Ferraro F, Scadden DT. The HSC niche concept has turned 31. Has our knowledge matured? *Ann N Y Acad Sci*. 2010; 1192:12–18.. This review article discusses the role of OBs as well as other cells in supporting HSCs.
 5. Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells*. 1978; 4:7–25. [PubMed: 747780]

6. Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003; 425:841–846. [PubMed: 14574413]
7. Zhang J, Niu C, Ye L, et al. Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*. 2003; 425:836–841. [PubMed: 14574412]
8. Kiel MJ, Morrison SJ. Uncertainty in the niches that maintain haematopoietic stem cells. *Nat Rev Immunol*. 2008; 8:290–301. [PubMed: 18323850] . This review nicely clarifies the confusing use of the word “osteoblast” when referring to cells on the surface of bone within the HSC niche. *This review nicely clarifies the confusing use of the word “osteoblast” when referring to cells on the surface of bone within the HSC niche.*
9. Porter RL, Calvi LM. Communications between bone cells and hematopoietic stem cells. *Arch Biochem Biophys*. 2008; 473:193–200. [PubMed: 18410740] . This review article discusses the role of OBs as well as other cells in supporting HSCs.
10. Purton, LE.; Scadden, DT. The hematopoietic stem cell niche. In: Silberstein, L., editor. *StemBook*. The Stem Cell Research Community, StemBook, 2008. 2008 November 15. <http://www.stembook.org>
11. Chitteti BR, Cheng YH, Streicher DA, et al. Osteoblast lineage cells expressing high levels of Runx2 enhance hematopoietic progenitor cell proliferation and function. *J Cell Biochem*. 2010; 111:284–294. [PubMed: 20506198]
12. Cheng YH, Chitteti BR, Streicher DA, et al. Impact of osteoblast maturational status on their ability to enhance the hematopoietic function of stem and progenitor cells. *J Bone Miner Res*. 2010 [Epub ahead of print].
13. Chitteti BR, Cheng YH, Poteat B, et al. Impact of interactions of cellular components of the bone marrow microenvironment on hematopoietic stem and progenitor cell function. *Blood*. 2010; 115:3239–3248. [PubMed: 20154218]
14. Weber JM, Forsythe SR, Christianson CA, et al. Parathyroid hormone stimulates expression of the notch ligand Jagged1 in osteoblastic cells. *Bone*. 2006; 39:485–493. [PubMed: 16647886]
15. Silverman GJ, Carson DA. Roles of B cells in rheumatoid arthritis. *Arthritis Res Ther*. 2003; 5 Suppl 4:S1–S6. [PubMed: 15180890]
16. Shu ST, Martin CK, Thudi NK, et al. Osteolytic bone resorption in adult T-cell leukemia/lymphoma. *Leuk Lymphoma*. 2010; 51:702–714. [PubMed: 20214446]
17. Silvestris F, Cafforio P, Tucci M, et al. Upregulation of osteoblast apoptosis by malignant plasma cells: a role in myeloma bone disease. *Br J Haematol*. 2003; 122:39–52. [PubMed: 12823344]
18. Hayer S, Polzer K, Brandl A, et al. B-cell infiltrates induce endosteal bone formation in inflammatory arthritis. *J Bone Miner Res*. 2008; 23:1650–1660. [PubMed: 18505368] . This article shows that reduced numbers of B cells in mice having inflammatory arthritis are associated with reduced OB number and bone formation.
19. Mohanty ST, Kottam L, Gambardella A, et al. Alterations in the self-renewal and differentiation ability of bone marrow mesenchymal stem cells in a mouse model of rheumatoid arthritis. *Arthritis Res Ther*. 2010; 12:R149. [PubMed: 20649960]
20. Udagawa N, Kotake S, Kamatani N, et al. The molecular mechanism of osteoclastogenesis in rheumatoid arthritis. *Arthritis Res*. 2002; 4:281–289. [PubMed: 12223101]
21. Edwards CM, Mundy GR. Eph receptors and ephrin signaling pathways: a role in bone homeostasis. *Int J Med Sci*. 2008; 5:263–272. [PubMed: 18797510]
22. Sanchez-Fernandez MA, Gallois A, Riedl T, et al. Osteoclasts control osteoblast chemotaxis via PDGF-BB/PDGF receptor beta signaling. *PLoS ONE*. 2008; 3:e3537. [PubMed: 18953417]
23. Luiz de Freitas PH, Li M, Ninomiya T, et al. Intermittent PTH administration stimulates pre-osteoblastic proliferation without leading to enhanced bone formation in osteoclast-less c-fos(–/–) mice. *J Bone Miner Res*. 2009; 24:1586–1597. [PubMed: 19419301]
24. Zhao C, Irie N, Takada Y, et al. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab*. 2006; 4:111–121. [PubMed: 16890539]
25. Pennisi A, Ling W, Li X, et al. The ephrinB2/EphB4 axis is dysregulated in osteoprogenitors from myeloma patients and its activation affects myeloma bone disease and tumor growth. *Blood*. 2009; 114:1803–1812. [PubMed: 19597185]

