Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Identification of osteogenic differentiation-related miRNA-mRNA regulatory network of human periodontal ligament stem cells

Zhenrong Chen[1], Lulu Long[2], Mianjia Wan[1], Min Huang[2], Haijuan Wu[1], Qun Huang[1,*]

[1] *Department of Stomatology, Guangdong Women and Children Hospital, Guangzhou, Guangdong, China*
[2] *Department of Gynecology, Guangdong Women and Children Hospital Guangzhou, Guangdong, China*

Article Info

Article history:

Received: December 10, 2023
Accepted: February 22, 2024
Published: March 31, 2024

Use your device to scan and read the article online

Abstract

This study aimed to investigate the hub genes and miRNA–mRNA regulatory network around periodontal ligament stem cells (PDLSC) for osteogenic differentiation through bioinformatic analysis. The dataset with osteogenic differentiation of human PDLSC was downloaded from the GEO database. The Weighted gene coexpression network analysis (WGCNA) was performed to identify key modules and hub genes. In addition, differentially expressed genes (DEGs) analysis was conducted with limma. The functional enrichment of differentially expressed hub genes was implemented with KEGG and GSEA analysis. The targeted genes of differentially expressed miRNA were predicted based on miRWalk database. The miRNA-mRNA interaction network of osteogenic differentiation of PDLSC was constructed and visualized. The WGCNA results showed that the light-cyan module was positively correlated with osteogenic differentiation ($r=0.98$, $P<0.05$). A total of 3125 hub genes and 1426 differentially expressed hub genes were detected in OG group. Innate immune-related signaling pathways and metabolic pathways were involved in the osteogenic differentiation. In addition, total of 2 upregulated miRNAs with 63 targeted DEGs and 6 downregulated miRNAs with 214 targeted DEGs were detected, which contributed to osteogenic differentiation by regulating amino acid metabolism signaling pathway. We identified hub genes and miRNA–mRNA regulatory network contributing to osteogenic differentiation of human PDLSC, which will provide novel strategy for periodontal disease therapy.

Keywords: Periodontitis, periodontal ligament stem cells, osteogenic differentiation, post-transcriptional regulation, metabolic changes.

1. Introduction

Periodontitis, the primary cause of tooth loss, has become a widespread public health problem and affects 42.2% of the population of US adults[1, 2]. Periodontitis is caused by a periodontal pathogen and can lead to loss of soft tissue attachment and alveolar bone resorption[3]. Various regenerative strategies, such as cytokine therapies, guided tissue regeneration (GTR) and guided bone regeneration (GBR) are applied to regain the supportive tissues, especially the alveolar bone around the tooth. However, their treatment effects remain suboptimal due to the complicated periodontal microenvironment[4, 5]. It is widely known that mesenchymal stem cells (MSC) play a critical role in tissue regeneration and function as a promising target for the repair of tissue defects [6]. Human periodontal ligament stem cell (PDLSC), as one of the MSC in dental tissues, has the potential to differentiate into several types of cells, including osteoblasts, fiber- and cementum-forming cells, adipocytes and even neuronlike cells[7, 8]. Hence, the PDLSC has become an appealing source for periodontal regeneration therapy. However, the mechanism of PDLSC for osteogenic differentiation remains unclarified. Hence, we aimed to investigate the hub genes and signaling pathways around PDLSC for osteogenic differentiation through bioinformatic analysis.

2. Methods and Materials

2.1. Data collection

The gene expression profile regarding the osteogenic differentiation of human PDLSCs was queried and downloaded from GEO database (https://www.ncbi.nlm.nih.gov/geo/). The data set (GSE159507) was included in our further analysis, which consists of human PDLSC under osteogenic induction for 14 days (n=3) (OG group) and human PDLSC(n=3) (control group). After normalization and normalization, the gene expression profile was applied to identify differentially expressed genes (DEG) in PDLSC during osteogenic differentiation.

