Epigenetic regulation of dental-derived stem cells and their application in pulp and periodontal regeneration

Yuyang Chen[1,*], Xiayi Wang[1,*], Zhuoxuan Wu[1], Shiyu Jia[1] and Mian Wan[1,2]

[1] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & West China School of Stomatology, Sichuan University, Chengdu, Sichuan, People's Republic of China

[2] State Key Laboratory of Oral Diseases & National Clinical Research Center for Oral Diseases & Department of Cariology and Endodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, Sichuan, People's Republic of China

[*] These authors contributed equally to this work.

ABSTRACT

Dental-derived stem cells have excellent proliferation ability and multi-directional differentiation potential, making them an important research target in tissue engineering. An increasing number of dental-derived stem cells have been discovered recently, including dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHEDs), stem cells from apical papilla (SCAPs), dental follicle precursor cells (DFPCs), and periodontal ligament stem cells (PDLSCs). These stem cells have significant application prospects in tissue regeneration because they are found in an abundance of sources, and they have good biocompatibility and are highly effective. The biological functions of dental-derived stem cells are regulated in many ways. Epigenetic regulation means changing the expression level and function of a gene without changing its sequence. Epigenetic regulation is involved in many biological processes, such as embryonic development, bone homeostasis, and the fate of stem cells. Existing studies have shown that dental-derived stem cells are also regulated by epigenetic modifications. Pulp and periodontal regeneration refers to the practice of replacing damaged pulp and periodontal tissue and restoring the tissue structure and function under normal physiological conditions. This treatment has better therapeutic effects than traditional treatments. This article reviews the recent research on the mechanism of epigenetic regulation of dental-derived stem cells, and the core issues surrounding the practical application and future use of pulp and periodontal regeneration.

Subjects Cell Biology, Genetics, Molecular Biology, Dentistry

Keywords Dental-derived stem cells, Epigenetic regulation, Dental pulp regeneration, Periodontal regeneration

Submitted 29 July 2022
Accepted 20 November 2022
Published 3 January 2023

Corresponding author
Mian Wan, mianwan@scu.edu.cn

Academic editor
Prashanth Ravishankar

Additional Information and Declarations can be found on page 22

DOI **10.7717/peerj.14550**

INTRODUCTION

Dental-derived stem cells are obtained from dental papilla or dental follicles and can be isolated and cultured in teeth or periodontal soft tissue. Dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (DFPCs), and stem cells from apical papilla (SCAPs) are all derived from dental papillae. Dental follicle precursor cells (DFPCs) and periodontal ligament stem cells (PDLSCs) are derived from dental follicles. In recent years, the discovery of dental-derived stem cells, their abundant sources, and their safety

and effectiveness have won them increasing attention in the field of tissue regeneration. Epigenetic regulation refers to the regulation of gene expression without changing the DNA sequence. This plays an important role in the self-renewal and differentiation capacity of adult and embryonic stem cells (*Chen et al., 2017*). Epigenetic regulation exists widely in natural organisms and participates in many biological processes, such as embryogenesis, germ cell formation, hematopoietic stem cell differentiation, and tumor formation (*Canovas et al., 2017*; *Chen, Yan & Duan, 2016*; *Mohammad, Barbash & Creasy, 2019*). More importantly, epigenetic modifications can not only regulate tooth formation, development, and aging, but also affect the differentiation of dental-derived stem cells (*Hodjat, Khan & Saadat, 2020*; *Liu et al., 2021*; *Townsend et al., 2009*). Epigenetic modifications are advantageous because they do not cause permanent DNA damage, off-target effects, or deleterious mutations. Therefore, an increasing number of studies have focused on the role of epigenetic modifications in regulating the proliferation and differentiation of dental-derived stem cells. This article focuses on the epigenetic regulation of dental-derived stem cells, describes the application of epigenetic regulation based on dental-derived stem cells in dental pulp and periodontal regeneration, summarizes the shortcomings of the existing research, and proposes possible future research directions.

Why this review is needed and who it is intended for

Endodontic and periodontal diseases are common and frequently-occurring diseases of the oral cavity. Traditional treatments for endodontics include root canal therapy and apical surgery. These treatments provide good relief of symptoms but cannot avoid tooth discoloration and pulp inactivation and necrosis. Periodontitis can cause destruction of the alveolar bone and also tooth loss. Traditional periodontal treatment may meet the needs of most patients with periodontitis, but fail to achieve periodontal tissue regeneration. In recent years, with the development of tissue engineering and the discovery of dental-derived stem cells, pulp and periodontal regeneration have become a potential treatment for these two diseases. The discovery of the mechanism of epigenetic modification of dental-derived stem cells has led to the potential use of epigenetic regulation in dental pulp and periodontal regeneration. However, no article targeting the same subject has been published to provide a clear guidance for clinic trials.

This article aims to provide perspectives for dentists and researchers investigating dental pulp and periodontal regeneration using epigenetic regulated dental-derived stem cells, in order to reduce failures and improve the prognosis for patients. It is our hope that these techniques can develop more secure and effective treatment approaches.

SURVEY METHODOLOGY

To ensure an inclusive and unbiased analysis of the literature and to accomplish the review's objectives, we searched the following literature databases: PubMed, Science Direct, Research Gate, and Google Scholar. The search terms included: dental-derived stem cells, epigenetic regulation, dental pulp regeneration, periodontal regeneration, DPSCs, SHEDs, SCAPs, DFPCs, PDLSCs, the targets were searched together with Boolean operators such as "AND" and "OR". It is important to note that the keywords used and their variants and relevant

words could be classified into categories and any combination of words from different categories was used for the search. The categories we used were as follows:

1. About dental-derived stem cells: dental-derived stem cells; DPSCs; SHEDs; SCAPs; DFPCs; PDLSCs; odontogenic stem cells.

2. About epigenetic regulation: epigenetic regulation; epigenetics; DNA methylation; histone modifications; noncoding RNA.

3. About pulp and periodontal regeneration: pulp regeneration; periodontal regeneration; odontogenic differentiation; nerve regeneration; angiogenesis; osteogenic differentiation.

This article is based on published literature. The aspects of the inclusion criteria are the retrieval keywords, information not covered by previous literature, and most importantly the use of a clear and credible source.

Properties of dental-derived stem cells

Stem cells have significant proliferative capacity and multi-directional differentiation potential. The development of regenerative medicine has led to the use of stem cells in the repair of damaged cells, tissues, and organs, which have low self-healing abilities, with excellent safety and efficacy. Dental-derived stem cells mediate the process of tooth regeneration by upregulating odontogenic and angiogenic capacity in the form of secreted exosomes (Exo) (*Mai et al., 2021*). Dental-derived stem cells have been used in relevant clinical trials in the fields of periodontal tissue, maxillofacial bone tissue repair, and apical pulp disease treatment (*Feng et al., 2010*; *Giuliani et al., 2013*; *Nakashima & Iohara, 2014*). However, the preclinical models for the use of stem cells in nerve regeneration, diabetes, and autoimmune diseases have only been preliminarily validated (*Kanafi et al., 2013*; *Mead et al., 2017*; *Shimojima et al., 2016*).

Properties and potential of DPSCs

Dental pulp stem cells have a relatively high proliferation rate, a low cellular senescence, multi-directional differentiation potential, and immunomodulatory properties (*Ma et al., 2019*; *Mortada & Mortada, 2018*). Damage to dental pulp causes dental pulp stem cells to induce the formation of various cellular components, including odontoblasts, to replenish damaged cells. In addition, *in vitro* studies have shown that DPSCs can differentiate into neural-like cells, osteoblasts, chondrocytes, adipocytes, muscle cells, endothelial cells, hepatocytes, and renal pericytes, *etc.* (*Barros et al., 2015*; *Gandia et al., 2008*; *Saito et al., 2015*). DPSCs can effectively promote pulp and periodontal regeneration. *Guo et al. (2021)* combined decellularized tooth matrix (DTM) with human dental pulp stem cells and successfully achieved the regeneration of dental pulp and periodontal tissue.

