Hematopoietic Stem Cells and Bone Regeneration

16

Reeva Aggarwal, Vincent J. Pompili, and Hiranmoy Das

Contents

Abstract

The mechanisms of therapeutic regeneration of osseous tissue in field of orthopedics still remain elusive. Not only cytokines, growth factors, terminally differentiated cells, and mechanical factors play role in etiology of bone functioning but also the numbers, and functionality of stem cells play critical role in bone regeneration and maintenance of bone health. Thus, regeneration of bone tissue using stem cells promises an efficient therapeutic approach for lost or traumatized bone tissue. This chapter emphasizes the factors regulating plasticity of hematopoietic stem cells to trans-differentiate into unconventional cell lineages and osteoblastic lineage. We discuss about the therapeutic application of stem cells of hematopoietic and non-hematopoietic origin for regeneration of bones in the preclinical and clinical setting. We also provide evidences of the use of hematopoietic stem cells for bone regeneration, particularly in osteoporotic bone diseases.

Introduction

Bone loss is one of the major public health issues and poses major morbidity and socioeconomic burden. Pathological degeneration, traumatization or nutritional deficiencies lead to unhealthy skeletal alterations and loss of integrity. These factors have been exacerbated due to

R. Aggarwal • V.J. Pompili • H. Das (✉)
Cardiovascular Stem Cell Research Laboratories, Davis Heart and Lung Research Institute, BRT, 460 W 12th Avenue, Columbus, OH, USA
e-mail: hiranmoy.das@osumc.edu

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rise in sedentary and ageing population. Orthopedic surgeries that involve replacement by an implant, tissue engineering with biocompatible scaffolds or bone grafting are rapidly on rise but some diseases such as non-union fractures, osteoporosis still remain untreatable. Thus, cell-based therapy may be considered as a strategy aimed at replacing, repairing, or enhancing the biological function of a damaged tissue or system by autologous or allogenic transplantation. In this chapter, we will briefly describe the cellular and molecular components in normal and osteoporotic bones. We will describe the existing treatments for osteoporosis and use of emerging stem cell therapy in osteoporotic bone disease.

Evolving concepts on stem cell plasticity challenges the previous views of the stem cell being destined to particular traditional lineages. Previously, adult stem cells such as hematopoietic stem cell (HSC)s were believed to give rise to hematopoietic lineage only, however, it has been reported by others and our lab that HSCs could also give rise to cells of non-hematopoietic lineages, such as muscle cells, vascular cells and osteoblasts (Asahara et al. 1997; Das et al. 2009a; Aggarwal et al. 2012). Not only HSCs, other stromal cells residing in the bone marrow were also found to give rise to several nontraditional cell types. In normal and pathological condition it was found that the stem cells egress from their resident tissue, specifically from bone marrow, circulate and migrate to the site of the injured tissue for repair. At the site of injury, these migrated stem cells could differentiate into the host organ, or fuse with the existing cells and may self-renew to generate more stem cells repertoire. As discussed later in this chapter, our lab has demonstrated that in vivo transplantation of $CD34^+$ cells, derived from hematopoietic stem cells, into an osteoporotic mouse was able to augment bone formation and inhibit bone resorption by affecting the activities of the bone cells (Aggarwal et al. 2012). Thus, stem cell biology may need to be viewed in different perspective to harness the full regenerative potential.

Hematopoietic Stem Cells and Their Lineages

HSC differentiate in vivo to mature blood lineages, which are generally categorized as lymphoid, myeloid and erythroid-megakaryocytic lineages. These progenitor cells then give rise to white blood cells and red blood cells by the process called hematopoiesis. In pre-natal life, hematopoiesis takes place in fetal liver and then shifts to bone marrow in the post-natal life. Rather, HSCs were shown to originate from either hemangioblasts or yolk sac or AGM regions in fetal life. Other progenitor cells such as endothelial progenitor cells were also shown to emerge from hemangioblasts. HSCs undergo self-renewal activities and give rise to either long-term self-renewing HSCs (LT-HSCs) or short-term self-renewing HSCs (ST-HSCs) and committed progenitor cells. LT-HSCs self-renew for life-time of the host while ST-HSCs self-renew for relatively short time (8 weeks) (Morrison and Weissman 1994). Since HSCs constitute only 0.05% of the total bone marrow cell population, these cells were isolated based on their surface markers and their self-renewal capacities, was shown by using limiting dilution assays. HSCs are isolated based on the expression of their surface markers (CD133 and/or CD34). CD133 or AC133 is cell surface marker that represents primitive HSC population and is believed to play a central role in asymmetric division that represents true stemness (Das et al. 2009a). CD133 is mostly expressed on premature HSC population that are $CD34^-$ Lin^- population and at later stages of HSC maturation, CD133 is co-expressed with CD34. This latter population is capable of giving rise to $CD34^+$ cells (Gallacher et al. 2000). CD34, a negatively charged transmembrane glycoprotein, was reported to have role in HSCs adhesion, homing in murine and human studies. It is of importance to current and past studies in our lab whereby we observed the maintenance of CD34 expression after ex vivo nanofiber expansion and also efficient bone marrow homing in animal models of osteoporosis,

