CHAPTER 11

BONE MARROW ADIPOGENESIS IN OSTEOPOROSIS

Chao Wan and Gang Li

Department of Trauma and Orthopaedic Surgery,
School of Medicine, Queen's University Belfast,
Musgrave Park Hospital, Belfast, BT9 7JB, UK

Bone marrow adipogenesis is a postnatal event during bone and marrow development. In the adult bone, marrow adipocytes occupy the largest space of the marrow cavity, and serve as a source of energy, autocrine and paracrine factors. Marrow adipocyte share a common multipotential mesenchymal stem cell with other marrow stomal lineages, and functional overlap exists among them. In the marrow stroma microenvironment, adipogenesis is closely associated with osteogenesis, hematopoiesis and osteoclastogenesis. With ageing, increased marrow adipocytes accompany with decreased trabecular bone volume. Marrow adipogenesis may be an important complication of osteoporosis. Many regulators including hormones, growth factors, proinflammatory cytokines and their receptors such as nuclear hormone receptors, trans-memberane kinase receptors and G-protein coupled receptors are involved in the signaling pathway of adipogenesis, and may present potential molecular targets for the manipulation of adipocyte differentiation. Marrow adipocyte may be considered as an important target cell for the therapeutic intervention in osteoporosis. The inhibition of marrow adipogenesis and concomitant enhancement in osteogenesis may provide a potential approach to increase bone formation and therefore provide more efficacious prevention or treatment of osteoporosis.

1. Introduction

Bone marrow stromal system is composed of different stromal cell lineages, the uncommitted mesenchymal stem cells (MSCs), committed precursors and differentiated osteoblasts, adipocytes, and hematopoietic support stromal cells, among which the adipocytes occupy the largest space in the marrow cavity. Accumulated clinical and experimental research have shown that an increase in marrow adipocytes is associated with conditions that lead to bone loss or osteoporosis, such as aging [1, 2], disuse [3, 4], long-term glucocorticoid use [5], and ovariectomy [6, 7]. Adipogenesis may be an important complication of osteopenia or osteoporosis. The elucidation of the mechanisms of marrow adipogenesis and its regulation has great importance not only for the understanding of bone cell biology, but also for possible therapeutic intervention in osteoporosis and other metabolic bone diseases.

2. Adipogenesis during bone marrow development

In the bone marrow (BM), there are two related systems: the hematopoietic system, which is the major source of adult hematopoietic stem cells (HSCs) that renew the circulating blood elements, and the stromal system, which contains mesenchymal stem cells (MSCs or bone marrow stromal stem cells, BMSSCs) and contributes to the regeneration or renewal of mesenchymal tissues such as bone, cartilage, fat, tendon, muscle, and marrow stroma [8]. The changes of different phenotypes in marrow stromal system during development, growth, and aging appear in

a temporal and spatial sequence along the direction of bone growth. It is well established that chondrogenesis, osteogenesis, pre-hematopoietic stroma, myelogenesis, and adipogenesis are subsequent phases in the history of bone and marrow development, among which only adipogenesis is a post-natal event [9]. According to Neumann's law, at birth, all bone marrow cavities are occupied by red haematopoietic marrow; at skeletal maturity, the whole of long bone diaphyseal marrow cavities is normally filled with yellow adipocytic marrow and red haematopoietic marrow is restricted to the cancellous bone of metaphyses and epiphyses. With aging, the number and size of marrow adipocytes increases in a linear manner [10]. It is estimated that approximately 30% of the proportion of marrow volume in the iliac crest is occupied by adipocytes in early adulthood, 60% or more at the age of 60 [11]. And up to 90% of the marrow cavity in long bones is occupied by adipocytes. Thus, bone marrow adipogenesis or adipocytic differentiation may be considered as the end point of bone development and aging. This also implies that there is a clinical correlation between the reduced bone forming capacity and the increased bone marrow adipogenesis.