2.2. Weighted genes correlation network analysis (WGCNA) for hub genes identification and functional enrichment

To further detect the osteogenesis-related genes, the WGCNA was applied to describe the correlation between clinical trait (osteogenic differentiation) and gene expression. To identify the soft threshold, the function “pickSoft-

* Corresponding author.
E-mail address: 2022760254@gzhmu.edu.cn (Q. Huang).
# These authors contributed equally
Doi: http://dx.doi.org/10.14715/cmb/2024.70.3.12

threshold" was applied to calculate the power (beta) from the WGCNA package. Hierarchical clustering and dynamic tree cutting were used to identify the gene modules. Subsequently, the modules whose eigengenes were highly correlated were merged with the merge module threshold was 0.25. Subsequently, the trait of osteogenic differentiation was correlated with the modules. In addition, the genes in the interesting module with the values of gene significance (GS) >0.1, the module membership (MM)>0.8 and weight >0.1 were considered hub genes. Gene Set Variation Analysis (GSVA) [9] was performed to detect the pathway activity, based on the hallmark gene sets that were downloaded from the Molecular Signatures Database (h.all.v7.4.symbols.gmt) [10]. The differential activity of the pathways between OG and the control group was analyzed by limma analysis. The activity of pathways with false discovery rates (FDR) <0.05 and |fold change| >1.5 was considered significant.

2.3. Identification of differentially expressed hub genes in PDLSCs with osteogenic differentiation

To compare the DEGs between the OG group and the control group, the limma analysis was conducted to calculate the DEGs. Gene expressed with FDR <0.05 and |fold change| >2 was considered significant DEGs. Subsequently, the differentially expressed hub genes were detected by intersecting DEGs and hub genes. To further understand the functional enrichment of differentially expressed hub genes, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis and Gene Set Enrichment Analysis (GSEA)[11] analysis were conducted. The subset (c2.cp.kegg.v7.4.symbols.gmt) was downloaded from the Molecular Signatures Database and the P value <0.05 was considered significant [11].

2.4. Identified differentially expressed miRNAs (DEM) and targeted gene prediction

To further investigate the miRNA regulation for osteogenic differentiation of PDLSC, the dataset (GSE159508) from GEO with human PDLSC under osteogenic induction for 14 days (n=3) and control human PDLSC(n=3) was included in our further analysis. To compare the DEMs between the OG group and the control group, the limma analysis was conducted to calculate the DEMs. The DEMs targeted genes were predicted by miRWalk database (http://mirwalk.umm.uni-heidelberg.de/) based on random forest [12]. Expression of genes with false discovery rates (FDR) <0.05 and |fold change| >2 was considered significant DEM.

2.5. Construction of the miRNA-mRNA interaction network of osteogenic differentiation of PDLSC

The predicted miRNA-targeted genes were intersected with differentially expressed hub genes. The Cytoscape software was used to construct and visualize the miRNA-mRNA interaction network of osteogenic differentiation of PDLSC [13]. To further investigate the effect of DEM on biological progress and molecular function of human PDLSC during osteogenic differentiation, functional enrichment analysis performed between intersected miRNA-targeted genes was conducted. The WikiPathways dataset was downloaded from the Molecular Signatures Database [10] (c2.cp.wikipathways.v7.4.symbols.gmt) for functional annotation.

3. Result

3.1. Identification of osteogenic differentiation-related hub genes through WGCNA

Figures 1A and 1B show that the network exhibited a scale-free topology with beta=9. The samples have been grouped into two clusters between the OG and the control group in Figure 1C. A total of 8 eigengene modules were detected in Figure 1D. In addition, the light-cyan module and brown module were positively correlated. Similarly, the green-yellow module and light-green were positively correlated in Figure 1E. Figure 2A verified the negative correlation between the light-cyan module and the brown module. The light-cyan module was positively correlated with osteogenic differentiation (r=0.98, P<0.05). A total of 3125 hub genes were clustered in the light-cyan module (Figure 2B).

3.2. Functional enrichment for WGCNA hub genes

The GSVA analysis showed that the apical surface, adipogenesis, fatty acid metabolism, reactive oxygen

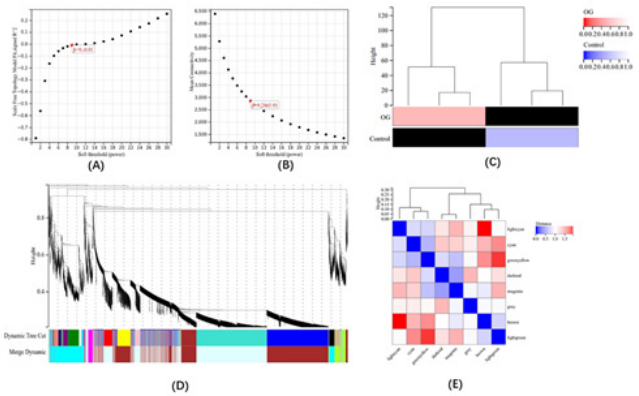

Fig. 1. WGCNA analysis for identifying the highly correlated gene modules. (A) Association between scale-free topology and soft threshold; (B) Association between mean connectivity and soft threshold; (C) Sample clustering diagram; (D) Hierarchical clustering dendrogram with merged dynamic tree cut plot; (E) Eigengene network heatmap.

