Current Molecular Biology Reports (2018) 4:1–7
https://doi.org/10.1007/s40610-018-0085-y

MOLECULAR BIOLOGY OF BONE MARROW FAT ADIPOSITY (B VAN DER EERDEN, SECTION EDITOR)

# Omics Contributions to the Molecular Mechanisms Regulating Bone Marrow Adipocyte Differentiation

Aline Clabaut [1,2] · Odile Broux [1,2,3]

Published online: 26 January 2018


## Abstract

**Purpose of Review** A shift in the commitment of human skeletal stem cells (SSCs) from the osteogenic lineage to the adipogenic lineage can result in increased marrow adiposity and bone loss. Advances in understanding the fate decision of SSCs and particularly the intracellular mechanisms controlling bone marrow adipocyte (BMA) differentiation have thus relevance to bone disorders. The aim of this review is to report the recent contributions of Omics studies to the understanding of mechanisms controlling human BMA differentiation.

**Recent Findings** Omics investigations allow the identification of factors involved in BMA differentiation, some of them already known to have relevance for adipogenesis and others not, and highlight the crucial role of epigenetic regulation in the control of SSCs lineage determination.

**Summary** There is a great potential in the use of Omics technologies for a better understanding of BMA differentiation. To draw a more complete picture of this process, efforts must be made in the standardization, compilation, and integration of data from different fields. Further expected outcome is the future identification of biomarkers or therapeutic targets in the context of bone disorders.

**Keywords** Omics · Skeletal stem cell · Bone marrow adipocyte · Differentiation

## Introduction

In recent years, more and more research groups from various scientific fields have been interested in the biology of bone marrow adipocyte (BMA) [1, 2]. BMAs, located in the human bone marrow cavity, increase in number and size with aging and may play important roles in the physiopathology of hematopoietic and musculoskeletal disorders [3, 4]. BMAs derive from multipotent progenitor cells of the bone marrow, the skeletal stem cells (SSCs) [also known as bone marrow stromal cells or mesenchymal stem cells (MSCs)] which can also give rise to osteoblasts [5]. The differentiation of SSCs into adipocytes and osteoblasts is regulated by mechanisms promoting cell fate into one lineage at the expense of the other [6, 7]. It has been suggested that the enhanced bone marrow adiposity associated with osteoporotic bone loss is due to a switch in the differentiation of SSCs in favor of adipogenesis [8]. Thus, cell fate determination of SSCs towards adipogenesis or osteogenesis represents an important area of investigation because of its potential impact on the neighboring cells of the bone marrow environment. The adipocyte/osteoblast balance is controlled by specific transcription factors, necessary to initiate and promote the differentiation process. RUNX2 and Osterix are the master transcriptional factors for the osteogenic pathway of SSCs [9]. The cascade that drives BMA differentiation is under the control of the same key regulators as extramedullary adipocytes, i.e., PPARγ and the members of the CCAAT/enhancer-binding proteins (C/EBP) family of transcription factors [10]. The commitment to mature adipocytes with successive stage-specific gene expression patterns involves complex signaling pathways, which are tightly controlled via endogenous and exogenous factors including

This article is part of the Topical Collection on *Molecular Biology of Bone Marrow Fat Adiposity*

✉ Odile Broux
broux@univ-littoral.fr

[1] Université de Lille, EA 4490, PMOI-Physiopathologie des Maladies Osseuses Inflammatoires, F-59000 Lille, France

[2] ULCO, PMOI, F-62200 Boulogne-sur-mer, France

[3] ULCO, PMOI, Bassin Napoléon, BP 120, F-62327 Boulogne-sur-Mer, France

hormones and cytokines. More detailed description of factors regulating this differentiation and their associated signaling pathways is summarized in a recently published review [11••]. The cascade of phenotypic modifications during cellular differentiation is also coupled to epigenetic changes which represent another fundamental mechanism of regulation, linking changes in chromatin states to the control of gene transcription [12–16].

Recent advances in the field of high-throughput techniques have helped to substantially increase the number of molecules that can be detected simultaneously. With the availability of these so-called "omics technologies," great progress has been made in understanding the molecular mechanisms behind the regulation of biological processes such as differentiation [17]. The term "Omics" generally makes reference to genomics, transcriptomics, proteomics, and epigenomics, the combination of these approaches allowing a more global comprehension of a complex cellular process [18••]. The aim of this review is to report the recent contributions of Omics studies to the understanding of intracellular mechanisms controlling BMA differentiation. The focus will be on the studies concerning human cells derived from SSCs, published within the past 5 years.