3. Function of marrow adipocytes

In the adult bone marrow, the adipocytes occupy the largest space of marrow cavity, playing an important role in maintaining the marrow stroma or marrow microenvironment. A variety of potential functions of marrow adipocytes have been proposed, as listed in several elegant

reviews [12, 13, 14], even though most of their functions need to be further investigated. It is hypothesized that adipocytes act as the "space fillers" for the marrow cavity, where is not required by active hematopoiesis. The changes in number and size of adipocytes occur as a function of changes in total hematopoietic component. As a component of hematopoietic supporting stroma, they exhibit an important role in the processes of lymphohematopoiesis [15-17]. Indeed, many adipocytic products including type 1 interferons (IFNs), prostaglandins (PGs), leptin, adiponectin, and sex steroids are known modulators of lymphohematopoiesis [18-25]. In addition to support lympho-hemaetopoiesis, preadipocytes or adipocytes support osteoclastogenesis [26-30], as several stromal adipocytic cell lines were shown to induce osteoclastogenesis. Adipocytes also play an active role in energy balance, they are not only lipid storing and mobilizing cells but produce or release a vast number of so called adipokines or adipocytokines, including metabolically active molecules belonging to different functional categories like endocrine function (leptin, sex steroids, various growth factors), metabolic function (fatty acids, adiponectin, resistin), and immunity (complement factors). Therefore, adipocytes in the marrow together with the extramedullary fat cells may serve as a source of energy, paracrine, or autocrine factors [31]. One of the examples, leptin, the adipocyte-derived hormone, has been identified as a powerful inhibitor of bone formation, and its effect is mediated via a brain relay, which suggests that a regulation network exists between the adipocytes and the brain [32, 33]. The marrow adipocytes also provide a localized energy reservoir for emergency situations such as blood loss which need

to be recovered by hematopoiesis, or fractures which needs to be reunited by endochondral or intramemberanous ossification processes. Adipocytes may act as support cells for the differentiation of hematopoietic cells and as a source of osteoblasts during bone regeneration. With age, marrow adipogenesis increases when osteogenesis decreases, as osteoblast and adipocyte share a common multipotential precursor, and functional overlap exists between adipocytes and other stromal cell lineages in the bone marrow.

4. Transcriptional regulation of adipocyte differentiation

It is well established that several transcription factors control the signaling pathway of adipocyte differentiation, among which peroxisome proliferator-activated receptor γ2 (PPARγ2) and CCAATT enhancer-binding protein (C/EBP) were best characterized. PPARγ2 has been shown to express early in adipogenesis and act synergistically with CCAATT enhancer-binding protein (C/EBP) to regulate the adipocyte differentiation cascades [34, 35, 36]. PPARγ2 plays important roles in the regulation of adipocyte differentiation, its overexpression in fibroblast cell lines initiates adipogenesis [37] and ES cells and embryonic fibroblastic cells from mice lacking PPARγ2 were unable to differentiate into adipocytes [38-40]. Expression of C/EBP and/or PPARγ2 in fibroblasts converts the cells into adipocytes [37, 41, 42]. A combined expression of PPARγ2 and C/EBPα in G8 myoblastic cells suppresses muscle phenotype and induces adipocyte differentiation [43]. Homozygous PPARγ-deficient ES cells failed to differentiate into

adipocytes, but spontaneously differentiated into osteoblasts, and the adipogenic potentials were restored by reintroduction of the PPARγ gene into the ES cells [44]. Another family of proteins are forkhead related activators (freac), among which the hepatic nuclear factor 3 (HNF3) controls the expression of lipoprotein lipase (LPL), an early adipocyte marker gene [45]. Adipocyte determination and differentiation-dependent factor 1 (ADDl) in the rat and sterol regulatory element binding protein 1 (SREBP-I) in the human [46], are shown to regulate transcription of the low density lipoprotein receptor gene. Recently, the transcription genes such as Zinc finger E-box binding protein (ZEB) and Zinc finger protein 145 (ZNF145) have been shown to regulate adipogenic differentiation of bone marrow derived MSCs [47]. A novel gene E2F5 transcriptional factor is also identified in differentiated adipocyte [48]. Adipocyte differentiation can not be identified solely by cellular morphological changes, but requires evidence of expression of phenotype specific genes. Several downstream adipocyte specific gene products are known to be involved in triglyceride synthesis, which include the early marker of adipocyte differentiation, LPL and the late markers such as glycerol-3-phosphate dehydrogenase (G-3-PD) [49] and the fatty acid binding protein aP2 [50, 51]. Using microarray technology, numbers of related genes are identified during adipogenesis [47, 48, 52-58].

