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New insights into the epigenetics of osteoporosis

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Abstract

Osteoporosis is characterized by reduced bone formation and accumulation of adipocytes in the bone marrow compartment. The decrease in bone mass results from an imbalance between osteoclast-mediated bone resorption and osteoblast-mediated bone formation. The deficiency of bone cells to replace the resorpted bone can be due to a preferential differentiation of bone marrow stromal cells into adipocytes at the expense of osteoblasts. Consequently, the processes that control the differentiation of osteoclasts, osteoblasts and adipocytes play a crucial role in bone metabolism. It is known that epigenetic mechanisms are critical regulator of the differentiation programs for cell fate and moreover are subject to changes during aging. Here, we summarize recent findings on the role of epigenetics in the modulation of mechanisms that may be associated with osteoporosis. In particular, we focus on disturbances in the bone remodeling process described in human studies that address the epigenetic regulation of the osteoblast/adipocyte balance.

Keywords: Human osteoporosis; Epigenetics; Osteoblast; Osteoclast; Adipocyte; Differentiation.

1. Introduction

Bone is under constant turnover throughout life in order to maintain a healthy bone mass. Bone homeostasis depends on the balance between bone resorption by osteoclast and bone formation by osteoblast. Osteoporosis (OP) results from the impairment of this balance leading to a reduction in bone mass associated with a high risk of fragility fractures. It is most often caused by an increase in osteoclastic activity that is not sufficiently compensated by an increase in bone formation. Recently another mechanism for OP involving the bone marrow adipocyte has been proposed. Indeed, evidence from numerous studies supports a link between bone loss and accumulation of medullary adipocytes [1, 2]. Osteoblasts and adipocytes are derived from the same multipotent precursor, the bone marrow mesenchymal stem cell (BMSC) [3, 4], and it has been suggested that the excessive accumulation of marrow adipocytes is caused by preferential differentiation of BMSCs into adipocytes at the expense of osteoblasts [5-7]. The processes which maintain in balance the differentiation and the activity of osteoclasts, osteoblasts and adipocytes are controlled by specific key genes and are tightly regulated by endogenous and exogenous factors. The phenotypic modifications during cellular differentiation are also driven by epigenetic changes which represent another fundamental mechanism of cell fate determination and commitment [8, 9]. The first use of the term 'epigenetic' was made by Conrad Waddington in 1942 to describe interactions between genotype and the environment, which together shape the expressed characteristic traits of an organism, defined as the phenotype [10]. It refers to potentially heritable changes in gene expression without modifications of the DNA sequence. At least, three mechanisms including DNA methylation, histone modifications and miRNA based pathways initiate epigenetic changes [11] (Figure 1). As these changes are influenced by factors such as age, gender and environment, it is not surprising that there is more and more interest in the study of epigenetics in the pathogenesis of OP. In this review, we summarize the impact of epigenetic modifications on the modulation of the bone remodeling process described in human clinical studies. In addition, we provide an overview of the recent *in vitro* findings on the epigenetic mechanisms that govern the osteoblast/adipocyte balance in human cells.

2. Epigenetic modifications

2.a. DNA methylation

DNA methylation is one of the most studied epigenetic mechanisms [12]. It consists of a methyl group being covalently bound to the 5' position of cytosine in palindromic cytosine-phosphate-guanine (CpG) dinucleotides, abbreviated to 5mC. CpG dinucleotides occur infrequently, only 2% of the mammalian genome contains short high-frequency stretches of CpGs called CpG islands that are mainly associated with gene promoters and regulatory regions [12]. The addition of methyl groups to cytosine is catalyzed by DNA methyltransferases (DNMTs). The predominant consequence of methylation is transcriptional repression, which can be mediated by directly or indirectly blocking the binding of essential transcription factors to their targets. However, DNA methylation pattern established early in vivo is often influenced by the external environment and small differences may have a big impact on the phenotype diversity that is linked to the onset of non-malignant diseases [12].

2.b. Histone modifications

The major protein complex around with DNA coiled and thereby condensed is called a nucleosome. Each nucleosome consists of eight proteins called histones (H): an H3-H4 tetramer and two H2A-H2B dimers. Histone methylation is one of the most common epigenetic modifications on histones. Lysine (K) and arginine (R) residues are the predominant sites of methylation, with the potential addition by the histone methyltransferases (KMTs) of one, two, or three methyl groups. These methyl groups regulate transcription mainly by blocking access to transcription factors and inhibiting gene transcription. The removal of methyl groups from histone lysine residues is catalyzed by histone lysine demethylases (KDMs) [13]. Acetylation is another well-known post translational modification of histones. In general, histone acetylation is associated with active gene transcription, whereas histone deacetylation is correlated with gene repression. Acetylation neutralizes the positive charge of histones, and thereby prevents the compaction of chromatin. On the contrary, histone deacetylation contributes to a more compacted chromatin, thus impeding transcription. The

dynamic balance between acetylation and deacetylation of histones is performed by two classes of enzymes: lysine/histone acetyltransferases (HATs) and lysine/histone deacetylases (HDACs) [13].

2.c. MicroRNAs

MicroRNAs (miRNAs) are a class of small (18-25 nucleotides) non-coding single-strand RNAs [14]. 28,645 miRNAs are referenced in the miRBase (<http://www.mirbase.org/release 17>; December 2017). Their role in gene regulation is essential as they are involved in a number of biological processes including cell proliferation, differentiation, developmental timing control, apoptosis and stress responses, as well as pathological states such as cancer [15]. miRNAs can regulate up to 60% of protein-coding genes at the post-transcriptional level by blocking messenger RNA (mRNA) translation or by inducing mRNA degradation, preventing protein production [14].