## Omics: Basic Aspects and Analytical Techniques

Since the word "genomics" appeared in the early 1990s, the new methods for a global analysis of a particular molecular type have been termed Omics technologies [17]. Major Omics include transcriptomics, proteomics, and epigenomics (Fig. 1a). Transcriptomics aims to analyze the full set of all genes being actively transcribed at a given point in time in one cell or a cellular population. The methods of choice for quantitative analysis of transcriptomes are DNA microarrays and next-generation sequencing (RNA Seq). Compared to microarrays, emerging RNA Seq techniques allow for the detection of different isoforms of a gene as well as previously unknown genes [19]. Complementary to transcriptomics, proteomics concerns the characterization and quantification of the entire set of proteins produced by a cell or tissue. High-throughput proteomic techniques are mainly based on mass spectrometry (MS) after initial separation by gel electrophoresis or by high-performance liquid chromatography (HPLC) [20]. Epigenomics refers to the study of epigenetic modifications on the genetic material of a cell. Changes in epigenetic marks have emerged as key modulators of cell fate determination, commitment, and differentiation. These modifications include histone modifications, DNA methylation, and non-coding RNA (ncRNA) modulation of gene expression [21••], which are further outlined below.

Histone tails are subjected to post-translational modifications including the two most common: acetylation and methylation. Histone acetylation is widely associated with a chromatin structure that is open and therefore accessible to transcription factors, which increases gene expression. The enzymes involved in histone acetylation are called histone acetyltransferases (HATs). The preferred targets for HATs are the lysine residues in the tails of histone H3 and H4. This acetylation is a reversible reaction, the removal of acetyl groups from histone lysine residues is catalyzed by histone deacetylases (HDACs). Histone methylation occurs on different lysine or arginine residues, with the potential addition by the histone lysine methyltransferase (KMT) of one, two, or three methyl groups. Lysine methylation of histones H3 and H4 is implicated in both transcriptional activation and repression depending on the methylation site and the degree of methylation while arginine methylation promotes transcriptional activation [22].

Histone modifications are tightly associated with DNA methylation, which correlates predominantly with gene silencing. It consists of a methyl group addition at the 5′ position of the cytosine ring within CpG dinucleotides to create a 5-methylcytosine (5mC) [23]. This modification is catalyzed by DNA methyltransferases (DNMTs).

Epigenetic-related ncRNAs, including microRNA (miRNA) and long non-coding RNA (lncRNA), are involved in heterochromatin formation, histone modification, DNA methylation targeting, and gene silencing. miRNAs generally bind to 3′ UTR of a specific target messenger RNA with a complementary sequence to induce cleavage, degradation, or block translation. Many miRNAs are epigenetically regulated by DNA methylation, histone modifications, or both. Inversely, miRNAs also control the expression of other epigenetic regulators such as HDACs and DNMTs [24]. lncRNAs are non-protein coding transcripts longer than 200 nucleotides. lncRNAs play possibly critical regulatory roles in chromatin remodeling, transcriptional regulation, and post-transcriptional regulation, and act as precursors for siRNAs [25].

Epigenetic research uses a wide range of molecular biological techniques [21••]: histone modifications are generally analyzed by chromatin immunoprecipitation combined with microarray technology (ChIP-chip) or new sequencing technology (ChIP-seq). Alternatively, post-translational modifications induce a mass shift that can be detected using mass spectrometry with high-resolution analyzers. High-throughput techniques to analyze DNA methylation are mainly based on bisulfite conversion followed by arrays or sequencing. Analysis of the expression of ncRNAs on a genome-wide scale is performed by microarrays and RNA Seq techniques.

