Published in final edited form as:

Curr Opin Organ Transplant. 2010 February ; 15(1): 73–78. doi:10.1097/MOT.0b013e328333d52c.

Prospects for Osteoprogenitor Stem Cells in Fracture Repair and Osteoporosis

Gregory A. Clines, M.D., Ph.D.[Assistant Professor of Medicine Division of Endocrinology and Metabolism]

The University of Virginia P.O. Box 801420 Charlottesville, VA 22908-1420 Office: (434) 982-6451 Fax: (434) 982-3314 gc4z@virginia.edu

Abstract

Purpose of review—Bone regeneration and fracture repair is a complex process of mesenchymal stem cell invasion, chondrogenesis, osteogenesis and angiogenesis. The coordinated actions of these principal processes results in the reconstruction of normal bone and restoration of a structural unit. However, these normal bone regenerative mechanisms breakdown during fracture repair failure and postmenopausal osteoporosis.

Recent findings—Recent discoveries of circulating multipotent stem cells with mixed characteristics of endothelial cell and osteogenic capacity have raised interest in new and potentially breakthrough therapies for fracture and pathologic bone loss. The cooperative actions of other mesenchymal stem cell lineage such as adipocytes and processes such as angiogenesis in bone repair could also serve as novel therapeutic targets. Recent data suggests that anabolic parathyroid hormone therapy, already approved for the treatment of osteoporosis, may recruit osteoprogenitor cells and also have a role in fracture repair.

Summary—This review will highlight recent information on stem cells and bone repair and examine potential avenues for future research.

Keywords

Fracture healing; osteoporosis; osteoblast; adipocyte; angiogenesis

Introduction

During development, two distinct mechanisms shape how bone is formed. Most of the skeleton is crafted by endochondral ossification, a process whereby an initial cartilage structure creates a backbone for osteoblasts to invade and secrete a bony matrix. Intramembranous bone is formed *de novo* from mesenchymal condensations that differentiate into mature osteoblasts to construct bones of the skull. These mechanisms of developmental bone modeling cease with the completion of adolescent growth and mark a transition to adult bone remodeling when bone activity is concentrated on maintenance and repair. Proper bone remodeling relies on a balance between the three principal cells in bone—osteoclasts, osteoblasts and osteocytes. The osteoclast is derived from hematopoietic monocytic cells whose formation is regulated by bone marrow stromal cells and early

The author has no conflicts of interest.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

osteoblasts (Figure). This multinucleated cell attaches to bone surfaces secreting hydrogen ions and hydrolytic enzymes that resorb bone. Osteoblasts are then recruited to resorption pits and secrete bone matrix proteins that will serve as the scaffold for new bone. The principal bone matrix protein is type I collagen (90%) but trace of amounts of other collagens such types III and V are present [1]. The osteoblast also secretes non-collagenous proteins that comprise about 10% of matrix. These include osteonectin, osteocalcin and osteopontin that bind calcium and likely regulate the local concentration of calcium and phosphate leading to precipitation and crystallization of hydroxyapatite on the collagen matrix. Osteoblasts also secrete growth factors that become immobilized in the bone matrix [2]. After completion of the bone remodeling cycle, most osteoblasts undergo apoptosis. Some cells will remain at the bone surface to form lining cells that may serve as osteoprogenitors or even prepare bone for osteoclast attachment [3]. A few osteoblasts become encased in their own matrix to become osteocytes, the most prevalent cell in bone. Only recently have the role of these cells as mechanical sensors been recognized [4].

Fracture Repair and the Clinical Problem

After a bone fracture, both endochondral and intramembranous ossification re-emerge. This process of fracture repair begins with the immediate formation of a hematoma at the fracture site. Through release of cytokines, cells are recruited to the fracture site that marks the site for repair. Hypoxia and vascular disruption at the fracture site are stimuli for chondrocyte recruitment and initiation of endochondral ossification. Adjacent to the fracture, fibroblastoid periosteal bone lining cells differentiate into osteoblasts and begin the process callus formation. The fracture gap is eventually filled with immature woven bone that is remodeled over time to more structurally sound lamellar bone.