5. Relationship between adipogenesis and osteogenesis

The relationship between adipogenesis and osteogenesis has confused the investigators for many years. Accumulated evidences have shown that an inverse relationship exists between adipocytes and osteoblasts. In 1970s, clinical studies on osteoporotic patients suggested that increased bone marrow adipocytes correlates with decreased trabecular bone volume [59]. Then it was found that ectopic bone formed by the "red" and "yellow" rabbit marrow are equally well, which suggested that both the "red" and "yellow" marrow might contain osteogenic cells [60]. Further investigation demonstrated that both rabbit adipocyte or fibroblast stromal colonies displayed an osteogenic capacity when implanted in diffusion chambers in vivo. The cells that have differentiated in an adipocytic direction are able to revert to a more proliferative stage and subsequently to differentiate along the osteogenic pathway [61]. In vitro, a large number of cell lines have been used to study adipogenesis, among them, the following are used extensively: BMS2 [62], UAMS33 [63], 2T3 [64], and the cell line derived from *p53* null mice [65]. In these cell models, an inverse relationship exists between the differentiation of adipocytic and osteogenic cells, enhanced expression of adipocytic phenotype is paralleled with decreased expression of osteoblastic phenotype [66]. Human cell lines that have been used include MG63, exhibiting a proven adipogenic phenotype in vitro [67, 68]; and those transformed from osteoblasts or MSCs [69-72], as well as the primary MSCs, which can be expanded rapidly in culture. The human bone-derived cells when cultured in the presence of dexamethasone (Dex) and 3-isobutyl-1-methylxanthine (IBMX) will undergo adipogenic differentiation [73]. Recent studies demonstrated that trabecular bone-

derived cells display stem cell-like capabilities, characterized by a stable undifferentiated phenotype as well as the ability to proliferate extensively while retaining the potential to differentiate along the osteoblastic, adipocytic, and chondrocytic lineages, even when maintained in long-term in vitro culture [74].

On the other hand, cloned adipocytes were found to be capable of dedifferentiation into fibroblast-like cells, and subsequently differentiate into two morphologically distinct cell types, osteoblasts and adipocytes [75]. Fat-derived stem cells were also successfully isolated from Lewis rats, and induced to differentiate along adipogenic and osteogenic lineages in vitro and in vivo [76]. These findings provide evidences of *trans*-differentiation between marrow adipocytes and osteoblasts. The terminally differentiated skeletal cells may be able to de-differentiate first, returning to the status of uncommitted stromal stem cells, then the cells differentiate along any other pathway. The plasticity and inter-relationship among the precursors or fully differentiated cells of the marrow stromal lineages may be of great important in understanding the progression of osteoporosis and other skeletal diseases.

6. Relationship between adipogenesis and hematopoiesis

As one of the major components of bone marrow stroma, adipocytes originate from the same MSCs that give rise to the hematopoiesis supporting stromal cells and have been suspected to influence hematopoiesis. The stromal cells secrete many extracellular matrix