myocardial and hind limb ischemia (Das et al. 2009a, b; Aggarwal et al. 2012). However, some reports suggested that HSCs may have heterogeneous expression of CD34 and those had low expression of CD34, were able to cause long-term reconstitution in vivo CD34 expression may be related to cell cycle status and represent a relatively committed progeny as it was found to be very low or undetectable in differentiated myeloid or lymphoid lineages (Morel et al. 1996; Sato et al. 1999; Nakamura et al. 1999). The major functions of CD34 are yet to be fully elucidated.

HSCs have been extensively studied for their transdifferentiation potential based on the evidences from in vitro and in vivo data. Although, HSCs give rise to blood cells in vivo, the former were also shown to give rise to endothelial progenitor cells (EPC), smooth muscle cells, neural cells or bone cells. Our laboratory and others have shown that HSCs could give rise to endothelial cells and smooth/skeletal muscle or osteoblastic cells in vitro when cultured in optimum conditioning media (Asahara et al. 1997; Das et al. 2009a, b; Aggarwal et al. 2012)

Transdifferentiation of Hematopoietic Stem Cells

Relevant to the context here, transdifferentiation of HSCs to osteogenic lineages is still largely unknown. Identification of a putative osteogenic stem cell that would self-renew and differentiate to osteogenic lineages is a field of vast interest to clinicians, biomedical engineers and basic research scientists. Osteoblastic cells were believed to originate only from bone marrow stromal cells, or mesenchymal stem cells (MSCs) defined by their adherence to the plastic culture dish in vitro. MSCs and whole bone marrow were shown to differentiate to adipocytes, chondrocytes and other cell types in vivo and in vitro (Pittenger et al. 1999). Indications of existence of single stem cells that can give rise to hematopoietic stem cells and non-hematopoietic stem cells was demonstrated by serial transplantation of single bone marrow cell, suggesting an existence of long term self-renewing stem cell that could give rise to various lineages of different organ systems (Krause et al. 2001; Jiang et al. 2002). Given, the micro-environmental effects, stem cells of one origin may trans-differentiate to cells of different lineages. Such as, our lab showed that CD34 positive cells differentiate towards osteoblasts in vitro under the influence of ascorbic acid (AA), β-glycerophosphate (BGP) (Aggarwal et al. 2012)

Transcriptional Factors in Osteoblastic Differentiation

Osteogenesis is a process of primary cell differentiation towards osteoblast lineage. Several extrinsic and intrinsic factors influence this process, including hormones and growth factors, which activate osteoblast specific transcription factors and signaling molecules as mentioned below (Fig. 16.1).

Core Binding Factor Alpha1

Core binding factor alpha1 (Cbfa1or Runx2/AML3; runt-related homeodomain/acute myeloid leukemia gene 3) belongs to the family of core binding factors that play key roles in hematopoiesis and osteogenesis. This family of transcription factor consists of DNA binding domains that have high degree of homology to murine runt-related transcription factor 2 (Runx 2) (a drosophila pair-rule gene product). Other members of the Cbfa family are Cbfa2 (AML1) and Cbfa3 (AML2). Cbfa1 was found to be most abundant in osteoblasts and is also present in thymocytes and T cells. It was shown that inherited mutations of Cbfa1 result in severe impairment of osteogenesis in human; this defect is called cleidocranial dysplasia. Absence of Cbfa1 in mice blocked the differentiation processes from the mesenchyme and lacked bone formation, and thus no ossification was observed. Heterozygous Cbfa1 mice showed bone defects similar to human suffering from cleidocranial dysplasia. In osteoblasts, Cbfa1 was found to bind to promoter regions of the osteocalcin gene, collagen type1a, bone sialoprotein