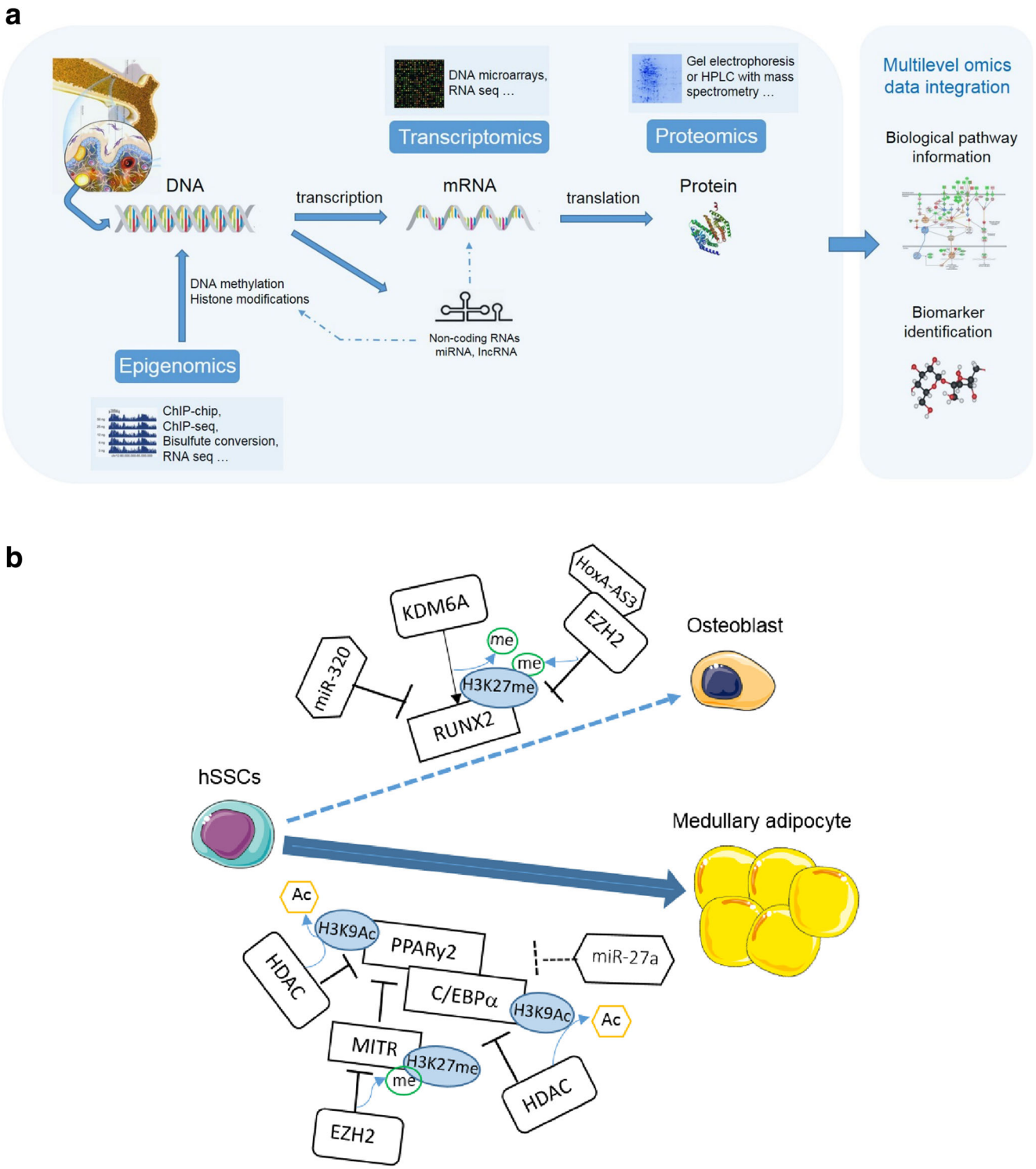


**Fig. 1** **a** Integration of different Omics data for the understanding of BMA differentiation. **b** Focus on some epigenetic mechanisms regulating SSCs differentiation into adipocytes versus osteoblasts. **a** Schematic representation of Omics approaches, and associated technologies, targeting different levels of cellular information. The experimental data can be integrated to support a better understanding of BMA differentiation and eventually the development of biomarkers. **b** The histone methyltransferase EZH2 facilitates adipogenesis (1) by trimethylation of histone H3 on lysine 27 at the promoter of MITR, resulting in suppression of its expression and reactivation of PPARγ2 expression and (2) by affecting, in association with the long non-coding RNA HoxA-AS3, the expression of the osteoblastic transcriptional factor RUNX2. Conversely, by removing this repressive mark, the lysine demethylase KDM6A inhibits adipogenesis and promotes osteogenesis. Other regulatory epigenetic factors are also indicated: Deacetylation at lysine H3K9 in the promoter region of PPARγ2 and C/EBPα by HDAC inhibits adipogenesis. Several members of the miR-320 family are potential regulator of adipogenesis by downregulating the transcription factor RUNX2. In contrast, miR-27 family negatively affects adipocyte differentiation but there are divergences concerning its targets

## Global Analysis of Transcriptome and Proteome during Adipogenesis

With the growing interest in the bone marrow adipose tissue, some large-scale studies were recently performed to analyze the global gene expression profiles during in vitro BMA differentiation.