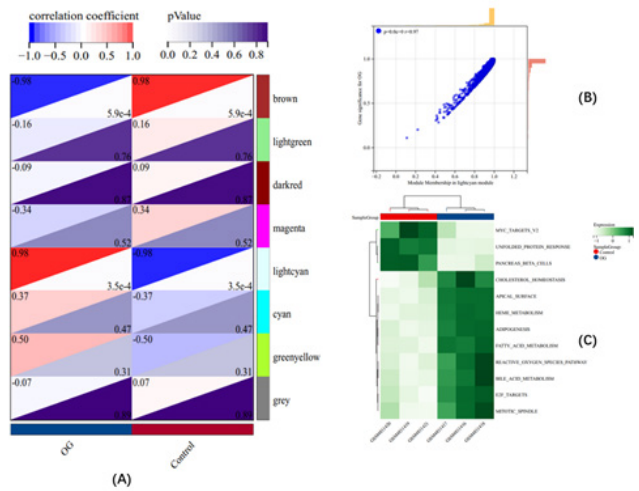

Fig. 2. Identification of interesting module and hub genes for functional enrichment. (A) Pearson's correlation analysis between the modules and the OG trait; (B) Pearson's correlation analysis between the genes significance for OG and the module membership in the light-cyan module; (C) Limma analysis of GSVA between the OG and control group.

species (ROS) pathway, E2F targets, heme metabolism, cholesterol homeostasis, bile acid metabolism and mitotic spindle-related signaling pathways were upregulated in the OG group compared with the control group in the light-cyan module. However, the Myc targets v2, unfolded protein response and pancreatic beta cells related signaling pathway were downregulated in the OG group compared with the control group in the light-cyan module (Figure 2C).

3.3. Identification of differentially expressed hub genes in the OG group

Compared with the control group, a total of 1307 upregulated and 1465 downregulated genes were detected in the OG group (Figure 3A). After the intersection with hub genes from the WGCNA module, a total of 1426 differentially expressed hub genes were detected in OG group (Figure 3B). Innate immune-related signaling pathways were involved in the osteogenic differentiation, including complement and coagulation cascades related signaling pathway, Toll-like receptor signaling pathway, natural killer cell-mediated cytotoxicity signaling pathway, and leukocyte transendothelial migration signaling pathway ($P<0.05$). In addition, the calcium signaling pathway, glycerolipid metabolism and amino sugar and nucleotide sugar metabolism were correlated with osteogenic differentiation of PDLSC ($P<0.05$) (Figure 3D). Similarly, the KEGG analysis showed that the metabolic pathways, AGE-RAGE signaling pathway in diabetic complications signaling pathway and fatty acid metabolism were associated with osteogenic differentiation of PDLSC ($P<0.05$) (Figure 3C).