Properties and potential of *SHEDs*

SHEDs are isolated from the pulp tissue of exfoliated deciduous teeth, whose expression level of osteocalcin and alkaline phosphatase activity are higher compared to DPSCs (*Koyama et al., 2009*). SHEDs can express osteocalcin and RUNX-2 markers, resulting in the differentiation potential of osteoblasts and odontoblasts (*Miura et al., 2003*). SHEDs can induce the migration of naive bone marrow mesenchymal stromal cells (BMSCs) by

secreting extracellular vesicles (EVs) containing various cytokines, thereby promoting the bone healing process (*Luo, Avery & Waddington, 2021*). SHEDs can also induce pulp and periodontal regeneration. *Yang et al. (2019)* combined SHEDs cell sheets and DFSCs cell sheets with dentin matrix (TDM) and implanted them into the orthotopic jawbone of nude mice. The results indicated that SHED/TDM successfully achieved periodontal tissue regeneration with better migration ability and neurogenic differentiation potential (*Yang et al., 2019*).

Properties and potential of SCAPs

SCAPs exist in the apex of the developing tooth before tooth eruption and differentiate to odontoblasts, which mainly secrete apical dentin. SCAPs are less resistant to immune cell-mediated toxicity compared with other dental-derived stem cells, but can induce high levels of pro-inflammatory cytokine secretion (*Whiting et al., 2018*). Since SCAPs are derived from developing odontogenic tissues, they are more widely used in the field of tissue regenerative. For example, in dental pulp engineering, the regeneration process of dental pulp can be achieved by inducing endogenous stem cells to move to the regeneration site (*Rombouts et al., 2017*). *Wei, Sun & Hou (2021)* successfully used the silk fibroin-RGD-stem cell factor scaffold (the RGD peptide was arginine-glycine-aspartic acid polypeptide) to promote the migration and proliferation of SCAPs. This approach is promising for the further use of cell homing in dental pulp regeneration.

Properties and potential of DFPCs

The dental follicle contains a large number of undifferentiated precursor cells. In 2005, DFPCs were first isolated from the dental follicles of human third molars (*Morsczeck et al., 2005*). DFPCs are derived from neural crest and are direct precursor cells of periodontal tissue (*Zhang et al., 2019a*). DFPCs can promote pulp regeneration through the paracrine pathway. *Hong et al. (2020)* found that DFPCs could effectively enhance the proliferation, migration, and odontogenic differentiation of inflammatory dental follicle cells (DPCs) *in vitro* and their ectopic dentinogenesis *in vivo*.

Properties and potential of PDLSCs

Periodontitis often leads to the destruction of periodontal tissue and may even lead to tooth loss. After root canal treatment, the periodontal tissue is the only source of nutrition for the root canals. Multiple preclinical studies have demonstrated the effectiveness of PDLSCs to restore damaged periodontal tissue in periodontal regenerative therapy (*Li et al., 2020a*). PDLSCs are mainly isolated from the periodontal tissue of permanent teeth and can be further differentiated into osteoblasts, chondrocytes, and adipocytes under appropriate conditions (*Deng et al., 2018a*). Studies have shown that PDLSCs have the strongest osteogenic ability, followed by DPSCs, and DFPCs (*Qu et al., 2021*).

Epigenetic regulations of dental-derived stem cells

Epigenetics refers to changing the expression level and function of a gene without changing its sequence. Its regulatory processes are regulated by signaling molecules whose interactions with neighboring cells induce appropriate transcriptional and epigenetic responses

(*Surani, Hayashi & Hajkova, 2007*). The way in which epigenetic mechanisms regulate gene expression related to environmental factors plays an important role in the development of various diseases, such as tumors and inflammation (*Yuan, Dong & Shen, 2022*; *Zarzour, Kim & Weintraub, 2019*). In addition, epigenetic regulation may also affect the tooth number, size, and shape (*Fernández et al., 2020*). The common epigenetic regulations include DNA methylation, histone modification, and non-coding RNA regulations. Tables 1–3 summarize the regulatory mechanisms and potential applications of DNA methylation, histone modifications, and ncRNAs in dental-derived stem cells.

DNA methylation

DNA methylation is one of the important epigenetic modifications and it is common in most eukaryotes (*Lin et al., 2018*). The formation of 5-methylcytosine (5mC) from cytosine-phosphorothioate-guanine (CpG) dinucleotides by DNA methyltransferase (DNMT) leads to the silencing of gene expression (*Radhakrishnan, Kabekkodu & Satyamoorthy, 2011*). The level of methylated CpG is regulated by DNMT and DNA demethylase ten-eleven translocation (TET) (*Ren, Gao & Song, 2018*; *Li et al., 2015*).

DNA methylation levels are associated with stemness and the differentiation potential of dental-derived stem cells. For instance, DPSCs exhibits low DNA methylation levels and repressive mark H3K9Me2 enrichment, which is mediated by increased DNMT3B and G9a expression, respectively. This leads to decreased AKT phosphorylation and promotes osteogenesis (*Shen et al., 2019*). The decreased expression of the serine metabolism-related enzyme phosphoserine aminotransferase 1 (PSAT1) provides less methyl donor S-adenosylmethionine (SAM) for the methylation of the aging marker p16 (CDNK2A), resulting in the reduced stemness and osteogenic differentiation capacity of DPSCs (*Yang et al., 2021*). Wnt can effectively regulate the epigenetic mechanism of DPSCs. The short-term activation of Wnt signaling by Wnt-3A can cause a decrease in the content of 5-methylcytosine (5-mC) in DPSCs, which reduces the ability of DPSCs to differentiate into osteoblasts (*Uribe-Etxebarria et al., 2020*).

Different odontogenic stem cell genes have varied methylation levels and differentiation potentials. In DPSCs, PDLSCs, and DFPCs, the methylation of genes CD109 and SMAD3 are significantly different. At the transcriptional level, PDLSCs showed significantly higher expression levels of CD109, SMAD3, ALP, and RUNX2, which were identical to the differences in their DNA methylation profiles. The transcription levels of osteogenic differentiation-related factors and their osteogenic differentiation potential are higher in PDLSCs (*Ai et al., 2018*). The osteogenic differentiation process can be altered by modulating the methylation levels of specific genes in PDLSCs. Advanced glycation end-products (AGE) can increase the expression of DNMT1 and inhibit the methylation activation of calcitonin-related polypeptide α (CALCA) promoter, which inhibits the osteogenic differentiation of PDLSCs (*Wang et al., 2022*). Additionally, periostin (POSTN) can reduce the level of AGE receptors and DNA methylation of the CALCA promoter, thereby attenuating the inhibitory effect of AGE induction (*Wang et al., 2022*).

Table 1 Regulation of DNA methylation in dental-derived stem cells.