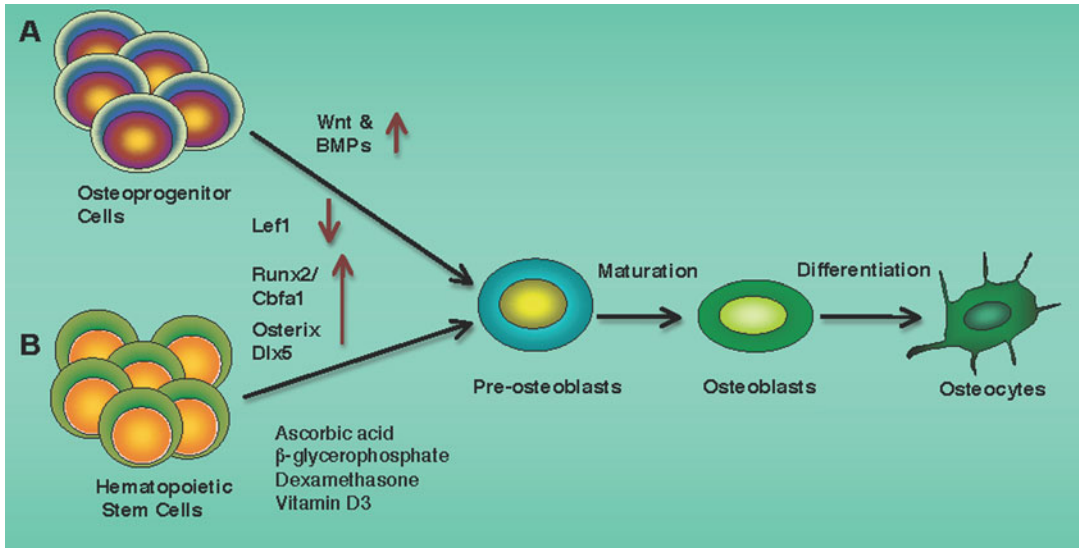

Fig. 16.1 Transcriptional regulation of HSC transdifferentiation. (**a**) Differentiation of osteoprogenitor cells. The commitment signal is initiated via BMPs and Wnt signaling molecular binding to the membrane receptors of osteoprogenitor cells. These signals mediate downregulation of transcription factor, such as Lef1, and upregulation of master regulators such as Runx2/Cbfa1, Osx, and Dlx5. These factors induce preosteoblastic differentiation and mediate cellular commitment towards maturation and differentiation towards osteocytes. (**b**) Hematopoietic stem cells when induced in vitro towards osteogenic lineage by using differentiation factors, such as, ascorbic acid, β-glycerophosphate, dexamethasone, and vitamin D also showed similar pattern in expression of regulatory transcription factor as observed in osteoprogenitor cells. Wnt: wingless; BMP: bone morphogenetic protein; Lef1: lymphocyte enhancer factor1; Runx2: runt-related transcription factor 2; Cbfa1: core binding factor alpha1; Osx: osterix; Dlx5: distal-less homeobox 5

and osteopontin (Ducy and Karsenty 1995). However, activation of Cbfa1 requires binding of bone morphogenetic protein (BMP2) to its cell membrane bound receptors that subsequently activates the cytoplasmic signaling molecules such as mothers against decapentaplegic (Smad)s, which enter the nucleus and complex with Cbfa1. Cbfa1 is critical for the induction of differentiation of stem cells to osteoblasts, however, in vitro differentiation of myoblast cell line (C2C12) could not be induced by activation of Cbfa1 alone (Lee et al. 1999). It is believed that the other transcription factors also play critical role in osteoblastic differentiation. It was suggested that although cbfa1 is a potent inducer of differentiation of progenitor cells to osteochondrogenic lineages, other transcription factors such as Osterix, is potentially involved in the guiding of osteochondrogenic cells towards osteogenic lineages.

Osterix

Osterix (Osx) is a zinc finger domain containing transcription factor, expressed in developing bones. In embryonic and post-natal life, Osx was shown to promote osteoblastic differentiation and formation of new bone. Osx was shown to be required for both endochondral and intramembranous ossification, and acts as a downstream factor of Cbfa1 gene (Nakashima et al. 2002). In Osx null mice, Cbfa1 expression was detected in the stem cells of mesenchymal origin in Osx null mice, however, stem cells were unable to differentiate to osteoblasts or deposit bone matrix, hence no bone formation occurred, and mice died at birth (Nakashima et al. 2002). It was also found that potent bone inducing proteins such as bone morphogenetic protein 2 (BMP2) induces expression of osterix, via induction of distal-less homeobox 5 (Dlx5), which binds to the homeodomain sequences on the proximal region of the osx

promoter. Furthermore, it was suggested that Cbfa1 is involved in the commitment of progenitor cells to osteochondro progenitor, however, Osx may have a role in segregation of osteoblasts from osteochondro progenitor cells and plays an essential role in genetic programming of osteocytes and osteoclasts. In post-natal life, Osx null mice showed hyper mineralized bones in the metaphysial region with limited bone marrow cells and smaller and lower number of osteoclast cells. Thus, since bone formation was affected in Cbfa and osterix null mice, these transcription factors were considered critical for healthy bone development in vivo.