3. Epigenetic mechanisms regulating bone remodeling in OP: Focus on clinical studies.

To maintain bone mass homeostasis, the bone remodeling cycle starts with the dissolution of bone mineral and enzymatic matrix degradation by osteoclasts. Subsequent bone formation is performed by osteoblasts, producing osteoid matrix which will mineralize. In order to keep both processes in balance, controlled differentiation of osteoblasts and osteoclasts from their precursors is essential. The transcriptional factors Runx2 (Runt-related transcription factor 2) and Osx (Osterix) are key regulators of all stages of osteoblast differentiation also controlled by critical signaling pathway including the Wnt pathway [16]. Once mature, osteoblasts that become entombed within the bone matrix, called osteocytes, also modulate the differentiation and activity of both cell types [16]. Osteoclast originates from the hematopoietic lineage and its bone-resorptive activity is mainly regulated by the RANK/RANKL/OPG (receptor activator of nuclear factor κ B /RANK ligand /osteoprotegerin) system. RANKL is produced by osteoblasts and its binding to RANK, located on the surface of osteoclasts, activates the expression of osteoclastogenic genes. In contrast, OPG, also secreted by osteoblasts, acts as a

decoy receptor by binding to RANKL and inhibits bone resorption. All these aforementioned factors interact with many transcriptional activators or repressors, increasing the complexity of these regulations [16]. Furthermore, it has been shown that epigenetic mechanisms take part in the control of osteoclastogenesis and osteoblastogenesis [17]. Thus it is expected that epigenetic changes may contribute to the altered bone remodeling associated with osteoporosis. Below are summarized the recent epigenetic findings reported in human studies with the exception of histone modifications for which no data were found. However, this epigenetic mechanism, explored *in vitro* and in animal experiments, are presented in recently published reviews [18-20].

3.a. DNA methylation

Due to its relevant roles in age-related diseases, some teams showed interest in the involvement of DNA methylation in the pathogenesis of OP. In 2013, Delgado-Calle and his colleagues compared the DNA methylation patterns of bone from patients with OP and osteoarthritis (OA). Thanks to an Illumina methylation arrays analysis, the authors found differentially methylated regions in whole genome between patients with OP and OA [21]. In another study by the same group, methylation of Wnt pathway genes was analyzed in postmenopausal bone tissue samples from patients with OP and OA. Several genes in the Wnt pathway presented differences in methylation between both groups, which could contribute to the higher Wnt pathway activity observed in osteoarthritis [22]. Another important actor of the Wnt pathway is sclerostin, the product of the SOST gene mainly secreted by osteocytes. A higher number of CpG methylations was found in the SOST promoter in bone biopsies of postmenopausal OP compared to normal controls, correlated with decreased SOST mRNA expression and serum level [23]. As SOST is described as an inhibitor of bone formation, the increase in SOST promoter methylation could be a counteracting mechanism to reduce the inhibition of wnt signaling in an attempt to promote bone. To go a step further, by combining transcript profiling with DNA methylation analyses in bone and blood of osteoporotic and healthy patients, they found other transcripts, inhibitors of bone formation, with methylation levels significantly different between osteoporotic and control patients [24]. This is in contrast with another genome-wide analysis which failed to detect associations

between DNA methylation changes in blood and bone mineral density [25]. Also paradoxically, increased SOST protein expression was observed at the femoral neck in women with osteoporotic fractures, concomitantly with reduced expression of the deacetylase Sirtuin 1, a negative regulator of SOST [26]. However, in this study bone samples from OA patients were used as surrogate controls instead of healthy controls. These observations highlight a critical need to take into account data heterogeneity according to experimental design (sample sizes, controls, tissue types and technologies) and analytical tools. Besides the involvement of DNA methylation in osteoblastogenesis, much less is known in the regulation of osteoclast differentiation. To our knowledge, only one study investigated the influence of methylation in the increase of resorption associated with osteoporosis, with a focus on the RANKL/OPG system in human bones [27]. Although the RANKL mRNA level and RANKL:OPG transcript ratio were significantly higher in bone from osteoporotic patients, there was no evidence for differential methylation of RANKL at the bone tissue level compared to control group. In this study, the experiments were conducted using bone samples from OA patients as controls and the biological interpretation of the results could be questioned. However, given the practical and ethical difficulties to obtain bone samples from healthy subjects, this limitation applies to most of the described studies. Notwithstanding these concerns, all the studies reported to date strongly suggest that perturbations in DNA methylation are present in osteoporosis and linked to altered bone remodeling.

3.b. miRNAs

Because of ethical and technical limitations, the number of studies investigating miRNAs expression in osteoporotic bone tissue is still limited. Li *et al.* first reported the association between the downregulation of miR-2861 and primary OP in 2 adolescents [28]. Two more recent studies compared bone samples from patients with hip fractures and non-fractured controls with osteoarthritis. Using micro-array technologies, Garmilla-Ezquerro *et al.* found significant down-regulation of miR-187-3p and up-regulation of miR-518f in osteoporotic bone while Zhang *et al.* focusing on the characterization of miR-221, showed its involvement in OP through regulating Runx2 [29,30]. Besides these few cell-specific studies, a higher number of reports investigated the differences in miRNAs signatures in human whole blood, serum,