Modification	Stem cell	Locus	Pathway mechanism	Target protein	Potential applications	Ref
DNA methylation	DPSCs	p16	PSAT1 provides reduced SAM and decreased p16 methylation	PSAT1, PHGDH	Improve the pulp regeneration potential of aging DPSCs	*Yang et al. (2021)*
DNA demethylation	DPSCs	Wnt	WNT-3A activates Wnt signaling by diminishing their 5mC content.	NNMT	Induce epigenetic remodeling and pulp regeneration potential of DPSCs	*Uribe-Etxebarria et al. (2020)*
DNA demethylation	DPSCs	OSX, DLX5, RUNX2	5-Aza-CdR induced the expression of OSX, DLX5 and RUNX2 by decreasing DNA methylation.	DSPP, DMP1	Promote the odontogenic growth and differentiation of DPSCs	*Zhang et al. (2015)*
DNA methylation	DPSCs	KDM6B	Alcohol suppressed KDM6B through dysregulating DNA methylation	ALP, BMP2, BMP4, DLX2, OCN, OPN	Promote osteogenic and odontogenic growth of dental mesenchymal stem cells	*Hoang et al. (2016)*
DNA methylation	PDLSCs	MIR31HG	Mechanical force downregulates MIR31HG through DNA methylation.	IL-6	Inhibite hPDLSCs proliferation	*Han et al. (2021)*
DNA demethylation	PDLSCs	CALAL	POSTN attenuated the AGE-induced CALAL methylation	RUNX2, OSX, OPEN, RANGE	inhibit the osteogenic differentiation of PDLSCs	*Wang et al. (2022)*
DNA methylation	PDLSCs	DKK-1	Downregulation of Tet1 and Tet2 leads to hypermethylation of DKK-1 promoter, activating WNT pathway.	FasL	Promote immunomodulation of PDLSCs	*Yu et al. (2019)*
DNA methylation	PDLSCs	TNFR-1	High-glucose upregulates TNFR-1 via CpG island hypomethylation.	TNFR-1 protein	Aggravate viability reduction in hPDLSCs	*Luo et al. (2020)*
DNA methylation	SHEDs	IGF2	IGF2 was induced via DNA methylation and RXR/RAR pathways activation.	RUNX2, ALP, BGLAP, DLX5	Promote osteogenic differentiation of SHED	*Fanganiello et al. (2015)*
DNA demethylation	DFSCs	HOXA2	HOTAIRM1 induced HOXA2 via DNA hypomethylation	DSPP, DMP1	Induce osteogenic differentiation of DFSCs	*Chen et al. (2020b)*

Notes.
DPSCs, dental pulp stem cells; PDLSCs, periodontal ligament stem cells; SHEDs, stem cells from exfoliated deciduous teeth; DFSCs, dental follicle stem cells; PSAT1, phosphoserine aminotransferase 1; PHGDH, phosphoglycerate; 5mC, 5methyl-cytosine; NNMT, Nicotinamide-N-methyltransferase; 5-Aza-CdR, 5-Aza-20-de-oxycytidine kinase 1; TNFR1, tumor necrosis factor-alpha receptor-1; RXR/RAR, Retinoid X Receptor/ Retinoic Acid Receptor; DSPP, dentin sialophosphoprotein; DMP1, dentin matrix protein 1; KDM6B, lysine (K)-specific demethylase 6B; IL-6, interleukin- 6; ALP, alkaline phosphatase.

Histone modifications

Histones are located in the nucleus of eukaryotic cells and can form nucleosomes, the basic structure of chromatin, when bound to DNA. Modifications of amino acid residues in histone tails can cause structural changes in histones, which provide sites that can be recognized by specific proteins (*Strahl & Allis, 2000*). The regulation of specific genes can

Table 2 Regulation of histone modifications in dental-derived stem cells.

Modification	Stem cell	Locus	Pathway mechanism	Target protein	Potential applications	Ref
Histone deacetylation	DPSCs	P21	IGFBP7 activated SIRT1, resulting in a deacetylation of H3K36ac and reduction of p21 transcription	SIRT1 deacetylase	Prevent DPSCs senescence and promote tissue regeneration	*Li et al. (2022)*
Histone acetylation	DPSCs	HAT/KAT8	WNT-3A activates Wnt by induces HAT expression and increased H3AC.	ACLY	Reduce the ability of DPSCs to differentiate into osteoblasts	*Uribe-Etxebarria et al. (2020)*
Histone acetylation and methylation	DPSCs	WNT3A, DVL3	Ferutinin regulates Wnt/β-catenin pathway by H3K9 acetylation and H3K4 trimethylation.	Osteocalcin, collagen 1A1	Direct DPSCs towards the osteogenic lineage	*Rolph et al. (2020)*
Histone demethylation	DPSCs	BMP2, HOX	KDM6B catalyzes the demethylation of H3K27me3 and activates BMP2 and HOX	ALP, BMP2, BMP4, DLX2, OCN, OPN	Promote osteogenic and odontogenic growth of dental mesenchymal stem cells	*Hoang et al. (2016)*
Histone demethylation	DFSCs	Wnt	Downregulated MEG3/EZH2 activated Wnt/β-catenin signaling pathway via demethylation on H3K27	β-catenin and Wnt5a protein	Promotes the osteogenesis of DPSCs and DFPCs	*Deng et al. (2018b)*
Histone methylation	DFSCs	PTH1R	CHD7 activates PTH/PTH1R signaling pathway and interaction with H3K4me	RUNX2, SP7, BGLAP, DLX5, BMP2, COL1A1	Promote osteogenic differentiation of DFSCs	*Liu et al. (2020a)*
Histone methylation	DFSCs	SFRP1	WAY-316606 inhibits SFRP1 via histone H3K4me3 and activates Wnt pathway	β-catenin, RUNX2, ALP, osteocalcin, collagen	Maintain the nonmineralized state of PDL Progenitors	*Gopinathan et al. (2019)*

(*continued on next page*)

Table 2 (*continued*)

Modification	Stem cell	Locus	Pathway mechanism	Target protein	Potential applications	Ref
Histone methylation	PDLSCs	COL1A1, RUNX2, IL-1β, CCL5	LPS downregulated COL1A1, COL3A1, RUNX2 by H3K4me3 and upregulated CCL5, DEFA4, IL-1β gene expression by H3K27me3	COL1A1, COL3A1, RUNX2, CCL5, DEFA4, IL-1β	Regulate periodontal lineage differentiation and the coordination of the periodontal inflammatory response	*Francis et al. (2019)*
Histone methylation	PDLSCs	RUNX2, MSX2, DLX5	The H3K4me3 active methyl mark globally switch to the H3K27me3 repressive mark under osteogenic induction conditions.	DSPP, DMP1	Induce osteogenic differentiation of DFSCs	*Francis et al. (2020)*
Histone demethylation	PDLSCs	IGFBP5	BCOR inhibits IGFBP5 through histone K27 methylation.	ALP	Promote the odontoblast differentiation, proliferation, migration and mineralization of PDLSCs	*Han et al. (2017)*
Histone methylation	SCAPs	$p15^{INK4B}$. $p27^{Kip1}$	KDM2A increased H3K4 trimethylation at loci p15 and p27	cyclin-CDK	Inhibite cell proliferation of SCAPs	*Gao et al. (2013)*

Notes.

DPSCs, dental pulp stem cells; DFSCs, dental follicle stem cells; PDLSCs, periodontal ligament stem cells; SCAPs, stem cells from apical papilla; H3AC, acetylated-Histone 3; H3K4me3, he histone H3 methylated at lysine 4; H3K27me3, the histone H3 methylated at lysine 27; H3K9me3, the histone H3 methylated at lysine 9; ACLY, ATP-citrate lyase enzyme; BMP2, bone morphogenic protein 2; HDAC3, histone deacetylase 3; MEG3, maternally expressed 3; EZH2, the enhancer of zeste homolog 2; PTH1R, parathyroid hormone receptor-1; PCNA, Proliferating cell nuclear antigen; CHD7, Chromodomain helicase DNA-binding protein 7; α-SMA, alpha-smooth muscle actin; TNNT2, cardiac muscle troponin T; ACTC1, cardiac muscle; IGFBP5, insulin-like growth factor binding protein 5; BCOR, BCL6 co-repressor; p15INK4B, cyclin-dependent kinase inhibitor 2B; p27Kip1, cyclin-dependent kinase inhibitor 1B.

