The Changing Balance Between Osteoblastogenesis and Adipogenesis in Aging and its Impact on Hematopoiesis

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Abstract

Osteoblasts (OBs) and adipocytes (APs) share a common mesenchymal ancestor. It is now clear that mesenchymal stem cell (MSC) maturation along the OB lineage comes at the expense of adipogenesis and vice versa. During aging, this balance increasingly favors the formation of APs. Hematopoiesis also slowly declines during the aging process. The role of OB lineage cells in hematopoiesis has been studied, but less is known about how APs regulate hematopoiesis. A few studies have demonstrated a negative relationship between APs and hematopoiesis; however, there is also evidence that brown adipose tissue (BAT) may promote hematopoiesis. This review will examine the current knowledge of how adipogenesis and osteogenesis change with aging and the implications of this changing environment on hematopoeisis.

Keywords

Mesenchymal stem cells; adipocyte; osteoblast; aging; osteoporosis; hematopoiesis

Introduction

It is an unfortunate fact of life that as humans age, they have a tendency to increase the proportion of their body weight made up by adipose tissue. In fact, it has been estimated that

between the ages of 20 and 50, total body fat content doubles in males and females [1]. While body fat is increasing, bone mineral density, which peaks in the third decade of life, gradually declines [2]. Concomitant with these changes, there is also a decline in hematopoiesis with aging affecting both myeloid and lymphoid lineage cells accompanied by a gradual replacement of bone marrow stromal cells with adipose tissue [3, 4]. As osteoblasts (OBs) and adipocytes (APs) share a common mesenchymal ancestor [5] and are in intimate contact with hematopoietic cells within the bone marrow, understanding how aging affects the development and interactions between these cell lineages may ultimately help older individuals combat bone loss diseases and hematopoietic disorders.

In this review, we will examine the current understanding of how bone and adipose tissue formation changes with aging. Since bone and adipose tissue are two main components of the hematopoietic niche, we will also examine the impact of age-related differentiation changes in mesenchymal stem cells (MSCs) on the functional capacity of the hematopoietic system.

Mesenchymal Stem Cell Differentiation

MSCs have the potential to differentiate into several cell types including OBs, APs, chondrocytes, tenocytes, and myocytes [6]. For OB development, activation of runt-related transcription factor 2 (Runx2) is an initiating event in the commitment of the MSC to the osteo/chondroprogenitor line [7]. Further downstream, members of the Smad family of proteins, β-catenin and Osterix are also involved in the formation of terminally differentiated OBs [8]. On the other hand, CCAAT enhancer binding protein alpha (C/EBPα) and peroxisome proliferatoractivated receptor gamma (PPARγ) are the key transcription factors involved in AP formation [9].

Other important factors in MSC differentiation include the canonical Wnt pathway (henceforth referred to as Wnt), which signals through elevations in intracellular levels of β-catenin. Wnt plays a critical role in MSC differentiation down the OB lineage by increasing expression of osteogenic genes, such as Runx2, in developing OBs [10]. In AP development, Wnt acts to increase the mitotic activity of preadipocytes but has a negative effect on their maturation through inhibition of C/EBPα and PPARγ [11, 12]. The Wnt pathway may also suppress adipogenesis via the actions of glycogen synthase kinase-3β (GSK-3β). In the absence of Wnt signaling, GSK-3β phosphorylates β-catenin and targets it for ubiquination, therefore increases in Wnt signaling inhibit this GSK-3β-mediated destruction of β-catenin allowing the accumulation of β-catenin within the cell [13]. We will now examine how these factors change with aging, and the influence these changes have on the balance between osteoblastogenesis and adipogenesis.

Osteoblastogenesis in Aging

Osteoporosis affects approximately 2.8 million men and 9.1 million women in the United States, and osteopenia, or low bone mass, is estimated to affect an additional 14.4 million men and 26 million women [14]. These staggering numbers have stimulated much research into the causes and treatment of bone loss[14]. The current understanding of the pathogenesis of age-related bone loss implicates decreased efficiency of the basic multicellular unit, in which the OB deposits less new bone than the osteoclast resorbs [15]. Over time, this imbalance in bone metabolism leads to trabecular thinning, porosity, and fragile bones that are more likely to fracture [15]. How does the aging process contribute to this dysfunction in OBs and/or their mesenchymal precursors?