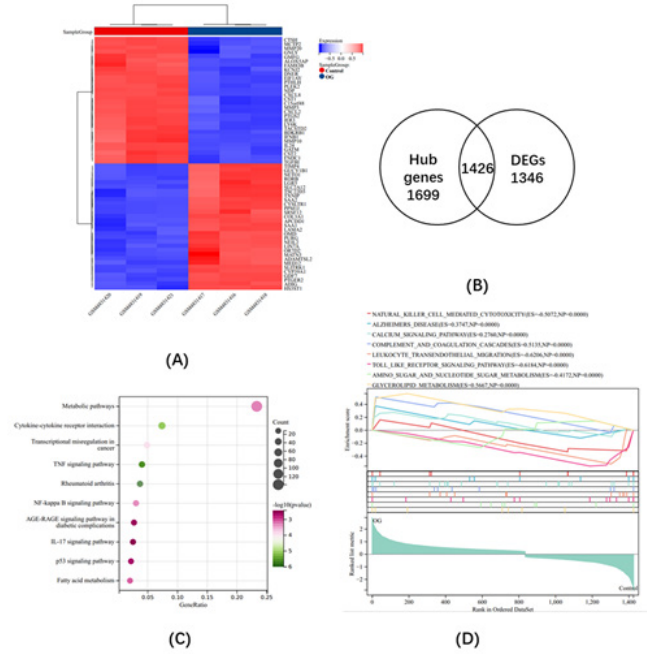

Fig. 3. Identification of differentially expressed hub genes and their function enrichment analysis. (A) Heat map of differentially expressed genes (DEGs) between OG and control group; (B) Venn plot of the intersection between hub genes and DEGs; (C) KEGG analysis of the differentially expressed hub genes; (D) GSEA analysis of the differentially expressed hub genes.

3.4. Construction of the miRNA-mRNA interaction network of osteogenic differentiation of PDLSC

Only 2 upregulated miRNAs (hsa-miR-143-3p and hsa-miR-379-3p) and 6 downregulated miRNAs (hsa-miR-4449, hsa-miR-4425, hsa-miR-124-5p, hsa-miR-663a, hsa-miR-4530, hsa-miR-4762-5p) were detected. In addition, a total of 1681 targeted genes of upregulated miRNAs and 6671 targeted genes of upregulated miRNAs were predicted. There were 63 intersecting genes between the targeted genes of upregulated miRNAs and downregulated differentially expressed hub genes (Figure 4A). Meanwhile, there were 214 intersecting genes between the targeted genes of downregulated miRNAs and upregulated differentially expressed hub genes (Figure 4B). The visualization for the miRNA-mRNA interaction network of osteogenic differentiation of PDLSC is shown in Figure 4C.

3.5. Functional enrichment of the differentially expressed targeted genes of miRNA in osteogenic differentiation of PDLSC

As shown in Figures 5A and 5B, the upregulated miRNAs targeted genes were enriched in the endochondral ossification-related signaling pathway, EGFR tyrosine kinase inhibitor resistance signaling pathway, ErbB signaling pathway and fibrin complement receptor 3 signaling pathway. In addition, the downregulated miRNAs targeted genes were enriched in the regulation of nuclear receptors meta-pathway, NRF2 pathway, TNF alpha signaling pathway, omega-9 FA synthesis, amino acid metabolism, eicosanoid metabolism via cyclooxygenases-Cox and sulfation biotransformation reaction in osteogenic differen-

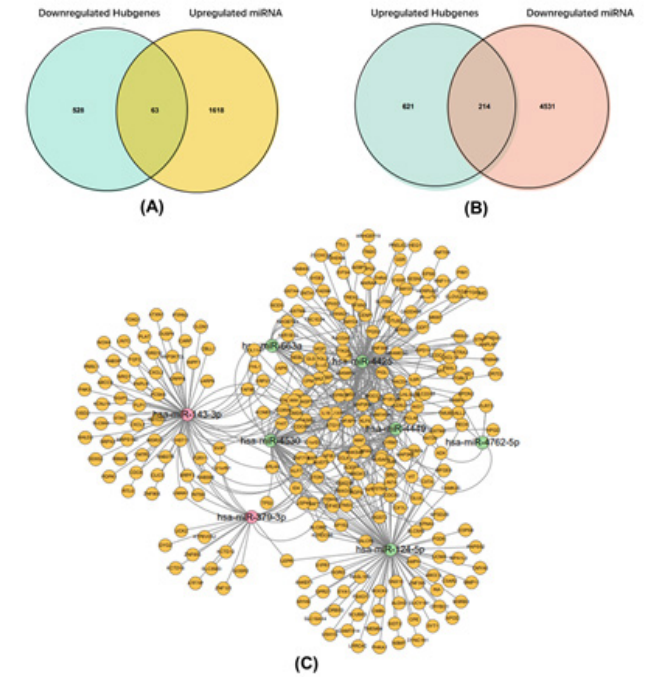

Fig. 4. Construction for the miRNA-mRNA interaction network of osteogenic differentiation of PDLSC. (A) The interaction between the targeted genes of upregulated miRNAs from miRWalk and downregulated differentially expressed hub genes; (B) The interaction between the targeted genes of downregulated miRNAs from miRWalk and upregulated differentially expressed hub genes; (C) Visualization for the miRNA-mRNA interaction network of PDLSC osteogenic differentiation of. The red nodes represent upregulated miRNA in osteogenic differentiation of PDLSC. Green nodes represent downregulated miRNA in the osteogenic differentiation of PDLSC. Yellow nodes represent the targeted genes of miRNA in the osteogenic differentiation of PDLSC. The edges represent the interaction between miRNAs and genes.