plasma or peripheral mononuclear cells between osteoporotic and non-osteoporotic subjects [31- 44]. In this way, the up or down regulation of circulating miRNAs linked to changes in bone metabolism was detected (Table 1). These miRNAs are potentially involved in the regulation of osteoblastogenesis, osteoclastogenesis or both but not all miRNA targets are biologically validated. Generally, the identity of regulated miRNAs varies significantly across studies, and for some of them there is even conflicting results. For example, Seelinger *et al.* demonstrated an upregulation of miR-21-5p in the serum of osteoporotic patients with bone fractures compared with patients with non-osteoporotic fractures [31]. This result was further confirmed by Panach *et al.* who found a strong correlation between the levels of mi-21-5p and CTx, a marker of bone resorption, in the serum of osteoporotic fractured patients compared with OA patients [32]. In contrast, two studies analyzing the plasma of osteoporotic patients and healthy controls demonstrated that the miR-21-5p levels were higher in the control groups [40, 44]. These discrepancies among studies are likely due to the different nature of patients' population (osteoporotic with or without fractures) and selected controls (healthy, OA or with non osteoporotic fractures). Moreover, given that fracture significantly affects bone homeostasis, one can assume that this has an impact on miRNA expression and can bias the results. Interestingly, a link between bone tissue and serum/blood expression patterns was found for some miRNAs, such as miR-125b upregulated in both serum and bone tissue of osteoporotic patients with fracture and in blood and bone tissue of osteoporotic women [32, 42]. This provides evidence that circulating miRNA levels may be directly correlated to cell-specific miRNA levels and thus to altered bone metabolism. Another evidence was provided by a recent study reporting substantial changes of several miRs that regulate osteoblast and osteoclast differentiation and function following treatment with anti-osteoporotic agents [45]. In particular, a decrease expression of miR-133a was found in the serum of osteoporotic postmenopausal women at 12 months of treatment with teriparatide, a bone anabolic drug .This finding is in line with two previous reports demonstrating an increase in miR-133a expression in the serum of osteoporotic patients [37, 40]. miR-133 directly targets Runx2 and therefore affects osteoblastogenesis, it is thus coherent that its expression was decreased following a treatment that stimulates bone formation. Despite some differences in the published data, taken all together, these studies suggest that miRNAs could be useful to

investigate alterations of bone metabolism and follow the response to anti-osteoporotic treatment.

4. Can epigenetic modifications also explain the increase in medullary adiposity observed in OP?

In bone loss, the deficiency of bone cells to replace the resorpted bone can be related to an insufficient differentiation of BMSCs into osteoblasts. In particular, it could be the reflection of the imbalance between adipogenesis and osteogenesis, lately proposed as another mechanism for OP [5-7]. Recent works, mostly performed through *in vitro* investigations on human cells, suggested that this perturbed BMSC fate decision and commitment could be directly driven by epigenetic mechanisms (Figure 2).

4.a. Histone modifications

Growing evidence suggests that histone modifications play a critical role in the balance between osteoblast and adipocyte and could explain the increase of bone marrow adiposity observed in OP [46-47]. First, the negative impact of histone deacetylation on osteogenesis was suggested by *in vitro* investigations using chemical inhibitors of histone deacetylases [48-49]. More recently, using an epigenetic library screen, another group investigated the effects of small-molecule inhibitors of histone deacetylase on BMSC differentiation [50-51]. They identified several novel compounds that promoted adipocyte differentiation through an increase in acetylation (particularly at lysine H3K9) in the promoter region of adipocyte-related genes, including the master adipogenic transcription factor PPAR γ (peroxisome proliferator-activated receptor). Moreover, several studies highlighted the importance of histone methylation and demethylation in the BMSC fate. For instance, Hemming and colleagues revealed that the combination of methylation and demethylation might function as an epigenetic switch for BMSC lineage determination [52]. Indeed, they showed that EZH2 (the Enhancer of Zeste Homolog 2), a histone

methyltransferase that catalyzes the trimethylation of histone H3 on lysine 27 (H3K27me3) on master regulatory genes, promoted adipogenic differentiation and inhibited osteogenesis. In contrast, by removing these repressive marks, lysine demethylase 6A (KDM6A) inhibited adipogenesis and promoted osteogenesis. Several EZH2 targets, involved in the regulation of BMSC fate have been identified [53]. Among these targets, HDAC9c, a non-catalytic HDAC protein, have the capacity to negatively regulate the transcriptional activity of PPARgamma. Interestingly, Chen *et al.* observed that, in aged BMSCs, adipogenesis is enhanced as a result of increased EZH2 and decreased HDAC9c expression [54]. Additional studies support the notion that EZH2 is a central key of complex epigenetic regulation of bone marrow adipogenesis. Phosphorylation of EZH2 inhibited its methyltransferase activity and resulted in promoted osteogenesis [55]. Furthermore, Zhu *et al.* showed that HoxA-AS3, a long non-coding RNA, interacted with EZH2 to modulate the status of H3K27me3 in the promoter region of the osteoblastic transcriptional factor Runx2 and repressed its transcription [56].

4.b. miRNAs

Studies addressing the role of miRNAs as mediators of the balance between osteogenesis and adipogenesis begin to emerge. By targeting different downstream targets, these miRNAs can affect both lineage commitment and terminal differentiation (Table 2). Indeed, it has been shown that miR-204, miR-211 and miR-320 inhibit osteogenesis via Runx2 repression while miR-637 down-regulates differentiation through repression of Osterix [57-59]. Qadir *et al.* demonstrated that miR-124 increases adipogenesis and negatively regulates osteogenesis by targeting several members of the Dlx (distal-less) family factors required for osteoblast differentiation and Runx2 expression [60]. Recently, Elsafadi *et al.* found that down-regulation of LRP3 (Low-density lipoprotein receptor-related protein 3) expression by miR-4739 suppresses osteogenic differentiation [61]. miRNAs can also promote a switch between osteogenesis and adipogenesis by targeting inhibitors of adipocyte differentiation. For instance, in BMSCs of aging mice and human, miR-188 activates PPARgamma by suppressing two of its inhibitors, HDAC9 and RICTOR (RPTOR-independent companion of MTOR complex), resulting in an increase number of adipocytes at the expense of osteoblasts [62]. Lastly and very interestingly,

perturbations in circulating miRNA levels in OP have been shown to be directly linked to *in vitro* altered adipocyte/osteoblast balance. Indeed, a microarray investigation of serum from osteoporotic patients and healthy controls revealed a significant reduction of miR-27a expression in postmenopausal osteoporotic patients compared to controls. Subsequent analysis on BMSCs from patients indicated that osteoblastogenesis was reduced, whereas adipogenesis was increased [63].