One of the mechanisms of OB activity decline appears to be a decrease in the pool and lifespan of MSCs from which they develop. It has been difficult to study human MSC

numbers by modalities such as flow cytometry as there is a lack of a well-defined subset of markers that can completely encompass the population of MSCs [16]. However, several studies have used indirect methods to quantitatively assess the number of MSCs in young and older individuals. In 1999 D'Ippolito et al cultured MSCs isolated from males and females aged 3–70 years. This group found that MSCs isolated from "older" donors (aged 41 and above) had about an 80 percent reduction in the number of alkaline phosphatase positive colonies formed in vitro, thus demonstrating the decreased osteogenic potential of aging MSCs [17]. More recently, Zhou et al further examined the intrinsic effects of aging on human MSCs in vitro. Using bone marrow from human patients aged 17–90 as a source of MSCs, the group found that MSCs from older patients were more apoptotic, had higher expression of p53, p53 pathway genes p21 and BAX, and also generated fewer OBs than their younger counterparts [18]. The relative importance of MSC number alone in age-related bone loss is unclear as studies have determined that the greatest loss of MSCs occurs after reaching skeletal maturity with little decline thereafter [19, 20].

In addition to the decline in the pool of precursor cells from which OBs can develop, there are also declines in the lifespan of these precursors in aging. Stenderup et al conducted long term cultures of MSCs derived from young and elderly donors (age 19–29 years, and 68–81 years, respectively) and found that MSCs from older donors had significantly decreased numbers of population doublings (24 vs 41 , $p<0.05$, [21]). Perhaps it is the combination of a smaller MSC pool and decreased longevity that contribute to the decreased bone formation in aging.

There are also age-related changes that directly affect OB differentiation and function. Rauner et al recently studied the expression of Wnt-related proteins in young, adult, and old C57BL/6 mice (6 weeks, 6 months, and 18 months of age, respectively). In human chronology, young mice represent humans who have not reached skeletal maturity, adult mice represent skeletally mature adults between the ages of 30 and 50 and old mice represent humans older than 50. In bone tissue from old mice, there was an overall decreased expression of Wnt related proteins and OB differentiation was reduced [22]. Interestingly, reactive oxygen species (ROS), substances which are known to damage cells and which are cleared less efficiently with age have been found to interfere with β-catenin driven Wnt signaling [23, 24]. Specifically, ROS activate Forkhead box O (FoxO) transcription factors which in turn increase the expression of free radical scavengers. This induces increased association of FoxO with β- catenin, thus reducing the availability of β-catenin to activate transcription factors involved in bone formation [23, 24].

Dysfunctional genome maintenance also plays a role in the decline of aging MSCs. Several authors have shown that lower levels of the enzymes telomerase (protects DNA from shortening after replication) and helicase (aids DNA repair) seen in older animals are associated with decreased bone mass [25–28]. In an in vitro model, a human mesenchymal cell line stably transduced with a gene for a subunit of the human telomerase gene maintained the ability to differentiate down the OB lineage over hundreds of population doublings, also forming more bone when implanted into immunodeficient mice [29]. Using murine models of accelerated aging including mice deficient in telomerase, Werner helicase, or both, Wang et al determined that shortened telomeres in MSCs led to decreased expression of Runx2, increased expression of p53 and p21, and the formation of fewer mature OBs [30].

The study of a particular human genetic disease, called Hutchinson-Gilford Progeria Syndrome, has also contributed new insights into how bone and fat are maintained in old age. Children affected with this syndrome have a mutation in the LMNA gene, which encodes a nuclear envelope protein, lamin A [31]. As a result of this mutation, processing of

lamin A is disturbed and the nuclei within the cells of these patients have altered shapes and strength, which disrupts mitosis and ultimately alters gene expression [32, 33]. The phenotypical manifestations of this disease include skeletal hypoplasia, short stature, pathologic fractures, hypertension, hyperlipidemia, arterial stenosis, lipodystrophy and shortened lifespan [33]. The Zmpste24-null mouse model of progeria lacks the enzyme to convert prelamin A to lamin A and therefore prelamin A accumulates within the nuclei of all cells [32]. In 2009, Rivas et al demonstrated that compared to age-matched wild-type mice, Zmpste24-null mice had a pattern of advanced osteopenia with increased levels of bone marrow fat [32]. These mice also had severely depressed levels of Runx2 expression and subsequently significantly fewer OBs and osteocytes while at the same time, expression of the adipogenic genes PPARγ and C/EBPα were elevated [32]. Similarly, Akter et al showed that knocking down lamin A expression in human MSCs, while it did not decrease Runx2 expression, did affect its nuclear binding ability, thus decreasing osteoblastogenesis and promoting adipogenesis [34]. These data implicate the involvement of lamin A, or deficiencies in the processing thereof, in age-related bone loss. Overall, it appears that in aging, there are multiple factors that decrease Runx2 activity, thus favoring PPARγ and driving the fate of MSCs towards adipogenesis.