Table 3 The regulatory role of ncRNAs in dental-derived stem cells.

Modification	Stem cell	Locus	Pathway mechanism	Target protein	Potential applications	Ref
miRNA	DPSCs	TLR-4	LPS activates lipopolysaccharide/TLR-4 signaling pathway by downregulating miR-140-5p.	TLR-4	Enhance differentiation of DPSCs and inhibit proliferation	*Sun et al. (2017a)*
miRNA	DPSCs	Rac1	miR-224-5p targets the 3′-untranslated region of Rac1 gene and downregulates Rac1.	MAPK8, caspase-3, caspase-9, Fas ligand	Potect DPSCs from apoptosis	*Qiao et al. (2020)*
miRNA	DPSCs	TGFBR1	miR-24-3p and LEF1-AS1 sponged to regulate TGFBR1 expression.	RUNX2, OSX, ALP	Promote osteogenic differentiation of DPSCs	*Wu, Lian & Sun (2020)*
miRNA	DPSCs	CAB39	miR-34a-3p activates AMPK/mTOR signaling pathway by downregulating CAB39	AMPK, mTOR	Downregulate alleviates senescence in DPSCs	*Zhang et al. (2021)*
miRNA	DPSCs	Foxq1	miR-320b mediated Foxq1 upregulation after calcium hydroxide stimulation.	cyclin E1, cyclin D1	Pomote proliferation of DPSCs	*Tu et al. (2018)*
miRNA	DPSCs	TLR4	lncRNA-Ankrd26 promotes migration and osteogenesis via regulating miR-150-TLR4 signaling in MSCs	OSX, ALP	Promote dental pulp repair	*Li & Ge (2022)*
miRNA	DFSCs	Runx2, ALP and SPARC	miR-204 negatively targets the gene of Runx2, ALP and SPARC.	Runx2, ALP and SPARC	Promote osteogenic induction in DFSCs	*Ito et al. (2020)*
miRNA	PDLSCs	IL-17, IL-35	Overexpression of miRNA-146a downregulates IL-17 and IL-35 expression under periodontitis	IL-17, IL-35	Inhibit proliferation of hPDLSCs	*Zhao, Cheng & Kim (2019)*
miRNA	PDLSCs	PTEN	miR-181b-5p regulates PTEN/AKT pathway and promotes BMP2/ Runx2	PKB, BMP2, Runx2	Promote hPDLSCs proliferation and osteogenic differentiation	*Lv et al. (2020)*
miRNA	PDLSCs	Satb2	miR-31 promotes Satb2 siRNA and inhibits osteogenic differentiation	Runx2	Promote osteogenic differentiation of PDLSCs	*Zhen et al. (2017)*
miRNA	PDLSCs	Spry1	Upregulating miR-21 repressing Spry1 and inhibits TNF- α	Spry1; TNF- α	Suppress adipogenic and osteogenic differentiation of PDLSCs	*Yang et al. (2017)*

(*continued on next page*)

Table 3 (*continued*)

Modification	Stem cell	Locus	Pathway mechanism	Target protein	Potential applications	Ref
miRNA	PDLSCs	Notch2	miR-758 regulated Notch2 and interacts with lncRNA-ANCR	Notch2	Regulate the osteogenic differentiation of PDLSCs	*Peng et al. (2018)*
circRNA	DPSCs	SATB2, RUNX2, OCN	Exosome circLPAR1 induced osteogenic differentiation via downregulation of hsa-miR-31.	SATB2, RUNX2, and OCN	Promote osteogenic differentiation of DPSCs	*Xie et al. (2020)*
circRNA	DPSCs	RUNX1, Beclin1	hsa_circ_0026827 promotes osteoblast differentiation via Beclin1 and the RUNX1 signaling pathways by sponging miR-188-3p	Beclin-1, RUNX1, ALP, OCN and OSX	Promote osteogenic differentiation of DPSCs	*Ji et al. (2020)*
circRNA	SCAPs	ALPL	CircSIPA1L1 is sponge for miR-204-5p, which upregulates ALPL.	ALPL	Promote the osteogenic differentiation of SCAPs	*Li et al. (2020b)*
circRNA	PDLSCs	SMAD5	circFAT1 inhibits miR-4781-3p targeting SMAD5.	SMAD5	Mediate the periodontal bone regeneration of PDLSCs	*Ye et al. (2021)*
circRNA	PDLSCs	ERK	CDR1as functioned as an miR-7 sponge to activate the ERK signal pathway.	ERK	Inhibites the proliferation of PDLSCs	*Wang et al. (2019b)*
lncRNA	SHEDs	BMP2	lncRNA C21orf121 competes with BMP2 binding to miR-140-5p, upregulates BMP2 expression.	BMP2, Nestin, βIII-tubulin, MAP2, NSE	Promote neurogenic differentiation of SHEDs	*Liu et al. (2018a)*
lncRNA	SCAPs	ALP, RUNX2	LncRNA-H19 bound to miR-141, elevating phosphorylated levels of p38 and JNK.	SPAG9	Promote the odontoblast differentiation of SCAPs	*Li et al. (2019b)*

Notes.

DPSCs, dental pulp stem cells; PDLSCs, periodontal ligament stem cells; SHEDs, stem cells from exfoliated deciduous teeth; DFSCs, dental follicle stem cells; SCAPs, stem cells from apical papilla; TLR-4, toll-like receptor 4; Rac1, the Rac family small GTPase 1; RUNX1, runt-related transcription factor 1; CAB39, calcium-binding protein 39; AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; BMP2, bone morphogenetic proteins 2; MAP2, microtubule-associated protein 2; MAPK8, mitogen-activated protein kinase 8; NSE, neuron-specific enolase; ALPL, alkaline phosphatase; SPARC, secreted protein acidic and rich in cysteine; ZEB2, zinc finger E-box binding homeobox 2; SMAD5, a receptor-regulated SMAD protein in SMAD family member; CDR1as, circRNA CDR1as; TNF-α, Tumor necrosis factor-alpha; PHD2, prolyl hydroxylase domain–containing protein 2; LY294402, small interfering RNA for AKT; AKT, a phosphoinositide 3 kinase (PI3K)-dependent serine/threonine.

also be achieved through the binding of specific proteins to sites. Many studies have been conducted on the modification of histones, specifically on methylation and acetylation.

Histone methylation

Histone methylation refers to the transfer of the methyl group of S-adenosylmethionine (SAM) to arginine or lysine site under the action of histone methyltransferases (HMTs) (*Wang & Jia, 2009*). The expression or repression of genes is associated with specific residues catalyzed by HMTs. For example, histone H3-lysine 4 (H3K4) methylation promotes gene expression, while H3K9 and H3K27 methylation inhibit gene expression (*Blanc & Richard, 2017*). However, histone demethylases can cause histone demethylation. For example, histone demethylase lysine (K)-specific demethylase 1A (KDM1A) targeting H3K4 and H3K9 can affect the differentiation of embryonic stem cells (*Pedersen & Helin, 2010*).

Histone modifications play key roles in the lineage commitment and differentiation of DFPCs and DPSCs. The H3K27me3 mark in DFSCs can strongly suppress the expression of two dentinogenic genes, dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP1), whereas the H3K27me3 mark is almost absent in the promoters of the genes DSPP and DMP1 in DPSCs and the gene expression levels are significantly higher (*Francis et al., 2020*; *Gopinathan et al., 2013*). The histone methylation-modifying enzyme enhancer of zeste homolog 2 (EZH2) mainly acts on H3K27 and regulates the osteogenic differentiation of DPSCs and DFPCs through the Wnt/ β-catenin signaling pathway. The reduction of EZH2 directly causes the downregulation of H3K27me3 and further leads to the accumulation of β-catenin, which activates the Wnt/β-canonical signaling pathway and ultimately promotes the osteogenesis of DPSCs and DFPCs (*Deng et al., 2018b*; *Li et al., 2018a*).