Two studies using human SSCs from bone marrow aspirates compared transcriptomes of cells differentiated to the adipogenic lineage for 14 days and undifferentiated cells. Xu et al. [26] used a full genome gene expression microarray system for a comparative analysis performed on cells from one donor. Next-generation sequencing was employed by Casadio-Diaz et al. [27•] to investigate mRNA and miRNA expression patterns in cells from 3 donors. Despite these differences in experimental design, the consistent results across the two experiments showed that most of the differentially expressed genes identified have well-defined roles in adipogenesis. The primary functional Gene Ontology (GO) category for upregulated genes was fat cell differentiation. Downregulated genes were mostly associated with cell division and proliferation. Processes related to extracellular matrix, calcium metabolism, and differentiation in osteoblasts were also found to be repressed during differentiation [27•]. To induce the in vitro adipocyte differentiation, culture media are supplemented by different stimulating factors including dexamethasone, indomethacin, isobutylmethylxanthine, and sometimes insulin. These inducers can affect processes other than adipogenesis and questions can arise about the specificity of the changes they cause in gene expression. In order to identify only genes related to the adipogenesis process, Ullah et al. [28] used reverse differentiation (also called dedifferentiation) as a filtering tool to narrow down the list of specific adipogenic markers. The comparison of microarray data obtained for undifferentiated, differentiated, and dedifferentiated cells allowed the filtration of 782 genes out of the 991 initially identified, most of them also known to have relevance for adipogenesis like PPARγ, C/EBPα, adiponectin, lipoprotein lipase, and fatty acid-binding protein. While these studies focused on events at rather late stage of differentiation, van de Peppel et al. [29] investigated genes controlling the early stages of human SSCs lineage commitment. Using human whole-genome expression array, they generated gene expression patterns of bone marrow-derived cells during the first 4 days of osteogenic and adipogenic differentiation. Data analysis showed that osteogenic and adipogenic lineages diverged within 2–3 h upon induction of differentiation and that three distinct phases were discriminated in both lineages. The first phase (0–3 h) represents the initiation of the differentiation program, the second one (6–24 h) corresponds to the lineage acquisition, and the third one (48–96 h) to the lineage progression. Most of the genes activated during the first phase were associated with regulation of transcription. Upstream regulator analysis identified transcription factors (TF) specifically regulated in adipogenic lineage, and homeobox TF not yet known to be involved in adipocyte differentiation. Interestingly and in line with the previous studies, downregulation of most of the genes associated with the cell cycle was observed within the third phase, reflecting the classical inverse correlation between cell proliferation and differentiation.

While numerous studies have characterized the proteome of adipose-derived stem cells or whole adipose tissue, proteomic investigations of BMAs remain scarce. Using two-dimensional (2D) gel electrophoresis and mass spectrometry, Lee et al. [30] identified 8 cytosolic proteins upregulated during the adipogenic differentiation of SSCs. Among these, four proteins (Syntaxin 3, OSBP-related protein 3, PPARγ, and glycophorin) were associated with adipogenesis [30]. This moderate divergence of the proteomic content during adipogenic conversion was pointed out in a 2D gel electrophoresis analysis of whole and membrane proteome [31]. However, a greater degree of difference in the membrane protein constituents between the native and adipogenic differentiated cells was revealed using a more sensitive non-gel-based spectrometry analysis. The results showed the emergence of several enzymes for lipid metabolism and trafficking associated with a loss of membrane proteins with ectoenzymatic activities [31]. In a methodological paper, the third category of protein isoforms, i.e., the secreted proteins, were investigated by LC-MS/MS in the culture supernatants of human adipocytes derived from SSCs. The great variations found in the secretome content according to the methods for preparing conditioned medium underlined the importance of procedure optimization prior to any proteomic investigation and the cautiousness to interpret proteomic outcomes [32].

## Epigenetic Mechanisms Regulating Adipogenesis

Despite their relatively small number, miRNAs are estimated to regulate hundreds of targets corresponding to around 60% of the human transcriptome [33]. The increasing number of studies addressing the role of miRNAs provided significant amount of data related to the regulation of SSCs differentiation. However, a survey of the literature evidences the heterogeneity of the published data. These variations are in part due to differences in the studied models and used methodologies. Moreover, while BMAs are considered to have specific phenotypic characteristics, data are sometimes combined with those concerning cells from primary adipose tissue or even preadipocyte cell lines [34–36]. Therefore, when focusing only on studies related to BMAs and that are obtained using high-throughput techniques, data are scarce. Casado-Diaz et al. combined transcriptome and miRNome analysis for

