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PERSPECTIVES

As a Matter of Fat: New Perspectives on the Understanding of Age-Related Bone Loss

Gustavo Duque

Division of Geriatric Medicine, McGill University and the Lady Davis Institute for Medical Research, Montreal, Quebec, Canada

Abstract

Aging is associated with increasing levels of bone marrow fat. This fat is the consequence of the predominant differentiation of mesenchymal stem cells into the adipocyte lineage at the expense of osteoblastogenesis. Furthermore, the reduction in osteoblastogenesis as well as the short life span seen in osteoblasts during aging in bone is the predominant pathophysiological mechanism of senile osteoporosis. In this review, the mechanisms of bone marrow fat infiltration and the potential toxic effects of fat accumulation in bone marrow will be considered. In addition, the potential therapeutic use of bone marrow fat for the prevention and treatment of senile osteoporosis is also proposed. *BoneKEy-Osteovision*. 2007 April;4(4):129-140.

Introduction

The three common findings in aging bone are: increasing levels of osteoclastogenesis, decreasing osteoblastogenesis and increasing bone marrow adipogenesis (1-4). The first is associated with a lack of estrogens and therefore is more commonly seen in women during their post-menopausal years (5;6). In contrast, the reduction in osteoblastogenesis, with the concomitant increase in adipogenesis, is the consequence of the aging process in bone that induces a shift in the differentiation of bone marrow cells predominantly into adipocytes. The consequence of these changes is a progressively fatty bone with reduced bone mass (Fig. 1).

Age-related bone loss is described as the physiological decline in bone mass associated with aging that starts after the third decade of life and continues at a rate of 0.5 to 1.5% of bone loss per year (7). Since this bone loss starts earlier than the menopausal years, and since estrogen supplementation in the post-menopausal years is not enough to stop a decline in bone mass (8), it is suggested that the cause of age-related bone loss is determined by age-related changes in bone cellularity as well as changes in the levels of calciotropic hormones (parathyroid hormone and vitamin D) independent of the serum levels of sex steroids.

The causes of cellular changes induced by aging in bone, such as the predominant differentiation of bone marrow cells into adipocytes, remain unexplained. In fact, several hypotheses regarding the role of bone marrow fat have been proposed since 1996 (9), when four important questions were asked concerning the role of bone marrow fat in the bone microenvironment:

1. Do bone marrow adipocytes occupy space that is no longer needed for hematopoiesis?

2. Does marrow fat participate in lipid metabolism?

3. Do marrow adipocytes act as an energy reservoir for emergency situations involving hematopoiesis or osteogenesis?

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4. Do bone marrow adipocytes act as a support in the maturation of other bone marrow cells, including osteoblasts?

While the past decade has provided us with enough evidence to answer some of these questions, others remain unsolved. This *Perspective* will review the current literature relevant to these issues. In addition, the regulation of bone marrow adipogenesis and its potential trans-differentiation into bone will be considered. Finally, the potential therapeutic benefits of preventing bone marrow adipogenesis will be discussed.

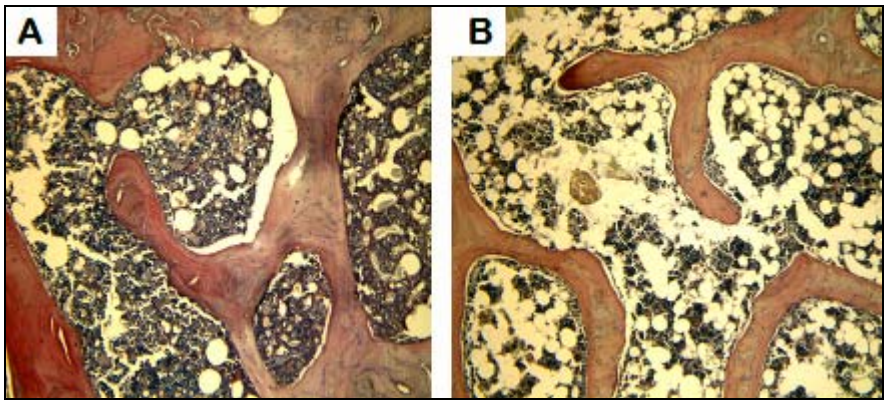

Figure 1. A comparison of bone marrow between young and old rats. The figure shows remarkably higher levels of bone marrow fat in a 24-month-old rat (B) as compared with a 4-month-old rat (A). In addition, the trabecular thickness is reduced in the old rat, as is the amount of hematopoietic tissue.