5. Conclusions

Although being actively studied, advances in understanding the role of epigenetic factors in the pathophysiology of skeletal diseases, such as OP, are still preliminary. Overall, experimental data presented in this review show that epigenetic events may have profound effect on commitment, differentiation and activity of the cells of the bone marrow environment and consequently may contribute to the pathophysiology of age-related bone loss. Studies that address the epigenetic regulation in the osteoblast/adipocyte balance are somewhat limited in mainly being performed *in vitro*. However, new high throughput technologies will likely contribute to increase the number of investigations, including those conducted on human samples. Promisingly, a link between bone and serum/blood expression patterns was found for some miRNAs, providing evidence that circulating miRNA levels may be directly correlated to altered bone metabolism. This finding together with the fact that miRNAs are stable in body fluids make them potential tools to detect early manifestations of OP. Given that epigenetics is recognized as a significant contributor to pathological conditions, efforts to investigate the epigenetic marks in OP could pave the way for new therapeutic advances.

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Disclosure of interest

The authors declare that they have no competing interest.

References

- [1] P. Meunier, J. Aaron, C. Edouard, G. Vignon, Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies, *Clin Orthop Relat Res.* 80 (1971)147-54.
- [2] J. Justesen, K. Stenderup, EN. Ebbesen, L. Mosekilde, T. Steiniche, M. Kassem, Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis, *Biogerontology* 2 (2001) 165-71.
- [3] M. Owen, Marrow stromal stem cells, *J Cell Sci.* 10 (1998) 63-76.
- [4] M.F. Pittenger, A.M. Mackay, S. Beck, *et al.*, Multilineage potential of adult human mesenchymal stem cells, *Science* 284 (1999) 143-7.
- [5] J.N. Beresford, J.H. Bennett, C. Devlin, P.S. Leboy, M.E. Owen, Evidence for an inverse relationship between the differentiation of adipocytic and osteogenic cells in rat marrow stromal cell cultures, *J Cell Sci.* 102 (1992) 341-51.
- [6] J.M. Gimble, S. Zvonic, Z.E. Floyd, M. Kassem, M.E. Nuttall, Playing with bone and fat, *J Cell Biochem* 98 (2006) 251-66.
- [7] G. Duque, Bone and fat connection in aging bone, *Curr Opin Rheumatol.* 20 (2008) 429-34.
- [8] C.M. Teven, X. Liu, N. Hu , *et al.*, Epigenetic regulation of mesenchymal stem cells: a focus on osteogenic and adipogenic differentiation, *Stem Cells Int.* 2011 (2011) 201371.
- [9] F.M. Perez-Campo and J.A. Riancho, Epigenetic mechanisms regulating Mesenchymal Stem Cell differentiation, *Curr Genomics* 16 (2015) 368-83.
- [10] C.H. Waddington, The Epigenotype, *Int J Epidemiol.* 41 (2012) 10- 3.
- [11] Y. Han, X. He, Integrating epigenomics into the understanding of biomedical insight, *Bioinform Biol Insights* 10 (2016) 267-89.
- [12] L.D. Moore, T. Le, G. Fan. DNA methylation and its basic function. *Neuropsychopharmacology* 38 (2013) 23–38.
- [13] T. Kouzarides, Chromatin modifications and their function, *Cell* 128 (2007) 693-705.

- [14] F. Sato, S. Tsuchiya, S.J. Meltzer, K. Shimizu, MicroRNAs and epigenetics, *FEBS Journal* 278 (2011) 1598-1609.
- [15] L.A. Macfarlane, P.R. Murphy, MicroRNA: Biogenesis, function and role in cancer. *Curr Genomics*, 11 (2010) 537-61.
- [16] C.S. Soltanoff, S. Yang, W. Chen, Y.P. Li, Signaling networks that control the lineage commitment and differentiation of bone cells, *Crit Rev Eukaryot Gene Expr.* 19 (2009) 1-46.
- [17] P. Vrtačnik, J. Marc, B. Ostanek. Epigenetic mechanisms in bone, *Clin Chem Lab Med*, 52 (2014) 589-608.
- [18] C. Gayor, F.E. Weber, Epigenetic regulation of bone remodeling and its impact on osteoporosis. *Int J Mol Sci*, 17 (2016) 1446.
- [19] F. Marini, L. Cianferotti, M.L. Brandi, Epigenetic regulation of bone remodeling and its impact on osteoporosis. *Int J Mol Sci*, 17 (2016) 1329.
- [20] A. Husein, M.A. Jeffries, Epigenetics and bone remodeling. *Curr Osteoporod Rep*, 5 (2017) 450-8.
- [21] J. Delgado-Calle, A.F. Fernández, J. Sainz, *et al.*, Genome-wide profiling of bone reveals differentially methylated regions in osteoporosis and osteoarthritis. *Arthritis Rheum*, 65 (2013)197-205.
- [22] C. García-Ibarbia, J. Delgado-Calle, I. Casafont, *et al.*, Contribution of genetic and epigenetic mechanisms to Wnt pathway activity in prevalent skeletal disorders, *Gene*. 532 (2013) 165-72.
- [23] S. Reppe, A. Noer, R.M. Grimholt, *et al.*, Methylation of bone SOST, its mRNA, and serum sclerostin levels correlate strongly with fracture risk in postmenopausal women, *J Bone Miner Res.* 30 (2015) 249-56.
- [24] S. Reppe, L.T. Lien TG, Y.H. Hsu, *et al.*, Distinct DNA methylation profiles in bone and blood of osteoporotic and healthy postmenopausal women, *Epigenetics*. 12 (2017) 674-687.
- [25] J.A. Morris, P.C. Tsai, R. Joehanes, *et al.*, Epigenome-wide association of DNA methylation in whole blood with bone mineral density, *J Bone Miner Res.* 32 (2017) 1644-1650.
- [26] M. El-Haj, I. Gurt , E. Cohen-Kfir, *et al.*, Reduced Sirtuin1 expression at the femoral neck in women who sustained an osteoporotic hip fracture, *Osteoporos Int.* 27 (2016) 2373-8.
- [27] J. Delgado-Calle, C. Sanudo, A.F. Fernández, *et al.*, Role of DNA methylation in the regulation of the RANKL-OPG system in human bone. *Epigenetics*, 1 (2012) 83-91.