Fracture repair in most situations is a predictable process. However, the risk of fracture repair failure increases with aging, diabetes and smoking [5-8]. Bone non-union with more extensive fractures and with bone lengthening procedures (distraction osteogenesis) are other more evident causes of failure. Bone morphogenetic protein-2 (BMP-2) by direct application to the fracture site is the only drug approved by the Food and Drug Administration to promote fracture repair [9*]. Anabolic parathyroid hormone, currently approved for treatment of osteoporosis [10], has been shown to accelerate fracture healing in animal models [11] but large clinical trials are lacking. Osteoporosis, from estrogen deficiency, glucocorticoids or inflammatory states, can also be viewed as a condition whereby bone fails to optimally ossify. Antiresorptive agents such as bisphosphonates are effective at reducing fracture risk but potential side effects have recently gained more attention [12]. Teriparatide (parathyroid hormone 1-34) is an effective anabolic agent that reduces vertebral fractures by 65% [10]. However, even with treatment advances, more than 2 million osteoporotic fractures occur each year in the U.S. costing more than \$17 billion [13]. Clearly, room exists for more effective therapies targeted at bone repair and regeneration.

Osteoblast Development

Much of the investigations on bone regeneration have focused on the osteoblast. During development, this bone-forming cell is derived from mesodermal sclerotome condensations that form MSCs, multipotent cells with the capacity to differentiate into other cell types including chondrocytes, myocytes, adipocytes and connective tissue fibroblasts. MSCs persist into adulthood and provide a supply of osteoblasts for normal adult bone remodeling. The majority of MSCs allocated for the osteoblast lineage likely reside in bone marrow and comprise approximately 1/100,000 bone marrow cells [14]. Other tissues that contain MSCs

include muscle, placenta, liver and spleen but how MSCs at these locations contribute to bone regeneration is unclear.

The “master switches” that determine MSC lineage have been identified (Figure). The basic helix-loop-helix MYOD/myogenin/MYF5/MRF4 transcription factor family specifies myogenic fate [15] and the fatty acid-sensing peroxisome proliferator-activated receptor- γ (PPAR γ) specifies an adipogenic fate [16]. SOX9 is a transcription factor with homology to SRY (sex-determining region Y) that regulates chondrogenesis and expression of the major cartilage collagen COL2a1. Conditional deletion of mouse *Sox9* gene in MSCs resulted in the absence of cartilage without defects in intramembranous bone formation [17].

The master switch that determines osteoblast fate is the transcription factor RUNX2 (Runt-related transcription factor 2, also known as Cbfa1 or AML3). The critical dependence of RUNX2 on osteoblast differentiation is demonstrated with *Runx2* deficient mice. These knockout mice have neither osteoblasts nor endochondral and intramembranous bone [18]. Mutations in *RUNX2* were identified as the cause for the human autosomal dominant condition cleidocranial dysplasia (CCD) that is characterized by hypoplastic clavicles, persistently open calvarial sutures and dental abnormalities [19]. CCD patients are haploinsufficient for *RUNX2* and it is unclear why only certain mineralized tissues are affected. A similar phenotype of *Runx2* deficient mice is seen with deletion of *Osterix* (Sp7), a downstream target of RUNX2 [20]. RUNX2 also cooperates with other critical osteoblast factors such as MSX2 [21], BMPs [22,23*], WNTs [24] and Hedgehog proteins [25].

During development, secreted factors in the primordial bone microenvironment activate osteoblastogenesis that ultimately leads to RUNX2 expression and bone mineral matrix formation. Multiple signaling pathways are able to individually activate RUNX2 expression. Well-studied activators of osteoblastogenesis are the bone morphogenetic proteins (BMPs), members of the transforming growth factor- β superfamily. They bind to the BMP type I and II receptor dimers, activate the BMP-specific Smads-1, -5 and -8 where they then are translocated to the nucleus and act as transcription enhancers [26]. It is likely that BMPs, secreted from mesenchymal condensations or vascular endothelium, are the initial inducers of osteoblastogenesis during development. Progressive differentiation of the osteoprogenitor cells causes expression of other osteoblast-specific factors such as alkaline phosphatase, type I collagen and osteocalcin.

