Cover Letter

Dear Editors and Reviewers,

Thank you for giving us the opportunity to revise our article entitled " **Epigenetic regulatory mechanisms of long noncoding RNAs in the osteo-/adipogenic differentiation of mesenchymal stem cells and the pathogenesis of degenerative bone diseases** " (Manuscript No.: 64620). We have highly regarded the insightful comments and suggestions, carefully responded to these suggestions point-by-point in this cover letter (see following "**responses**" part), and revised the manuscript accordingly. All changes made to the text are highlighted in red color so that they can be identified with ease.

Please contact me if you have any questions. We look forward to hearing from you. Thank you for your time.

Yours sincerely,

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Responses

Reviewer 1

Comment 1: *All latin words must be written in italic (via, in vivo, etc) - Them font should be consistent over the manuscript (e.g "As a critical transcription factor for adipogenesis, C/EBP-α was found to be upregulated via the recruitment of the MLL3/4 complex to its promoter, which is guided by the binding of PA1 (a component of the MLL3/4 complex).*

[Author response]

Thank the reviewer for this precious suggestion. We have italicized all Latin words and unified the font over the manuscript.

1. We have **revised** "via" **into** "*via*" **(See DNA METHYLATION Section, Page 5, Line 8)**

2. We have **revised** "via" **into** "*via*" **(See DNA METHYLATION Section, Page 5, Line 8)**

3. We have **changed the font** of "*PPAR-γ*" **into** Book Antiqua **(See DNA METHYLATION Section, Page 6, Line 15)**

4. We have **changed the font** of "*C/EBP-α*" **into** Book Antiqua **(See DNA METHYLATION Section, Page 6, Line 16)**

5. We have **changed the font** of "*Ppar-γ2*" **into** Book Antiqua **(See DNA METHYLATION Section, Page 6, Line 23)**

6. We have **revised** "via" **into** "*via*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 4)**

7. We have **revised** "via" **into** "*via*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 15)**

8. We have **changed the font** of "*C/EBP-α*" **into** Book Antiqua **(See DNA METHYLATION Section, Page 8, Line 18)**

9. We have **revised** "via" **into** "*via*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 19)**

10. We have **revised** "et al." **into** "*et al.*" **(See Table 1, Page 28)**

11. We have **revised** "et al." **into** "*et al.*" **(See Table 2, Page 29)**

12. We have **revised** "et al." **into** "*et al.*" **(See Table 3, Page 30)**

Comment 2: *Methylation is important to control gene expression, but the source of methyl donor is more important because deficiency of methyl donor or the alteration expression of enzyme involved in the methyl metabolism (GNMT, BHMT,...). The authors should explain methyl donors but also if lncRNA are involved in altering the expression of enzyme involved in methyl metabolism.*

[Author response]

We thank for the comment!

1. We have **added** "*Another potential involvement of lncRNA in epigenetics is the interaction with the key enzyme of methyl metabolism. It is known that DNMT and HMT utilize S-adenosylmethionine (SAM) as a major methyl-group donor in mammals, which is consumed and regenerated in one-carbon metabolism*[90,91]*. Several studies have shown that lncRNAs play a role in SAM-dependent methylation through regulating enzymes related to the metabolism*[92,93]*. However, similar studies on differentiation and bone diseases are lacking. Further studies are needed to assess the potential importance of lncRNAs on the methyl metabolism.*" **(See CONCLUSIONS AND PERSPECTIVES Section, Page 11, Line 26 - Page 12, Line 6)**

2. We have **added** "*90 **Johnson C**, Warmoes MO, Shen X, Locasale JW. Epigenetics and cancer metabolism. Cancer Lett. 2015; **356**: 309-314. [PMID: 24125862 DOI: 10.1016/j.canlet.2013.09.043]*" **(See REFERENCES Section, Page 25, Line 8-10)**

3. We have **added** "*91 **Mentch SJ**, Locasale JW. One-carbon metabolism and epigenetics: understanding the specificity. Ann N Y Acad Sci. 2016; **1363**: 91-98. [PMID: 26647078 DOI: 10.1111/nyas.12956]*" **(See REFERENCES Section, Page 25, Line 11-13)**

4. We have **added** "*92 **Guo T**, Gong C, Wu P, Battaglia-Hsu SF, Feng J, Liu PP, Wang HT, Guo DL, Yao Y, Chen BY, Xiao YS, Liu ZS, Li Z. LINC00662 promotes hepatocellular carcinoma progression via altering genomic methylation profiles. Cell*

Death & Differentiation. 2020; ***27****: 2191-2205. [PMID: 31959915 DOI: 10.1038/s41418-020-0494-3]*" **(See REFERENCES Section, Page 25, Line 14-18)**

5. We have **added** "*93* ***Zhou JC****, Yang LH, Zhong TY, Mueller M, Men Y, Zhang N, Xie JK, Giang K, Chung H, Sun XG, Lu LG, Carmichael GG, Taylor HS, Huang YQ. H19 lncRNA alters DNA methylation genome wide by regulating S-adenosylhomocysteine hydrolase. Nat Commun. 2015;* ***6****: 10221. [PMID: 26687445 DOI: 10.1038/ncomms10221]*" **(See REFERENCES Section, Page 25, Line 19-23)**

Comment 3: *It is true that it is generally accepted that methylation of DNA is associated with gene repression. However, it is not always the case. for example, imprinting of the H19 locus is related with the expression and the non expression of H19 and IGF2 (H19 is mentioned few times over the manuscript). it will be interesting that the authors wrote few sentences about the increase of gene expression because of DNA methylation (H19 loci, PMID: 31914996...). DNA methylation is also involved in the chromatin structure in the nucleus, that regulates genes expression by controlling the location of the genes in the nucleus.*

[Author response]

Thank the reviewer for this professional suggestion.

1. We have **added** "*Nevertheless, it is worth mentioning that DNA methylation is also associated with upregulated gene expression under certain circumstance* [29]." **(See DNA METHYLATION Section, Page 4, Line 17-19)**

2. We have **added** "*29* ***Rauluseviciute I****, Drabløs F, Rye MB. DNA hypermethylation associated with upregulated gene expression in prostate cancer demonstrates the diversity of epigenetic regulation. BMC Med Genomics. 2020;* ***13****: 6. [PMID: 31914996 DOI: 10.1186/s12920-020-0657-6]*" **(See REFERENCES Section, Page 16, Line 25-28)**

Editorial office

Comment 1: *In the main text, only the role of long non coding RNAs in MSCs differentiation process was in detail descibed. Only a small part (3.3 Involvement of*

lncRNAs in osteoarthritis through histone modifications) described the potential role of these RNAs in osteoarthritis. This section should be a seperate section from the 3. Histone Modifications, and should be stated as a new section (e.g. 4. Role of lnRNAs in bone degenerative diseases.). In this way, degenerative bone diseases such as osteoarthritis, spinal ostearthritis and cartilage damage and their potential relation with the long non coding RNAs should be presented.

[Author response]

We really appreciated this comment!

1. We have **added** "

4. ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES

More recently, epigenetic regulation of bone homeostasis has been considered as an important factor in the pathogenesis of degenerative bone diseases, such as osteoporosis, arthritis, post menopausal osteoporosis, etc.[69,70]. As mentioned above, lncRNAs have attracted considerable attention in the epigenetic regulation of bone homeostasis. The potential link between degenerative bone diseases and lncRNAs at the epigenetic level is also an intriguing area for exploration." **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 9, Line 12-19)**

2. We have **added** "*69 **Huang T**, Peng X, Li ZX, Zhou Q, Huang SS, Wang YT, Li J, Song YQ. Epigenetics and bone diseases. Genet Res (Camb). 2018; **100**: e6. [PMID: 30047344 DOI: 10.1017/s0016672318000034]*" **(See REFERENCES Section, Page 22, Line 14-16)**

3. We have **added** "*70 **Yang SQ**, Duan XH. Epigenetics, Bone Remodeling and Osteoporosis. Curr Stem Cell Res Ther. 2016; **13**: 101-109. [PMID: 28002993 DOI: 10.2174/1574888X11666161221125656]*" **(See REFERENCES Section, Page 22, Line 17-19)**

4. We have **added** "

4.1 LncRNAs regulate DNA methylation in osteoarthritis and osteoporosis

Osteoarthritis (OA) is a common degenerative joint disease that is associated with the impairment of cartilage regeneration, chondrocyte apoptosis, and the degradation of the cartilage extracellular matrix (ECM)[71,72]. In this sophisticated balance between

biosynthesis and degradation, lncRNAs play a role in the survival of chondrocytes and the regulation of arthritis-associated factors[73].

It has been reported that the overexpression of lncRNA CTBP1-AS2 downregulates miR-130a by increasing the methylation level of the miR-130a gene, which finally leads to a decreased proliferation rate of chondrocytes in OA patients[74].