- [28] H. Li, H. Xie, W. Liu, *et al.*, A novel microRNA targeting HDAC5 regulates osteoblast differentiation in mice and contributes to primary osteoporosis in humans, *J Clin Invest.* 119 (2009) 3666-77.
- [29] P. Garmilla-Ezquerria, C. Sañudo, J. Delgado-Calle, M.I. Pérez-Nuñez, M. Sumillera, J.A. Riancho, Analysis of the bone microRNome in osteoporotic fractures, *Calcif Tissue Int.* 96 (2015) 30-7.
- [30] Y. Zhang, Y. Gao, L. Cai, *et al.*, MicroRNA-221 is involved in the regulation of osteoporosis through regulates RUNX2 protein expression and osteoblast differentiation, *Am J Transl Res.* 9 (2017) 126-35.
- [31] C. Seeliger, K. Karpinski, A.T. Haug, *et al.*, Five freely circulating miRNAs and bone tissue miRNAs are associated with osteoporotic fractures, *J Bone Miner Res.* 29 (2014) 1718-28.
- [32] L. Panach, D. Mifsut, J.J. Tarín, A. Cano, M.A. García-Pérez, Serum circulating MicroRNAs as biomarkers of osteoporotic fracture, *Calcif Tissue Int.* 97 (2015) 495-505.
- [33] S. Weilner, S. Skalicky, B. Salzer, *et al.*, Differentially circulating miRNAs after recent osteoporotic fractures can influence osteogenic differentiation, *Bone.* 79 (2015) 43-51.
- [34] R. Kocijan, C. Muschitz, E. Geiger, *et al.*, Circulating microRNA signatures in patients with idiopathic and postmenopausal osteoporosis and fragility fractures, *J Clin Endocrinol Metab.* 101 (2016) 4125-34.
- [35] U. Heilmeyer, M. Hackl, S. Skalicky *et al.*, Serum miRNA signatures are indicative of skeletal fractures in postmenopausal women with and without type 2 diabetes and influence osteogenic and adipogenic differentiation of adipose tissue-derived Mesenchymal Stem Cells in vitro, *J Bone Miner Res.* 31 (2016) 2173-92.
- [36] L. De-Ugarte, E. Caro-Molina, M. Rodríguez-Sanz, *et al.*, SNPs in bone-related miRNAs are associated with the osteoporotic phenotype, *Sci Rep.* 7 (2017) 516.
- [37] Y. Wang, L. Li, B.T. Moore, *et al.*, MiR-133a in human circulating monocytes: a potential biomarker associated with postmenopausal osteoporosis, *PLoS One.* 7 (2012) e34641.
- [38] Z. Cao, B.T. Moore, Y. Wang, *et al.*, MiR-422a as a potential cellular microRNA biomarker for postmenopausal osteoporosis, *PLoS One.* 9 (2014) e97098.
- [39] C. Chen, P. Cheng, H. Xie, *et al.*, MiR-503 regulates osteoclastogenesis via targeting RANK, *J Bone Miner Res.* 29 (2014) 338-47.
- [40] H. Li, Z. Wang, Q. Fu, J. Zhang, Plasma miRNA levels correlate with sensitivity to bone mineral density in postmenopausal osteoporosis patients, *Biomarkers* 19 (2014) 553-6.

- [41] J. Meng, D. Zhang, N. Pan, *et al.*, Identification of miR-194-5p as a potential biomarker for postmenopausal osteoporosis, *Peer J.* 21 (2015) e971.
- [42] H. Chen, H. Jiang, D. Can, *et al.*, Evaluation of MicroRNA 125b as a potential biomarker for postmenopausal osteoporosis, *Trop J Pharm Res.* 16 (2017) 641.
- [43] C. Wang, H. He, L. Wang, *et al.* Reduced miR-144-3p expression in serum and bone mediates osteoporosis pathogenesis by targeting RANK, *Biochem Cell Biol*, (2018). doi.org/10.1139/bcb-2017-0243.
- [44] A.D. Yavropoulou, A.D. Anastasilakis P. Makras, *et al.* Expression of microRNAs that regulate bone turnover in the serum of postmenopausal women with low bone mass and vertebral fractures, *Eur J Endocrinol*, 2 (2017) 169-76.
- [45] A.D. Anastasilakis, P. Makras, M. Pikilidou, *et al.* Changes of Circulating MicroRNAs in Response to Treatment With Teriparatide or Denosumab in Postmenopausal Osteoporosis, *J Clin Endocrinol Metab*, 3 (2018) 1206-13.
- [46] P. Deng P, Q.M. Chen, C. Hong, C.Y. Wang, Histone methyltransferases and demethylases: regulators in balancing osteogenic and adipogenic differentiation of mesenchymal stem cells, *International Journal of Oral Science* 7 (2015) 197–204.
- [47] B. Huang, G. Li, X.H. Jiang, Fate determination in mesenchymal stem cells: a perspective from histone-modifying enzymes, *Stem Cell Res Ther.* 19 (2015) 6:35.
- [48] J. de Boer, R. Licht, M. Bongers, *et al.*, Inhibition of histone acetylation as a tool in bone tissue engineering, *Tissue Eng.* 12 (2006) 2927-37.
- [49] H.H. Cho, H.T. Park, Y.J. Kim, Y.C. Bae, K.T. Suh, J.S. Jung, Induction of osteogenic differentiation of human mesenchymal stem cells by histone deacetylase inhibitors, *J Cell Biochem.* 96 (2005) 533-42.
- [50] D. Ali, R. Hamam, M. Halfayez, M. Kassem, A. Aldahmash, N.M. Alajez, Epigenetic library screen identifies Abexinostat as novel regulator of adipocytic and osteoblastic differentiation of human skeletal (mesenchymal) stem cells, *Stem Cells Translational Medicine* 5 (2016) 1036-47.
- [51] D. Ali, H. Alshammari, R. Vishnubalaji, E.P. Chalisserry, R. Hamam, M. Alfayez *et al.* CUCDC-907 promotes bone marrow adipocytic differentiation through inhibition of histone deacetylase and regulation of cell cycle, *Stem Cells Dev.* 26 (2017) 353-62.
- [52] S. Hemming, D. Cakouros, S. Isenmann, *et al.* EZH2 and KDM6A act as an epigenetic switch to regulate mesenchymal stem cell lineage specification, *Stem Cells* 32 (2014) 802-15.
- [53] Y.H. Chen, F.L. Yeh, S.P. Yeh, H.T. Ma, S.C. Hung, M.C. Hung, *et al.* Myocyte Enhancer Factor-2 Interacting Transcriptional Repressor (MITR) is a switch that promotes osteogenesis and inhibits adipogenesis of Mesenchymal Stem Cells by inactivating Peroxisome Proliferator-activated Receptor 2, *The journal of biological chemistry* 286 (2011):10671-80.