proteins including proteoglycans, fibronectin, tenascin, laminin, and express cell surface transmembrane proteins including CD36, CD44, integrins and vascular cell adhesion molecule (V-CAM), mediating adhesion between the stroma and the various blood cell lineages [77, 78]. The preadipocytes display some common marks with the stromal cells, and several preadipocyte stromal cell lines support both lymphopoiesis and myelopoiesis in vitro. In fact, many factors produced by adipocyte, such as type 1 IFNs, PGs, leptin, adiponectin, and sex steroids are known modulators of lymphohematopoiesis [18-25]. Recently, it is reported that adiponectin is produced by adipocytes within human bone marrow and has an inhibitory effect on adipocyte differentiation through a paracrine mechanism [79]. The protein suppresses myelomonocytic progenitor growth and macrophage functions in culture [80] and negatively and selectively influence lymphopoiesis through induction of PG synthesis [24]. These findings suggest new mechanisms for functional interactions between adipogenesis and hematopoiesis within bone marrow. However, patterns of cytokines made by mature adipocytes and preadipocyte stromal cells differ substantially [81]. The functions of these cytokines are partially affected by adipocyte differentiation, adipogenesis alters the expression of the extracellular matrix, membrane proteins, and cytokines in stromal cells. Adipocytes within bone marrow cavities interact with surrounding cells and support the microenvironment that regulate the differentiation of hematopoietic cells.

7. Relationship between adipogenesis and osteoclastogenesis

In addition to supporting hemaetopoiesis, marrow adipocytes support osteoclastogenesis. Osteoclasts are generally believed to derive from hemopoietic precursors in the bone marrow, but their differentiation pathway is complex. Several stromal adipocytic cell lines were employed to investigate the relationship between adipocytogenesis and osteoclastogenesis. When cocultured with preadipocyte or adipocyte-enriched BMS2 stromal layers, primary bone marrow cells undergo osteoclast differentiation and maturation [82]. TMS-14 is a line of preadipocytes that supports osteoclast-like cell formation without any other bone resorbing factors. When treated with thiazolidinedione, a ligand and activator of PPARγ, the ability of TMS-14 cells to support osteoclastogenesis was prevented, together with inhibiting gene expression of osteoclast differentiation factor (ODF, also called OPGL, RANKL, and TRANCE) [83]. Using the myeloblast (M1) cells and the 14F1.1 endothelial-adipocyte stromal cell line coculture system, it was demonstrated that marrow endothelial-adipocytes may play a role in regulating the differentiation of myeloblasts into osteoclasts [84]. MC3T3-G2/PA6 cells are preadipocytes similar to bone marrow derived stromal cells, and their adipose conversion is induced by glucocorticoids. The research on coculture of PTH-prestimulated long bone cells and MC3T3-G2/PA6 cells suggested that stromal preadipocytes may create a microenvironment conductive to osteoclastogenesis through direct cell-to-cell contact and communication [85]. The soluble factors released by stromal cell lines such as M-CSF and complement component C3 are also involved in osteoclastogenesis. The presence of 1,25 dihydroxyvitamin or adipogenic agonists (hydrocortisone, indomethacin,

methylisobutylxanthine) induces stromal cell production of complement C3 [86-88]. The adipocytes may also serve an active role in the energy metabolism of the resorbing osteoclasts where fatty acid oxidation appears to be the major source of acetyl-CoA to support a predominantly oxidative metabolism [89].

Recently, α-Melanocyte-stimulating hormone (α-MSH), a 13-amino acid peptide produced in the brain and pituitary gland, stimulated osteoclastogenesis, whereas its production is regulated by leptin, a factor that is secreted by adipocytes [90]. The P6 strain of senescence-accelerated mice (SAM) exhibit an early decrease in bone mass with a reduction in bone remodeling. In the bone marrow, suppressed osteoblastogenesis and osteoclastogenesis with enhanced adipogenesis are observed in SAM mice[91]. Interleukin-11 (IL-11) has been shown to potentialy inhibit adipogenesis and to stimulate osteoclastogenesis. Menatetrenone (MK4), a vitamin K(2) with four isoprene units specifically inhibit adipogenesis and osteoclastogenesis of bone marrow cells[92]. Thus, a complex regulation network exists between marrow adipocyte and osteoclastogenesis.