As a natural inhibitor of matrix metalloproteinases (MMPs), TIMP-3 deficiency can lead to mild cartilage degeneration in patients with OA[75]. lncRNA XIST is capable of downregulating the expression of TIMP-3 through the recruitment of DNMT1, DNMT3A, and DNMT3B, which increased the methylation ratio of the CpG island in the TIMP-3 promoter region, and consequently increased collagen degradation in OA chondrocytes[76].

Increasing evidence suggests that small nucleolar RNA host gene (SNHG) family members are involved in the pathogenesis of OA[77-79]. The overexpression of lncRNA SNHG15 alleviated ECM degradation and promoted chondrocyte formation via competing endogenous RNA (ceRNA) SNHG15/miR-7/KLF4 axis[33]. In human OA cartilage tissues, however, the promoter region of lncRNA SNHG15 had a higher level of methylation than in normal cartilage tissues, and this might be a promising therapeutic target for OA[33]. Another SNHG family member, lncRNA SNHG9, was found to be downregulated in chondrocytes from OA patients[80]. Functional studies indicated that the overexpression of SNHG9 led to a decreased apoptotic rate through increased methylation of the miR-34a gene that suppressed the expression of miR-34a[80].

Osteoporosis is characterized by a loss of bone mass and microarchitectural deterioration of the skeletal structure[81]. The imbalance of bone homeostasis between osteoblastic bone formation and osteoclastic bone resorption plays a fundamental role in the pathogenesis of osteoporosis[82]. Emerging evidence suggests that epigenetic modifications are deeply involved in bone metabolism, which contributes to the development of osteoporosis.

The ERK-MAPK signaling pathway is a well-established pathway with critical roles in immune responses and embryonic development, including the regulation of bone mass

via controlling osteoblast differentiation[83]. A previous study suggested that lncRNA H19 promoted tension-induced osteogenesis of hBMSCs through the FAK-ERK1/2-RUNX2 signaling pathway[84]. Likewise, an alteration in H19 methylation may also be involved in the disruption of bone formation in disuse osteoporosis. It has been shown that DNMT1-induced hypermethylation of the H19 promoter results in H19 downregulation and ERK-MAPK signaling inhibition, which leads to osteogenesis impairment both in vivo and in vitro (rat osteoblast/osteocyte-like UMR-106 cells)[85]."

(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 9, Line 20 - Page 11, Line 6)

5. We have **deleted** "

2.3 LncRNAs regulate DNA methylation in osteoarthritis and osteoporosis

Osteoarthritis (OA) is a common degenerative joint disease that is associated with the impairment of cartilage regeneration, chondrocyte apoptosis, and the degradation of the cartilage extracellular matrix (ECM)[49,50]. In this sophisticated balance between biosynthesis and degradation, lncRNAs play a role in the survival of chondrocytes and the regulation of arthritis-associated factors[51].

It has been reported that the overexpression of lncRNA CTBP1-AS2 downregulates miR-130a by increasing the methylation level of the miR-130a gene, which finally leads to a decreased proliferation rate of chondrocytes in OA patients[52].

As a natural inhibitor of matrix metalloproteinases (MMPs), TIMP-3 deficiency can lead to mild cartilage degeneration in patients with OA[53]. lncRNA XIST is capable of downregulating the expression of TIMP-3 through the recruitment of DNMT1, DNMT3A, and DNMT3B, which increased the methylation ratio of the CpG island in the TIMP-3 promoter region, and consequently increased collagen degradation in OA chondrocytes[54].

Increasing evidence suggests that small nucleolar RNA host gene (SNHG) family members are involved in the pathogenesis of OA[55-57]. The overexpression of lncRNA SNHG15 alleviated ECM degradation and promoted chondrocyte formation via competing endogenous RNA (ceRNA) SNHG15/miR-7/KLF4 axis[30]. In human OA cartilage tissues, however, the promoter region of lncRNA SNHG15 had a higher level

of methylation than in normal cartilage tissues, and this might be a promising therapeutic target for OA[30]. Another SNHG family member, lncRNA SNHG9, was found to be downregulated in chondrocytes from OA patients[58]. Functional studies indicated that the overexpression of SNHG9 led to a decreased apoptotic rate through increased methylation of the miR-34a gene that suppressed the expression of miR-34a[58].

Osteoporosis is characterized by a loss of bone mass and microarchitectural deterioration of the skeletal structure[59]. The imbalance of bone homeostasis between osteoblastic bone formation and osteoclastic bone resorption plays a fundamental role in the pathogenesis of osteoporosis[60]. Emerging evidence suggests that epigenetic modifications are deeply involved in bone metabolism, which contributes to the development of osteoporosis.

The ERK-MAPK signaling pathway is a well-established pathway with critical roles in immune responses and embryonic development, including the regulation of bone mass via controlling osteoblast differentiation[61]. A previous study suggested that lncRNA H19 promoted tension-induced osteogenesis of hBMSCs through the FAK-ERK1/2-RUNX2 signaling pathway[62]. Likewise, an alteration in H19 methylation may also be involved in the disruption of bone formation in disuse osteoporosis. It has been shown that DNMT1-induced hypermethylation of the H19 promoter results in H19 downregulation and ERK-MAPK signaling inhibition, which leads to osteogenesis impairment both in vivo and in vitro (rat osteoblast/osteocyte-like UMR-106 cells)[63]."

6. We have **added** "

4.2 LncRNAs regulate histone modifications in osteoarthritis

An abnormality of cartilage regeneration can be related to attenuated chondrogenic differentiation of MSCs in OA patients[8]. Similar to other MSCs derived from other tissues, synovium-derived mesenchymal stromal cells (SMSCs) are multipotent but have the greatest chondrogenesis potential, representing a promising stem cell source for cartilage repair in OA patients[86]. LncRNA MEG3 was reported to have the ability to inhibit the chondrogenic differentiation of SMSCs and the expression of cartilage-associated genes (aggrecan and Col2A1) by inhibiting TRIB2 expression through

EZH2-mediated H3K27me3[87]." **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 11, Line 7-16)**

7. We have **deleted** "

3.3 Involvement of lncRNAs in osteoarthritis through histone modifications

An abnormality of cartilage regeneration can be related to attenuated chondrogenic differentiation of MSCs in OA patients[6]*. Similar to other MSCs derived from other tissues, synovium-derived mesenchymal stem cells (SMSCs) are multipotent but have the greatest chondrogenesis potential, representing a promising stem cell source for cartilage repair in OA patients*[81]*. LncRNA MEG3 was reported to have the ability to inhibit the chondrogenic differentiation of SMSCs and the expression of cartilage-associated genes (aggrecan and Col2A1) by inhibiting TRIB2 expression through EZH2-mediated H3K27me3*[82]."

Comment 2: *In addition, and based to the criteria of MSCs as has been outlined by the ISCT (Mesenchymal stem versus stromal cells: International Society for Cellular Therapy Mesenchymal Stromal Cell committee position statement on nomenclature. (Cytotherapy. 2019 Sept 13; DOI: https://doi.org/10.1016/j.jcyt.2019.08.002 and Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement (Cytotherapy 2006, 8(4): 315-317) the acronym of MSCs refers to Mesenchymal Stromal Cells and not to Mesenchymal Stem Cells. This change must be performed in the title and also in the whole manuscript. The properties of MSCs should be clearly presented in the introduction section of the manuscript, as described in the above references.*

[Author response]

We thank for this precious comment! We have added the properties of MSCs in the introduction section. According to the criteria proposed by the ISCT, we have revised the non-standard use of "mesenchymal stem cells" in the included articles into "mesenchymal stromal cells", as the authors did not use the recommended methods to identify the MSCs being isolated from the tissue.

1. We have **added** "*Mesenchymal stromal cells (MSCs) refer to a heterogeneous unfractionated population of cells, which include fibroblasts, myofibroblasts, and progenitor cells*[2,3]" **(See INTRODUCTION Section, Page 3, Line 5-7)**

2. We have **revised** "*During this process, mesenchymal stem cells (MSCs) differentiate into chondrocytes or osteoblasts...*" **into** "*MSCs are able to differentiate into chondrocytes or osteoblasts...*" **(See INTRODUCTION Section, Page 3, Line 7-9)**

3. We have **added** "*2* ***Viswanathan S****, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, Nolta J, Phinney DG, Sensebe L. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. Cytotherapy. 2019;* ***21****: 1019-1024. [PMID: 31526643 DOI: 10.1016/j.jcyt.2019.08.002]*" **(See REFERENCES Section, Page 13, Line 9-13)**

4. We have **added** "*3* ***Dominici M****, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;* ***8****: 315-317. [PMID: 16923606 DOI: 10.1080/14653240600855905]*" **(See REFERENCES Section, Page 13, Line 15-19)**

5. We have **revised** "*mesenchymal stem cells (MSCs)*" **into** "*mesenchymal stromal cells (MSCs)*" **(See ABSTRACT Section, Page 2, Line 4)**

6. We have **revised** "*human dental pulp stem cells*" **into** "*human dental pulp stromal cells*" **(See DNA METHYLATION Section, Page 5, Line 7)**