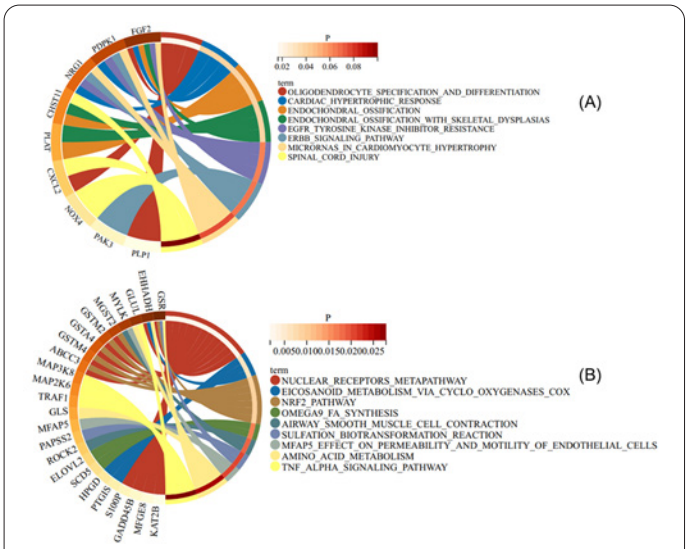

Fig. 5. Functional enrichment of the differentially expressed miRNA targeted genes in PDLSC. (A) Functional enrichment of 2 upregulated miRNA targeted genes by Wiki Pathways. (B) Functional enrichment of 6 downregulated miRNA targeted genes by Wiki Pathways.

tiation of PDLSC.

4. Discussion

Human PDLSC, as multipotential stem cells, can differentiate into the neurogenic, cardiomyogenic, chondrogenic and osteogenic lineages. Energy metabolism is highly related to osteogenic differentiation of MSC. Consistent with our study, differentially expressed enzymes about glycolysis, citric acid cycle, mitochondrial activity, and lipid metabolism were detected during osteogenic induction of dental follicle cells (DFC). A lipid with a lower number of C-atoms and double bonds was upregulated in DFC during osteogenic differentiation [14]. In addition, higher levels of (R, R)-tartaric acid, D-glucarate, glycerol, hydantoin-5-propionate, vinylacetylglycine, hydroxy-phenyllactate and 4-Imidazolone-5-propanoate were detected in osteogenic MSC differentiation [15]. Therefore, metabonomic analysis plays a crucial role in the periodontal regenerativeness of MSC. Future research on metabolic profile of osteogenic differentiation of PDLSC is warranted [16].

Epigenetic regulation by miRNA contributes to the phenotypic differences among species without alteration of gene sequence. MiRNA is implicated in bone remodeling and inflammatory mediation in periodontal tissue [17]. Salivary miRNAs could function as potential noninvasive biomarkers for early diagnosis of periodontal diseases [18]. Several studies have demonstrated that the miR-143-3p could function as a proinflammatory miRNA. MiR-143-3p was highly expressed in lipopolysaccharide (LPS)-induced periodontal ligament cells (PDLC) and periodontitis tissues. Knockdown of miR-143-3p reduced the apoptosis and inflammatory response in PDLC with LPS stimuli [19]. However, other studies have verified that miR-143-3p inhibited osteogenic differentiation in human PDLC through targeting KLF5 [20]. In addition, the miR-143-3p inhibited osteogenic differentiation in dental pulp stem cells by binding to TNF-α via inactivating the NF-κB signaling pathway [21]. Interestingly, the miR-143-3p facilitated self-renewal and enhanced the expression of pluripotency genes in mouse embryonic stem cells, which revealed that hsa-miR-143-3p could maintain the stemness in corneal epithelial stem cells (CESCs) [22]. MiR-143-3p also suppressed glycolysis mentalism and reduced tumor formation via targeting HK2 [23]. Higher expression of miR-379 was detected in inflamed gingival tissues compared with noninflamed tissues [24]. The miR-379 plays a vital role in tumor suppressor, which can inhibit the epithelial-mesenchymal transition process and suppress cell proliferation and migration. The exact mechanism of the effect of miR-379 on osteogenic differentiation in PDLSC remains unclear. Several evidences confirmed that miR-124 negatively regulates the osteogenic differentiation of MSC [25, 26]. The miR-124 directly targeted Dlx3, Dlx5, and Dlx2 and inhibited the expression of osteogenic marker genes, the activity of alkaline phosphatase and matrix mineralization [26]. The miR-124, derived from exosomes of osteocytes, has an implication for the development of diabetes mellitus-related bone loss in periodontal tissue [27]. However, miR-124-3p also facilitated osteogenesis in bone marrow-derived mesenchymal stem cells (BMSC) by regulating the GSK-3β/β-catenin signaling pathway [28].