Histone demethylases such as KDM6B, KDM1A, and KDM2A also play a regulatory role in the gene expression of dental-derived stem cells. KDM6B catalyzes the demethylation of histone H3K27me3 located near the promoter of bone morphogenetic protein-2 (BMP2). This activates BMP2 expression and promotes osteogenic and odontogenic growth of dental mesenchymal stem cells (*Hoang et al., 2016*; *Liu et al., 2022*). In addition, KDM6B decreases the level of histone K27 methylation in the promoter of insulin-like growth factor binding protein 5 (IGFBP5), thereby promoting the odontoblast differentiation, proliferation, migration and mineralization of PDLSCs (*Han et al., 2017*).

KDM1A can cooperate with 2-oxoglutarate 5-dioxygenase 2 (PLOD2) to regulate the differentiation process of SACPs (*Wang et al., 2018a*). The knockdown of KDM1A or PLOD2 reduces ALP activity, promotes the expression of DSPP, DMP1 and RUNX2, and enhances bone/dentin production in SCAPs (*Wang et al., 2018a*). Homeobox C8 (homeobox, HOXC8) significantly inhibits the osteogenic differentiation ability of SCAPs by directly binding to the KDM1A promoter and enhancing its transcription (*Yang et al., 2020b*).

KDM2A is able to increase histone H3 lysine 4 (H3K4) trimethylation at the $p15^{INK4B}$ (cyclin-dependent kinase inhibitor 2B) and $p27^{Kip1}$ (cyclin-dependent kinase inhibitor 1B) loci (*Gao et al., 2013*). On the other hand, the attenuation of KDM2A prevents cell cycle

progression in the G1/S phase of SCAPs (*Gao et al., 2013*). Inflammation and hypoxia can also cause the upregulation of KDM2A expression and repress the secreted frizzled-related protein 2 (SFRP2) transcription by reducing histone methylation in the SFRP2 promoter (*Yang et al., 2020a*). SFRP2 can inhibit the Wnt/β-catenin signaling pathway and further inhibit the target genes of the nuclear factor kappa B (NF-kB) signaling pathway. This enhances the bone/odontogenic differentiation capacity of SCAPs (*Yang et al., 2020a*). Similarly, histone demethylase KDM3B is also capable of regulating the bone/dental differentiation, cell proliferation, and migratory potential of SCAPs (*Zhang et al., 2020*).

Histone acetylation

Histone acetylation is mainly related to histone acetyltransferases (HATs) and histone deacetylases (HDACs). Under the catalysis of HDACs, the acetyl group of acetyl-CoA is transferred to the amino acid residues of histone tails and promotes gene transcription (*Galvani & Thiriet, 2015*). In addition, histone deacetylases cause chromatin condensation by deacetylating amino acids in histone tails, thereby repressing gene transcription (*Meier & Brehm, 2014*).

Histone acetylation regulates the stemness and differentiation process of dental-derived stem cells. For instance, histone acetyltransferases such as p300, general control non-arrestin 5 (GCN5), and lysine acetyltransferase 6B (KAT6B, also known as MORF) regulate the stemness or osteogenic differentiation of cells by modifying histones on target genes of DPSCs and PDLSCs. Among them, p300 can regulate the expression of genes DMP-1, DSPP, DSP, NANOG, and SOX2 in different ways. p300 promotes the odontogenic differentiation of DPSCs by catalyzing acetylation and promoting the expression of the histone, H3K9, within the promoter regions of DMP-1, DSPP, and DSP (*Liu et al., 2015b*). Furthermore, MORF and GCN5 are mainly involved in the osteogenic differentiation process of PDLSCs under inflammatory conditions, among which GCN5 regulates DKK1 expression through the acetylation of the H3K9 and H3K14 promoter regions (*Li et al., 2016*). DKK1 can inhibit the Wnt/β-catenin pathway and promote the osteogenic differentiation of PDLSCs. Chronic periodontal inflammation reduces the expression of MORF in PDLSCs (*Xue et al., 2016*). Methoxy-parvacrol (osthole) upregulates MORF in PDLSCs and catalyzes the acetylation of H3K9 and H3K14, which promotes the osteogenic differentiation of PDLSCs under inflammatory conditions (*Sun et al., 2017b*).

In addition, silencing HDAC expression or using histone deacetylase inhibitor (HDACi) can regulate gene expression by inhibiting HDAC activity. The inhibition of HDAC1, HDAC3, and HDAC6 expression can contribute to the odontogenic differentiation of DPSCs. The HDAC inhibitor MS-275 can act on HDAC1 and HDAC3, causing the up-regulation of the gene expression of odontogenic differentiation-related proteins in DPSC, including RUNX2, DMP1, ALP, and DSPP (*Lee et al., 2020*). Similarly, silencing HDAC6 can induce the expression of odontogenic marker genes such as OSX, OCN, and OPN in DPSCs, while inhibiting osteoclast differentiation (*Wang et al., 2018c*). In addition, HDAC6 is also involved in the development and differentiation of PDLSCs. For instance, HDAC6 participates in the aging process of PDLSCs by regulating the acetylation of $p27^{Kip1}$. The inhibition of HDAC6 promotes senescence in PDLSCs and attenuates their osteogenic

differentiation and migration abilities (*Li et al., 2017*). HDAC9, which is mainly involved in the osteogenic differentiation of PDLSCs under inflammatory conditions, impairs the osteogenic differentiation capacity of PDLSCs, whereas miR-17 induces osteogenic differentiation by inhibiting HDAC9 (*Li et al., 2018b*). Finally, HDACi can regulate the differentiation process of dental-derived stem cells by inhibiting HDAC. *Luo et al. (2018)* found that HDACi, trichostatin A, and valproic acid could enhance the acetylation of histones H3 and H4, to promote the proliferation, migration, and adhesion of DPSCs.

Noncoding RNA

Non-coding RNAs (ncRNA) are transcripts with no or low coding potential, including ribosomal RNA (rRNA), transfer RNA (tRNA), and microRNA (miRNA) (*Ren & Wang, 2021*). miRNA is the most studied in the field of epigenetics (*Ren & Wang, 2021*). miRNAs directly interact with partially complementary target sites located in the 3′ untranslated region of target mRNAs and repress their expression (*Hombach & Kretz, 2016*). Endogenous competing RNAs (ceRNAs) mainly regulate gene expression by competitively binding to miRNA (*Qi et al., 2015*). ceRNAs typically include long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs).

miRNA

miRNAs regulate dental-derived stem cells by affecting the expression of related genes in RUNX2, BMP, Wnt, MAPK, and Notch1 signaling pathways. miRNAs regulate the differentiation process of SCAPs, DPSCs, and PDLSCs by affecting the expression of the gene RUNX2, which mainly mediates osteogenic/odontogenic differentiation in stem cells (*Hussain, Tebyaniyan & Khayatan, 2022*). miR-450a-5p and miR-28-5p can affect the expression of signal transducer and activator of transcription 1 (STAT1), which is mainly involved in the negative regulation of RUNX2 (*Dernowsek et al., 2017*). An *in vitro* model system study found that STAT1 mRNA was gradually down-regulated and RUNX2 mRNA was gradually up-regulated as SHEDs differentiated into osteoblasts (*Dernowsek et al., 2017*). Similarly, miR-218 regulated the mineralization and differentiation process of DPSCs through the ERK1/2 pathway. ERK1/2 signaling converge at Runx2 to control the differentiation of DPSCs (*Chang et al., 2019*).