Circulating osteoprogenitor Cells

Conventional methods of measuring osteoprogenitor cells in bone marrow are to identify the population of cells that adhere to plastic and either express alkaline phosphatase or are able to mineralize. The addition of osteoblast differentiation agents ascorbic acid, β -glycerophosphate, dexamethasone and 1,25(OH) $_2$ vitamin D further support osteoblast development. These cells initially have a fibroblastoid appearance but differentiation and confluence transforms these osteoprogenitors into a cuboidal shape. Adherence to plastic was once considered a prerequisite of osteoprogenitor cells. However, Long *et al* identified a population of bone marrow cells that expressed the late osteoblast marker osteocalcin but was small and non-adherent to plastic [27]. This non-adherent bone marrow population was able to generate both osteoblasts and hematopoietic cells and suggested that osteoblasts and hematopoietic cells have a common lineage [28]. Other groups have further characterized these non-adherent cells in the basis of express of additional cell surface proteins such as alkaline phosphatase, CD34, STRO-1, CD106 and CD146 [29]. However, there is no consensus on the MSC cell surface expression signature and may suggest that osteoblasts may not differentiate in a stepwise fashion or that different subsets of MSCs are able to generate osteoblasts of differing characteristics.

In recent work, flow cytometry of circulating monocytic cells using antibodies to osteocalcin and bone alkaline phosphatase demonstrated that osteocalcin and alkaline phosphatase expressing cells comprised 1% of circulating mononuclear cells [30]. Furthermore, this population of cells formed mineralized nodules in culture and co-expressed other typical osteoblast markers such as type I collagen and formed mineralized tissue after implantation into immunocompromised mice. Comparison of adolescent versus aged subjects revealed higher numbers (5-fold) of circulating osteoprogenitor cells in adolescent individuals likely reflecting the bone growth at that age. Moreover, circulating osteoprogenitors were also higher in subjects who had experienced a recent fracture [30]. Expression of the chemokine receptor CXCR4 on the circulating osteoprogenitor may assist in homing to bone [31**]. These studies raise the possibility that osteoprogenitors may not only be recruited locally but recruited from distant sites during times of heightened bone formation or fracture. A different interpretation is that the increase in osteoprogenitors is only a consequence of bone damage and release of osteoprogenitors into the circulation.

In recent work, a unique “pulse-chase” system to mark pre-osteoblasts uncovered the location of osteoprogenitors during early mouse development [32,33]. Even before mineralization of the bone template, osteoblast precursors co-localized with blood vessels in the perichondrium. At later stages, fewer labeled osteoblast precursors remained associated with blood vessels and became progressively associated with mineralized bone surfaces. This suggested that the origin of trabecular osteoblasts during development is perichondrial mesenchymal condensations that gain access into bone via perichondrial vessels that traverse the cortical bone surface. These results are again suggestive that osteoprogenitor cells can be transported via vessels to distant tissues. However, whether such a mechanism occurs in adults or during fracture repair is uncertain.

Angiogenesis and Adipogenesis

Angiogenesis coordinates and fat indirectly assists in the optimal osteoblastic response, each representing a potential therapeutic target. Angiogenesis is coupled to bone formation especially during times of enhanced bone remodeling and fracture healing [34,35]. The growth of new vessels likely precedes bone formation and suggests that vascular endothelium itself regulates the invasion of osteoprogenitor cells into the fracture. The discovery that bone remodeling compartments (BRCs) are bone structural units that where bone remodeling takes place in adjacent to vascular structures [36] supports this coupling mechanism. The BRC structure consists of a roof of flattened vascular endothelial cells and a vascular space that is adjacent to the marrow space with the bone remodeling surface on the opposite side next to ossified bone matrix. The vascular space provides a mechanism whereby both osteoclast and osteoblast precursors are transported to areas of bone turnover. Cooperative secreted factors support the BRC—BMP-2 is expressed in endothelial progenitor cells, which then stimulates osteoblasts to produce vascular endothelial growth factor (VEGF) [37]. Moreover in the initial stages of BRC formation, hypoxia itself stimulates expression of both of these factors via upregulation of the hypoxia-inducible factor-1 alpha (HIF-1 α) pathway [38**, 39,40]. Targeting VEGF and HIF-1 α has been proposed as a strategy to accelerate fracture healing and enhance bone formation in osteoporosis [41].