The miR-663 plays an important role in the innate immune mediation in human oral epithelial cells. The overexpression of miR-663 was detected in epithelial cells treated with *Porphyromonas gingivalis.* The miR-663 promoted the apoptosis of epithelial cells by targeting AATF [29]. Similarly, cell apoptosis induced by miR-4530 was verified in human umbilical vein endothelial cells [30]. The miR-4425 promoted cell proliferation and led to ovarian cancer progression by targeting the FDFT1 [31]. Similarly, The miR-4425 promoted the cell proliferation of colorectal cancer by activating the STAT3 signaling pathway [32]. The miR-4449 aggravated the development of diabetic kidney disease through increasing the level of reactive oxygen species (ROS) and pro-inflammatory cytokines [33]. In addition, the miR-4449 expedited the proliferation of colorectal cancer via activating the STAT3 signaling pathway [32]. In multiple myeloma, higher expression of miR-4449 was observed compared with health control [34]. However, no published research investigating the effects of hsa-miR-4762 on cell biological progress has been found at present.

5. Conclusion

Our study identified a possible hub gene and miRNA-mRNA regulatory network that contribute to the osteogenic differentiation of human PDLSC, which provides a novel strategy for the treatment of periodontal disease therapy.

Informed consent

The authors report no conflict of interest.

Availability of data and material

We declared that we embedded all data in the manuscript.

Authors' contributions

CZ conducted the experiments and wrote the paper; LL, WM, HM and WH analyzed and organized the data; HQ conceived, designed the study and revised the manuscript.

Funding

None.

Acknowledgements
We thanked Guangdong Women and Children hospital approval our study.