Interestingly, Osx overexpression in stem cells induces them to differentiate towards hematopoietic lineages instead of osteoblastic lineages. It was shown that higher levels of Osx in stem cells indeed lead to upregulation of CD34, hematopoietic stem cells markers, and GATA1 (marker of erythroid lineages), even though the stem cells were cultured in the known osteoblastic conditioning media containing ascorbic acid, β-glycerophosphate, and dexamethasone. Conversely, overexpression of homeobox B4 (HoxB4), a well-known transcription factor of HSCs, lead to osteogenic differentiation of stem cells of embryonic origin, evident by the upregulation of osteoblast specific proteins, such as osteocalcin, bone sialoprotein, and collagen type I. Also, expression of Osx at low level induced transcription of HoxB4, which perhaps may support the idea that cell-cell interaction between HSCs and pre-osteoblasts is required for hematopoiesis. On the other hand, higher levels of HoxB4 in adult CD34 cells may be an underlying cause of pathological mineralization in arteries via expression of bone specific genes such as bone sialoprotein. Thus, these studies definitely point towards a possibility of a close relationship in origin and development of bone and blood lineages at an earlier stage of commitment.

Lymphocyte Enhancer Binding Protein1

Lymphocyte enhancer binding protein1 (Lef1), also known as T cell factor (TCF) is a DNA binding high mobility group (HMG) transcription factor that associates with β-catenin and acts as nuclear effectors in Wnt pathways, essential for maintaining bone homeostasis (van Genderen et al. 1994). However, Lef1 was shown to have less important role in osteoblastic differentiation unlike Cbfa1 and osterix. Although Lef1-deficient mice were found smaller in size and have numerous skeletal deformities compared to littermate controls, however, they still have osteoblasts and mineralized skeletal structures (van Genderen et al. 1994; Galceran et al. 2004), Lef1 regulates osteoblastic differentiation by controlling the expression of extracellular matrix proteins, which are essential for matrix mineralization and terminal osteoblastic differentiation. The lack of proliferation capacity may be due to the fact that these cells are already destined to osteoblastic fate (Kahler et al. 2006). Also, mutations in Wnt pathways such as Wnt3a, Wnt10b, their receptors Lrp6, inhibitors dickkopf (Dkk1, Dkk2) were shown to affect osteoblast functioning, bone formation, and total bone mass (Bennett et al. 2005; Holmen et al. 2004; Morvan et al. 2006). Lef1 is also expressed in lymphocytes and their progenitor cells, such as hematopoietic stem cells (HSCs). Lef1 has been shown to regulate the self-renewal activities of HSCs and the dysregulation of the expression level has been linked to malignancy. However, studies on the murine blood precursors has shown that Lef1 expression is limited to precursor cells only and is turned off at the late stage of lymphocytic development. Similarly, high expression of Lef1 was observed in the pre-osteoblastic, undifferentiated cell lines (MC3T3-E1). Lef1 was found to be downregulated upon osteogenic induction in vitro. It was found that overexpression of Lef1 (almost 140-fold) inhibited the terminal osteoblastic differentiation as was evident by the expression of late bone specific proteins such as alkaline phosphatase, osteocalcin and calcium incorporation. However, reports showed that both upregulation or downregulation of Lef1 affected the proliferation rate of the pre-osteoblastic cells (MC3T3-E1) (Kahler et al. 2006) The evidences of similar activities of Lef1 in two different types of progenitor cells indicate that progenitor cells could be induced towards different lineages, or there is

a possibility that these progenitor cells have a common origin. Furthermore, genetic studies are needed to address specific questions regarding the effects of transcriptional factors in Wnt signaling pathways, which controls progenitor cell differentiation towards osteoblastic lineages and their maturation. The transcription factors and their roles were described above to establish an understanding of the osteoblastic origin and their differentiation from stem cells into mature functional osteoblasts. The above-mentioned transcription factors, reviews the current literature that may provide evidences of stem cell plasticity such as HSCs and their transcriptional regulation dictated by the microenvironment.

To understand the regenerative potential of bone, understanding of cellular components of the bone is important. Bone is being recognized as a regenerative organ as it harbors stem cells in its anatomical structures, such as periosteum (outer most layers of the bones), and bone marrow, however, its regenerative potential declines with age, and is influenced by other risk factors. Major cellular compartment of bone consists of the osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells), and an imbalance in their cellular activities results in various bone disorders such as osteoporosis.