The Process Of Bone Marrow Cell Differentiation

Both osteoblasts and adipocytes share the same precursor known as the mesenchymal stem cell (MSC). MSCs are not only able to differentiate into these two cell types, but also into fibroblasts, chondrocytes, myocytes and other cells of the mesenchymal type (10;11), when the appropriate factors and environment are present. MSC differentiation into either adipocytes or osteoblasts requires multiple steps as well as the presence of several growth factors and hormones. These steps have been analyzed recently by Kratchmarova *et al*. (12). This review will summarize some of the critical steps, focusing on the ones that determine MSC differentiation into one specific phenotype, either the osteoblast or the adipocyte (Fig. 2). In addition, the changes induced by aging in each of these steps will be described.

Step 1: MSC Mobility and Confluence

The bone marrow contains multiple cell lines in constant interaction. Cells from the hematopoietic lineage share their space with mesenchymal precursors as well as cells of the macrophage lineage (13). Factors that are known to induce MSC mobilization and confluence include: insulin growth factor I (IGF-1), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-alpha (TGF-α), hepatocyte growth factor (HGF), fibroblast growth factor-2 (FGF-2), and thrombin (14-20).

This process of MSC migration and confluence enhances proliferation as well as differentiation. In the case of aging, migration and confluence are affected not only by a lower number of MSCs available to differentiate within the bone marrow, but also by decreasing levels of osteogenic growth factors within the local microenvironment (14;15). Additionally, aging induces changes in the gap-junctions of MSCs that affect their capacity to differentiate into osteoblasts (16;21). The gap-junction is dependent on the proximity

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between the MSCs, since this, in turn, is also dependent on the capacity of the MSCs to migrate (22); a vicious cycle is established that affects osteogenesis as a whole.

Step 2: Fat or Bone? That Is the Question.

Once MSCs are both confluent and under the right conditions, cells committed to differentiate into osteoblasts must express high levels of osteogenic transcription factors (23). Among those factors, core binding factor 1 (Cbfa1) is the master regulator of osteogenesis. The predominant expression of Cbfa1 by MSCs will determine their differentiation into osteoblasts (24). In contrast, the predominant expression of the adipogenic transcription factors peroxisome proliferator-activated receptor-gamma 2 (PPARγ2) and CCAAT/enhancer binding protein alpha (CEBPα1) will induce adipogenic differentiation of MSCs (25).