8. Regulators and receptors for regulating adipogenesis and their therapeutic potentials for osteoporosis

Based on the fact that aging is associated with a reciprocal decrease of osteogenesis and an increase of adipogenesis in bone marrow and that adipocytes and osteoblasts share a common multipotential mesenchymal

stem cell, with a conversion relationship existing between them, marrow adipocyte may be considered as a target cell for the prevention and treatment of osteoporosis. However, the regulation of adipogenesis is very complex, and many factors are involved in the regulation pathway of adipocyte differentiation. A series of studies have shown that several hormones, cytokines or growth factors are important regulators for adipocyte differentiation, such as steroid hormones, estrogen, androgen, growth hormone (GH), leptin , 1,25 dihydroxyvitamin D, transforming growth factor-β (TGF-β) related cytokines, and some proinflammatory cytokines. These regulators may mediate cytokine communication networks between adipocytes and osteoblasts. Some receptors involved in the regulation pathway, such as nuclear hormone receptors, trans-memberane kinase receptors, and G-protein coupled receptors are also found expressed on marrow stromal cells and adipocytes, these may present potential molecular targets for the manipulation of adipogenesis. In particular, in vitro and in vivo studies have demonstrated that ligands binding to the PPARs, glutococorticoid, estrogen, androgen, and vitamin D_3 receptors regulate bone marrow stromal cell adipogenesis and osteogenesis [17, 62, 93-95].

8.1 Glucocorticoids and glucocorticoid receptor

It is well konwn that high dose glucocorticoid treatment results in osteopenia or osteonecrosis in vivo. Dexamethasone and other glucocorticoids stimulate adipocyte differentiation in vitro [96, 97]. For example, human trabeculae derived osteoblasts and marrow derived

MSCs are induced to express adipocyte phenotype by Dex and IBMX treatment [73, 98]. However, dexamethasone also induce expression of osteoblast-specific mRNAs including alkaline phosphatase, osteopontin, osteocalcin, decorin, biglycan [99, 100]. The complex effects of glucocorticoid on osteogenesis and adipogenesis suggest that physiological or pharmacological glucocorticoid activities may play important roles in maintaining bone health.

Glucocorticoid receptor may also mediate the process of adipocyte differentiation. It is demonstrated that glucocorticoids potentiate the early steps of preadipocyte differentiation and promote obesity in Cushing's syndrome and during prolonged steroid therapy. In vitro, glucocorticoids stimulate extrameullary 3T3 L1 preadipocyte differentiation through a non-transcriptional mechanism, mediated through the ligand-binding domain of the glucocorticoid receptor. This enhanced the onset of CCAAT/enhancer binding protein (C/EBPα) expression by potentiating its initial transcriptional activation by C/EBPβ [101]. It is postulated that the same response exist for the preadipocytes in the marrow. The modulation of glucocorticoid receptor signaling may contribute to the therapeutic intervention in osteoporosis.

8.2 1, 25 dihydroxyvitamin D and vitamin D receptor (VDR)

Though 1,25 dihydroxyvitamin D was identified as a osteoblast-inducing factor, the effects of 1,25 dihydroxyvitamin D on adipogenesis remain controversial. In primary rat calvarial cultures, 1,25 dihydroxyvitamin D,

alone or in combination with dexamethasone stimulates adipogenesis [102]. In contrast, the addition of 1,25 dihydroxyvitamin D with glucocorticoids inhibits adipogenesis in MC3T3-G2/PA6 (PA6) cells, a preadipocytic stromal cell line from newborn mouse [103]. Using the multipotent murine bone marrow stromal cell line, BMS2, and its subclones, as well as primary-derived murine bone marrow stromal cell cultures, it shows that 1,25(OH)2D3 blocked adipogenesis induced by hydrocortisone, IBMX, and indomethacin. At nanomolar concentrations, 1,25(OH)2D3, completely inhibits murine bone marrow stromal cell differentiation into adipocytes in response to glucocorticoid-based adipogenic agonists [62]. Like PA6 and 3T3-L1 cells, adipogenesis in stromal ST2 cells was inhibited by 1,25-dihydroxyvitamin D3, as well as retinoic acid, tumour necrosis factor-α (TNF-α), and TGF-β [104]. However, studies on the effects of nuclear vitamin D(3) receptor (VDR) on adipogenesis are lacking. Some reported that VDR represses the transcriptional activity of PPARα but not PPARγ in a 1,25(OH)2D3-dependent manner, VDR signaling might be considered as a factor regulating lipid metabolism via PPARα pathway [105]. This suggests that VDR signaling may be involved in the regulation of adipogenesis, and it needs further investigation.