Bone Degenerative Disorder: Osteoporosis

Osteoporosis is a major health problem, affecting almost 75 million people worldwide, especially women and elderly people (WHO 1994). It is a chronic disease that is characterized by low bone density, brittle bones and is caused due to an imbalance of bone formation and resorption activities of the bone cells. This skeletal disorder increases the risk of skeletal fractures, high morbidity and mortality. Multiple factors such as genetics, age, hormonal, drug usage, and environmental factors are responsible for osteoporosis. From various reports it is evident that osteoporosis may also be developed by an age related decline in number, and functionality of stem cells (that give rise to osteoblasts) in rodents, primates, and humans, confirmed by in vitro colony forming assays, mineralization, and alkaline phosphatase staining. Certain pathological conditions or age may induce the differentiation of osteoblastic progenitor cells towards adipocytes rather than osteoblasts (Moerman et al. 2004). Thus, due to the potential limitations of body's own stem/progenitor cells to maintain bone homeostasis, stem cell based alternative therapies are being investigated to provide long-term self-renewing source of bone forming cells. In this section we will summarize the function of osteoblastic and osteoclastic cells and current therapies for osteoporosis followed by emerging new cell-based therapies.

In adult skeleton, bone forming cells or osteoblasts make up to 4–6% of all the bone, whereas, osteocytes make up to 90–95% of the bone. However, all these cells play distinct roles in the initiation and regulation of mineralization of bone matrix. Osteoblasts are mononuclear cells that are recruited to the site of bone formation mainly from stem cells of mesenchymal origin via osseous vasculature. Once, osteoblasts reach the bone surface or bone-remodeling sites, osteoblastic cells produce bone matrix and get mineralized. Later osteoblasts either get embedded within the matrix to become osteocytes or die by apoptosis, thereby releasing signals for resorption, which activates bone resorbing (osteoclasts) cells that targets their removal of dead osteocytes. Osteocyte cell death also occurs in association with pathological conditions, such as osteoporosis leading to skeletal fragility; which results in the loss of the ability to sense microdamage and signal repair. Thus osteocyte viability plays a significant role in maintaining healthy bone.

Osteoclasts or bone resorbing cells are specialized multinucleated cells, derived from the mononuclear monocytic/macrophage precursors, and comprise 1–2% of bone. These mononuclear cells differentiate and fuse to form multinuclear osteoclastic cells in presence of the two differentiating factors, receptor activator of nuclear factor kappa- β ligand (RANKL) and macrophage colony-stimulating factor (MCSF). Bone resorption by osteoclast requires a unique polarization of its cytoskeleton to form ruffled bordered

membrane over the bone matrix. In addition to ruffled border membrane, contact with the bone also causes the reorganization of actin cytoskeleton to form sealing zone or actin ring with the help of integrins. These acidified compartments enables the osteoclast for resorptive purposes by release of vesicles that contain matrix metalloproteases (MMPs), cathepsin K and hydrochloric acid (HCl) to dissolve the underlying type1 I collagen in the bone matrix (Teitelbaum 2000).

Current Therapies for Osteoporosis

As indicated earlier, metabolic disorders such as osteoporosis results from the imbalance between bone resorption and formation, understanding and restoring the balancing mechanisms are important for the development, efficacy, and long-term benefits of therapeutic agents. Current available treatments are largely anti-resorptive, which help to inhibit the resorptive activities of the osteoclasts, however, simultaneously suppress bone formation thus reducing their bone remodeling and thus lowering the overall performance of the drug. So far, only one anabolic treatment mediated via parathyroid hormone is FDA approved for osteoporotic patients and once patients are off- the medication, the osteoporotic symptoms re-appear (Kawai et al. 2011). Thus, it has become paramount to identify molecules and/or therapeutic strategies that could regulate both resorption and formation in synchrony. Newer research suggests that cell therapy may be an option that may restore the balance between bone formation and resorption, and restore the normal bone homeostasis. This section will highlight the current treatments in clinic and development of newer therapies for osteoporosis.