Mature APs can also inhibit osteogenic function; therefore, the increased numbers of APs in aging may directly inhibit bone formation. In recent in vitro experiments, Lui et al showed that primary fat cells or fully differentiated 3T3-L1 cells (a pre-adipocyte cell line [35, 36]) co-cultured with partially differentiated OBs decreased Runx2 expression and alkaline phosphatase activity. These changes were attributed to substances released by APs that activate PPARγ within the OB, as silencing of PPARγ in the OB cell line rescued Runx2 expression [35]. There were also increases in adiponectin, which is a cytokine highly expressed by APs . In these experiments, adiponectin alone had no effect on Runx2, osteocalcin or alkaline phosphatase expression in OBs [35]. These results suggest that PPARγ influences the balance between OBs and APs even among more differentiated cells.

In vitro, exposure of MSCs to 1,25-dihydroxyvitamin D3 (1,25-VitD), the active form of vitamin D, increases their differentiation to OBs [37]. 25-hydroxyvitamin D3 (25-VitD), the inactive form of vitamin D, also has this effect on MSCs [38]. This discovery led to the finding that MSCs manufacture the 1α-hydroxylase CYP27B1, which can activate 25-VitD into the active form [38]. Interestingly, when exposed to 25-VitD, MSCs from older humans (age greater than 55) have a decreased ability to produce CYP27B1 [39]. Geng et al showed that in vitro, this decline can be rescued by administration of parathyroid hormone (PTH) [39]. PTH was able to upregulate the expression and activity of CYP27B1 in MSCs. This effect explains, at least in part, the efficacy of PTH in osteoporosis treatment. We will revisit the role of 1,25-VitD in MSC differentiation later, as it also appears to play a role in inhibiting adipogenesis.

Adipogenesis in Aging

Unlike osteoblastogenesis, which declines with age, adipogenesis seems to accelerate with aging. This paradigm was first demonstrated in the 1990's in the senescence accelerated mice-P6 (SAMP6). The SAMP6 mouse begins developing osteoporosis within a few months of its birth [40]. Three years after the introduction of the SAMP6 mouse strain, Kajkenova et al, determined that SAMP6 mice not only had decreased osteoblastogenesis, but also increased numbers of mature APs within their bone marrow [41]. This finding of increased bone marrow fat with aging was soon translated to humans. In 2001, Justesen et al compared iliac crest bone biopsies of healthy individuals with osteoporotic individuals: compared to the total volume of the specimen, they found an age-related decrease in bone volume (BV/TV) concurrently with an increase in adipose tissue volume [4]. As the evidence mounted of

an inverse relationship between osteoblastogenesis and adipogenesis, the search was on for the underlying mechanisms behind this shift in the balance.

With the increases in fat deposition with aging, it would be expected that the expression of adipogenic transcription factors would also increase. In 2004, Moerman et al demonstrated exactly this in mice. Specifically, this study showed that PPARγ expression was elevated in 26 week-old mice compared to 8 week-old mice. Furthermore, MSCs obtained from the bone marrow of the older mice were better able to spontaneously differentiate into APs in vitro, without requiring any stimulus [42]. What is it about aging that activates the adipogenic pathway in MSCs? The answer may lie with the increased oxidative stress that accompanies aging. As discussed above, these stresses reduce osteoblastogenesis in favor of adipogenesis.