SSCs from bone marrow aspirates at day 14 of adipogenic differentiation versus undifferentiated [27•]. They identified 46 miRNAs to be upregulated and 55 downregulated during the process of differentiation. Analysis of mRNA-miRNA interactions using the web server omiRAS (http://tools.genxpro.net/omiras) showed that PPARγ-related genes, in particular the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), were the most relevant induced genes while the key target in downregulated networks was transforming growth factor beta 1 (TGFB1). In a similar study, Tsai et al. [37] provided comprehensive miRNome profiles for SSCs differentiated into adipocytes or not, including 40 unreported miRNAs, 9 of which were overexpressed in differentiated cells. Using telomerized human MSC line (hMSC-TERT) as a model for primary SSCs, Hamam et al. identified several members of the miR-320 family as potential regulator of adipogenesis by downregulating especially the transcription factor Runx2 [38]. In addition to this in vitro study, You et al. used microarray analyses to investigate miRNA expression profiles in the serum of osteoporotic patients and healthy controls [39•]. They observed a reduction of miR-27a expression in osteoporotic patients. Subsequent analyses on SSCs derived from patients corroborated this finding with a decrease in osteoblastogenesis and an increase in adipogenesis. The myocyte-specific enhancer factor 2c gene (MEF2C), a transcriptional activator involved in the control of myogenesis was subsequently identified as a target gene. Interestingly, two previous studies reported the miR-27 family to negatively affect white adipocyte differentiation by targeting the transcription factors PPARγ and C/EBPα [40,41] while another identified the human Prohibitin (PHB) gene, essential in mitochondrial function and adipocyte differentiation, as a direct target of miR-27a and miR-27b [42]. These discrepancies emphasize that the essential process of miRNA target identification is a challenging task because one gene can be regulated by several miRNAs, one miRNA can regulate a large number of genes, and miRNA-mRNA relationships differ among tissues, cells, and conditions.

Growing evidence suggests that histone modifications, by affecting the activation or repression of key transcriptional factors, play a pivotal role in the regulation of SSC fate determination [43, 44]. Using an epigenetic library screen, Ali and his colleagues investigated the effects of chemical inhibitors of histone deacetylase on bone marrow adipocyte differentiation [45, 46]. Among eight compounds promoting adipocyte differentiation, they showed that abexinostat induced a marked increase in acetylation particularly at lysine H3K9 in the promoter region of adipocyte-associated transcription factors such as C/EBPα and PPARγ2, leading to an upregulation of their expression [45]. Several studies also highlight the importance of histone methylation, mainly through the action of the enhancer of zeste homolog 2 (EZH2), a histone methyltransferase that catalyzes the trimethylation of histone H3 on lysine 27 (H3K27me3), resulting in gene silencing. Using a ChIP-on-chip assay, Chen et al. identified differential EZH2 targets in osteogenesis and adipogenesis of SSCs on a genome-wide scale [47]. A non-catalytic HDAC protein, myocyte enhancer factor-2 interacting transcriptional repressor (MITR or HDAC9c), having the capacity to negatively regulate PPARγ2 transcriptional activity, is one of these targets. By binding to and methylate H3K27 at the MITR promoter region, EZH2 promotes adipogenic differentiation. Hemming and colleagues further revealed that modifications of H3K27 by both methylation and demethylation determine SSCs fate [48]. Indeed, they showed that the EZH2-dependent H3K27me3 repressive mark was removed by lysine demethylase 6A (KDM6A). Consequently, while EZH2 positively regulates adipogenesis, KDM6A promotes osteogenesis and inhibits adipogenesis, by affecting the same master regulatory genes. In addition, using EZH2 ChIP-PCR experiments, Zhu et al. showed that HoxA-AS3, a long non-coding RNA, interacts with EZH2 to modulate the status of H3K27me3 in the promoter region of the osteoblastic transcriptional factor Runx2 [49]. Altogether, these findings support the notion that EZH2, implying multiple epigenetic mechanisms, is a key regulator of bone marrow adipogenesis. More generally, epigenetic regulation controls the early stages of human SSC differentiation into one phenotype much of the time by blocking the alternative fate (Fig. 1b).

## Conclusions

SSCs commitment to osteoblastogenesis or adipogenesis is a complex and sequential process that is regulated by a large number of bioactive molecules. Although being actively studied, advances in understanding the fate decision of SSCs and particularly the intracellular mechanisms controlling BMA differentiation are still preliminary. Overall, even if data are still scarce, studies highlighted in this review show that the use of Omics technologies will likely contribute to draw a more complete picture of this process and demonstrate the necessity to increase the number of such investigations. In addition, more studies aiming to identify how epigenetic elements are coordinated to achieve lineage commitment are necessary. To fully explore their potential, screening efforts must be accompanied by compilation and integration of data from different Omics fields. In other words, we are far from understanding the role of each factor alone, and one of the challenges is to integrate transcriptomics, proteomics, and epigenomics information to elucidate the spatiotemporal molecular network controlling the process of adipogenesis. This requires powerful bioinformatics tools for target prediction or database development as well as mathematical modeling to connect the vast amount of data generated. One critical step in tackling this goal is the standardization of data, in its generation, taking into

account sample source and processing techniques, and in its informatics integration. Although the challenge is considerable, the constant development of experimental tools offers a much more ambitious objective with the recent emergence of single-cell Omics and the perspective to overcome the limitation of cellular heterogeneity, particularly important for stem cells [50, 51].