The transforming growth factor beta (TGF-β)/ BMP signaling pathway plays an important role in the odontogenic/osteogenic differentiation of dental-derived stem cells. miR-132 inhibits the growth differentiation factor 5 (GDF5) of the TGF-β family and activates the NF-κB axis, which attenuates the osteogenic differentiation ability of PDLSCs (*Xu et al., 2019*). CD105 is a co-receptor for the type I transmembrane glycoprotein and TGF β-1, which is associated with the osteogenic differentiation of cells. *Ishiy et al. (2018)* compared the mineralization degree of the SHED matrix with the low/high expression of CD105 and found that the high expression of CD105 reduced osteogenic potential, while miR-1287 was negatively correlated with CD105. miRNA can affect cell differentiation by regulating the expression of the Smad gene, which is an essential transcription factor in the TGF-β/BMP signaling pathway. miR-135b can inhibit the expression of the Smad4 and Smad5 genes, which hinder the odontoblast-like differentiation of dental pulp cells

(*Song et al., 2017*). In PDLSCs, miR-23a acts on the bone morphogenetic protein receptor type 1B (BMPR1B) gene and inhibits the phosphorylation of Smad1/5/9, which attenuates the osteogenic differentiation of PDLSCs (*Zhang et al., 2019b*). The Smad ubiquitination regulator (Smurf) regulates TGF-β/BMP signaling through ubiquitination, causing the degradation of signaling molecules and preventing the overactivation of TGF-β/BMP signaling (*Kushioka et al., 2020*). In SCAPs, miR-497-5p promotes bone/odontogenic differentiation by targeting SMAD-specific E3 ubiquitin protein ligase 2 (Smurf2) and regulating the Smad signaling pathway (*Liu et al., 2020b*). Furthermore, the expression of miR-26a can be upregulated in the exosomes secreted from SHED, and miR-26a can improve angiogenesis in SHED by regulating TGF-β/SMAD2/3 signaling (*Wu et al., 2021*).

Wnt/β-catenin signaling can regulate the proliferation, development, and cell fate aspects of dental-derived stem cells. The overexpression of miR-140-5p represses the Wnt1 gene, which affects Wnt/β-catenin signaling and ultimately inhibits the odontoblast differentiation of DPSCs (*Lu et al., 2019*). Chromodomain helicase DNA-binding protein 8 (CHD8) plays an essential role in maintaining the active transcription of nerve-specific genes and can be targeted and regulated by miR-221 (*Wen et al., 2020*). For example, in SHED, upregulated miR-221 activates the Wnt/β-catenin pathway by inhibiting CHD8, which promotes the neurogenic differentiation of cells (*Wen et al., 2020*).

In addition, both p38-mitogen-activated protein kinase (MAPK) and neurogenic locus Notch homolog 1 (Notch1) signaling pathways are involved in the osteogenic/odontogenic differentiation process of dental-derived stem cells. miR-143-5p can regulate the expression of MAPK pathway-related genes in DPSCs. To be specific, the downregulation of miR-143-5p increased the expression of p38 MAPK signaling pathway-related genes such as MAPK14 and MKK3/6, and odontoblast differentiation markers such as ALP and OCN (*Wang et al., 2019a*). IGF-I can enhance the odontogenic/osteogenic differentiation ability of mesenchymal stem cells (MSCs) by activating the MAPK pathway, while the IGFBPs/IGF-I complex is regulated by matrix metalloproteinase 1(MMP1) (*Wang et al., 2018b*). In SCAPs, miRNA let-7b inhibits bone/odontogenic differentiation of SCAP by targeting MMP1 (*Wang et al., 2018b*). Notch1 is a transmembrane receptor, and the downregulation of Notch signaling inhibits self-renewal of DPSCs and induces their differentiation (*Wang et al., 2011*). miR-146a-5p can inhibit the expression of Notch1 and regulate the osteogenic/odontogenic differentiation process of DPSCs (*Qiu et al., 2019*).

ceRNA

lncRNA. lncRNA can regulate the differentiation of dental-derived stem cells by directly acting on GDF5, distal-less homeobox 3 (DLX3), and Kruppel-like factor 2 (KLF2). lncRNA growth arrest specific transcript 5 (GAS5) can enhance the expression of GDF5 in cells and promote the phosphorylation of the p38 MAPK/JNK signaling pathway, which enhances the osteogenic differentiation of PDLSCs (*Yang et al., 2020c*). lncRNA H19 inhibits DNMT3B-mediated methylation of the DLX3 gene through S-adenosyl-L-homocysteine hydrolase (SAHH), which regulates odontoblast differentiation of DPSCs (*Zeng et al., 2018a*). The direct interaction of lncRNA SNHG1 with EZH2 regulates KLF2 promoter H3K27me3

methylation and inhibits the differentiation of PDLSCs to osteoblasts (*Li, Guo & Wu, 2020c*).

By inhibiting the expression of miRNAs, lncRNAs can also play a regulatory role. During the osteogenic differentiation of PDLSCs, lncRNAs can act as ceRNAs and form networks to regulate the Wnt/β-catenin signaling pathway (*Lai et al., 2022*). lncRNA-ANCR competitively binds miR-758 and inhibits the expression of Notch2, which further affects the Wnt/β-catenin signaling pathway and inhibits the osteogenic differentiation of PDLSCs (*Peng et al., 2018*). FoxO1 promotes bone formation in PDLSCs by competing with TCF-4 for β-catenin and inhibiting the Wnt pathway (*Wang et al., 2016*). lncRNA-POIR can inhibit the expression of the miR-182 target gene FoxO1 and affect the osteogenic differentiation process of PDLSCs (*Wang et al., 2016*). As ceRNAs, lncRNAs can affect the expression of genes related to the MAPK and BMP signaling pathways. LncRNA-H19 can competitively bind to miR-141 and prevent the miRNA-mediated degradation of SPAG9, thereby increasing the phosphorylation levels of p38 and JNK, which promotes the bone/odontogenic differentiation of SCAPs (*Li et al., 2019b*). In SHEDs, lncRNA C21 or f121 can compete with BMP2 to bind with miR-140-5p and promote the neurogenic differentiation of SHEDs by upregulating BMP2 expression (*Liu et al., 2018a*). lncRNA-CCAT1 combined with miR-218, and lncRNA G043225 combined with miR-588 can promote the odontogenic differentiation of DPSCs (*Chen et al., 2020a*; *Zhong et al., 2019*).

circRNA

In PDLSCs, circRNAs can indirectly regulate osteogenic differentiation by binding to miRNAs (*Gu et al., 2017*). circRNA cerebellar degeneration-related protein 1 transcript (CDR1as) and miR-7 can regulate the osteogenic differentiation and stemness of PDLSCs. CDR1as may promote the upregulation of GDF5 and the phosphorylation of Smad1/5/8 and p38 MAPK by inhibiting the expression of miR-7, inducing the differentiation of PDLSCs to osteoblasts. In addition, the interaction of CDR1as with miR-7 can also upregulate the expression of KLF4 to maintain the stemness of PDLSCs, while RNA-binding protein hnRNPM regulates its expression in PDLSCs by interacting with CDR1as (*Gu et al., 2021*). During the osteogenic differentiation of SCAPs, the expression profiles of circRNAs are significantly altered, and circRNAs mainly function as ceRNAs (*Li et al., 2019a*). circ SIPA1L1 can promote the expression of the gene ALPL (alkaline phosphatase alkaline phosphatase) by binding to miR-204-5p, which causes the osteogenic differentiation of SCAPs (*Li et al., 2020b*).