Antiresorptive Drugs

Bisphosphonates are potent inhibitors of bone resorption, and were shown to increase bone mineral density (BMD) and reduce the risk for osteoporotic fractures with reduction in bone turnover in numerous clinical trials. Bisphosphonates chemically bond to the calcium hydroxyapatite in the bone and thus decrease bone resorption by blocking the function and survival of osteoclasts. For example, in Fracture Intervention Trial (FIT), postmenopausal women with low femoral neck BMD were enrolled in randomized, double blind, placebo controlled multi center studies, were shown to have significantly reduced rates of vertebral fractures when compared to placebo controls (Black et al. 1996). However, potential adverse effects (many women with subtrochanteric or typical fractures) of bisphosphonates after a treatment for continued long period of time has been reported. Newer antibody based anti-resorptive drugs, such as denosumab are still under investigation. It is a human monoclonal antibody against the RANK ligand and thus prevents its binding to its receptor RANK, on the osteoclasts and its precursor cells, and thus, has been claimed to reversibly inhibit osteoclast-mediated resorption. In contrast to bisphosphonates, denosumab acts by blocking the formation, function, and survival of osteoclasts. The major concerns of denosumab were related to an increase in overall incidence of cancer, infection or skin problems, as RANK and RANKL are expressed on the members of the lymphoid family. In a separate study, increased incidences in osteonecrosis of jaw (ONJ) caused by both of the above-mentioned anti-resorptive therapies were reported. However, no cases of ONJ were reported in patients on selective estrogen receptor modulators (SERMS) (Nalliah 2012).

SERMS are non-steroidal synthetic compounds that mimic estrogen effects in tissue-specific manner. Since, estrogens causes increase in bone density and inhibits bone turnover, estrogen deficiency in postmenopausal women increases bone turnover and resorption, leading to bone loss. Thus, hormone replacement therapy reverses bone loss in early and late phases of postmenopausal period. However, use of certain SERMS for bone therapy may exert cancerous effects on extra-skeletal tissues, such as breast and uterus, raising concerns for their long-term safety (Rodan and Martin 2000).

Other antiresoprtive drugs such as odanacatib selectively and reversibly inhibits cathepsin K, a cysteine protease expressed in osteoclasts, degrades type I collagen. However, it was found that effects on the bone formation markers of this drug were lesser compared to other anti-resorptive drugs, such as bisphosphonates, and their potential side effects are still unknown (Bone et al. 2010).

Anabolic Drugs

Anabolic drugs were designed to increase bone mineral density by stimulating bone formation and increasing bone remodeling. There are number of anabolic therapies, including bone morphogenetic proteins (BMP 2, 7), insulin-like growth hormone (IGH), vascular endothelial growth factor, parathyroid hormone (PTH), statins, and strontium fluoride. Anabolic agents, such as PTH have the ability to restore bone mass, restoring bone homeostasis, thus lowering the risk of fractures, more than that of anti-resorptive agents. PTH and its analogs, BMP2 and BMP7 were one the FDA approved anabolic drugs for the treatment of osteoporosis. Low levels of PTH directly increase bone mass stimulating osteoblastic survival and activities. Indirectly, PTH regulates skeletal growth factors that induces IGH synthesis, inhibits sclerostin expression (antagonist of BMPs), and activates Wnt signaling pathways. The limitation of PTH are that they need to be administered everyday (Jilka 2007). PTH was shown to increase BMD as well as bone strength in osteoporotic women and men. Statins, a class of lipid lowering drugs, used for the treatment of cardiovascular disease was also shown to reduce the risk of fractures by stimulating the production of BMPs and endothelial nitric oxide synthase (eNOS). However, inhibition of osteoblastic eNOS did not prevent statins in bone formation (van't Hof and Ralston 2001). Newer drugs such as, monoclonal antibody (AMG 785), to sclerostin (antagonists of BMP), induced a dose dependent increase in the bone formation markers, as well as decrease in the bone resorption markers. However, other effects are largely unknown as potential drugs are still under investigation. Newer research has shown that semaphorin 3A (Sema 3A), an axon guidance molecule, expressed by osteoblasts, has a distinct osteoprotective effects in osteoporotic murine models. Sema 3A was shown to have crucial role in bone formation by osteoblast cells, and at the same time limiting the migration, and activities of osteocalsts to the bone formation sites through inhibiting the expression of cytoskeletal protein RhoA and immunoreceptor tyrosine-based activation motif (ITAM) (Hayashi et al. 2012). Interestingly, it was pointed that as mice ages, their serum level of Sema 3A reduced and this could be one of the factors for bone loss due to age. Thus, Sema 3A could be used as a potential bone formation biomarker. Above studies direct towards a possible development of therapies that would restore coupling of bone cells (Hayashi et al. 2012).