7. We have **revised** "*human dental follicle stem cells*" **into** "*human dental follicle stromal cells*" **(See DNA METHYLATION Section, Page 6, Line 6)**

8. We have **revised** "*BMSCs*" **into** "*bone marrow stromal cells*" **(See DNA METHYLATION Section, Page 6, Line 22)**

9. We have **revised** "*human periodontal ligament stem cells*" **into** "*human periodontal ligament stromal cells*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 12)**

10. We have **revised** "human adipose-derived stem cells" **into** "*human adipose-derived stromal cells*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 22)**

11. We have **revised** "synovium-derived mesenchymal stem cells" **into** "*synovium-derived mesenchymal stromal cells*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 11, Line 10)**

12. We have **revised** "*human dental pulp stem cells*" **into** "*human dental pulp stromal cells*" **(See Table 1, Page 28)**

13. We have **revised** "*human dental follicle stem cells*" **into** "*human dental follicle stromal cells*" **(See Table 1, Page 28)**

14. We have **revised** "*human periodontal ligament stem cells*" **into** "*human periodontal ligament stromal cells*" **(See Table 1, Page 28)**

15. We have **revised** "human adipose-derived stem cells" **into** "*human adipose-derived stromal cells*" **(See Table 2, Page 29)**

16. We have **revised** "synovium-derived mesenchymal stem cells" **into** "*synovium-derived mesenchymal stromal cells*" **(See Table 3, Page 30)**

Comment 3: *Also, the title may be need some adjustments e.g. Epigenetic regulatory mechanisms of long noncoding RNAs in the osteo-/adipogenic differentiation of mesenchymal stem cells. A potential link to the pathogenesis in degenerative bone diseases.*

[Author response]

We thank for the comment! However, according to the guidelines, the title should be no more than 18 words. In order to meet this comment, we have added the following sentence in the main text.

1. We have **added** "*As mentioned above, lncRNAs have attracted considerable attention in the epigenetic regulation of bone homeostasis. The potential link between degenerative bone diseases and lncRNAs at the epigenetic level is also an intriguing area for exploration.*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 9, Line 15-19)**

2. We have **revised** "*Epigenetic regulatory mechanisms of long noncoding RNAs in the osteo-/adipogenic differentiation of mesenchymal stem cells and the pathogenesis of degenerative bone diseases*" **into** "*Epigenetic regulation by lncRNAs in osteo-*

/adipogenic differentiation of mesenchymal stromal cells and the pathogenesis of degenerative bone diseases" **(See TITLE PAGE, Title, Page 1)**

Comment 4: *Acadrmic Norms and rules: The authors should place the date and sign the Copyright License Agreement.*

[Author response]

We thank for the comment! All the authors have signed the Copyright License Agreement.

Comment 5: *Issues raised: The authors should check the typesetting of the references. Please use the recommended font as described in the guidelines of the World Journal of Stem Cells.*

[Author response]

We thank for the comment! We have changed the format of the references into "Book Antiqua font and 1.5 line spacing with ample margins" according to the guidelines of the World Journal of Stem Cells.

And more

1. We have **revised** "***Chen Y**, Guo H, Li L, Bao D, Gao F, Li Q, Huang Q, Duan X, Xiang Z. Long Non-Coding RNA (lncRNA) Small Nucleolar RNA Host Gene 15 (SNHG15) Alleviates Osteoarthritis Progression by Regulation of Extracellular Matrix Homeostasis. Med Sci Monit. 2020; **26**: e923868. [PMID: 32643707 DOI: 10.12659/msm.923868]*" **into** "***Chen YP**, Guo HN, Li L, Bao DS, Gao F, Li Q, Huang Q, Duan X, Xiang Z. Long Non-Coding RNA (lncRNA) Small Nucleolar RNA Host Gene 15 (SNHG15) Alleviates Osteoarthritis Progression by Regulation of Extracellular Matrix Homeostasis. Med Sci Monit. 2020; **26**: e923868. [PMID: 32643707 DOI: 10.12659/msm.923868]*" **(See REFERENCES Section, Page 17, Line 10-14)**

2. We have **revised** "***Chen H**, Yang S, Shao R. Long non-coding XIST raises methylation of TIMP-3 promoter to regulate collagen degradation in osteoarthritic chondrocytes after tibial plateau fracture. Arthritis Res Ther. 2019; **21**: 271. [PMID: 31815654 DOI:*

10.1186/s13075-019-2033-5]" **into** "***Chen*** *HW, Yang SD, Shao RY. Long non-coding XIST raises methylation of TIMP-3 promoter to regulate collagen degradation in osteoarthritic chondrocytes after tibial plateau fracture. Arthritis Res Ther. 2019;* ***21****: 271. [PMID: 31815654 DOI: 10.1186/s13075-019-2033-5]*" **(See REFERENCES Section, Page 23, Line 10-13)**

3. We have **revised** "***Chen Y****, Lin Y, Bai Y, Cheng D, Bi Z. A Long Noncoding RNA (lncRNA)-Associated Competing Endogenous RNA (ceRNA) Network Identifies Eight lncRNA Biomarkers in Patients with Osteoarthritis of the Knee. Med Sci Monit. 2019;* ***25****: 2058-2065. [PMID: 30890688 DOI: 10.12659/msm.915555]*" **into** "***Chen YX****, Lin Y, Bai Y, Cheng DL, Bi ZG. A Long Noncoding RNA (lncRNA)-Associated Competing Endogenous RNA (ceRNA) Network Identifies Eight lncRNA Biomarkers in Patients with Osteoarthritis of the Knee. Med Sci Monit. 2019;* ***25****: 2058-2065. [PMID: 30890688 DOI: 10.12659/msm.915555]*" **(See REFERENCES Section, Page 26, Line 1-5)**

Comment 6: *The title of the manuscript is too long and must be shortened to meet the requirement of the journal (Title: The title should be no more than 18 words).*

[Author response]

We thank for the comment! The title has been shortened within 18 words.

1. We have **revised** "*Epigenetic regulatory mechanisms of long noncoding RNAs in the osteo-/adipogenic differentiation of mesenchymal stem cells and the pathogenesis of degenerative bone diseases*" **into** "*Epigenetic regulation by lncRNAs in osteo-/adipogenic differentiation of mesenchymal stromal cells and the pathogenesis of degenerative bone diseases*" **(See TITLE PAGE, Page 1)**

Others

1. We have **revised** "*..., which is orchestrated by histone methyltransferases and demethylases*" **into** "*..., which is orchestrated by histone methyltransferases (HMTS) and histone demethylases (HDMs)*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 10)**

2. We have **revised** "*4. CONCLUSIONS AND PERSPECTIVES*" **into** "*5. CONCLUSIONS AND PERSPECTIVES*" **(See CONCLUSIONS AND PERSPECTIVES Section, Page 11, Line 18)**