References

1. Papathanasiou E, Conti P, Carinci F, Lauritano D, Theoharides TC (2020) IL-1 Superfamily Members and Periodontal Diseases. J Dent Res 99 (13): 1425-1434. doi: 10.1177/0022034520945209
2. Eke PI, Borgnakke WS, Genco RJ (2020) Recent epidemiologic trends in periodontitis in the USA. Periodontol 2000 82 (1): 257-267. doi: 10.1111/prd.12323
3. Carlisle MD, Srikantha RN, Brogden KA (2009) Degradation of human alpha- and beta-defensins by culture supernatants of Porphyromonas gingivalis strain 381. J Innate Immun 1 (2): 118-122. doi: 10.1159/000181015
4. Zhu S, Liu Z, Yuan C, Lin Y, Yang Y, Wang H, Zhang C, Wang P, Gu M (2020) Bidirectional ephrinB2EphB4 signaling regulates the osteogenic differentiation of canine periodontal ligament stem cells. Int J Mol Med 45 (3): 897-909. doi: 10.3892/ijmm.2020.4473
5. Sano K, Usui M, Moritani Y, Nakazawa K, Hanatani T, Kondo H, Nakatomi M, Onizuka S, Iwata T, Sato T, Togari A, Ariyoshi W, Nishihara T, Nakashima K (2020) Co-cultured spheroids of human periodontal ligament mesenchymal stem cells and vascular endothelial cells enhance periodontal tissue regeneration. Regen Ther 14: 59-71. doi: 10.1016/j.reth.2019.12.008
6. Wang Y, Jia Z, Diao S, Lin X, Lian X, Wang L, Dong R, Liu D, Fan Z (2016) IGFBP5 enhances osteogenic differentiation potential of periodontal ligament stem cells and Wharton's jelly umbilical cord stem cells, via the JNK and MEK/Erk signalling pathways. Cell Prolif 49 (5): 618-627. doi: 10.1111/cpr.12284
7. Zhou Q, Zhao ZN, Cheng JT, Zhang B, Xu J, Huang F, Zhao RN, Chen YJ (2011) Ibandronate promotes osteogenic differentiation of periodontal ligament stem cells by regulating the expression of microRNAs. Biochem Biophys Res Commun 404 (1): 127-132. doi: 10.1016/j.bbrc.2010.11.079
8. Liu J, Zhao Z, Ruan J, Weir MD, Ma T, Ren K, Schneider A, Oates TW, Li A, Zhao L, Xu HHK (2020) Stem cells in the periodontal ligament differentiated into osteogenic, fibrogenic and cementogenic lineages for the regeneration of the periodontal complex. J Dent 92: 103259. doi: 10.1016/j.jdent.2019.103259
9. Hanzelmann S, Castelo R, Guinney J (2013) GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 14: 7. doi: 10.1186/1471-2105-14-7
10. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdottir H, Tamayo P, Mesirov JP (2011) Molecular signatures database (MSigDB) 3.0. Bioinformatics 27 (12): 1739-1740. doi: 10.1093/bioinformatics/btr260
11. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102 (43): 15545-15550. doi: 10.1073/pnas.0506580102
12. Sticht C, De La Torre C, Parveen A, Gretz N (2018) miRWalk: An online resource for prediction of microRNA binding sites. PLoS One 13 (10): e0206239. doi: 10.1371/journal.pone.0206239
13. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13 (11): 2498-2504. doi: 10.1101/gr.1239303
14. Pieles O, Horing M, Adel S, Reichert TE, Liebisch G, Morsczeck C (2022) Energy Metabolism and Lipidome Are Highly Regulated during Osteogenic Differentiation of Dental Follicle Cells. Stem Cells Int 2022: 3674931. doi: 10.1155/2022/3674931
15. Surrati A, Evseev S, Jourdan F, Kim DH, Sottile V (2021) Osteogenic Response of Human Mesenchymal Stem Cells Analysed Using Combined Intracellular and Extracellular Metabolomic Monitoring. Cell Physiol Biochem 55 (3): 311-326. doi: 10.33594/000000377
16. Fujihara C, Nantakeeratipat T, Murakami S (2022) Energy Metabolism in Osteogenic Differentiation and Reprogramming: A Possible Future Strategy for Periodontal Regeneration. Frontiers in Dental Medicine 3. doi: 10.3389/fdmed.2022.815140
17. Irwandi RA, Vacharaksa A (2016) The role of microRNA in periodontal tissue: A review of the literature. Arch Oral Biol 72: 66-74. doi: 10.1016/j.archoralbio.2016.08.014
18. Schmalz G, Li S, Burkhardt R, Rinke S, Krause F, Haak R, Ziebolz D (2016) MicroRNAs as Salivary Markers for Periodontal Diseases: A New Diagnostic Approach? Biomed Res Int 2016: 1027525. doi: 10.1155/2016/1027525
19. Dong Y, Feng S, Dong F (2020) Maternally-Expressed Gene 3 (MEG3)/miR-143-3p Regulates Injury to Periodontal Ligament Cells by Mediating the AKT/Inhibitory kappaB Kinase (IKK) Pathway. Med Sci Monit 26: e922486. doi: 10.12659/MSM.922486
20. Wangzhou K, Lai Z, Lu Z, Fu W, Liu C, Liang Z, Tan Y, Li C, Hao C (2020) MiR-143-3p Inhibits Osteogenic Differentiation of Human Periodontal Ligament Cells by Targeting KLF5 and Inactivating the Wnt/beta-Catenin Pathway. Front Physiol 11: 606967. doi: 10.3389/fphys.2020.606967
21. Zhang P, Yang W, Wang G, Li Y (2018) miR-143 suppresses the osteogenic differentiation of dental pulp stem cells by inactivation of NF-kappaB signaling pathway via targeting TNF-alpha. Arch Oral Biol 87: 172-179. doi: 10.1016/j.archoralbio.2017.12.031
22. Kalaimani L, Devarajan B, Namperumalsamy VP, Veerappan M, Daniels JT, Chidambaranathan GP (2022) Hsa-miR-143-3p inhibits Wnt-β-catenin and MAPK signaling in human corneal epithelial stem cells. Sci Rep 12 (1): 11432. doi: 10.1038/s41598-022-15263-x
23. Fang R, Xiao T, Fang Z, Sun Y, Li F, Gao Y, Feng Y, Li L, Wang Y, Liu X, Chen H, Liu XY, Ji H (2012) MicroRNA-143 (miR-143) regulates cancer glycolysis via targeting hexokinase 2 gene. J Biol Chem 287 (27): 23227-23235. doi: 10.1074/jbc.M112.373084
24. Ogata Y, Matsui S, Kato A, Zhou L, Nakayama Y, Takai H (2014) MicroRNA expression in inflamed and noninflamed gingival tissues from Japanese patients. J Oral Sci 56 (4): 253-260. doi: 10.2334/josnusd.56.253
25. Tang JZ, Lin X, Zhong JY, Xu F, Wu F, Liao XB, Cui RR, Li F, Yuan LQ (2019) miR124 regulates the osteogenic differentiation of bone marrowderived mesenchymal stem cells by targeting Sp7. Mol Med Rep 19 (5): 3807-3814. doi: 10.3892/mmr.2019.10054
26. Qadir AS, Um S, Lee H, Baek K, Seo BM, Lee G, Kim GS, Woo KM, Ryoo HM, Baek JH (2015) miR-124 negatively regulates osteogenic differentiation and in vivo bone formation of mesenchymal stem cells. J Cell Biochem 116 (5): 730-742. doi: 10.1002/jcb.25026
27. Wang J, Li Y, Zhao P, Tian Y, Liu X, He H, Jia R, Oliver BG, Li J (2020) Exposure to Air Pollution Exacerbates Inflammation in Rats with Preexisting COPD. Mediators of inflammation 2020: 4260204. doi: 10.1155/2020/4260204
28. Li Z, Zhao H, Chu S, Liu X, Qu X, Li J, Liu D, Li H (2020) miR-124-3p promotes BMSC osteogenesis via suppressing the GSK-3beta/beta-catenin signaling pathway in diabetic osteoporosis rats. In Vitro Cell Dev Biol Anim 56 (9): 723-734. doi: 10.1007/s11626-020-00502-0
29. Benakanakere MR, Zhao J, Finoti L, Schattner R, Odabas-Yigit