Epigenetic regulatory network

In the epigenetic regulation of dental-derived stem cells, there are multiple links between histone modifications, DNA methylation, and ncRNA, which interact with each other and participate in genetic regulation together. ncRNAs participate in the regulation of gene expression in stem cells by regulating DNA methylation. For example, lncRNA H19 can inhibit the activity of DNMT3B, which reduces the methylation of the distal-less homeobox (DLX3) of the gene, thereby promoting the odontogenic differentiation of DPSCs (*Zeng et al., 2018a*). Similarly, miR-675 can also promote the odontogenic differentiation of

DPSCs by inhibiting DNMT3B (*Zeng et al., 2018b*). The lncRNA HOTAIRM1 inhibits the expression and enrichment of DNMT1 on the HOXA2 promoter and mechanically binds to the CpG island in the HOXA2 promoter region, leading to hypomethylation and the induction of HOXA2 and DFSC differentiation into osteoblasts (*Chen et al., 2020b*).

ncRNAs can also play a role in histone modifications, including histone methylation, histone acetylation, and histone deacetylation. miR-153-3p inhibits the transcription of ALP, Runx2, and OPN by targeting KDM6A, which results in the attenuated osteogenic differentiation of PDLSCs (*Jiang & Jia, 2021*). miRNAs are involved in the aging and differentiation process of dental-derived stem cells by regulating the expression of HAT or HDAC. The upregulation of miR-152 represses HAT sirtuinc 7 (SIRT7) expression and affects the degree of histone acetylation, which accelerates the aging process of DPSCs (*Gu et al., 2016*). The upregulation of miRNA-383-5p can promote the down-regulation of the HDAC9 mRNA level, which leads to increased alkaline phosphatase activity, mineral node formation, and the expressions of RUNX2, osteocalcin, and Smad4 in PDLSCs and other osteogenic markers (*Ma & Wu, 2021*). Similarly, miR-22 can inhibit HDAC6 expression and promote the osteogenic differentiation of PDLSCs (*Yan et al., 2017*).

Therapeutic application of dental-derived stem cells in dental pulp and periodontal regeneration

In 1971, *Nygaard-Ostby & Hjortdal (1971)* proposed the concept of pulp tissue regeneration. Pulp regeneration refers to the formation of new pulp tissue through tissue engineering to replace the infected or necrotic pulp tissue, thereby restoring the structure and function of the pulp-dentin complex under physiological conditions. Conventional apexogenesis may result in the thinning of the dentin wall and underdevelopment of the root, which greatly increases the risk of long-term root fracture. However, pulp regeneration can effectively form healthy pulp tissue and promote the formation of dentin. Periodontitis, a chronic inflammation of the periodontal tissue caused by dental plaque, can cause the destruction and absorption of the alveolar bone and tooth loss. Traditional periodontal treatments, such as scaling, focuses on controlling the occurrence of inflammation, but fails to restore the structure and function of periodontal tissue entirely. Periodontal tissue regeneration reconstructs periodontal tissue damaged by periodontitis and restores its structure and function by means of tissue engineering (*Chen et al., 2010*). The key elements of tissue regeneration are stem cells, scaffolds, and signaling molecules. The biological behavior of stem cells is regulated by epigenetics. Further research will likely lead to the discovery of an increasing number of new factors. These factors may regulate the development of stem cells towards odontogenic differentiation, angiogenesis, neurogenesis, and osteogenic differentiation through epigenetic mechanisms, and may facilitate the application of pulp regeneration and periodontal regeneration. Figure 1 demonstrates the epigenetic regulation of dental-derived stem cell differentiation and its application in pulp regeneration and periodontal regeneration.

Odontogenic differentiation

Transcription factor RUNX2 mainly mediates osteogenic/odontogenic differentiation and may effectively promote the expression of dentin matrix proteins or induce the

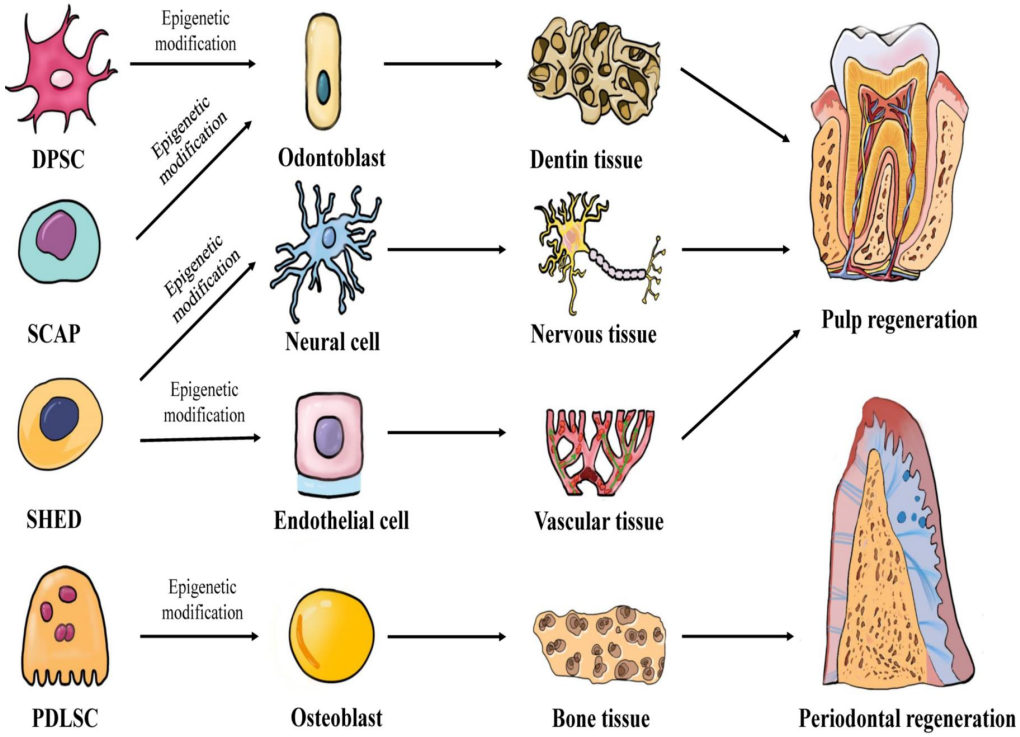

Figure 1 **Multilineage potential of human dental-derived stem cells.** Four kinds of human dental-derived stem cells have the capacity to differentiate under epigenetic modification into different somatic cell and tissue types, and finally contribute to regeneration of pulp or periodontal tissue. DPSC, Dental pulp stem cell; SCAP, Stem cells from apical papilla; SHED, Stem cells from human exfoliated deciduous teeth; PDLSC, Periodontal ligament stem cell.

Full-size DOI: 10.7717/peerj.14550/fig-1

transdifferentiation of cells into osteoblasts (*Li et al., 2011*). HDACi can affect the expression of the RUNX2 gene in stem cells by acting on HDAC. MS-275 can inhibit the expression of HDAC1 and HDAC3 and may induce the up-regulation of odontogenic related proteins in DPSCs, including RUNX2, DMP1, ALP, and DSPP, which promotes the odontogenic differentiation of DPSCs (*Lee et al., 2020*). *Sultana et al. (2021)* showed that without the induction of mineralized medium, MS-275 alone could increase the expression levels of BMP2, DMP1, DSPP, and Runx2 mRNA of mouse odontoblast-like cell line MDPC-23, and improved ALP activity. Therefore, MS-275 can effectively promote the odontogenic differentiation of DPSCs.

BMPs signal through canonical Smad and non-Smad signaling pathways, in which BMP-Smad signaling can be involved in the formation of coronal dentin (*Omi et al., 2020*). The HDACi inhibitor TSA can significantly upregulate the levels of Smad and NFI-C in DPSCs by inhibiting HDAC3. *Jin et al. (2013)* treated DPSCs with TSA and found that the expression of BSP, DMP1, and DSPP was significantly increased compared with the control group; the level of Smad2/3 was also significantly up-regulated 21 days after mineralization induction. In contrast, neonatal mice that were maternally exposed to TSA exhibited thicker dentin and more dentin cells in their postpartum molars, with a

greater ability to secrete DSP (*Jin et al., 2013*). In addition to regulating gene expression in DPSCs, *Duncan et al. (2017)* demonstrated that TSA could also promote the release of dentin matrix components from dentin. These two studies show that HDACi can promote the differentiation of DPSCs into odontoblasts as well as the release of the dentin matrix, which is very beneficial to the repair of dental pulp-dentin complex.