3. We have **revised** "*[2]*" **into** "*[4]*" **(See INTRODUCTION Section, Page 3, Line 9)**

4. We have **revised** "*[3, 4]*" **into** "*[5,6]*" **(See INTRODUCTION Section, Page 3, Line 12)**

5. We have **revised** "*[5,6]*" **into** "*[7,8]*" **(See INTRODUCTION Section, Page 3, Line 14)**

6. We have **revised** "*[7-9]*" **into** "*[9-11]*" **(See INTRODUCTION Section, Page 3, Line 17)**

7. We have **revised** "*[10,11]*" **into** "*[12,13]*" **(See INTRODUCTION Section, Page 3, Line 20)**

8. We have **revised** "*[12]*" **into** "*[14]*" **(See INTRODUCTION Section, Page 3, Line 23)**

9. We have **revised** "*[12]*" **into** "*[14]*" **(See INTRODUCTION Section, Page 3, Line 23)**

10. We have **revised** "*[13-15]*" **into** "*[15-17]*" **(See INTRODUCTION Section, Page 3, Line 26)**

11. We have **revised** "*[16-19]*" **into** "*[18-21]*" **(See INTRODUCTION Section, Page 4, Line 1)**

12. We have **revised** "*[20-22]*" **into** "*[22-24]*" **(See DNA METHYLATION Section, Page 4, Line 12)**

13. We have **revised** "*[23,24]*" **into** "*[25,26]*" **(See DNA METHYLATION Section, Page 4, Line 14)**

14. We have **revised** "*[25]*" **into** "*[27]*" **(See DNA METHYLATION Section, Page 4, Line 15)**

15. We have **revised** "*[26]*" **into** "*[28]*" **(See DNA METHYLATION Section, Page 4, Line 17)**

16. We have **revised** "*[27]*" **into** "*[30]*" **(See DNA METHYLATION Section, Page 4, Line 22)**

17. We have **revised** "*[27,28]*" **into** "*[30,31]*" **(See DNA METHYLATION Section, Page 4, Line 25)**

18. We have **revised** "*[29,28]*" **into** "*[30,31]*" **(See DNA METHYLATION Section, Page 4, Line 25)**

19. We have **revised** "*[29,30]*" **into** "*[32,33]*" **(See DNA METHYLATION Section, Page 5, Line 1)**

20. We have **revised** "*[31-34]*" **into** "*[34-37]*" **(See DNA METHYLATION Section, Page 5, Line 6)**

21. We have **revised** "*[29]*" **into** "*[32]*" **(See DNA METHYLATION Section, Page 5, Line 9)**

22. We have **revised** "*[29]*" **into** "*[32]*" **(See DNA METHYLATION Section, Page 5, Line 10)**

23. We have **revised** "*[35]*" **into** "*[38]*" **(See DNA METHYLATION Section, Page 5, Line 13)**

24. We have **revised** "*[36]*" **into** "*[39]*" **(See DNA METHYLATION Section, Page 5, Line 14)**

25. We have **revised** "*[37]*" **into** "*[40]*" **(See DNA METHYLATION Section, Page 5, Line 16)**

26. We have **revised** "*[37]*" **into** "*[40]*" **(See DNA METHYLATION Section, Page 5, Line 18)**

27. We have **revised** "*[38]*" **into** "*[41]*" **(See DNA METHYLATION Section, Page 5, Line 23)**

28. We have **revised** "*[39]*" **into** "*[42]*" **(See DNA METHYLATION Section, Page 5, Line 25)**

29. We have **revised** "*[38]*" **into** "*[41]*" **(See DNA METHYLATION Section, Page 5, Line 28)**

30. We have **revised** "*[38]*" **into** "*[41]*" **(See DNA METHYLATION Section, Page 6, Line 1)**

31. We have **revised** "*[40-42]*" **into** "*[43-45]*" **(See DNA METHYLATION Section, Page 6, Line 5)**

32. We have **revised** "*[15]*" **into** "*[17]*" **(See DNA METHYLATION Section, Page 6, Line 9)**

33. We have **revised** "*[43]*" **into** "*[46]*" **(See DNA METHYLATION Section, Page 6, Line 12)**

34. We have **revised** "*[44,45]*" **into** "*[47,48]*" **(See DNA METHYLATION Section, Page 6, Line 20)**

35. We have **revised** "*[46]*" **into** "*[49]*" **(See DNA METHYLATION Section, Page 6, Line 24)**

36. We have **revised** "*[47]*" **into** "*[50]*" **(See DNA METHYLATION Section, Page 6, Line 26)**

37. We have **revised** "*[47]*" **into** "*[50]*" **(See DNA METHYLATION Section, Page 6, Line 28)**

38. We have **revised** "*[47,48]*" **into** "*[50,51]*" **(See DNA METHYLATION Section, Page 7, Line 2)**

39. We have **revised** "*[64]*" **into** "*[52]*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 6)**

40. We have **revised** "*[65,66]*" **into** "*[53,54]*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 10)**

41. We have **revised** "*[65]*" **into** "*[53]*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 12)**

42. We have **revised** "*[67]*" **into** "*[55]*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 17)**

43. We have **revised** "*[65,68]*" **into** "*[53,56]*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 19)**

44. We have **revised** "*[35]*" **into** "*[38]*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 26)**

45. We have **revised** "*[35]*" **into** "*[38]*" **(See HISTONE MODIFICATIONS Section, Page 7, Line 29)**

46. We have **revised** "*[69]*" **into** "*[57]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 3)**

47. We have **revised** "*[70]*" **into** "*[58]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 5)**

48. We have **revised** "*[70,71]*" **into** "*[58,59]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 8)**

49. We have **revised** "*[71]*" **into** "*[59]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 9)**

50. We have **revised** "*[72]*" **into** "*[60]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 13)**

51. We have **revised** "*[73]*" **into** "*[61]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 14)**

52. We have **revised** "*[74]*" **into** "*[62]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 15)**

53. We have **revised** "*[75]*" **into** "*[63]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 22)**

54. We have **revised** "*[76,77]*" **into** "*[64,65]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 24)**

55. We have **revised** "*[78,79]*" **into** "*[66,67]*" **(See HISTONE MODIFICATIONS Section, Page 8, Line 27)**

56. We have **revised** "*[14]*" **into** "*[16]*" **(See HISTONE MODIFICATIONS Section, Page 9, Line 1)**

57. We have **revised** "*[80]*" **into** "*[68]*" **(See HISTONE MODIFICATIONS Section, Page 9, Line 6)**

58. We have **revised** "*[49,50]*" **into** "*[71,72]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 9, Line 23)**

59. We have **revised** "*[51]*" **into** "*[73]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 9, Line 26)**

60. We have **revised** "*[52]*" **into** "*[74]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 10, Line 1)**

61. We have **revised** "*[53]*" **into** "*[75]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 10, Line 3)**

62. We have **revised** "*[54]*" **into** "*[76]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 10, Line 7)**

63. We have **revised** "*[55-57]*" **into** "*[77-79]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 10, Line 9)**

64. We have **revised** "*[30]*" **into** "*[33]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 10, Line 12)**

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66. We have **revised** "*[58]*" **into** "*[80]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 10, Line 16)**

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71. We have **revised** "*[62]*" **into** "*[84]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 11, Line 1)**

72. We have **revised** "*[63]*" **into** "*[85]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 11, Line 6)**

73. We have **revised** "*[6]*" **into** "*[8]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 11, Line 9)**

74. We have **revised** "*[81]*" **into** "*[86]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 11, Line 12)**

75. We have **revised** "*[82]*" **into** "*[87]*" **(See ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES Section, Page 11, Line 16)**

76. We have **revised** "*[83,84]*" **into** "*[88,89]*" **(See CONCLUSIONS AND PERSPECTIVES Section, Page 11, Line 24)**

77. We have **revised** "*[85]*" **into** "*[94]*" **(See CONCLUSIONS AND PERSPECTIVES Section, Page 12, Line 9)**

78. We have **revised** "*[86]*" **into** "*[95]*" **(See CONCLUSIONS AND PERSPECTIVES Section, Page 12, Line 13)**

79. We have **revised** "*[87-89]*" **into** "*[96-98]*" **(See CONCLUSIONS AND PERSPECTIVES Section, Page 12, Line 20)**

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81. We have **revised** "*2*" **into** "*4*" **(See REFERENCES Section, Page 13, Line 20)**

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85. We have **revised** "*6*" **into** "8" **(See REFERENCES Section, Page 14, Line 7)**

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106. We have **revised** "*27*" **into** "30" **(See REFERENCES Section, Page 16, Line 29)**

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110. We have **revised** "*31*" **into** "34" **(See REFERENCES Section, Page 17, Line 15)**

111. We have **revised** "*32*" **into** "35" **(See REFERENCES Section, Page 17, Line 21)**

112. We have **revised** "*33*" **into** "36" **(See REFERENCES Section, Page 17, Line 25)**

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114. We have **revised** "*35*" **into** "38" **(See REFERENCES Section, Page 18, Line 4)**

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118. We have **revised** "*39*" **into** "42" **(See REFERENCES Section, Page 18, Line 23)**

119. We have **revised** "*40*" **into** "43" **(See REFERENCES Section, Page 18, Line 28)**

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128. We have **revised** "*49*" **into** "71" **(See REFERENCES Section, Page 22, Line 20)**

129. We have **revised** "*50*" **into** "72" **(See REFERENCES Section, Page 22, Line 24)**

130. We have **revised** "*51*" **into** "73" **(See REFERENCES Section, Page 22, Line 29)**

131. We have **revised** "*52*" **into** "74" **(See REFERENCES Section, Page 23, Line 3)**

132. We have **revised** "*53*" **into** "75" **(See REFERENCES Section, Page 23, Line 7)**

133. We have **revised** "*54*" **into** "76" **(See REFERENCES Section, Page 23, Line 10)**

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136. We have **revised** "*57*" **into** "79" **(See REFERENCES Section, Page 23, Line 22)**

137. We have **revised** "*58*" **into** "80" **(See REFERENCES Section, Page 23, Line 26)**

138. We have **revised** "*59*" **into** "81" **(See REFERENCES Section, Page 24, Line 2)**

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141. We have **revised** "*62*" **into** "84" **(See REFERENCES Section, Page 24, Line 12)**

142. We have **revised** "*63*" **into** "85" **(See REFERENCES Section, Page 24, Line 16)**

143. We have **revised** "*64*" **into** "52" **(See REFERENCES Section, Page 20, Line 8)**

143. We have **revised** "*65*" **into** "53" **(See REFERENCES Section, Page 20, Line 11)**

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145. We have **revised** "*67*" **into** "55" **(See REFERENCES Section, Page 20, Line 16)**

146. We have **revised** "*68*" **into** "56" **(See REFERENCES Section, Page 20, Line 19)**

147. We have **revised** "*69*" **into** "57" **(See REFERENCES Section, Page 20, Line 22)**

148. We have **revised** "*70*" **into** "58" **(See REFERENCES Section, Page 20, Line 26)**

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153. We have **revised** "*75*" **into** "63" **(See REFERENCES Section, Page 21, Line 17)**