- [54] Y.H. Chen, C.C. Chung, Y.C. Liu, S.P. Yeh, J.L. Hsu, M.C. Hung, *et al.* Enhancer of Zeste Homolog 2 and Histone Deacetylase 9c regulate age-dependent mesenchymal stem cell differentiation into osteoblasts and adipocytes, *Stem Cells* 34 (2016) 2183-93.
- [55] Y. Wei, Y.H. Chen, L.Y. Li, *et al.*, CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells, *Nat Cell Biol.* 1 (2011) 87-94.
- [56] X.X. Zhu, Y.W. Yan, D. Chen, *et al.*, Long non-coding RNA HoxA-AS3 interacts with EZH2 to regulate lineage commitment of mesenchymal stem cells, *Oncotarget* 7 (2016) 63561-70.
- [57] J. Huang, L. Zhao, L. Xing, D. Chen, MicroRNA-204 regulates Runx2 protein expression and mesenchymal progenitor cell differentiation, *Stem Cells* 28 (2010) 357-64.
- [58] D. Hamam, D. Ali, R. Vishnubalaji, *et al.*, microRNA-320/RUNX2 axis regulates adipocytic differentiation of human mesenchymal (skeletal) stem cells, *Cell Death Dis.* 5 (2014) e1499.
- [59] J.F. Zhang, W.M. Fu, M.L. He, *et al.*, MiR-637 maintains the balance between adipocytes and osteoblasts by directly targeting Osterix, *I Biol Cell.* 22 (2011) 3955-61.
- [60] A.S. Qadir, S. Um S, H. Lee, *et al.*, miR-124 negatively regulates osteogenic differentiation and in vivo bone formation of mesenchymal stem cells, *J Cell Biochem.* 116 (2015) 730-42.
- [61] M. Elsafadi, M. Manikandan, N.M. Alajez, *et al.*, MicroRNA-4739 regulates osteogenic and adipocytic differentiation of immortalized human bone marrow stromal cells via targeting LRP3, *Stem Cell Res.* 20 (2017) 94-104.
- [62] C.J. Li , P. Cheng, M.K. Liang, *et al.*, MicroRNA-188 regulates age-related switch between osteoblast and adipocyte differentiation, *J Clin Invest.* 125 (2015) 1509-22.
- [63] L. You, L. Pan, L. Chen, W. Gu, J. Chen, MiR-27a is essential for the shift from osteogenic differentiation to adipogenic differentiation of Mesenchymal Stem Cells in postmenopausal osteoporosis, *Cell Physiol Biochem* 39 (2016) 253-65.

Legends

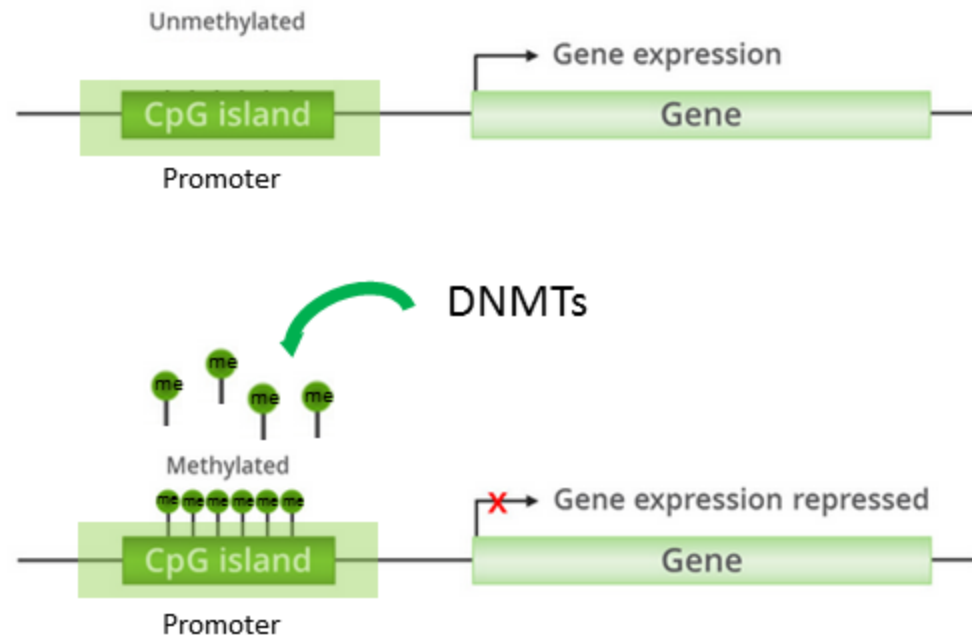
Figure 1: Mechanisms of epigenetic regulations.