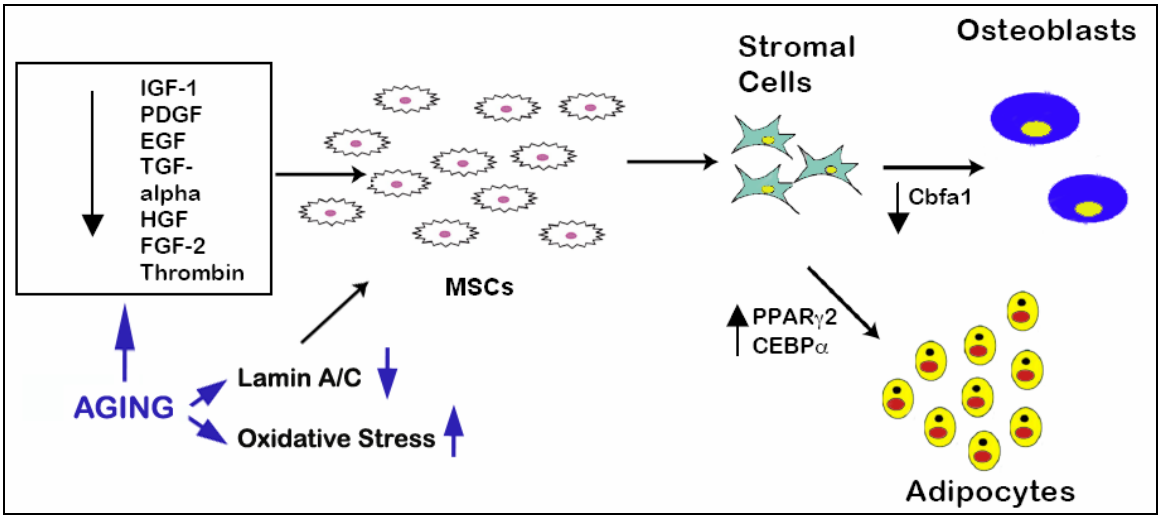

Figure 2. Aging is associated with a reduction in the secretion of growth factors required for an appropriate number and confluence of MSCs. Additionally, aging induces higher levels of oxidative stress as well as decreasing lamin A/C expression within the bone marrow. Finally, a reduction in osteoblastogenesis and Cbfa1 expression leads to fewer active osteoblasts, and increasing levels of adipogenic differentiation as well as increased PPARγ2 and CEBPα expression lead to fewer differentiated osteoblasts and increased adipogenesis.

The question of what determines whether a MSC differentiates into either an adipocyte or osteoblast remains unknown and is the focus of intense research (26). However, it is known that this process is affected by aging in multiple ways. Since the levels of Cbfa1 expression in MSCs are reduced with aging while levels of PPARγ2 increase (1;26), it is tempting to suggest that aging could induce a shift from osteogenesis to adipogenesis within the bone marrow through the up- or down-regulation of the genes that codify their expression. Additionally, with aging, the presence of growth factors that stimulate osteogenesis is decreased. Among these factors, IGF-1 levels in the bone marrow are markedly reduced (27), as are components of the TGF-β and BMP 2/4 signaling pathways (28).

Although these changes in transcription and growth factors have been associated with aging, the trigger that activates this shift from predominant osteoblastogenesis in young bone to adipogenesis in old bone remains unknown. After examining the aging theories that may explain these age-related changes in MSC differentiation, the author has selected two that may account for this potential trigger:

Oxidative stress: D'Ippolito *et al*. (29) have

reported that osteogenic differentiation of MSCs is affected by low oxygen tension (pO2). Additionally, other studies support the hypothesis that there is a significant reduction in pO2 within the bone marrow with aging as demonstrated by increasing levels of markers for oxidative stress (30). This reduction in pO2 is probably due to low levels of blood supply within the aging bone marrow that have been documented by magnetic resonance (31). In fact, although both adipogenesis and osteoblastogenesis require sufficient levels of pO2, osteoblast precursors seem to be more sensitive to oxidative stress (32;33) than adipocyte precursors (34), which may explain why with lower blood supply and therefore lower pO2 within the bone marrow, adipogenic precursors are predominant over osteogenic ones.