154. We have **revised** "*76*" **into** "64" **(See REFERENCES Section, Page 21, Line 21)**

155. We have **revised** "*77*" **into** "65" **(See REFERENCES Section, Page 21, Line 24)**

156. We have **revised** "*78*" **into** "66" **(See REFERENCES Section, Page 21, Line 28)**

157. We have **revised** "*79*" **into** "67" **(See REFERENCES Section, Page 22, Line 4)**

158. We have **revised** "*80*" **into** "68" **(See REFERENCES Section, Page 22, Line 9)**

159. We have **revised** "*81*" **into** "86" **(See REFERENCES Section, Page 24, Line 20)**

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161. We have **revised** "*83*" **into** "88" **(See REFERENCES Section, Page 24, Line 29)**

162. We have **revised** "*84*" **into** "89" **(See REFERENCES Section, Page 25, Line 4)**

163. We have **revised** "*85*" **into** "94" **(See REFERENCES Section, Page 25, Line 24)**

164. We have **revised** "*86*" **into** "95" **(See REFERENCES Section, Page 25, Line 27)**

165. We have **revised** "*87*" **into** "96" **(See REFERENCES Section, Page 26, Line 1)**

166. We have **revised** "*88*" **into** "97" **(See REFERENCES Section, Page 26, Line 6)**

167. We have **revised** "*89*" **into** "98" **(See REFERENCES Section, Page 26, Line 9)**

168. We have **revised** "*90*" **into** "99" **(See REFERENCES Section, Page 26, Line 12)**

169. We have **revised** "*[29]*" **into** "*[32]*" **(See Table 1, Page 28)**

170. We have **revised** "*[35]*" **into** "*[38]*" **(See Table 1, Page 28)**

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172. We have **revised** "*[38]*" **into** "*[41]*" **(See Table 1, Page 28)**

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177. We have **revised** "*[43]*" **into** "*[46]*" **(See Table 2, Page 29)**

178. We have **revised** "*[46]*" **into** "*[49]*" **(See Table 2, Page 29)**

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182. We have **revised** "*[80]*" **into** "*[68]*" **(See Table 2, Page 29)**

183. We have **revised** "*[52]*" **into** "*[74]*" **(See Table 3, Page 30)**

184. We have **revised** "*[54]*" **into** "*[76]*" **(See Table 3, Page 30)**

185. We have **revised** "*[30]*" **into** "*[33]*" **(See Table 3, Page 30)**

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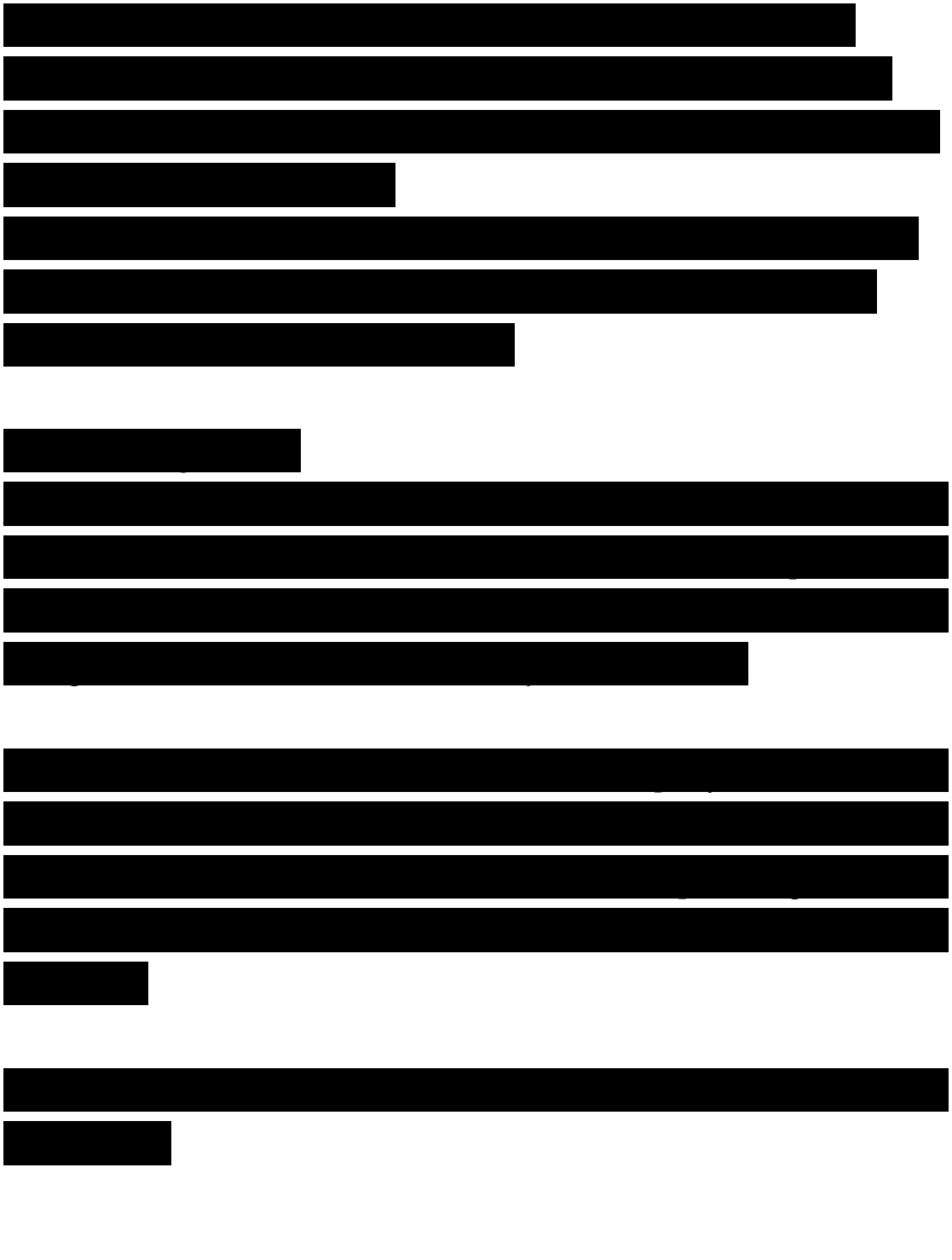
189. We have **deleted** "*MD.*" **(See TITLE PAGE, Page 1)**

Epigenetic regulation by lncRNAs in osteo-/adipogenic differentiation of mesenchymal stromal cells and the pathogenesis of degenerative bone diseases



Abstract

Bone is a complex tissue that undergoes constant remodeling to maintain homeostasis, which requires coordinated multilineage differentiation and proper proliferation of mesenchymal stromal cells (MSCs). Mounting evidence indicates that a disturbance of bone homeostasis can trigger degenerative bone diseases, including osteoporosis and osteoarthritis. In addition to conventional genetic modifications, epigenetic modifications (*i.e.*, DNA methylation, histone modifications, and the expression of noncoding RNAs) are considered to be contributing factors that affect bone homeostasis. Long noncoding RNAs (lncRNAs) were previously regarded as 'transcriptional noise' with no biological functions. However, substantial evidence suggests that lncRNAs have roles in the epigenetic regulation of biological processes in MSCs and related diseases. In this review, we summarized the interactions between lncRNAs and epigenetic modifiers associated with osteo-/adipogenic differentiation of MSCs and the pathogenesis of degenerative bone diseases and highlighted promising lncRNA-based diagnostic and therapeutic targets for bone diseases.

Keywords: RNA, long noncoding; Epigenetics; DNA methylation; Histones; Cell differentiation; Bone diseases

Core tip: In this review, we summarized the roles of lncRNAs played in MSC differentiation and common degenerative bone diseases through reciprocal interactions between lncRNAs and epigenetic modifiers, focusing on the most common epigenetic mechanisms: DNA methylation and histone modifications. It is our hope that this review may provide an updated summary that sheds light on the lncRNA-based precise regulation of the MSC differentiation process and highlights possible therapeutic targets of degenerative bone diseases.

1. INTRODUCTION

The skeletal system contains bones, joints, and ligaments that function together as a locomotive organ and provide structural support. Originating from mesenchymal progenitors during embryogenesis, the skeletal system undergoes modeling and remodeling throughout life[1]. Mesenchymal stromal cells (MSCs) refer to a heterogeneous unfractionated population of cells, which include fibroblasts, myofibroblasts, and progenitor cells[2,3]. MSCs are able to differentiate into chondrocytes or osteoblasts to comply with bone formation and regeneration needs[4]. It is worth mentioning that adipocytes, as well as osteoblasts, are derive from the same population of MSCs. A shift in the osteoadipogenic differentiation balance may lead to bone diseases, such as osteoporosis, which typically manifests as a shift toward adipogenesis[5,6]. Likewise, osteoarthritis is usually characterized by impairment of cartilage regeneration due to the attenuated chondrogenic capacity of MSCs[7,8]. Therefore, the differentiation of MSCs, which proceeds under the control of various transcription factors, influences the pathogenesis of common bone diseases[9-11].