8.3 Estrogen, its analogs, and estrogen receptor

Estrogen exhibits osteogenic agonist and adipogenic antagonist properties. In ovariectomized rat and canine models, decreased estrogen levels result in reduced bone volume, increased bone erosion surfaces,

and increased marrow fat volume [106, 107]. Few studies have addressed the effects of exogenous estrogen on bone marrow adipocytes. However, treatment with exogenous estrogen reduces the size and metabolic activity of rat extramedullary adipocytes [108]. It is postulated that arrow adipocytes may exhibit a similar response. Using a mouse clonal cell line KS483, it showed that 17 β-estradiol (E2) stimulates the differentiation of progenitor cells into osteoblasts and concurrently inhibits adipocyte formation in an estrogen receptor (ER)-dependent way [109]. Treatment with bone morphogenetic protein-2 (BMP-2) stimulated both osteoblastic and adipocytic differentiation in a mouse bone marrow stromal cell line, ST-2, overexpressing either human ER α (ST2ERα) or ER β (ST2ER β). When treated with E2, alkaline phosphatase activity was enhanced and lipid accumulation suppressed in these cells, and these effects were completely reversed by an ER antagonist, ICI182780 [110].

Recently, phytoestrogens have been considered as a canditate for the purposes of intervention in osteoporosis. Phytoestrogen genistein was shown to enhance the differentiation of bone marrow stromal cells to the osteoblast lineage while reducing adipogenic differentiation and maturation via an ER-dependent mechanism, involving autocrine or paracrine TGF-β1 signaling [111]. However, the biological actions of genistein are complex. At low concentrations, genistein acts as estrogen, stimulating osteogenesis and inhibiting adipogenesis. At high concentrations, it acts as a ligand of PPARγ, leading to up-regulation of adipogenesis and down-regulation of osteogenesis [112]. Daidzein, one of the main soy phytoestrogens, was also shown to activate different

amounts of ERs and PPARs, and the balance of the divergent actions of ERs and PPARs determines daidzein-induced osteogenesis and adipogenesis [113]. These findings may explain distinct effects of phytoestrogen in different tissues.

8.4 Growth hormone (GH) and growth hormone receptor (GHR)

Growth hormone (GH) has diverse effects on adipose tissue. GH inhibits adipocyte differentiation, reduces triglyceride accumulation and increases lipolyses, which in turn reduce adipose tissue mass [114]. Also, GH stimulates bone growth and mineralization by direct effects on chondrocytes and osteoblasts. In GH-deficient dwarf (*dw/dw*) rats, marrow adipocyte numbers were increased 5-fold and adipocyte cell size was also increased by 20%, these values returned toward normal in *dw/dw* rats when given GH treatment but not with insulinlike growth factor-1 (IGF-1) treatment [115]. These results suggest that GH has a specific action on marrow adipocytes that is not simply due to altered bone or fat metabolism. The adipocyte population in bone marrow is an important primary target for GH. Administration of GH to a patient with severe osteopenia resulted in an increase in biochemical markers of bone formation, osteoid and osteoblast surface and also clearly decreased adipocyte number and size [116]. Both preadipocytes and mature adipocytes possess specific GH receptors; GH may mediate its actions via these receptors, but some effects are indirectly mediated through the secretion of IGF-1 [115]. In long-term marrow stromal cells culture system, it shows that GH-receptor is present in proliferating progenitor

cells, myofibroblast-like cells, large reticular fibroblast cells, adipocytes and endothelial cells [117]. This implies that GH may regulate marrow stromal cell lineages directly via its receptor pathway.