As previously discussed, mammalian cells are under constant attack by free radicals, and the ability of our bodies to neutralize these threats decreases with aging [43]. Interestingly, besides the indirect effects on osteogenesis through binding β-catenin, evidence is emerging that these free radicals may directly induce PPARγ expression and stimulate adipogenesis. Recently, Almeida et al found that levels of oxidized lipids, which can act as free radicals, were elevated in older vs. younger mice. Furthermore, these oxidized lipids increased PPARγ expression and promoted apoptosis of OB lineage cells [44]. In addition, heme oxygenase-1 (HO-1), an agent known to neutralize oxidative stress [45], has been found to influence MSC differentiation. Suppression of HO-1 was found to strongly increase PPARγ expression and adipogenesis in human MSCs [46]. Taken together, it seems that increasing intracellular oxidative stress may be one of the major drivers of the switch from osteogenesis to adipogenesis during the aging process. Other factors, although contributing less, may also be regulating the balance between fat and bone in aging.

Even in healthy elderly individuals, low levels of vitamin D are commonly seen [47]. As discussed previously, vitamin D plays a role in stimulating OB differentiation. Moreover, vitamin D has been shown to directly inhibit adipogenesis in vitro using wild-type murine bone marrow stromal cells [48]. The mechanism of this inhibition was found to be suppression of PPARγ type 2 expression in the SAMP6 mouse model [49]. These data suggest that in the elderly population, vitamin D deficiency may mediate a synergistically negative effect on bone health by failing to stimulate osteoblastogenesis and enhancing adipogenesis. Also of importance when discussing osteoporosis, and perhaps the most widely thought of cause of osteoporosis in aging, is reduced estrogen levels (postmenopausal osteoporosis). Estrogen has also been implicated in increased bone marrow adiposity with aging.

In an abstract presented at the 2012 annual meeting of the American Society of Bone and Mineral Research, Krum and Wend found that estrogen receptor alpha knockout mice (ERαKO) had increased APs in the bone marrow compared to wild-type [50]. Ovariectomy was also able to induce this effect. Further analysis showed that the ERαKO mice had smaller, but more numerous lipid droplets within AP and that these mice also had higher levels of perilipin, an enzyme that promotes adipogenesis. These results suggest that estrogen may help to minimize AP accumulation in the bone marrow, an effect that would be diminished in the postmenopausal female. While it is clear that all of the above factors are converging to increase adipose tissue within the marrow cavity with age, the type of fat that is forming is also critical.

Not all fat is created equally. There are deposits of white and brown adipose tissue in the human, and each has different metabolic activities and functions. White adipose tissue (WAT) mainly stores energy in the form of triglycerides and cholesterol, with the individual

cells containing few mitochondria. Conversely, brown adipose tissue (BAT) is composed of cells containing many mitochondria (hence the brown color), and act to dissipate energy in the form of heat [51]. Krings et al studied murine bone marrow adipose tissue in normal and diabetic mice, and determined that fat cells within the bone marrow possess characteristics of both white and brown adipose tissue. Specifically, marrow fat expressed gene markers associated with WAT, such as adiponectin and leptin, as well as markers for thermogenic proteins associated with BAT, such as deiodinase 2 and PPARγ coactivator 1α [52]. In this same study, the investigators also showed that with aging and diabetes, the expression of BAT-type gene markers in bone marrow decreased [52]. There has not been any specific link between BAT and osteogenesis, however, as discussed above, WAT may have direct inhibitory effects on OBs. Furthermore, there is some evidence that this progression of bone marrow fat away from the BAT phenotype may have implications for hematopoiesis as well.

Hematopoiesis in Aging

As the bone marrow also houses hematopoietic stem cells (HSCs), how does the aging bone marrow microenvironment affect the hematopoietic system? The deleterious effects of aging causes increases in anemia and weakened adaptive immune responses [53–57]. As both the myeloid and lymphoid lineages are affected, this suggests that HSCs, like MSCs, are also negatively affected by aging [58]. With HSCs, MSCs, OBs and adipose tissue all in close proximity within the bone marrow, how might their interactions influence hematopoiesis in old age?

In one of the earlier studies of interactions between hematopoietic lineage cells and APs, Belaid-Choucair et al showed that fibroblast like fat cells (FLFCs), which share many primary characteristics to unilocular fat cells, when co-cultured with monocyte and granulocyte precursors, were able to inhibit granulopoiesis [59]. They found that this inhibition was secondary to FLFC suppression of granulocyte-monocyte colony stimulating factor (GM-CSF) secretion by monocytes [59]. Addition of anti-NP1 antibody into these co-cultures abrogated this effect. NP1 is one of the receptors for vascular endothelial growth factor, is known to be expressed in WAT and bone marrow (BM) APs, and is known to suppress granulopoiesis [5, 60, 61].