In addition to conventional genetic and environmental factors, epigenetic modifications can influence the bone phenotype and the development of skeletal diseases[12,13]. Epigenetic mechanisms alter gene expression patterns without changing the DNA sequence by three major mechanisms, including DNA methylation, histone modifications, and altered expression of noncoding RNAs[14]. With the rapid development of next-generation sequencing (NGS) and advanced bioinformatic tools, the crucial roles of epigenetic mechanisms in the differentiation of MSCs and the pathogenesis of bone diseases have begun to be elucidated[15-17].

Long noncoding RNAs (lncRNAs) are defined as a set of noncoding RNAs longer than 200bp that have no protein-coding ability. Evidence is rapidly accumulating on the functions of lncRNAs in epigenetic regulation in the

differentiation of MSCs and the occurrence of many diseases[18-21]. In this review, we revisit the epigenetic regulatory mechanisms of lncRNAs involved in DNA methylation and histone modifications and summarize the biological functions of lncRNAs in regulation crucial differentiation- and bone disease-related genes by interacting with key epigenetic modifiers. It is our hope that this review may provide an updated summary that sheds light on the lncRNA-based precise regulation of the MSC differentiation process and highlights possible therapeutic targets of degenerative bone diseases.

2. DNA METHYLATION

DNA methylation functions as a regulator of osteogenesis and adipogenesis of MSCs and is involved in common bone diseases[22-24]. In humans, the majority of DNA methylation occurs at cytosines in cytosine-phospho-guanosine (CpG) dinucleotides[25,26]. Approximately 75% of all gene promoters are within CpG-rich regions, known as CpG islands, that are mostly unmethylated[27]. It is generally accepted that the methylation of these CpG islands is associated with the repression of gene expression[28]. Nevertheless, it is worth mentioning that DNA methylation is also associated with upregulated gene expression under certain circumstances[29].

As writer enzymes, DNA methyltransferases (DNMTs) catalyze DNA methylation by transferring a methyl group onto the C5 position of a cytosine at CpG dinucleotide sites to form 5mCpG[30]. A member of the DNMT family, DNMT1, which is also called the maintenance DNMT, maintains the original methylation pattern during DNA replication, while DNMT3a and DNMT3b are involved in *de novo* methylation[30,31]. The interaction of lncRNAs with DNMTs is varied and reciprocal. For example, lncRNAs can recruit DNMTs to the promoters of target genes and regulate their expression patterns. In turn, the changes in the methylation level of specific lncRNA gene promoters can alter the expression of lncRNAs, including downstream lncRNA-regulated

genes[32,33]. In MSCs, lncRNAs, as regulators of DNA methylation, have received increasing attention due to their great importance in the regulation of differentiation and bone-related diseases (Figure 1).

2.1 LncRNAs regulate DNA methylation during osteogenic differentiation

H19, a well-known lncRNA, plays a crucial role in embryo development, cell differentiation, and the occurrence and development of bone diseases[34-37]. In human dental pulp stromal cells (hDPSCs), H19 positively regulates odontogenic differentiation *via* hypomethylation of distal-less homeobox 3 (*DLX3*), a key factor in odontogenic differentiation[32]. H19 decreases SAHH and DNMT3B activity, consequently promoting the expression of *DLX3*[32]. In turn, a mutation of *DLX3* identified in dentin hypoplasia patients could increase DNMT3B activity, and the subsequently repressed H19/miR-675 axis impairs the odontoblastic differentiation of hDPSCs[38]. Similarly, in valve interstitial cells (VICs), which have a mesenchymal origin[39], the knockdown of H19 attenuated their osteogenic differentiation capacity by increasing the transcription of *NOTCH1* and decreasing the levels of RUNX2 and BMP2[40]. In mineralized aortic valve tissue, H19 was upregulated as a result of hypomethylation of CpG in its promoter region[40]. These results suggest the possibility that H19 forms a positive feedback loop with DNMTs and promotes the osteogenic differentiation of MSCs.

Another study found an inverse association between the methylation level of perinatal *CDKN2A*, which encodes the lncRNA antisense noncoding RNA in the INK4 locus (ANRIL), and bone mass at ages 4 and 6 years[41]. Considering that transitional hypomethylation of *CDKN2A* has been identified in human bone marrow stromal cells (hBMSCs) during osteogenic differentiation[42], the authors further verified that the methylation of *CDKN2A* decreased the binding of transcription factors SMAD3/4 and consequently downregulated the expression of ANRIL[41]. In terms of the functional mechanism of ANRIL, it has been demonstrated that the knockdown of ANRIL decreased the number of

live cells and induced cell apoptosis of SaOS-2 cells[41].

Given the crucial roles of *HOX* genes in development and differentiation, it is reasonable to believe that the lncRNAs encoded by the *HOX* gene cluster could also exert their function as critical biological regulators (*i.e.*, *HOTAIR* in the *HOXC* cluster and *HOTAIRM1* in the *HOXA* cluster)[43-45]. In human dental follicle stromal cells (hDFSCs), lncRNA HOTAIRM1 promoted osteogenesis by inhibiting the enrichment of DNMT1 in the *HOXA2* promoter region and subsequently maintaining two CpG islands in a hypomethylated state, which guaranteed the transcriptional activation of *HOXA2*[17].

2.2 LncRNAs regulate DNA methylation during adipogenic differentiation

lncRNA HOTAIR, encoded by the *HOXC* gene cluster as mentioned above, could also inhibit the adipogenic differentiation of hBMSCs[46]. In this process, HOTAIR probably directly interacts with DNMTs or is involved in gene regulation by triple helix formation[46].

Peroxisome proliferator-activated receptor-gamma (PPAR-γ) and CCAAT enhancer binding protein-alpha (C/EBP-α) are key transcription factors involved in adipogenesis. They synergistically promote the transcriptional activation of genes that induce the adipocyte phenotype and maintain their expression throughout the entire differentiation process and the entire life of the adipocytes[47,48]. In mouse ST-2 cells (bone marrow stromal cells), 3T3-L1 cells (committed preadipocytes derived from MSCs), and C3H10T1/2 cells (embryonic stem cells) as well as in bone marrow stromal cells, lncRNA Plnc1 promotes adipogenesis by increasing *Ppar-γ2* transcription through reducing the DNA methylation level on its promoter[49].

Upregulation of lncRNA slincRAD is also observed in the early stages of adipocyte differentiation in 3T3-L1 cells[50]. LncRNA slincRAD guides Dnmt1 to translocate to the perinuclear region in S phase and direct Dnmt1 to the promoter of cell cycle-related genes, including p21 (Cdkn1a)[50]. As p21 is a cyclin-dependent kinase inhibitor that play an important role in the

differentiation of 3T3-L1 cells, this effect facilitates the progression of differentiation[50,51].

3. HISTONE MODIFICATIONS

The building block of chromatin is the nucleosome, which consists of a complex of DNA and four types of core histone subunits (H2A, H2B, H3, and H4)[52]. Histone proteins are subject to a variety of modifications, with most studies focusing on methylation and acetylation. Lysine (K) residues in histone H3 are commonly modified by methylation, which is orchestrated by histone methyltransferases (HMTS) and histone demethylases (HDMs)[53,54]. Previous studies have revealed that trimethylation of H3K4 (H3K4me3) promotes transcription, whereas H3K9me3 and H3K27me3 restrict gene expression[53]. Likewise, acetylation and deacetylation of lysine residues in histones are regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. It is believed that the addition of an acetyl group to lysine residues alters the structure and folding of the nucleosome and consequently loosens the chromatin to enable transcription[55]. During cellular biological and pathologic processes, including cell differentiation, bone regeneration and disease, histone modifications are dynamically changed[53,56]. This process is at least in part mediated by lncRNAs that recruit histone-modifying enzymes to targeted gene promoters and alter histone modification enrichment (Figure 1).

3.1 Involvement of lncRNAs in osteogenic differentiation through histone modifications

As mentioned earlier, a mutation of *DLX3* identified in dentin hypoplasia patients could increase DNMT3B activity[38]. This study also reported that this mutation was capable of repressing H19 expression by increasing the enrichment of H3K9me3 in the promoter region of the H19 gene and retarding the odontoblastic differentiation of hDPSCs[38].

Similar to RUNX2, Osterix (OSX) is considered a master transcription factor that regulates the osteogenic differentiation of MSCs and it is required for the maturation of functional osteoblasts[57]. lnc-OB1 promotes osteogenic differentiation of MSCs, probably by upregulating OSX *via* the inhibition of H3K27me3 in the OSX promoter region[58]. In human osteoblast cells, this regulation might be mediated by an interaction between lnc-OB1 and SUZ12, which is an integral component of polycomb repressive complex 2 (PRC2), responsible for H3K27me3[58,59].