Stem Cell Therapies for Bone Regeneration in Osteoporosis

Stem cells have taken a center-stage of the field of regenerative medicine, owing to their self-renewal capacity and ability to differentiate towards various lineages, depending on their potency. In particular, two types of stem cells have been used in bone related pathologies: embryonic stem cells (ESCs) and adult stem cells (ASCs). Adult stem cells particularly comprise of hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and could be derived from various tissues such as bone marrow, umbilical cord blood (UCB), fat tissue or peripheral blood. In particular, UCB-derived stem cells, such as MSCs and HSCs transplantation are promising for various systemic and local disorders. Adult stem cells have been documented to present minimal graft vs. host disease and are multipotent in nature. ESCs are totipotent and were shown to be tumorigenic in vivo and are bound by ethical issues. Recently, it was shown in an osteoporotic individual that MSCs transdifferentiate towards non-bone forming cells, such as adipocyte by switching towards the expression of adipocytic transcriptional factor peroxisome proliferator-activated receptor gamma (PPARγ) from cbfa1. MSCs were tested for their ability to restore bone formation in

murine models of fracture and bone pathologies, however, only few studies have focused on their ability to restore skeletal bone micro-architecture in osteoporotic conditions. Potential drawback of using MSCs, in vivo studies were the lack of bone marrow homing markers, which would be needed to guide the MSCs to mobilize towards their target site for bone regeneration. Recently, a study showed that the use of MSCs in combination with overexpression of homing receptors (CXCR4; ligand of stromal-derived factor 1, SDF1) and osteogenic transcriptional factor (Cbfa1) (Lien et al. 2009). Although, this approach increases stem cell's capacity of bone marrow engraftment but techniques, such as transfection of MSCs were of potential concerns. MSCs could also be derived from adipose tissue by liposuction techniques. It was shown that adipose tissue-derived MSCs (AT-MSCs) transduced with CXCR4 showed enhanced capacity of in vitro differentiation towards osteoblastic lineages and low engraftment abilities were overcome by transduction of CXCR4 (Bobis-Wozowicz et al. 2011). There are limited examples of HSC-mediated regeneration of bones. One of the major limitations of HSCs derived from UCB is low number of available stem cells for the clinical applications. Various protocols for ex-vivo expansion of the stem cells are being extensively explored but have relatively lower expansion rates, which preclude the possibility of their use in clinical applications. Our laboratory has established a nanofiber-based ex vivo expansion technology. We reported that human UCB-derived HSCs could be expanded substantially on nanofiber-coated plates supplemented with cytokine cocktail in serum-free media within a very short period of time. The expanded stem cells were shown to differentiate towards various lineages (Das et al. 2009a; Aggarwal et al. 2012). UCB and adipocyte tissue are being investigated as potential sources of adult stem cells, as they are easy to harvest, no ethical concerns, less discomfort to the donor, and almost no hospital stay time. Furthermore, stem cells could be used in variety of ways to treat the bone related disorders. First, isolated stem cells could be directly transplanted into the injured tissue, thereby allowing the stem cells to be differentiated in vivo into any lineage. Second, stem cells could be transfected with osteoinductive genes to direct towards osteoblastic lineages, and transplanted into the injured part of the body. Third, stem cells could be differentiated in vitro into desired lineage under the influence of certain cytokines, growth factors, and then transplanted into the patients. Most of the stem cell-based therapies used non-hematopoietic stem cells and use of hematopoietic stem cells is still being investigated for bone related disorders. Recently, we have shown that transplantation of nanofiber-expanded $CD34^+$ cells ameliorates bone formation and suppresses the bone resorbing activities of the osteoclastic cells. Nanofiber-expanded $CD34^+$ cells constitutively express high levels of pro-migratory (CXCR4) and pro-adhesive (LFA-1) thus circumventing the need of transduction and enabling efficient homing into bone marrow. We also found that nanofiber-expanded $CD34^+$ cells when induced with osteoblastic induction media were able to upregulate osteoblast related genes (such as bone morphogenetic proteins, Type I collagen, osteocalcin) and differentiate into osteoblastic lineages in vitro. Moreover, we showed that transplantation of these cells into a murine model of osteoporosis increases the serum levels of bone formation markers such as osteocalcin, and simultaneously decrease the serum levels of resorptive chemokines such as monocyte chemotactic protein (MCP-1). Systemic delivery of $CD34^+$ cells increases bone remodeling and concurrently inhibits osteoclastic differentiation and activities in osteoporotic mice. Thus, it is possible that $CD34^+$ cells offer an osteoprotective role by synergistic mechanisms that may help to regenerate bone and inhibit the pathological resorptive activities of the osteoclasts (Fig. 16.2) (Aggarwal et al. 2012).