Data generated from several other studies also support the hypothesis that APs do influence hematopoiesis. A 2009 study by Naveiras demonstrated several findings that implicate APs as negative regulators of hematopoiesis: in bone marrow regions of mice where APs are predominant, there were decreased numbers of HSCs and short-term repopulating cells; furthermore, transplantation of wild-type HSC into "fatless" mice (which cannot form APs) or treatment of transplanted wild-type mice with the PPARγ inhibitor bisphenol A diglycidyl ether (BADGE), resulted in enhanced repopulation of the hematopoietic system within the recipient mice [62]. More recently, Poncin et al studied the effects of the OB/AP balance within the marrow environment on hematopoietic recovery after irradiation in mice. These investigators found that while Runx2 expression peaked in MSCs one day after radiation treatment, PPARγ expression remained unchanged and then decreased significantly by day 7 [61]. The changes in Runx2 and PPARγ expression corresponded to hematopoietic precursor cell numbers, which reached a nadir by day 3 and were beginning to rebound by day 7 [61].

Along the same line, in 2012, Zhu et al examined hematopoietic recovery in mice following chemotherapy. Treatment of C57BL/6J mice with the PPARγ inhibitor BADGE after chemotherapy resulted in faster recovery of leukocytes, greater numbers of colony forming units in culture and increased numbers of HSCs within the bone marrow of these animals

[63]. Taken together, these results suggest that adipogenesis is suppressed during times of increased hematopoietic demand.

Our laboratories recently studied the effects of APs on murine HSC maintenance. Here we cultured Lin-Sca+c-Kit+ cells for seven days (LSK cells, commonly regarded as HSCs [64]) with a mesenchymal stromal cell line that contained either low numbers of APs (GZL) or one that contained high numbers of APs (GZL/Adi) [65]. Co-culture of LSK cells with the GZL/Adi cells resulted in significantly fewer viable LSK cells after 7 days than co-culture with the GZL line [65]. There was also a significant elevation of NP1 expression in the GZL/Adi line compared to the GZL line [65]. Our findings are consistent with other studies that have found a negative relationship between APs and hematopoeisis, perhaps mediated by NP1. While these studies clearly implicate APs as negative regulators of hematopoiesis, other studies, involving BAT show an instance in which adipose tissue may promote hematopoiesis.

It is well-established that the bone marrow gradually fills with fat over time in humans. It is also known that humans rapidly lose deposits of BAT after birth, but it never completely disappears. As previously discussed, BAT is present within the bone marrow, and it too, appears to be lost in aging. Interestingly, very recent data are emerging that BAT is connected to hematopoiesis, although the mechanisms are incompletely understood. In 2012, using immunodeficient NOG mice, Nishio et al showed better engraftment of HSCs that had been cultured with human embryonic stem cell derived BAT (hESCdBA) than HSCs alone [66]. This hESCdBA was also found to express the hematopoietic cytokines thrombopoietin, interleukin-6, interleukin-3, colony-stimulating factors 3 and 2, as well as erythropoietin [66]. These studies highlight the complex interactions occurring in the BM microenvironment and how much work needs to be done to fully elucidate the mechanisms by which adipose tissue may be regulating hematopoiesis.

While the connections between adipose tissue and hematopoiesis are developing, the involvement of OBs in the HSC niche and hematopoeisis is much better understood [67–69]. In turn, several investigators recently reported that HSCs participate in OB differentiation [70, 71]. This reciprocal relationship may play a role in the decline of both hematopoiesis and osteogenesis with aging. Our laboratory recently demonstrated that the maturational status of OBs is important in their ability to stimulate HSCs. Specifically, we showed that OBs with higher Runx2 expression and low osteocalcin expression (i.e., less mature OBs) co-cultured with LSK cells significantly enhanced the in vitro generation of phenotypically defined progenitor cells and colony forming units (CFUs) compared to co-culture with more mature OBs [72]. As previously discussed, MSC differentiation in aging favors the AP lineage, therefore leaving fewer early OBs to support hematopoiesis.