Although a connection between bone and fat has been proposed for many years, only until recently have mechanisms have been elucidated [42*]. Aged subjects with lower bone density have higher bone marrow fat; and conversely, subjects with normal bone density have lower amounts of marrow fat. Although these two cell types are derived from the same MSC precursor, does one cell type form at the expense of another due to limiting numbers of MSCs? Glucocorticoids and PPAR γ agonists in the thiazolidinedione (TZD) class of

diabetes medications reduce bone mass and increase bone marrow fat by promoting adipogenesis in bone marrow MSCs [43,44]. Furthermore, TZDs have been associated with an increased fracture risk [45]. Factors have been identified that promote osteoblastogenesis at the expense of adipogenesis such as WNTs [46], BMPs, TGF β and MSX2 [47], as well as mechanical stimulation [48]. Conversely, PPAR γ promotes adipogenesis at the expense of osteoblastogenesis. A critical question is can adipocytes transdifferentiate into osteoblasts? Previous work has shown that stem cells derived from adipose tissue retain the capacity to form osteoblasts [49]

The Future

Conventional treatment for bone fracture is to join or fixate the fractured ends and allow physiologic ossification and remodeling to occur. The application of BMP-2 or biodegradable scaffolds enhance repair times. However, failure of fracture repair is still common especially when associated with chronic disease. Can strategies to manipulate MSCs be harnessed to treat disorders of bone mineralization? Discoveries that circulating osteoprogenitor cells are able to differentiate into mature osteoblasts and mineralize suggest that a pool of bone forming cells exist outside of bone and are able to mobilize during times of need [50]. Or, are these circulating cells released from the fracture site and are only a consequence of fracture? Do endocrine signals emanate from a fracture, recruiting osteoprogenitor cells from distant tissues to be released into the circulation and then migrate to the fracture site? Inflammatory cytokines are certainly released into the circulation but how and which of these contribute to the systemic osteoblastogenesis are unclear. Does there exist an immediate supply of osteoprogenitors in periosteum that are recruited during a fracture? Similar questions can be posed for osteoporosis. Although linked with normal aging, osteoporosis can also be considered a failure of optimal bone remodeling and repair. Can osteoprogenitor cells be infused and migrate to sites of fractures or osteoporosis? Potential allogeneic sources MSCs include peripheral [30] and umbilical cord blood [51], amniotic fluid [52], and teeth [53]. Such MSC targeted therapies could be enhanced with local delivery of VEGF or HIF-1 α pathway stimulators in bone.

Irrespective of how osteoprogenitor cells arrive in bone, preliminary evidence suggests that direct application of these cells may have benefit. In a case series of three patients with large bone defects, osteoprogenitor cells were isolated, expanded from bone marrow and then placed into bone matrix scaffolds. Although the treatment was reported to reduce healing time, this was not a controlled trial [54]. A similar strategy was examined for treatment of osteogenesis imperfecta but again a control group was not examined [55]. Recent developments in bone scaffolds such as electrospun nanofibers [56], biodegradable polymers like poly lactic-co-glycolic acid (PLGA) [57] and other biodegradable polymers will likely enhance novel strategies involving osteoprogenitor cells.

Mobilizing MSCs from bone marrow fat represent another strategy of bone regeneration. Insight into the connection between bone and fat came from the thiazolidinediones (TZDs), a class of drugs first introduced in the 1990s for treatment of type 2 diabetes. These PPAR γ agonists switch the developmental programming of MSCs towards adipogenesis. Therefore, could PPAR γ antagonists be developed to increase bone mass, reduce marrow fat but without reducing insulin resistance and causing diabetes? Another class of medication, teriparatide (parathyroid hormone 1-34), is the only bone anabolic agent approved for treatment of osteoporosis. Parathyroid hormone, normally associated with bone loss, when administered in intermittent pulses, acts as an osteoblast stimulator [10,58]. A recent study suggests that teriparatide may reduce fracture healing time. Aspenberg *et al*, studied 102 postmenopausal women with distal radial fractures. Time to complete cortical bridging in the teriparatide group was 7.4 week compared to 9.1 weeks in the control group [59**].

Conclusion

The next ten years will likely see the development of novel strategies to enhance fracture repair, treat fracture failure and promote bone formation in osteoporosis. It is likely that combinations of systemic therapies to enhance MSCs recruitment to bone and local treatments to enhance angiogenesis and prepare the bone microenvironment for ossification will be the most effective.

Acknowledgments

The author is supported by grants from the National Institutes of Health (CA118428) and the Department of Defense (PC0737756).