8.5 PTH, PTHrP, and PTH/PTHrP receptor or G-protein coupling receptor

PTH and PTHrP have been shown to have potent anabolic effects on bone. Clinically relevant doses of PTH have been documented to increase the rate of bone turnover in vivo by stimulating new bone formation in rats, monkeys, and humans, with little or no stimulation of bone resorption activity [118-123]. Young heterozygous mice carrying a targeted PTHrP-null allele display a premature form of osteoporosis characterized by decreased trabecular bone volume and increased bone marrow adiposity [124]. Both PTHrP and PTH/PTHrP receptor are expressed in cells of the adipocytic lineage [125]. In a nonhuman primate ovariectomy (OVX) model, teriparatide PTH(1-34) increased bone mass, enhanced bone structural architecture, and strengthened the hip, despite increasing cortical porosity; at the cellular level, adipocyte number reduced and osteoblast number increased [126]. In vitro, PTHrP could direct osteoblastic commitment of pluripotent mesenchymal cell line C3H10T(1/2) by increasing the expression of markers of the osteoblastic phenotype. Also, PTHrP can increase MAPK activity in 3T3-L1 cells via the PKA pathway, thereby enhancing PPARγ phosphorylation and inhibiting the expression of adipocyte-specific genes [127, 128]. Signal transduction through the parathyroid hormone receptor requires G-

protein coupling. In early studies, modulation of Gs alpha activity has been shown to effect differentiation of fibroblasts to adipocytes [129]. Some rare metabolic disorders, such as progressive osseous heteroplasia (POH), exhibiting heterotopic bone formation in subcutaneous adipose tissue [130], and McCune-Albright syndrome, displaying impaired stroma development including adipogenesis [131, 132], are caused by the mutations in G-protein. Taken together, a novel mechanism for the anabolic action of PTH, PTHrP, and their analogs may be the inhibition of adipogenesis within the bone marrow. G-proteins, may therefore, be a potential target for therapeutic intervention in osteoporosis.

8.6 Leptin and leptin receptor

Bone marrow adipocytes may also provide a source of paracrine factors, regulating osteoblastogenesis and adipogenesis [133, 134]. The obvious example is leptin, which is known to be secreted by both extramedullary adipocytes and marrow adipocytes, a cytokine that activates a stromal cell transmembrane tyrosine kinase receptor [135, 136]. Leptin exerted a negative correlation with bone mass, dependent on serum insulin levels, whereas adiponectin did not exert any effect on bone mineral density (BMD); adipocyte derived circulating leptin is considered as a determinant of bone mass [137, 138]. Increasing serum leptin level dramatically reduces bone mass, while reducing serum leptin level by overexpressing a soluble receptor for leptin increases bone mass [136]. In vivo studies using leptin (*ob/ob*) and leptin receptor (*db/db*) -deficient mice show that functional blockade of the leptin-signaling pathway in

db/db mice is associated with increased bone mass. Intracerebroventricular infusion of leptin to the leptin-deficient (*ob/ob*) mice causes bone loss, suggesting that leptin regulates bone formation through a central nervous system pathway [32, 33]. Moreover, marrow tissue from the femora of ob/ob mice also shows a marked increase in adipocyte number compared to that of normal mice, and few adipocytes are observed in bone marrow from lumbar vertebrae, suggesting that the ob/ob mouse may be a useful animal model for studying the relationship between bone marrow adipogenesis and osteopenia or osteoporosis [139]. In vitro studies demonstrated that leptin promotes differentiation of human mesenchymal stem cells into osteoblasts rather than adipocytes [136, 140]. This may suggest that the effect of leptin on bone is mediated through both the central nervous system and peripheral receptors at the stromal cell level.