26. Chang MK, Raggatt LJ, Alexander KA, et al. Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. *J Immunol.* 2008; 181:1232–1244. [PubMed: 18606677] . This article describes the ability of macrophages to modulate OB mineralization.
27. Kacena MA, Shivdasani RA, Wilson K, et al. Megakaryocyte-osteoblast interaction revealed in mice deficient in transcription factors GATA-1 and NF-E2. *J Bone Miner Res.* 2004; 19:652–660. [PubMed: 15005853]
28. Ciovacco WA, Goldberg CG, Taylor AF, et al. The role of gap junctions in megakaryocyte-mediated osteoblast proliferation and differentiation. *Bone.* 2009; 44:80–86. [PubMed: 18848655]
29. Lemieux JM, Horowitz MC, Kacena MA. Involvement of integrins alpha(3)beta(1) and alpha(5)beta(1) and glycoprotein IIb in megakaryocyte-induced osteoblast proliferation. *J Cell Biochem.* 2010; 109:927–932. [PubMed: 20052668]
30. Ciovacco WA, Cheng YH, Horowitz MC, Kacena MA. Immature and mature megakaryocytes enhance osteoblast proliferation and inhibit osteoclast formation. *J Cell Biochem.* 2010; 109:774–781. [PubMed: 20052670]
31. Frey BM, Rafii S, Teterson M, et al. Adenovector-mediated expression of human thrombopoietin cDNA in immune-compromised mice: Insights into the pathophysiology of osteomyelofibrosis. *J Immunol.* 1998; 160:691–699. [PubMed: 9551904]
32. Yan XQ, Lacey D, Hill D, et al. A model of myelofibrosis and osteosclerosis in mice induced by overexpressing thrombopoietin (mpl ligand): reversal of disease by bone marrow transplantation. *Blood.* 1996; 88:402–409. [PubMed: 8695786]
33. Villeval JL, Cohen-Solal K, Tulliez M, et al. High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. *Blood.* 1997; 90:4369–4383. [PubMed: 9373248]
34. Suva LJ, Hartman E, Dilley JD, et al. Platelet dysfunction and a high bone mass phenotype in a murine model of platelet-type von willebrand disease. *Am J Pathol.* 2008; 172:430–439. [PubMed: 18187573]
35. Kacena MA, Nelson T, Clough ME, et al. Megakaryocyte-mediated inhibition of osteoclast development. *Bone.* 2006; 39:991–999. [PubMed: 16782418]
36. Beeton CA, Bord S, Ireland D, Compston JE. Osteoclast formation and bone resorption are inhibited by megakaryocytes. *Bone.* 2006; 39:985–990. [PubMed: 16870519]
37. Dominici M, Rasini V, Bussolari R, et al. Restoration and reversible expansion of the osteoblastic hematopoietic stem cell niche after marrow radioablation. *Blood.* 2009; 114:2333–2343. [PubMed: 19433859] . This article shows that following lethal irradiation, MKs migrate to endosteal bone surfaces and stimulate OB proliferation.
38. Kacena MA, Gundberg CM, Nelson T, Horowitz MC. Loss of the transcription factor p45 NF-E2 results in a developmental arrest of megakaryocyte differentiation and the onset of a high bone mass phenotype. *Bone.* 2005; 36:215–223. [PubMed: 15780947]
39. Bord S, Vedi S, Beavan SR, et al. Megakaryocyte population in human bone marrow increases with estrogen treatment: a role in bone remodeling? *Bone.* 2000; 27:397–401. [PubMed: 10962351]
40. Bord S, Frith E, Ireland DC, et al. Synthesis of osteoprotegerin and RANKL by megakaryocytes is modulated by oestrogen. *Br J Haematol.* 2004; 126:244–251. [PubMed: 15238146]
41. Heiss CJ, Sanborn CF, Nichols DL, et al. Associations of body fat distribution, circulating sex hormones, and bone density in postmenopausal women. *J Clin Endocrinol Metab.* 1995; 80:1591–1596. [PubMed: 7745005]
42. Thiele J, Kvasnicka HM, Fischer R. Histochemistry and morphometry on bone marrow biopsies in chronic myeloproliferative disorders—aids to diagnosis and classification. *Ann Hematol.* 1999; 78:495–506. [PubMed: 10602893]
43. Chagraoui H, Wendling F, Vainchenker W. Pathogenesis of myelofibrosis with myeloid metaplasia: Insight from mouse models. *Best Pract Res Clin Haematol.* 2006; 19:399–412. [PubMed: 16781480]

44. Miao D, Murant S, Scutt N, et al. Megakaryocyte-bone marrow stromal cell aggregates demonstrate increased colony formation and alkaline phosphatase expression in vitro. *Tissue Eng.* 2004; 10:807–817. [PubMed: 15265298]
45. Willecke K, Eiberger J, Degen J, et al. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol Chem.* 2002; 383:725–737. [PubMed: 12108537]
46. Krenacs T, Rosendaal M. Connexin43 gap junctions in normal, regenerating, and cultured mouse bone marrow and in human leukemias: Their possible involvement in blood formation. *Am J Pathol.* 1998; 152:993–1004. [PubMed: 9546360]
47. Donahue HJ. Gap junctions and biophysical regulation of bone cell differentiation. *Bone.* 2000; 26:417–422. [PubMed: 10773579]
48. Schmitz B, Thiele J, Otto F, et al. Evidence for integrin receptor involvement in megakaryocyte-fibroblast interaction: a possible pathomechanism for the evolution of myelofibrosis. *J Cell Physiol.* 1998; 176:445–455. [PubMed: 9699497]
49. Wickenhauser C, Schmitz B, Baldus SE, et al. Selectins (CD62L, CD62P) and megakaryocytic glycoproteins (CD41a, CD42b) mediate megakaryocyte-fibroblast interactions in human bone marrow. *Leuk Res.* 2000; 24:1013–1021. [PubMed: 11077115]
50. Bord S, Frith E, Ireland DC, et al. Megakaryocytes modulate osteoblast synthesis of type-I collagen, osteoprotegerin, and RANKL. *Bone.* 2005; 36:812–819. [PubMed: 15794927]