Lamins and premature aging: After the recent discovery of mutations in the gene encoding lamin A/C in cases of Hutchinson-Gilford progeria syndrome (HGPS) (35), a type of progeria with severe bone changes (36), several groups including ours are looking at lamins as the potential link between aging and changes in bone marrow cellularity. Lamins form the lamina that keeps the shape and strength of the nucleus. They also play a role in a number of nuclear processes including DNA replication and transcription (37). Lamins are grouped into two classes, A-type (A, AΔ10, and C) and B-type (B1 and B2). While B-type lamins are found in all nucleated somatic cells, the expression of A-type lamins is developmentally regulated (38). A-type lamins are absent from all pre-implantation stage embryonic cells with their synthesis commencing at about day 9 within the visceral endoderm and trophoblast (38). Subsequently, A-type lamins appear asynchronously in various tissues (37;38). Truncation in lamin A/C results in progeroid phenotypes in both mice and humans (35;36;39;40) where the mutations in nuclear proteins induce increased DNA damage and chromosome aberrations that make the cells more sensitive to DNA-damaging agents (41). This recent evidence suggests that lamins serve as “guardians of the soma” (38) and that the absence of lamin A/C affects more significantly the group of mesenchymal cells, including those located within the bone marrow (42). Previous reports on defects in lamin A/C processing and expression in mice have described defects associated with alterations in the differentiation of MSCs, including muscular dystrophy, lipodystrophy and spontaneous bone fractures due to a deficit in osteoblastic activity (39;40). Indeed, there is evidence that lamin A/C plays a role in the differentiation and stability of MSCs (42). Lammdering *et al*. (43) analyzed cultured embryonic fibroblasts from lamin A/C null mice. These cells showed mechanical strain-induced deformations and reduced viability. This report suggest that lamins act as cell protectors from physical damage while maintaining the function of transcription factors required for the differentiation of MSCs. From the point of view of MSC differentiation, there are two studies that have found changes in lamin A/C expression in normal models of adipocyte differentiation (44;45). The first one identified lamin expression in human adipose cells both in relation to anatomical site and differentiation state, finding that lamin A/C and B1, but not B2, were expressed in mature human adipocytes, whereas pre-adipocytes expressed all four lamins (44). A second study looked at proteomic changes in adipocyte differentiation of cells obtained from subcutaneous fat. Among the 170 protein features found in their study at day 9 of differentiation, lamin A/C expression was included in the group of proteins of the cytoskeleton with a more than 3-fold reduction in its expression (45).

Furthermore, our group assessed the levels of expression of lamin A/C in osteogenic differentiating MSCs *in vitro* (46) as well as in differentiated osteoblasts *in vivo* (47). We found that in contrast to embryonic stem cells (38) where lamin A/C is not expressed, MSCs express low levels of lamin A/C. Additionally, we found that lamin A/C expression levels are increased during the osteogenic differentiation of MSCs *in vitro* and that those levels are reduced when MSCs are committed to differentiate into adipocytes (46). Our data suggest that lamin A/C is required for the normal differentiation of both osteoblasts and adipocytes, with

higher levels required for successful osteoblastic differentiation. Finally, our *in vivo* data showed that expression of lamin A/C by osteoblasts is reduced in samples obtained from aging mice (47).

Taken together, this evidence suggests that lamin A/C could play a role as one of the potential triggers for age-related bone loss, as suggested by evidence that osteogenic differentiation of MSCs requires higher levels of lamin A/C expression than adipogenesis (43;45). This role could be explained by either the direct role of lamin A/C on cell differentiation, or by changes in cell shape induced by lower levels of lamin A/C in differentiating cells where MSCs allowed to adhere, flatten, and spread underwent osteogenesis, while unspread, round cells became adipocytes (48). The direct role of lamin A/C in these specific cell shapes remains to be identified.

Step 3: Calciotropic Hormones

In addition to the differentiation of MSCs determined by the presence of transcription and growth factors, calciotropic hormones (vitamin D and parathyroid hormone [PTH]) play an important role in the regulation of bone turnover. Although vitamin D is known to be essential for calcium absorption in the gut (49), new regulatory roles of vitamin D in MSC differentiation and osteoblast survival have been reported in recent years. Vitamin D is well known as an inducer of osteogenic differentiation of MSCs (50). This osteogenic effect is explained by up regulation of both transcription (i.e., Cbfa1) and growth factors (i.e., BMP 2 and 6) that are required for osteogenesis and matrix formation (51). Additionally, vitamin D has been shown to inhibit apoptosis in mature osteoblasts (52), which may preserve the number of osteoblasts available for effective bone formation.