Conclusions and Future Directions

The potential of stem cell based therapies for various bone degenerative diseases is attractive because the existing treatments suppress the disease symptoms, and are unable to regenerate bone tissues at genetic and cellular level to offer longer disease free periods. The demographic

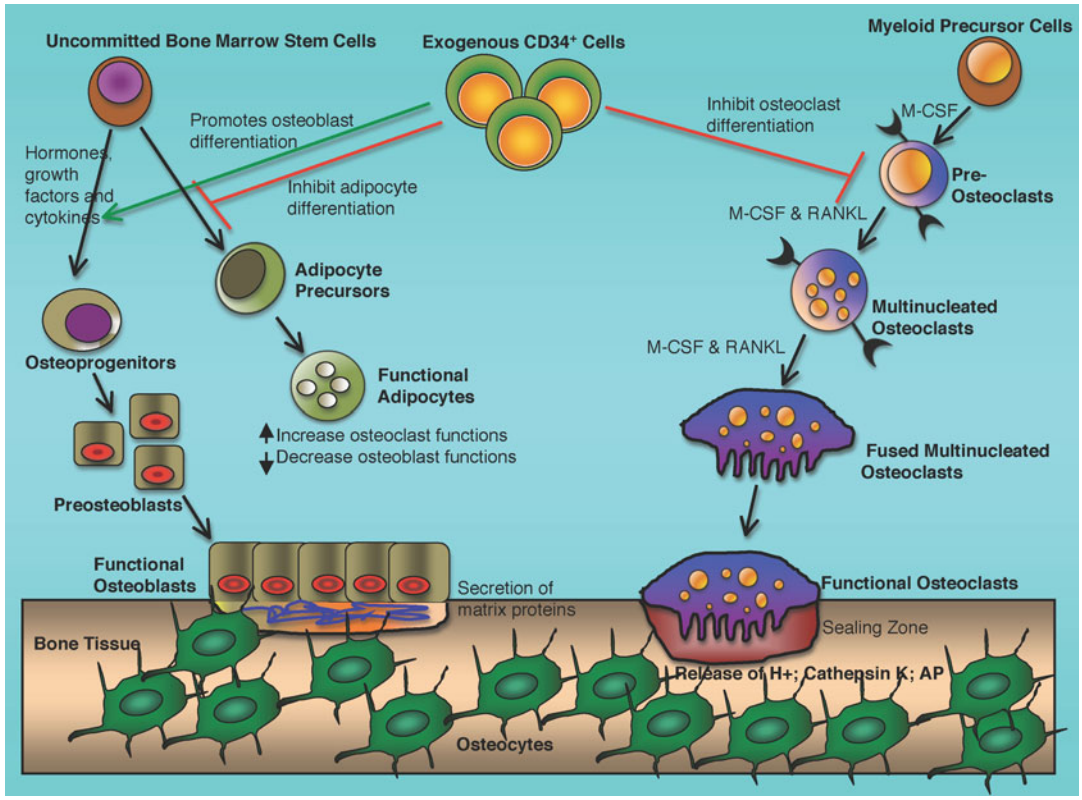

Fig. 16.2 Mechanisms of exogenous CD34⁺ stem cell functions. Exogenous CD34⁺ cells inhibit differentiation of stromal cells towards adipocytic lineages by downregulating the expression of adipocytic master regulator (PPAR γ) in the uncommitted bone marrow stem cells. Thus, bone marrow stromal cells differentiate towards their destined osteoblastic lineages via upregulation of the osteoblast specific transcriptional factors such as Cbfa1/Runx2. Exogenous CD34⁺ cells concomitantly inhibit the differentiation and impair functions of progenitor cells of monocytic-macrophagic lineages, which differentiate towards osteoclasts that resorb the bone matrix and decreases the bone mineral density

challenges presented by the aging population worldwide emphasize the need for innovative research and approaches to address the issues of skeletal restoration. Osteoporosis raises substantial risk of fractures with age. A continuous blood supply is essential to regenerate functional bone tissues, as degeneration of vessels and bones are evident in osteoporosis. So far, vascular supply restoration was shown to be achieved by using stem cells of hematopoietic and non-hematopoietic origins at preclinical levels. Essentially, it was been shown that adult stem cells and embryonic stem cells are able to differentiate into bone cells in vitro and in vivo. However, lot of work still has to be done to understand the mechanistic basis of differentiation and their functionality. These mechanistic studies not only, help us to reveal the origin of the stem cells, but hopefully mirror their functionality in vivo and to find a possible cure for life-long disease free survival. Also, tumorigenic potential in long-term studies, and in vivo fate of therapeutic stem cells after transplantation is largely unknown, and need to be determined prior to therapeutic consideration.

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