Further expected outcome of the use of Omics data is the potential identification of biomarkers or therapeutic targets in pathological contexts. It is interesting to note that perturbations in circulating miRNA levels in serum of osteoporosis patients have been shown to be directly linked to in vitro-altered adipocyte/osteoblast balance [39•]. This finding together with the fact that miRNAs are stable in body fluids make them potential tools to specifically target bone marrow adipocytes in bone loss and bone-related diseases.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflicts of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance


1. Hardouin P, Marie PJ, Rosen CJ. New insights into bone marrow adipocytes: report from the first European meeting on bone marrow adiposity (BMA 2015). Bone. 2016;93:212–5. https://doi.org/10.1016/j.bone.2015.11.013.
2. van der Eerden B, van Wijnen A. Meeting report of the 2016 bone marrow adiposity meeting. Adipocyte. 2017;6(4):1–10. https://doi.org/10.1080/21623945.2017.1313374.
3. Veldhuis-Vlug AG, Rosen CJ. Mechanisms of marrow adiposity and its implications for skeletal health. Metabolism. 2017;67:106–14. https://doi.org/10.1016/j.metabol.2016.11.013.
4. Hardouin P, Pansini V, Cortet B. Bone marrow fat. Joint Bone Spine. 2014;81(4):313–9. https://doi.org/10.1016/j.jbspin.2014.02.013.
5. Bianco P, Robey PG. Skeletal stem cells. Development. 2015;142(6):1023–7. https://doi.org/10.1242/dev.102210.
6. Chen Q, Shou P, Zheng C, Jiang M, Cao G, Yang Q, et al. Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ. 2016;7(7):1128–39. https://doi.org/10.1038/cdd.2015.168.
7. Gimble JM, Zvonic S, Floyd ZE, Kassem M, Nuttall ME. Playing with bone and fat. J Cell Biochem. 2006;98(2):251–66. https://doi.org/10.1002/jcb.20777.
8. Abdallah BM, Kassem M. New factors controlling the balance between osteoblastogenesis and adipogenesis. Bone. 2012;50(2):540–5. https://doi.org/10.1016/j.bone.2011.06.030.
9. Muruganandan S, Sinal CJ. The impact of bone marrow adipocytes on osteoblast and osteoclast differentiation. IUBMB Life. 2014;66(3):147–55. https://doi.org/10.1002/iub.;1254.
10. Sadie-Van Gijsen H, Hough FS, Ferris WF. Determinants of bone marrow adiposity: the modulation of peroxisome proliferator-activated receptor- γ2 activity as a central mechanism. Bone. 2013;56(2):255–65. https://doi.org/10.1016/j.bone.2013.06.016.
11. •• Tencerova M, Kassem M. The bone marrow-derived stromal cells: commitment and regulation of adipogenesis. Front Endocrinol (Lausanne). 2016;7:127. https://doi.org/10.3389/fendo.2016.00127. **This review presents a summary of the information regarding the bone marrow adipocyte characterization and provides a comprehensive overview of mechanisms of SSCs lineage commitment to adipocyte lineage.**
12. Teven CM, Liu X, Hu N, Tang N, Kim SH, Huang E, et al. Epigenetic regulation of mesenchymal stem cells: a focus on osteogenic and adipogenic differentiation. Stem Cells Int. 2011;2011:201371–18. https://doi.org/10.4061/2011/201371.
13. Berendsen AD, Olsen BR. Osteoblast-adipocyte lineage plasticity in tissue development, maintenance and pathology. Cell Mol Life Sci. 2014;71(3):493–7. https://doi.org/10.1007/s00018-013-1440-z.
14. Perez-Campo FM, Riancho JA. Epigenetic mechanisms regulating mesenchymal stem cell differentiation. Curr Genomics. 2015;16(6):368–83. https://doi.org/10.2174/1389202916666150817202559.
15. Tye CE, Gordon JA, Martin-Buley LA, Stein JL, Lian JB, Stein GS. Could lncRNAs be the missing links in control of mesenchymal stem cell differentiation? J Cell Physiol. 2015;230(3):526–34. https://doi.org/10.1002/jcp.24834.
16. Yoshioka H, Yoshiko Y. The roles of long non-protein-coding RNAs in osteo-adipogenic lineage commitment. Int J Mol Sci. 2017;18(6):1236. https://doi.org/10.3390/ijms18061236.
17. Debnath Mousumi, Prasad GBKS, Bisen PS. Omics technology. In: Molecular Diagnostics: Promises and Possibilities. Dordrech Heidelberg London: Springer, 2010 p. 11–31. Doi: https://doi.org/10.1007/978-90-481-3261-4.
18. •• Manzoni C, Kia DA, Vandrovcova J, Hardy J, Wood NW, Lewis PA, et al. Genome, transcriptome and proteome: the rise of omics data and their integration in biomedical sciences. Brief Bioinform 2016:1–17. doi: https://doi.org/10.1093/bib/bbw114. **This study provides an overview of the current strengths and weaknesses of Omics technologies.**
19. Wang Z, Gerstein M, Snyder M. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 2009;10(1):57–63. https://doi.org/10.1038/nrg2484.
20. Graves PR, Haystead TAJ. Molecular biologist's guide to proteomic. Microbiol Mol Biol Rev. 2002;66(1):39–63. https://doi.org/10.1128/MMBR.66.1.39-63.2002.
21. •• Han Y, He X. Integrating epigenomics into the understanding of biomedical insight. Bioinform Biol Insights. 2016;10:267–89. https://doi.org/10.4137/BBI.S38427. **This paper provides a comprehensive review of epigenetic mechanisms, epigenomics data generation, and integrative analysis approaches.**
22. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693–705. https://doi.org/10.1016/j.cell.2007.02.005.
23. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology. 2013;38(1):23–38. https://doi.org/10.1038/npp.2012.112.
24. Sato F, Tsuchiya S, Meltzer SJ, Shimizu K. MicroRNAs and epigenetics. FEBS J. 2011;278(10):1598–609. https://doi.org/10.1111/j.1742-4658.2011.08089.
25. Cao J. The functional role of long non-coding RNAs and epigenetics. Biological Procedures Online. 2014;16(1):11. https://doi.org/10.1186/1480-9222-16-11.
26. Xu X, Li X, Yan R, Jiang H, Wang T, Fan L, et al. Gene expression profiling of human bone marrow-derived mesenchymal stem cells