In contrast, vitamin D has an inhibitory effect on adipogenesis. This effect is partially explained by suppressing the expression of inhibitors of the canonical Wnt signaling pathway (53;54) or by direct inhibition on PPARγ2 expression (55) and activation (56). In fact, vitamin D has been demonstrated not only to induce bone formation in a model of senescence-accelerated mice (SAMP/6) (57) but also to change their predominant bone marrow adiposity into new bone mass, suggesting a potential role for vitamin D as a "trans-differentiation" factor between fat and bone through the induction of osteogenic differentiation of adipocyte precursors (58).

Similar to vitamin D, PTH has an osteogenic effect through the induction of MSC differentiation into osteoblasts (59). Additionally, PTH inhibits the adipogenic differentiation of MSCs and suppresses markers of differentiated adipocytes such as lipoprotein lipase and PPARγ (60).

The subject of age-related changes in the levels of calciotropic hormones has been extensively reviewed in the literature (61). Briefly, serum levels of vitamin D decrease in aging due to multiple factors such as lower sun exposure and poor oral intake. In addition to the cellular effects of vitamin D deficiency within the bone marrow, the lack of appropriate levels of this vitamin will decrease calcium absorption in the gut, which subsequently activates the release of PTH in the circulation. Chronically high levels of PTH will increase osteoclastic activity, inducing bone loss. Indeed, the chronic effect of PTH in bone is different than the intermittent effect of therapeutic PTH that stimulates osteoblastic differentiation and function (61).

The Role Of Bone Marrow Fat: Friend Or Foe?

The question of whether bone marrow fat is good or bad should be answered from two perspectives: first, its potential metabolic role and second, its potential toxic effect on neighboring cells.

The metabolic role of marrow fat has been partially tested by our group using a model of aging rats exposed to caloric restriction. We have found that after 12 months of caloric restriction, no differences in the amount of bone marrow fat were found between the restricted and the ad-libitum controls (Duque *et al*., submitted for publication). In contrast, these rats show significant weight loss and a marked decline in their serum levels of leptin. Other studies

have used dynamic labeling of bone marrow fat to identify the level of metabolic activity. In agreement with our findings, these other investigations have also found that bone marrow fat is metabolically inactive (62). Taken together, these results suggest that bone marrow fat is not used as a source of energy except in states of extreme starvation and that its role is probably the occupation of space that has been left empty by decreasing hematopoiesis and altered bone mass architecture.

Concerning the potential toxicity of fat on neighboring cells, the pancreas provides an example. Progressive fat infiltration leads to β-cell toxicity, and this is known as one of the mechanisms of type II diabetes in older age (63;64).

In the case of bone, marrow fat has been associated with the release of adipokines and fatty acids that have potential toxic effects in neighboring cells (65). *In vitro* experiments using co-cultures of adipocytes and osteoblasts have shown that osteoblast function and proliferation is affected by adipocytes (65). Although there is evidence to suggest that fatty acids are involved in this toxicity, further research is required to test this hypothesis.

Another potential mechanism that explains lipotoxicity in bone is its potential induction of "lipoapoptosis" (63). This type of programmed cell death that has been described in the aging pancreas and muscle is the consequence of the activation of apoptotic pathways by either adipokines or fatty acids (63). Among the most common adipokines, TNF-α is known to be closely involved in the induction of apoptosis in osteoblasts (63;64). Since osteoblast apoptosis is a major feature in senile osteoporosis, it would be tempting to suggest that this is due to lipotoxicity secondary to the progressive infiltration of bone marrow fat with age. As for previous issues concerning fat and bone, further studies are required to test this hypothesis.

Finally, there is a probability that bone marrow fat acts as a promoter of adipogenesis by inducing PPARγ2 expression by MSCs. Studies by Lecka-Czernik *et al*. (66) have demonstrated that increasing levels of oxidized fatty acids within the aging bone marrow have a stimulatory effect on PPARγ2 expression, thereby promoting bone marrow adipogenesis.