Conclusions

There has been a recent explosion of interest in the interactions between bone, fat, and blood. We now understand that there is a balance between osteogenesis and adipogenesis that changes as we grow older. As the overall population ages, obesity rates increase and the incidence of osteoporosis and diabetes rises. At the same time, hematopoiesis in the elderly is not as robust as it is in younger individuals suggesting that the hematopoietic microenvironment mediates a negative regulatory effect on hematopoiesis (Figure 1 is a cartoon model illustrating these changes with age). In this review, we examined many of the complex mechanisms governing the relationships between bone, fat, and blood formation and how the aging process changes these processes; however, there is still much that remains unknown. Understanding how these tissues develop and change with time and defining how OB and AP interact during aging to impact bone health and hematopoiesis will

be critically important to developing new and better therapies for disorders of bone, metabolism and hematopoiesis in the elderly.

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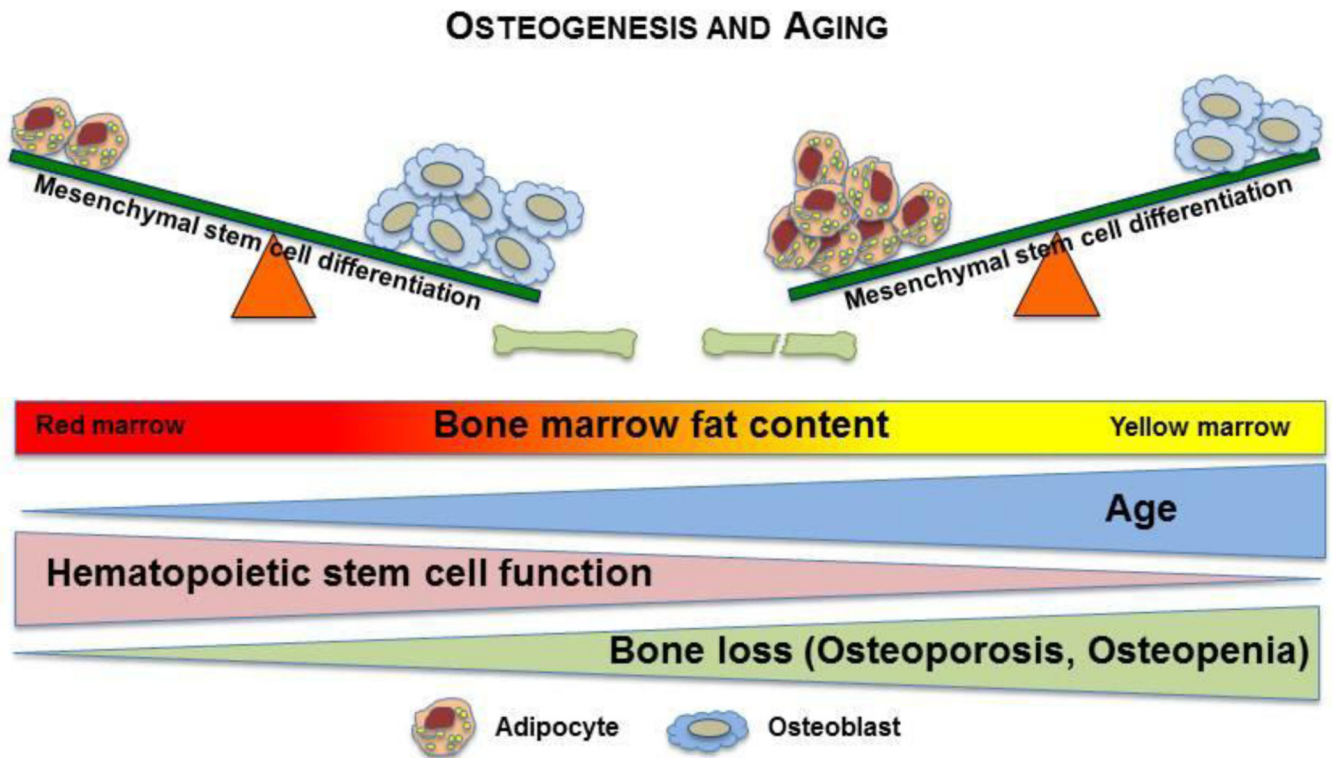
Figure 1.
Bone marrow microenvironment changes with aging. MSCs, which reside in the bone marrow, are common ancestors of both APs and OBs. In youth, MSC differentiation favors osteoblastogenesis; however; in aging, this balance progressively leans towards adipogenesis. This mirrors the gradual accumulation of APs within the bone marrow. Concurrently, HSC function, and subsequently hematopoiesis, declines with aging.