8.7 TGF-βs, BMPs and their receptors

Transforming growth factor-Beta (TGF-β) family members, including TGF-βs and bone morphogenetic proteins (BMPs), play important roles in directing committment and differentiation of mesenchymal stem cells. TGF-β family members signal via specific serine/threonine kinase receptors and their nuclear effectors, named Smad proteins. While TGF-β is a potent adipogenic antagonist at all concentration [141], BMPs exhibit a dose dependent action in vitro [142, 143]. It is demonstrated that both bone marrow stromal preadipocytes and adipocytes express receptors for the BMPs and other related TGF-β family members [144,

145]. Recent evidence shows that TGF-β provide competence for early stages of chondroblastic and osteoblastic differentiation, with inhibiting myogenesis, adipogenesis, and late-stage osteoblast differentiation, and BMPs also inhibit adipogenesis and myogenesis [146]. Another TGF-β family member, Myostatin, was demonstrated to prevent BMP7 but not BMP2 mediated adipocytic differentiation by binding to its receptors. BMP7-induced heteromeric receptor complex formation was blocked by myostatin through competition for the common type II receptor, ActRIIB, suggesting that myostatin may be an important regulator of adipogenesis [147]. BMP Type IB and IA receptors play essential roles for commitment and differentiation of osteoblasts and adipocytes. Introduction of a constitutively active BMP type IB receptor forces stromal cells to differentiate into osteoblasts. In contrast, introduction of the constitutively active BMP type IA receptor forces the same cells to undergo adipocyte differentiation. Moreover, expression of truncated BMPR-IA suppresses PPARγ mRNA expression, and expression of truncated BMPR-IB enhances PPARγ mRNA expression [148]. These findings suggest that PPARγ may be one of the important downstream target genes for BMPR-IA signaling during adipocyte differentiation.

8.8 Proinflammatory factors and gp130 containing receptors

In the bone marrow microenvironment, many proinflammatory cytokines are involved in regulating adipogenesis, bone resorption and remodeling [149]. Among them, TNFα and IL-1 are mainly from macrophage-lineage cells, and both of them are potent adipogenic antagonists. Recent

evidence shows that expression of IL-1 and TNF-α in bone marrow may alter the fate of pluripotent mesenchymal stem cells, directing cellular differentiation towards osteoblasts rather than adipocytes by suppressing PPARγ function through NF-kappaB activated by the TAK1/TAB1/NIK cascade [150]. Cytokines that share the gp130 protein in their receptor complex, including IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M and ciliary neurotrophic factor, are mainly secreted by stromal-derived cells. These cytokines exhibit a potential autocrine effect, inhibiting stromal cell adipogenesis in a dose dependent manner in vitro [151]. It is demonstrated that IL-11 stimulated transcription of the target gene for BMP via STAT3, leading to osteoblastic differentiation in the presence of BMP-2, but inhibited adipogenesis in bone marrow stromal cells [152]. Preadipocyte, bone marrow and calvarial-derived stromal cells express receptor complexes containing the gpl30 protein [153], suggesting that some of the proinflammatory cytokines may serve as stimulatory factors for bone formation and inhibitors for adipogenesis, possibly via gp130 containing receptors signaling pathway.

9. Summary

Based on the evidence presented, osteoporosis may be attributable to increased adipogenesis at the expense of osteogenesis. Marrow adipogenesis exhibits a complex relationship with osteogenesis, hematopoiesis, and osteoclastogenesis. Marrow adipocyte may be considered as a crucial target cell for the therapeutic intervention in

osteoporosis, and the signaling pathways implicated in the process of adipogenesis may become potential molecular targets for drug design. The key point, however, is to identify specific molecular targets that inhibit marrow adipogenesis and concomitantly increase osteoblastogenesis with no adverse effects on extramedullary tissues. The inhibition of marrow adipogenesis and concomitant enhancement in osteogenesis may provide a potential approach to enhance overall osteogenesis and bone mass, and therefore, as a stand-alone therapy or in combination with an antiresorptive medication, may provide more efficacious prevention or treatment of osteoporosis.

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