Another core part of PRC2, EZH2[59], was also found to interact with lncRNAs and regulate osteogenic differentiation. It has been shown that lncRNA SNHG1 inhibits the osteogenic differentiation of human periodontal ligament stromal cells by repressing the expression of KLF2, a positive regulator of osteoblast differentiation[60], through EZH2-mediated H3K27me3 of its promoter[61]. Likewise, lncRNA HOXA-AS3 inhibits hBMSC osteogenesis, possibly *via* EZH2-dependent H3K27me3, and represses RUNX2 expression[62].

3.2 Involvement of lncRNAs in adipogenic differentiation through histone modifications

As a critical transcription factor for adipogenesis, C/EBP-α was found to be upregulated *via* the recruitment of the MLL3/4 complex to its promoter, which is guided by the binding of PA1 (a component of the MLL3/4 complex) to lncRNA ADINR during adipogenic differentiation of human adipose-derived stromal cells (hASCs)[63]. It is believed that MLL3/4 complexes are involved in the maintenance of H3K4me3 and the removal of H3K27me3, thereby regulating downstream gene expression[64,65].

Adipocyte fatty acid-binding protein (A-FABP, also known as FABP4 or aP2), a downstream target gene of PPAR-γ and C/EBP-α, is considered a marker of adipogenic differentiation[66,67]. The knockdown of lncRNA MIR31HG suppressed FABP4 expression by reducing the enrichment of acetylated histone 3 (AcH3) and H3K4me3 in the FABP4 promoter, leading to

the inhibition of adipogenic differentiation of hASCs[16].

H19 and miR-675 (derived from H19) inhibited the adipogenic differentiation of hBMSCs through the miRNA-mediated repression of HDAC4, 5 and 6. In turn, the inhibition of HDACs decreased CCCTC-binding factor (CTCF) occupancy on the imprinting control region (ICR) of H19 and reduced H19 expression[68]. This evidence, combined with that mentioned in an earlier section that H19 is considered a positive regulator of osteogenic differentiation, suggests that DNA methylation and histone modifications might be linked together by H19 and shift the osteoadipogenic differentiation balance toward osteogenesis.

4. ROLE OF LNCRNAS IN DEGENERATIVE BONE DISEASES

More recently, epigenetic regulation of bone homeostasis has been considered as an important factor in the pathogenesis of degenerative bone diseases, such as osteoporosis, arthritis, post menopausal osteoporosis, *etc.*[69,70]. As mentioned above, lncRNAs have attracted considerable attention in the epigenetic regulation of bone homeostasis. The potential link between degenerative bone diseases and lncRNAs at the epigenetic level is also an intriguing area for exploration.

4.1 LncRNAs regulate DNA methylation in osteoarthritis and osteoporosis

Osteoarthritis (OA) is a common degenerative joint disease that is associated with the impairment of cartilage regeneration, chondrocyte apoptosis, and the degradation of the cartilage extracellular matrix (ECM)[71,72]. In this sophisticated balance between biosynthesis and degradation, lncRNAs play a role in the survival of chondrocytes and the regulation of arthritis-associated factors[73].

It has been reported that the overexpression of lncRNA CTBP1-AS2 downregulates miR-130a by increasing the methylation level of the *miR-130a* gene, which finally leads to a decreased proliferation rate of chondrocytes in

OA patients[74].

As a natural inhibitor of matrix metalloproteinases (MMPs), TIMP-3 deficiency can lead to mild cartilage degeneration in patients with OA[75]. lncRNA XIST is capable of downregulating the expression of TIMP-3 through the recruitment of DNMT1, DNMT3A, and DNMT3B, which increased the methylation ratio of the CpG island in the *TIMP-3* promoter region, and consequently increased collagen degradation in OA chondrocytes[76].

Increasing evidence suggests that small nucleolar RNA host gene (*SNHG*) family members are involved in the pathogenesis of OA[77-79]. The overexpression of lncRNA SNHG15 alleviated ECM degradation and promoted chondrocyte formation *via* competing endogenous RNA (ceRNA) SNHG15/miR-7/KLF4 axis[33]. In human OA cartilage tissues, however, the promoter region of lncRNA SNHG15 had a higher level of methylation than in normal cartilage tissues, and this might be a promising therapeutic target for OA[33]. Another *SNHG* family member, lncRNA SNHG9, was found to be downregulated in chondrocytes from OA patients[80]. Functional studies indicated that the overexpression of SNHG9 led to a decreased apoptotic rate through increased methylation of the *miR-34a* gene that suppressed the expression of miR-34a[80].

Osteoporosis is characterized by a loss of bone mass and microarchitectural deterioration of the skeletal structure[81]. The imbalance of bone homeostasis between osteoblastic bone formation and osteoclastic bone resorption plays a fundamental role in the pathogenesis of osteoporosis[82]. Emerging evidence suggests that epigenetic modifications are deeply involved in bone metabolism, which contributes to the development of osteoporosis.

The ERK-MAPK signaling pathway is a well-established pathway with critical roles in immune responses and embryonic development, including the regulation of bone mass *via* controlling osteoblast differentiation[83]. A previous study suggested that lncRNA H19 promoted tension-induced osteogenesis of

hBMSCs through the FAK-ERK1/2-RUNX2 signaling pathway[84]. Likewise, an alteration in H19 methylation may also be involved in the disruption of bone formation in disuse osteoporosis. It has been shown that DNMT1-induced hypermethylation of the H19 promoter results in H19 downregulation and ERK-MAPK signaling inhibition, which leads to osteogenesis impairment both *in vivo* and *in vitro* (rat osteoblast/osteocyte-like UMR-106 cells)[85].

4.2 LncRNAs regulate histone modifications in osteoarthritis

An abnormality of cartilage regeneration can be related to attenuated chondrogenic differentiation of MSCs in OA patients[8]. Similar to other MSCs derived from other tissues, synovium-derived mesenchymal stromal cells (SMSCs) are multipotent but have the greatest chondrogenesis potential, representing a promising stem cell source for cartilage repair in OA patients[86]. LncRNA MEG3 was reported to have the ability to inhibit the chondrogenic differentiation of SMSCs and the expression of cartilage-associated genes (aggrecan and Col2A1) by inhibiting TRIB2 expression through EZH2-mediated H3K27me3[87].

5. CONCLUSIONS AND PERSPECTIVES

lncRNAs are extensively involved in various types of epigenetic modifications, including DNA methylation, histone modifications, and ncRNA interactions, during MSC differentiation and the occurrence and progression of degenerative bone diseases. Concerning the large body of available literature and comprehensive reviews on the RNA-RNA interactions of lncRNAs (*i.e.*, ceRNA mechanisms)[88,89], this topic of epigenetics is not discussed in this review, but it is worth mentioning that in some cases, ceRNA mechanisms act as mediators between lncRNAs and epigenetic modifiers. Another potential involvement of lncRNA in epigenetics is the interaction with the key enzyme of methyl metabolism. It is known that DNMT and HMT utilize S-adenosylmethionine (SAM) as a major methyl-group donor in mammals,

which is consumed and regenerated in one-carbon metabolism[90,91]. Several studies have shown that lncRNAs play a role in SAM-dependent methylation through regulating enzymes related to the metabolism[92,93]. However, similar studies on differentiation and bone diseases are lacking. Further studies are needed to assess the potential importance of lncRNAs on the methyl metabolism.

Although it seems that DNA methylation and histone modification are two different types of epigenetic modification, these two systems can be dependent on and influence one another during organism development[94]. However, the underlying molecular mechanisms are complicated and remain vague. Intriguingly, lncRNAs are capable of regulating gene expression either in a cis- or trans- manner by guiding or serving as scaffolds for transcription factors or epigenetic modifiers to specific gene loci[95]. This raises the possibility that lncRNAs could be coordinator of these processes. In this review, we summarized the roles of lncRNAs played in MSC differentiation and common degenerative bone diseases through reciprocal interactions between lncRNAs and epigenetic modifiers. A complete list of the epigenetic regulatory mechanisms of lncRNAs discussed in this review is available in Table 1, 2, and 3.

Taken in combination with previous studies[96-98], the present evidence indicates that lncRNAs could be diagnostic and prognostic biomarkers in degenerative bone diseases. Moreover, as lncRNAs can be manipulated pharmacologically to modulate epigenetic modifications[99], this also opens new avenues for future therapeutic interventions. However, multiple challenges need to be overcome before clinical applications can be achieved. Given that lncRNAs have complex secondary structures, one of the challenges that lies ahead is the off-target possibilities, as a single lncRNA is capable of binding to multiple epigenetic modifiers and targeting several genes. Therefore, more reliable bioinformatic tools in terms of *in silico* algorithms for

comprehensive lncRNA interaction prediction and sequencing technologies are required. Despite these impediments, lncRNA-based epigenetic interventions have shown potential in the regulation of MSC differentiation and therapeutic strategies for bone diseases.