Boning Up From Fat Precursors: A New Approach To The Treatment Of Senile Osteoporosis

Most current treatments for osteoporosis focus on the reduction of osteoclastic activity, which is a major feature in post-menopausal osteoporosis (4), but not the predominant mechanism in senile osteoporosis. In fact, in the case of senile osteoporosis, the efficacy of these treatments may be less, since most of the treatments have been shown to be effective in the reduction of vertebral and wrist fractures, the typical fractures seen in post-menopausal osteoporosis, while showing lower efficacy for the prevention of non-vertebral fractures (67). Therefore, from a pathophysiological point of view, optimal treatment for senile osteoporosis should be directed to stimulate osteoblastogenesis, decrease osteoblast/osteocyte apoptosis and inhibit adipogenesis.

Two examples of treatments that act on the osteoblastic lineage are PTH and vitamin D. As previously mentioned, PTH has been shown to have an anabolic effect through the stimulation of osteoblast activity, and an inhibitory effect on adipogenesis (59) and osteoblast apoptosis *in vitro* (68).

The active form of vitamin D ($1,25(OH)_2D_3$) has also been shown to be effective as an inhibitor of osteoblast apoptosis *in vitro* (53). Interestingly, $1,25(OH)_2D_3$ also has an anabolic effect *in vivo* through both the stimulation of osteoblastogenesis (57) and the inhibition of adipogenesis (58). Overall, vitamin D has been demonstrated to be effective not only in increasing hip bone mass in human subjects treated at sub-therapeutic doses (400 IU/day) (69) but also in reducing the incidence of non-vertebral fractures in an elderly population under appropriate dosing (70).

However, the effectiveness of vitamin D in the prevention of fractures is not superior to the effectiveness of the current antiresorptives (71). Therefore, new therapeutic choices should be developed with the understanding that the goals of the treatment are to provide the bone marrow with a number of active osteoblasts sufficient to regulate bone turnover by appropriately coupling with osteoclasts.

One innovative approach would be the utilization of the fat that is already within the bone marrow as the source of new osteoblasts. The concept of trans-differentiation of fat into bone, although controversial, has been extensively studied (72;73). This process involves the conversion of either a mature adipocyte or its precursor into a mature osteoblast. In the case of the differentiation of mature adipocytes into mature osteoblasts, it would involve a process known as “metaplasia” (73;74). There are concerns over the reports on metaplasia of adipocytes into osteoblasts because of the possibility of progenitor cell contamination and cell fusion. Recently, Song *et al*. (74) showed that fully differentiated cells from hMSCs, including adipocytes, were capable of de-differentiation and trans-differentiation into cells of another developmental lineage at single cell levels. Although the evidence is limited, their study and other recent work (75) demonstrate that it may be possible to use differentiated adipocytes within the bone marrow as the source of new osteoblasts. However, the predominant adipose cells within the bone marrow do not seem to be mature adipocytes but rather a different type of cell known as the “Mesenchymal Adipocytic Default” (MAD) cell (76). According to Kirkland *et al*. (76), these cells are in a permanent stage of de-differentiation acting mostly as pre-adipocytes. The fact that the cells are in this stage would make them more susceptible to trans-differentiate into mature osteoblasts and would also eliminate the risk of trans-differentiating unwanted fat in other organs.

A final potential and, in the author's opinion, expensive approach, is the injection of new pluripotent cells into the bone marrow and their stimulation to produce bone by either chemical or genetic means. This approach has shown some limited effectiveness in SAMP6 mice (77). Thus far, no studies have been pursued in human subjects where ethical and procedural limitations must be considered.

In summary, bone marrow fat could be converted into a source of new bone if the appropriate factors are used. Targeting endogenous MAD cells that are already inside the bone marrow is probably the safer and more “biologically clean” approach. When fat accumulates in older bone, it affects bone mass and bone marrow biology, thus becoming an “enemy inside”. However, this enemy has a weakness due to its capacity to differentiate into bone cells if exposed to the appropriate factors. In this case, our enemy’s weakness could provide us with a major strength.

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