during adipogenesis. Folia Histochem Cytobiol. 2016;54(1):14–24. https://doi.org/10.5603/FHC.a2016.0003.

27.• Casado-Diaz A, Anter J, Müller S, Winter P, Quesada-Gomez JM, Dorado G. Transcriptomic analyses of adipocyte differentiation from human mesenchymal stromal-cells (MSC). J Cell Physiol. 2016;9999(4):1–14. https://doi.org/10.1002/jcp.25472. **This study describes a combine transcriptome and miRNAOme investigation of adipocyte differentiation from SSCs, associated with networks analysis.**

28. Ullah M, Stich S, Häupl T, Eucker J, Sittinger M, Ringe J. Reverse differentiation as a gene filtering tool in genome expression profiling of adipogenesis for fat marker gene selection and their analysis. PLoS One. 2013;8(7):e69754. https://doi.org/10.1371/journal.pone.0069754.

29. van de Peppel J, Strini T, Tilburg J, Westerhoff H, van Wijnen AJ, van Leeuwen JP. Identification of three early phases of cell-fate determination during osteogenic and adipogenic differentiation by transcription factor dynamics. Stem Cell Reports. 2017;8(4):947–60. https://doi.org/10.1016/j.stemcr.2017.02.018.

30. Lee HK, Lee BH, Park SA, Kim CW. The proteomic analysis of an adipocyte differentiated from human mesenchymal stem cells using two-dimensional gel electrophoresis. Proteomics. 2006;6(4):1223–9. https://doi.org/10.1002/pmic.200500385.

31. Jeong JA, Ko KM, Park HS, Lee J, Jang C, Jeon CJ, et al. Membrane proteomic analysis of human mesenchymal stromal cells during adipogenesis. Proteomics. 2007;7(22):4181–91. https://doi.org/10.1002/pmic.200700502.

32. Clabaut A, Grare C, Leger T, Hardouin P, Broux O. Variations of secretome profiles according to conditioned medium preparation : the example of human mesenchymal stem cell-derived adipocytes. Electrophoresis. 2015;36(20):2587–93. https://doi.org/10.1002/elps.201500086.

33. Friedman RC, Farh KKH, Burge CB, Bartel DP. Mammalian mRNAs are conserved targets of MicroRNAs. Genome Res. 2009;19(1):92–105. https://doi.org/10.1101/gr.082701.108.

34. Kang H, HATA A. The role of microRNAs in cell fate determination of mesenchymal stem cells: balancing adipogenesis and osteogenesis. BMB Rep. 2015;48(6):319–23. https://doi.org/10.5483/BMBRep.2015.48.6.206.

35. Hamam D, Ali D, Kassem M, Aldahmash A, Alajez NM. microRNAs as regulators of adipogenic differentiation of mesenchymal stem cells. Stem Cells Dev. 2015;24(4):417–25. https://doi.org/10.1089/scd.2014.0331.