M, Kinane DF (2019) MicroRNA-663 antagonizes apoptosis antagonizing transcription factor to induce apoptosis in epithelial cells. Apoptosis 24 (1-2): 108-118. doi: 10.1007/s10495-018-01513-9

30. Jing L, Li H, Zhang T, Lu J, Zhong L (2019) MicroRNA4530 suppresses cell proliferation and induces apoptosis by targeting RASA1 in human umbilical vein endothelial cells. Mol Med Rep 19 (5): 3393-3402. doi: 10.3892/mmr.2019.10000
31. Lu J, Zhou Y, Zheng X, Chen L, Tuo X, Chen H, Xue M, Chen Q, Chen W, Li X, Zhao L (2020) 20(S)-Rg3 upregulates FDFT1 via reducing miR-4425 to inhibit ovarian cancer progression. Arch Biochem Biophys 693: 108569. doi: 10.1016/j.abb.2020.108569
32. Yan Z, Hong S, Song Y, Bi M (2021) microR-4449 Promotes Colorectal Cancer Cell Proliferation via Regulation of SOCS3 and Activation of STAT3 Signaling. Cancer Manag Res 13: 3029-3039. doi: 10.2147/CMAR.S266153
33. Gao C, Wang B, Chen Q, Wang M, Fei X, Zhao N (2021) Serum exosomes from diabetic kidney disease patients promote pyroptosis and oxidative stress through the miR-4449/HIC1 pathway. Nutr Diabetes 11 (1): 33. doi: 10.1038/s41387-021-00175-y
34. Shen X, Ye Y, Qi J, Shi W, Wu X, Ni H, Cong H, Ju S (2017) Identification of a novel microRNA, miR-4449, as a potential blood based marker in multiple myeloma. Clin Chem Lab Med 55 (5): 748-754. doi: 10.1515/cclm-2015-1108