References

1. Robey, PG.; Boskey, AL. The composition of bone. In: Rosen, CJ., editor. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*. American Society for Bone and Mineral Research; Washington, D.C.: 2008. p. 32-38.
2. Hauschka PV, Mavrakos AE, Iafrazi MD, et al. Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-Sepharose. *Journal of Biological Chemistry*. 1986; 261:12665–12674. [PubMed: 3745206]
3. Everts V, Delaisse JM, Korper W, et al. The bone lining cell: its role in cleaning Howship's lacunae and initiating bone formation. *J Bone Miner Res*. 2002; 17:77–90. [PubMed: 11771672]
4. Tatsumi S, Ishii K, Amizuka N, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab*. 2007; 5:464–475. [PubMed: 17550781]
5. Lu C, Hansen E, Sapozhnikova A, et al. Effect of age on vascularization during fracture repair. *J Orthop Res*. 2008; 26:1384–1389. [PubMed: 18464248]
6. Gruber R, Koch H, Doll BA, et al. Fracture healing in the elderly patient. *Exp Gerontol*. 2006; 41:1080–1093. [PubMed: 17092679]
7. Gandhi A, Liporace F, Azad V, et al. Diabetic fracture healing. *Foot Ankle Clin*. 2006; 11:805–824. [PubMed: 17097518]
8. Castillo RC, Bosse MJ, MacKenzie EJ, Patterson BM. Impact of smoking on fracture healing and risk of complications in limb-threatening open tibia fractures. *J Orthop Trauma*. 2005; 19:151–157. [PubMed: 15758667]
- 9 *. Dean DB, Watson JT, Moed BR, Zhang Z. Role of bone morphogenetic proteins and their antagonists in healing of bone fracture. *Front Biosci*. 2009; 14:2878–2888. [PubMed: 19273241]
This review highlights the development and current trends of BMPs in fracture healing.
10. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med*. 2001; 344:1434–1441. [PubMed: 11346808]
11. Komatsubara S, Mori S, Mashiba T, et al. Human parathyroid hormone (1-34) accelerates the fracture healing process of woven to lamellar bone replacement and new cortical shell formation in rat femora. *Bone*. 2005; 36:678–687. [PubMed: 15781006]
12. Khosla S, Burr D, Cauley J, et al. Bisphosphonate-associated osteonecrosis of the jaw: Report of a task force of the American Society for Bone and Mineral Research. *Journal of Bone & Mineral Research*. 2007; 22:1479–1491. [PubMed: 17663640]
13. Burge R, Dawson-Hughes B, Solomon DH, et al. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res*. 2007; 22:465–475. [PubMed: 17144789]
14. Kassem M, Abdallah BM. Human bone-marrow-derived mesenchymal stem cells: biological characteristics and potential role in therapy of degenerative diseases. *Cell Tissue Res*. 2008; 331:157–163. [PubMed: 17896115]
15. Olson EN. MyoD family: a paradigm for development? *Genes Dev*. 1990; 4:1454–1461. [PubMed: 2253873]

16. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell*. 1994; 79:1147–1156. [PubMed: 8001151]
17. Mori-Akiyama Y, Akiyama H, Rowitch DH, de Crombrughe B. Sox9 is required for determination of the chondrogenic cell lineage in the cranial neural crest. *Proc Natl Acad Sci U S A*. 2003; 100:9360–9365. [PubMed: 12878728]
18. Otto F, Thornell AP, Crompton T, et al. Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. *Cell*. 1997; 89:765–771. [PubMed: 9182764]
19. Mundlos S, Otto F, Mundlos C, et al. Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. *Cell*. 1997; 89:773–779. [PubMed: 9182765]
20. Nakashima K, Zhou X, Kunkel G, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*. 2002; 108:17–29. [PubMed: 11792318]
21. Towler DA, Rutledge SJ, Rodan GA. Msx-2/Hox 8.1: a transcriptional regulator of the rat osteocalcin promoter. *Mol Endocrinol*. 1994; 8:1484–1493. [PubMed: 7877617]
22. Javed A, Afzal F, Bae JS, et al. Specific residues of RUNX2 are obligatory for formation of BMP2-induced RUNX2-SMAD complex to promote osteoblast differentiation. *Cells Tissues Organs*. 2009; 189:133–137. [PubMed: 18728344]
- 23 *. Javed A, Bae JS, Afzal F, et al. Structural coupling of Smad and Runx2 for execution of the BMP2 osteogenic signal. *J Biol Chem*. 2008; 283:8412–8422. [PubMed: 18204048] This report establishes the mechanism of BMP and Runx2 coupling.
24. Gaur T, Lengner CJ, Hovhannisyan H, et al. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. *J Biol Chem*. 2005; 280:33132–33140. [PubMed: 16043491]
25. Lanske B, Karaplis AC, Lee K, et al. PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. *Science*. 1996; 273:663–666. [PubMed: 8662561]
26. Li B. Bone morphogenetic protein-Smad pathway as drug targets for osteoporosis and cancer therapy. *Endocr Metab Immune Disord Drug Targets*. 2008; 8:208–219. [PubMed: 18782017]
27. Long MW, Robinson JA, Ashcraft EA, Mann KG. Regulation of human bone marrow-derived osteoprogenitor cells by osteogenic growth factors. *J Clin Invest*. 1995; 95:881–887. [PubMed: 7860771]
28. Dominici M, Pritchard C, Garlits JE, et al. Hematopoietic cells and osteoblasts are derived from a common marrow progenitor after bone marrow transplantation. *Proc Natl Acad Sci U S A*. 2004; 101:11761–11766. [PubMed: 15282377]
29. Kassem M, Abdallah BM, Saeed H. Osteoblastic cells: differentiation and trans-differentiation. *Arch Biochem Biophys*. 2008; 473:183–187. [PubMed: 18406334]
30. Eghbali-Fatourehchi GZ, Lamsam J, Fraser D, et al. Circulating osteoblast-lineage cells in humans. *New England Journal of Medicine*. 2005; 352:1959–1966. [PubMed: 15888696]
- 31 **. Otsuru S, Tamai K, Yamazaki T, et al. Circulating bone marrow-derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway. *Stem Cells*. 2008; 26:223–234. [PubMed: 17932420] This preclinical study examined the mechanism behind homing of circulating osteoprogenitor cells to bone.
32. Maes C, Kobayashi T, Kronenberg HM. A novel transgenic mouse model to study the osteoblast lineage in vivo. *Ann N Y Acad Sci*. 2007; 1116:149–164. [PubMed: 18083926]
33. Maes C, Kobayashi T, Paruch J, Kronenberg HM. Characterization of the osteoblast lineage in vivo during early bone development. *Journal of Bone and Mineral Research*. 2006; 21:S3.
34. Brandi ML, Collin-Osdoby P. Vascular biology and the skeleton. *J Bone Miner Res*. 2006; 21:183–192. [PubMed: 16418774]
35. Towler DA. The osteogenic-angiogenic interface: novel insights into the biology of bone formation and fracture repair. *Curr Osteoporos Rep*. 2008; 6:67–71. [PubMed: 18778566]
36. Hauge EM, Qvesel D, Eriksen EF, et al. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. *J Bone Miner Res*. 2001; 16:1575–1582. [PubMed: 11547826]