36. Saidi N, Ghalavand M, Hashemzadeh MS, Dorostkar R, Mohammadi H, Mahdian-Shakib A. Dynamic changes of epigenetic signatures during chondrogenic and adipogenic differentiation of mesenchymal stem cells. Biomed Pharmacother. 2017;89:719–31. https://doi.org/10.1016/j.biopha.2017.02.093.

37. Tsai CH, Liao KH, Shih CC, Chan CH, Hsieh JY, Tsai CF, et al. Small RNA and RNA-IP sequencing identifies and validates novel MicroRNAs in human mesenchymal stem cells. OMICS. 2016;20(3):191–8. https://doi.org/10.1089/omi.2015.0136.

38. Hamam D, Ali D, Vishnubalaji R, Hamam R, Al-Nbaheen M, Chen L, et al. MicroRNA-320/RUNX2 axis regulates adipocytic differentiation of human mesenchymal (skeletal) stem cells. Cell death and Dis. 2014;5(10):e1499. https://doi.org/10.1038/cddis.2014.462.

39.• You L, Pan L, Chen L, Gu W, Chen J. MiR-27a is essential for the shift from osteogenic differentiation to adipogenic differentiation of mesenchymal stem cells in postmenopausal osteoporosis. Cell Physiol Biochem. 2016;39(1):253–65. https://doi.org/10.1159/000445621. **The only study showing a link between perturbations in circulating miRNA levels in serum of osteoporosis patients and in vitro altered adipocyte/osteoblast balance.**

40. Lin Q, Gao Z, Alarcon RM, Ye J, Yun Z. A role of miR-27 in the regulation of adipogenesis. FEBS J. 2009;276(8):2348–58. https://doi.org/10.1111/j.1742-4658.2009.06967.x.

41. Karbiener M, Fischer C, Nowitsch S, Opriessnig P, Papk C, Ailhaud G, et al. MicroRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma. Biochem Biophys Res Commun. 2009;390(2):247–51. https://doi.org/10.1016/j.bbrc.2009.09.098.

42. Kang T, Lu W, Xu W, Anderson L, Bacanamwo M, Thompson W, et al. MicroRNA-27 (miR-27) targets Prohibitin and impairs adipocyte differentiation and mitochondrial function in human adipose-derived stem cells. J Biol Chem. 2013;288(48):34394–402. https://doi.org/10.1074/jbc.M113.514372.

43. Deng P, Chen QM, Hong C, Wang CY. Histone methyltransferases and demethylases: regulators in balancing osteogenic and adipogenic differentiation of mesenchymal stem cells. Int J Oral Sci. 2015;7(4):197–204. https://doi.org/10.1038/ijos.2015.41.

44. Huang B, Li G, Jiang XH. Fate determination in mesenchymal stem cells: a perspective from histone-modifying enzymes. Stem Cell Res Ther. 2015;6(1):35. https://doi.org/10.1186/s13287-015-0018-0.

45. Ali D, Hamam R, Halfayez M, Kassem M, Aldahmash A, Alajez NM. Epigenetic library screen identifies Abexinostat as novel regulator of adipocytic and osteoblastic differentiation of human skeletal (mesenchymal) stem cells. Stem Cells Transl Med. 2016;5(8):1036–47. https://doi.org/10.5966/sctm.2015.0331.

46. Ali D, Alshammari H, Vishnubalaji R, Chalisserry EP, Hamam R, Alfayez M, et al. CUCDC-907 promotes bone marrow adipocytic differentiation through inhibition of histone deacetylase and regulation of cell cycle. Stem Cells Dev. 2017;26(5):353–62. https://doi.org/10.1089/scd.2016.0183.

47. Chen YH, Yeh FL, Yeh SP, Ma HT, Hung SC, Hung MC, 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. J Biol Chem. 2011;286(12):10671–80. https://doi.org/10.1074/jbc.M110.199612.

48. Hemming S, Cakouros D, Isenmann S, Cooper L, Menicanin D, Zannettino A, et al. EZH2 and KDM6A act as an epigenetic switch to regulate mesenchymal stem cell lineage specification. Stem Cells. 2014;32(3):802–15. https://doi.org/10.1002/stem.1573.

49. Zhu XX, Yan YW, Chen D, Ai CZ, Lu X, Xu SS, et al. Long non-coding RNA HoxA-AS3 interacts with EZH2 to regulate lineage commitment of mesenchymal stem cells. Oncotarget. 2016;7(39):63561–70. https://doi.org/10.18632/oncotarget.11538.

50. Bian Q, Cahan P. Computational tools for stem cell biology. Trends Biotechnol. 2016;34(12):993–1009. https://doi.org/10.1016/j.tibtech.2016.05.010.

51. Kelsey G, Stegle O, Reik W. Single-cell epigenomics: recording the past and predicting the future. Science. 2017;358(6359):69–75. https://doi.org/10.1126/science.aan6826.