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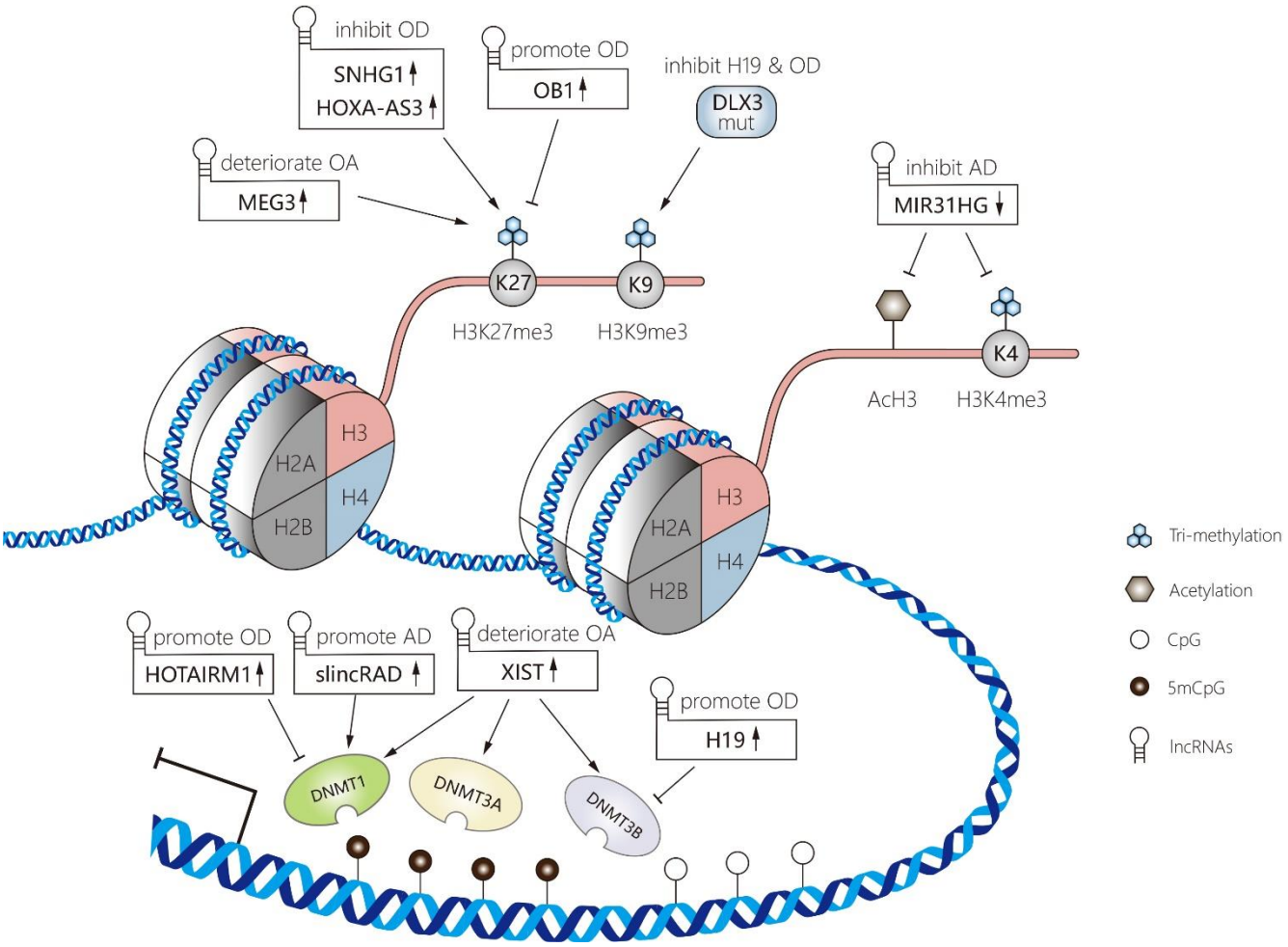

Figure 1 A Brief Illustration of the interactions between lncRNAs and the epigenetic modification associated with osteo-/adipogenic differentiation of MSCs and osteoarthritis. Histone acetylation and H3K4me3 are believed to promote transcription, whereas DNA methylation, H3K9me3 and H3K27me3 restrict gene expression. OD: osteogenic differentiation; AD: adipogenic differentiation; OA: osteoarthritis.

Table 1 Interactions between lncRNAs and epigenetic modifiers during osteogenic differentiation of MSCs

LncRNAs	Samples	Expression	Epigenetic regulatory mechanisms	Target genes	Effects	Ref.
H19	hDPSCs	up	decreasing DNMT3B activity	*DLX3*	promote odontogenic differentiation	Zeng *et al.*[32]
H19	hDPSCs	down	H19 was inhibited by the recruitment of DNMT3B and the enrichment of H3K9me3 in its promoter	*miR-675 (derived from H19)*	inhibit odontogenic differentiation	Zeng *et al.*[38]
H19	VICs	up	H19 was upregulated by hypomethylation of its promoter	NR	promote osteogenic differentiation	Hadji *et al.*[40]
ANRIL	umbilical cord	down	ANRIL was inhibited by methylation of its promoter	NR	decrease bone mass	Curtis *et al.*[41]
HOTAIRM1	hDFSCs	up	inhibiting the recruitment of DNMT1	*HOXA2*	promote osteogenic differentiation	Chen *et al.*[17]
HOXA-AS3	hBMSCs	up	facilitating EZH2-mediated H3K27me3	*RUNX2*	inhibit osteogenic differentiation	Zhu *et al.*[62]
SNHG1	hPDLSCs	up	facilitating EZH2-mediated H3K27me3	*KLF2*	inhibit osteogenic differentiation	Li *et al.*[61]
OB1	human osteoblasts	up	inhibiting H3K27me3 by interacting with SUZ12 (a core part of PRC2)	*Osterix*	promote osteogenic differentiation	Sun *et al.*[58]

hDPSCs: human dental pulp stromal cells; VICs: valve interstitial cells; hDFSCs: human dental follicle stromal cells; hBMSCs: human bone marrow stromal cells; hPDLSCs: human periodontal ligament stromal cells.

Table 2 Interactions between lncRNAs and epigenetic modifiers during adipogenic differentiation of MSCs

LncRNAs	Samples	Expression	Epigenetic regulatory mechanisms	Target genes	Effects	Ref.
HOTAIR	hBMSCs	up	interacting with DNMTs	NR	inhibit adipogenic differentiation	Kalwa *et al.*[46]
Plnc1	BMSCs	up	reducing the DNA methylation level	*Ppar-γ2*	promote adipogenic differentiation	Zhu *et al.*[49]
slincRAD	3T3-L1	up	facilitating the recruitment of Dnmt1	*Cdkn1a*	promote adipogenic differentiation	Yi *et al.*[50]
ADINR	hASCs	up	facilitating the recruitment of MLL3/4 complex (involved in the maintenance of H3K4me3 and the removal of H3K27me3) by binding PA1	*C/EBP-α*	promote adipogenic differentiation	Xiao *et al.*[63]
MIR31HG	hASCs	down	reducing the enrichment of AcH3 and H3K4me3	*FABP4*	inhibit adipogenic differentiation	Huang *et al.*[16]
H19	hBMSCs	up	facilitating miR-675-mediated repression of HDACs	NR	inhibit adipogenic differentiation	Huang *et al.*[68]

hBMSCs: human bone marrow stromal cells; BMSCs: bone marrow stromal cells; hASCs: human adipose-derived stromal cells.

Table 3 Interactions between lncRNAs and epigenetic modifiers in degenerative bone diseases

LncRNAs	Samples	Expression	Epigenetic regulatory mechanisms	Target genes	Effects	Ref.
CTBP1-AS2	OA chondrocytes	up	increasing the methylation level of target gene	*miR-130a*	decease proliferation rate of OA chondrocytes	Zhang *et al.*[74]
XIST	OA chondrocytes	up	facilitating the recruitment of DNMT1, DNMT3A, and DNMT3B	*TIMP-3*	raise collagen degradation in OA chondrocytes	Chen *et al.*[76]
SNHG15	OA cartilage tissues	down	SNHG15 was inhibited by methylation of its promoter	*miR-7/KLF4*	affect ECM homeostasis	Chen *et al.*[33]
SNHG9	OA chondrocytes	down	altering the methylation level of target gene	*miR-34a*	affect apoptotic rate of chondrocytes	Zhang *et al.*[80]
H19	UMR-106 & bone tissues from osteoporosis rat model	down	H19 was inhibited by DNMT1-induced hypermethylation of its promoter	ERK-MAPK signaling-related genes	impair osteogenic differentiation	Li *et al.*[85]
MEG3	SMSCs	up	facilitating EZH2-mediated H3K27me3	*TRIB2*	inhibit chondrogenic differentiation	You *et al.*[87]

OA: osteoarthritis; SMSCs: synovium-derived mesenchymal stromal cells.