Prospects for Osteoprogenitor Stem Cells in Fracture Repair and Osteoporosis

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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 monocyctic cells whose formation is regulated by bone marrow stromal cells and early

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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 (PPARγ) 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 (Runx-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.

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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-1α) pathway [38**, 39,40]. Targeting VEGF and HIF-1α 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 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 PPARγ 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, TGFβ and MSX2 [47], as well as mechanical stimulation [48]. Conversely, PPARγ 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-1α 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 PPARγ agonists switch the developmental programming of MSCs towards adipogenesis. Therefore, could PPARγ antagonists be developed to increased 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 woman 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.

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References


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, PPARγ directs adipocyte differentiation and SOX9 regulates chondrogenesis. RUNX2 expression promotes osteoblastogenesis. Bone remodeling is divided into a 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.