(a) DNA methylation is a process catalyzed by DNA methyltransferases (DNMTs), preventing the binding of regulatory transcription factors to the promoter regions thereby repressing gene expression. (b) Histone methylation by histone methyltransferases (KMTs) and the removal of acetyl groups by histone deacetylases (HDACs) induce the closing of chromatin conformation and consequently transcriptional repression. In contrast, acetylation of lysine residues on histone tails, regulated by histone acetyltransferases (HATs), prevents the compaction of chromatin and promote transcriptional activation. (c) miRNAs negatively regulate gene expression by blocking mRNA translation or by inducing mRNA degradation.

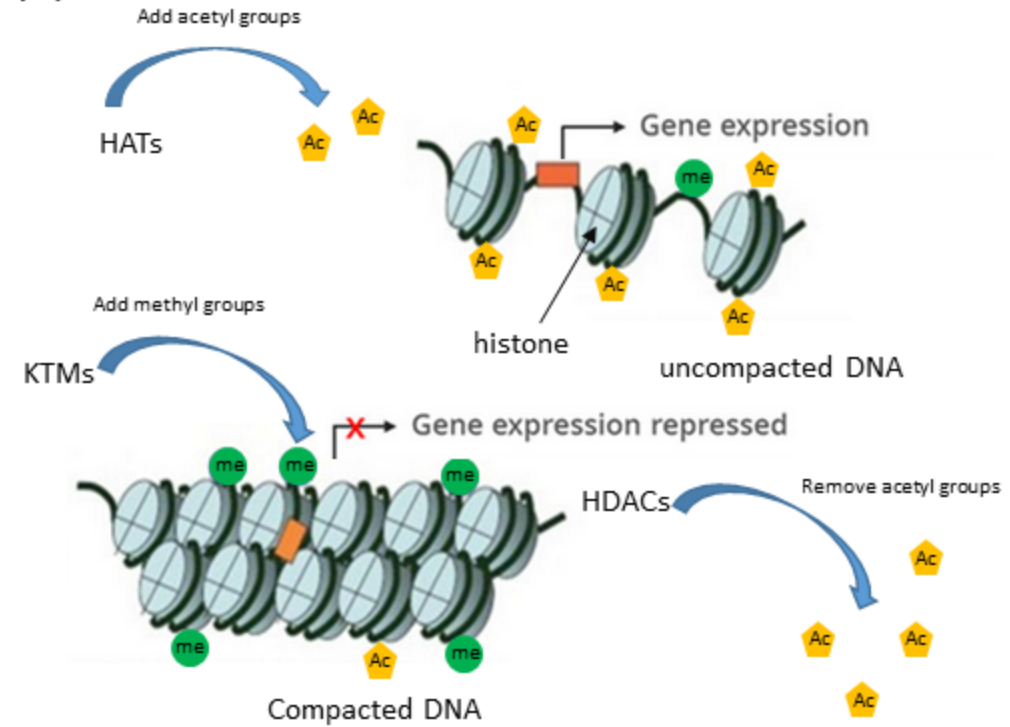
Figure 2: Epigenetic mechanisms regulating preferential human BMSC differentiation into adipocytes.

Here are schematized the epigenetic modifications involved in the perturbations of cell fate commitment that favor adipogenesis at the expense of osteogenesis. The histone methyltransferase EZH2 facilitates adipogenesis (1) by trimethylation of histone H3 on Lysine 27 at the promoter of HDAC9c, resulting in suppression of its expression and reactivation of PPARgamma expression and (2) by downregulating, in association with the long non-coding RNA HoxA-AS3, the expression of the osteoblastic transcriptional factor Runx2. In addition, by targeting genes required for osteoblast differentiation, such as Runx2, miRNAs and histone deacetylases (HDACs) negatively regulate osteogenesis and increase adipogenesis. There is no available data concerning the role of DNA methylation in the increase of bone marrow adiposity observed in human OP.