37. Deckers MM, van Bezooijen RL, van der Horst G, et al. Bone morphogenetic proteins stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A. *Endocrinology*. 2002; 143:1545–1553. [PubMed: 11897714]
- 38 **. Wan C, Gilbert SR, Wang Y, et al. Activation of the hypoxia-inducible factor-1alpha pathway accelerates bone regeneration. *Proc Natl Acad Sci U S A*. 2008; 105:686–691. [PubMed: 18184809] This important study uncovered a critical mechanism of vasculature-bone coupling.
39. Bouletreau PJ, Warren SM, Spector JA, et al. Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: implications for fracture healing. *Plast Reconstr Surg*. 2002; 109:2384–2397. [PubMed: 12045566]
40. Wang Y, Wan C, Deng L, et al. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. *Journal of Clinical Investigation*. 2007; 117:1616–1626. [PubMed: 17549257]
41. Riddle RC, Khatri R, Schipani E, Clemens TL. Role of hypoxia-inducible factor-1alpha in angiogenic-osteogenic coupling. *J Mol Med*. 2009; 87:583–590. [PubMed: 19415227]
- 42 *. Rosen CJ, Klibanski A. Bone, fat, and body composition: evolving concepts in the pathogenesis of osteoporosis. *Am J Med*. 2009; 122:409–414. [PubMed: 19375545] This review highlights the recent data on the fat-bone connection.
43. Gimble JM, Robinson CE, Wu X, et al. Peroxisome proliferator-activated receptor-gamma activation by thiazolidinediones induces adipogenesis in bone marrow stromal cells. *Mol Pharmacol*. 1996; 50:1087–1094. [PubMed: 8913339]
44. Lecka-Czernik B, Moerman EJ, Grant DF, et al. Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2 ligands on adipocyte versus osteoblast differentiation. *Endocrinology*. 2002; 143:2376–2384. [PubMed: 12021203]
45. Grey A. Thiazolidinedione-induced skeletal fragility--mechanisms and implications. *Diabetes Obes Metab*. 2009; 11:275–284. [PubMed: 18671797]
46. Ross SE, Hemati N, Longo KA, et al. Inhibition of adipogenesis by Wnt signaling. *Science*. 2000; 289:950–953. [PubMed: 10937998]
47. Ichida F, Nishimura R, Hata K, et al. Reciprocal roles of MSX2 in regulation of osteoblast and adipocyte differentiation. *J Biol Chem*. 2004; 279:34015–34022. [PubMed: 15175325]
48. Luu YK, Capilla E, Rosen CJ, et al. Mechanical stimulation of mesenchymal stem cell proliferation and differentiation promotes osteogenesis while preventing dietary-induced obesity. *J Bone Miner Res*. 2009; 24:50–61. [PubMed: 18715135]
49. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002; 13:4279–4295. [PubMed: 12475952]
50. Matsumoto T, Kuroda R, Mifune Y, et al. Circulating endothelial/skeletal progenitor cells for bone regeneration and healing. *Bone*. 2008; 43:434–439. [PubMed: 18547890]
51. Rosada C, Justesen J, Melsvik D, et al. The human umbilical cord blood: a potential source for osteoblast progenitor cells. *Calcif Tissue Int*. 2003; 72:135–142. [PubMed: 12457262]
52. De Coppi P, Bartsch G Jr, Siddiqui MM, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol*. 2007; 25:100–106. [PubMed: 17206138]
53. Miura M, Gronthos S, Zhao M, et al. SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A*. 2003; 100:5807–5812. [PubMed: 12716973]
54. Quarto R, Mastrogiacomo M, Cancedda R, et al. Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med*. 2001; 344:385–386. [PubMed: 11195802]
55. Horwitz EM, Gordon PL, Koo WK, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cell therapy of bone. *Proc Natl Acad Sci U S A*. 2002; 99:8932–8937. [PubMed: 12084934]
56. Gupta D, Venugopal J, Mitra S, et al. Nanostructured biocomposite substrates by electrospinning and electrospraying for the mineralization of osteoblasts. *Biomaterials*. 2009; 30:2085–2094. [PubMed: 19167752]
57. Ngiam M, Liao S, Patil AJ, et al. The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering. *Bone*. 2009; 45:4–16. [PubMed: 19358900]

58. Kaback LA, Soung do Y, Naik A, et al. Teriparatide (1-34 human PTH) regulation of osterix during fracture repair. *J Cell Biochem.* 2008; 105:219–226. [PubMed: 18494002]
- 59 **. Aspenberg P, Genant HK, Johansson T, et al. Teriparatide for Acceleration of Fracture Repair in Humans: A Prospective, Randomized, Double-blind Study of 102 Postmenopausal Women with Distal Radial Fractures. *J Bone Miner Res.* 2009 epub ahead of date. This is the first prospective clinical trial to examine teriparatide in fracture healing.

Figure. MSC lineage allocation and bone remodeling

Lineage specification of MSCs is regulated by transcription factors that act as “master switches”. Members of the MYOD family specify a muscle fate, PPAR γ directs adipocyte differentiation and SOX9 regulates chondrogenesis. RUNX2 expression promotes osteoblastogenesis. Bone remodeling is divided into an osteoclast-dependent resorption phase, which is followed by recruitment of osteoblast precursors to the resorption pit. Osteoblasts then secrete a mineralizing matrix. Angiogenesis is intimately connected with bone formation and regulated by BMPs and VEGF.