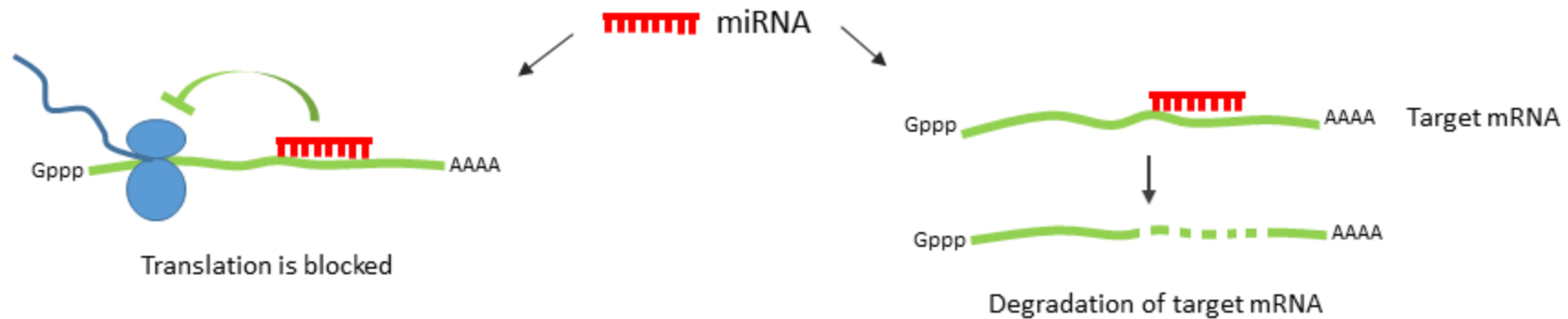
(a) DNA methylation



(b) Histone modifications



(c) miRNA expression



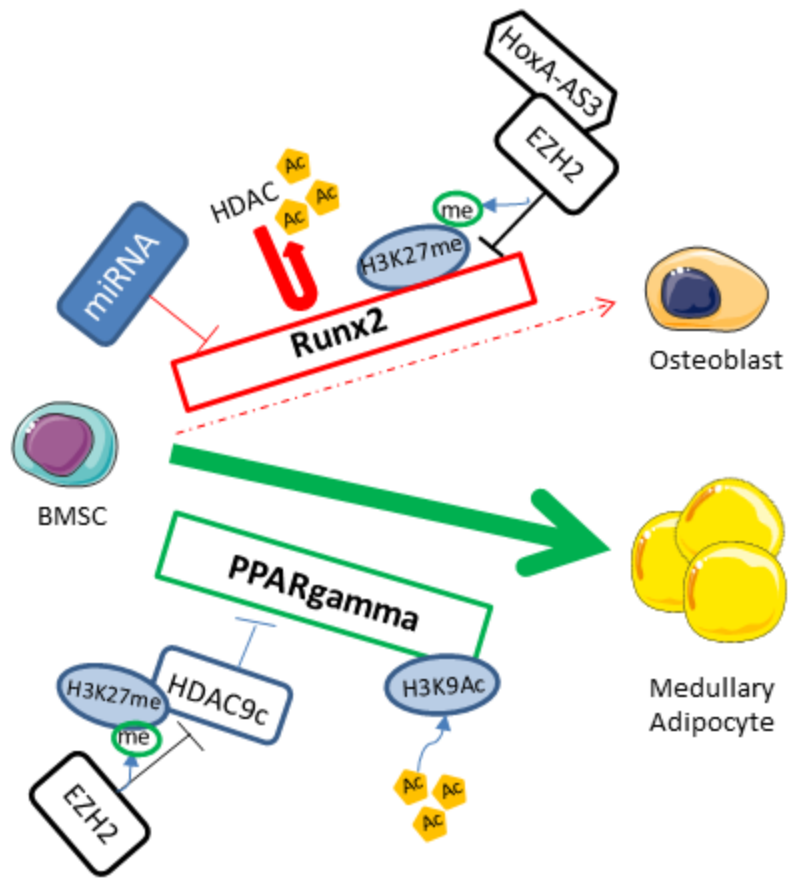


Table 1. Human clinical studies identifying miRNAs involved in the pathogenesis of osteoporosis

a. miRNAs expression upregulated in patients with osteoporosis

miRNA	Study design	References
miR-518f-3p	Bone samples from women with hip fractures (n=18) and non-fractured control group with osteoarthritis (n=18)	29
miR-21-5p, miR-23-3p, miR-24-3p, miR-25-3p, miR-100-5p, miR-125b-5p	Serum and bone samples from osteoporotic fractured patients and non-osteoporotic fractured controls (n=30 per group/serum and n=20 per group/bone tissue)	31
miR-122-5p, miR-125b-5p, miR-21-5p	Serum samples from osteoporotic fractured patients (n=15) and osteoarthritic controls (n=12)	32
miR-3679, miR-4274	Bone samples from postmenopausal women with hip fracture (n=10) and osteoarthritis (n=10)	36
miR-133a	Circulating monocytes from postmenopausal women with high (n=10) and low (n=10) BMD	37
miR-422	Circulating monocytes from postmenopausal women with high (n=10) and low (n=10) BMD	38
miR-133a-3p	Plasma samples from postmenopausal normal (n=40), osteopenic (n=40) and osteoporotic women (n=40)	40
miR-130b-3p, miR-151a-3p, miR-151b, miR194-5p, miR-590-5p	Blood samples from osteopenic (n=23) and osteoporotic women (n=25)	41
miR-125b	Serum and bone samples from postmenopausal normal (n=30) and osteoporotic patients (n=30)	42
miR-124, miR-2861	Serum from postmenopausal women with low BMD with (n=35) and without (n=35) fractures and healthy control group (n=30)	44

b. miRNAs expression downregulated in patients with osteoporosis

miRNA	Study design	References
miR-2861	Adolescents with primary osteoporosis (n=2)	28
miR-187-3p	Bone samples from women with hip fractures (n=18) and non-fractured control group with osteoarthritis (n=18)	29
miR-221	Bone sample from osteoporotic (n=12) and osteoarthritis patients (n=12)	30
miR-22-3p, miR-328-3p, miR-let-7g-5p	Serum samples from osteoporotic fractured women (n=12) and non osteoporotic controls (n=11)	33
miR-382-3p, miR-188-3p	Blood samples from postmenopausal fractured women (n=20) and controls (n=20)	35
miR-503	Peripheral blood mononuclear cells from normal (n=10) and osteoporotic (n=10) postmenopausal women	39
miR-21-5p	Plasma samples from postmenopausal normal (n=40) , osteopenic (n=40) and osteoporotic women (n=40)	40
miR-144-3p	Blood and bone samples from osteoporotic fractured patients (n=45) and non-osteoporotic fractured controls (n=15)	43
miR-21-5p, miR-23, miR-29	Serum from postmenopausal women with low BMD with (n=35) and without (n=35) fractures and healthy control group (n=30)	44

Table 2. miRNAs involved in the osteoblast/adipocyte balance

miRNA	Cellular model	Target	References
miR-204/211	Human BMSCs	Runx2	57
miR-320	Human BMSCs	Runx2	58
miR-637	miR-637	Osx	59
miR-124	Human maxillary BMSCs	Dlx5, Dlx3, Dlx2	60
miR-4739	Immortalized human BMSCs	LRP3	61
miR-188	Human BMSCs	HDAC9, RICTOR	62
miR-27a	Human BMSCs	Mef2c	63

Runx2, Runt-related transcription factor 2; Osx, Osterix; Dlx, Distal-Less Homeobox; LRP3, low-density lipoprotein receptor-related protein 3; HDAC9, Histone Deacetylase 9; RICTOR, RPTOR Independent Companion Of MTOR Complex 2; Mef2c, Myocyte Enhancer Factor 2C.