The Wnt/β-catenin signaling pathway may regulate the process of dentin formation and tooth development and the amplification of Wnt signaling can significantly improve the survival rate of damaged dental pulp cells and promote tertiary dentin formation (*Hunter et al., 2015*). miR-140-5p can repress the Wnt1 gene and affect the Wnt/β-catenin signaling process (*Lu et al., 2019*). *Lu et al. (2019)* collected impacted third molars from patients aged 14–22 years and divided the extracted DPSCs into an miR-140-5p inhibition group, a negative control group (NC), and a blank control group. After 14 days of inducing cells to differentiate into odontoblasts, Alizarin Red S staining showed that the mineralized matrix deposition was greatest in the inhibitor group and least in the mock group. Western blotting showed that the inhibitor group had the highest expressions of DSPP and DMP-1 proteins while the mock group had the lowest (*Lu et al., 2019*). These results indicate that miR-140-5p can affect the odontoblast differentiation process of DPSCs.

The p38 MAPK pathway is central to the transcriptional control of odontoblasts and its activation is critical for apical morphogenesis and enamel secretion (*Greenblatt et al., 2015*). The activation of the MAPK signaling pathway is also associated with the osteogenic/odontogenic differentiation of DPSCs (*Wu et al., 2019*). lncRNA-H19 can competitively bind to miR-141 and upregulate the phosphorylation levels of p38 and JNK. *Li et al. (2019b)* induced transfected SCAPs in an osteoblast differentiation medium, and the Western blot results showed that the protein expressions of OCN, RUNX2, ALP, and DSP in the H19-infected SCAP group were significantly higher than those in the control group. The SCAP that had a stable expression of H19, and the control group, were further loaded on Bio-Oss collagen scaffolds and implanted in the subcutaneous tissue of nude mice. H&E and Masson staining showed that the abundance of bone-like structures, collagen deposition, and dentin-like structures in the H19-infected SCAP group was higher than that in the control group (*Li et al., 2019b*). This indicates that lncRNA-H19 can promote the odontoblast differentiation process of SCAPs by activating the p38 and JNK signaling pathways.

Nerve regeneration

CHD8 can affect neural progenitor cells and neurons, and also plays a role in maintaining the active transcription of neural-specific genes (*Wilkinson et al., 2015*). Meanwhile, CHD8 can also alter neurogenesis and cortical development by regulating the Wnt/β-catenin signaling pathway (*Platt et al., 2017*). In SHEDs, upregulated miR-221 can bind to CHD8 and activate the Wnt/β-catenin pathway (*Wen et al., 2020*). *Wen et al. (2020)* divided the SHEDs in the third-generation logarithmic growth phase into six groups: blank group, NC group (transfected with miR-221 negative sequence), miR-221 mimic group (transfected with miR-221 mimic), miR-221 inhibitor group (transfected with miR-221 inhibitor), siRNA-CHD8 group (transfected with siRNA into CHD8 vector), and miR-221 inhibitor

+ siRNA-CHD8 group (co-transfected with miR-221 inhibitor and siRNA-CHD8). The results of Western blot analysis revealed that the expressions of NSE, NESTIN, MAP-2, NF-M, and TH in the miR-221 inhibitor group were significantly lower than those in the NC group, while the miR-221 mimic group and siRNA-CHD8 group were both lower than those in the NC group. Immunofluorescence examination showed that the expressions of NSE and MAP-2 in the miR-221 inhibitor + siRNA-CHD8 group were higher than that in the miR-221 inhibitor group (*Wen et al., 2020*). Among them, neuron-specific enolase (NSE) was a highly specific marker of neurons and peripheral neuroendocrine cells, NESTIN was a key early neural progenitor cell marker, NF-M and microtubule-associated protein 2 (MAP2) was a neuron-associated marker, and TH was the rate-limiting enzyme in dopamine neurotransmitter biosynthesis. These results suggest that miR-221 can promote SHED differentiation into neurons by inhibiting CHD8.

BMP2 is a neurotrophic factor that induces the growth of brain dopaminergic (DA) neurons *in vitro* and *in vivo*, whose induction depends on the Smad signaling pathway (*Hegarty, Sullivan & O'Keeffe, 2013*). In SHEDs, lncRNA C21 or f121 competitively binds to miR-140-5p and upregulates BMP2 expression (*Liu et al., 2018a*). The results of bioinformatics analysis conducted by *Liu et al. (2018a)* showed that there was a targeting relationship between the second spliceosome of lncRNA C21 or f121 and miR-140-5p, the same as miR-140-5p and BMP2. This suggested that lncRNA C21 or f121 competed with BMP2 to bind to miR-140-5p. *Liu et al. (2018a)* grouped and experimented with SHEDs in the third-generation logarithmic growth phase. The results showed that the protein expressions of both Nestin and βIII-tubulin decreased, but increased in the transfected miR-140-5p inhibitor group compared with the NC group (transfected with lncRNA C21 or f121 negative sequence), the BMP2 and MAP2 in the si-C21 or f121 group, the miR-140-5p group, and the si-C21 or f121+miR-140-5p group. Further experiments showed that the up-regulation of lncRNA C21 or f121 or down-regulation of miR-140-5P increased the frequency of social behavior in rats and decreased the cumulative time of repetitive stereotyped movements in young rats (*Liu et al., 2018a*). All of the abovementioned studies show that lncRNA C21 or f121 can effectively promote the neurogenic differentiation of SHEDs.

Finally, some HDACi have the effect of inducing neurogenic differentiation of cells. For instance, *Okubo et al. (2016)* found that the total number of mRNAs of mature neuronal markers, neurofilament medium polypeptide (NeFM), and microtubule-associated protein 2 (MAP2) significantly increased to approximately 80% in VPA-treated rats compared with untreated rats (*Okubo et al., 2016*). Other studies have shown that the neurite number on the cells increased and branched processes were elongated after treating MSCs with combinations of MS-275 or NaB (a kind of HDACi). The cells were visualized by immunofluorescence staining of the neuronal markers (*Jang et al., 2019*). The above studies demonstrate the role of HDACi in inducing neurogenic differentiation. Further research may reveal whether dental-derived stem cells can be inducted to differentiate into neural cells.

Angiogenesis

TGF-β/SMAD2 signaling can promote angiogenesis and the secretion of vascular endothelial growth factor (*Ji et al., 2014*). *Wu et al. (2021)* discovered that the expression of miR-26a was up-regulated in SHED-secreted exosomes (SA-Exo), and miR-26a could promote the expression of TGF-β/SMAD2/3 signaling. The expression of angiogenesis-related proteins (VEGF, angiopoietin 2 and PDGF) of SHEDs was up-regulated, and the endothelial differentiation potential was increased after being treated with SA-Exo. SA-Exo treatment also increased the expression levels of angiogenesis-related proteins in HUVECs. *Wu et al. (2021)* implanted SHED aggregates into immunodeficient mice and performed histological analysis, which revealed the formation of a new, continuous dentin layer and blood vessels, and the regeneration of the dentin-pulp complex. In addition, dentin and blood vessel formation were enhanced by the combined implantation of SHED aggregates and SA-Exo. The expression level of the angiogenic marker CD31 was also higher. The inhibition of SA-Exo repressed dentin-pulp complex regeneration; however, supplementation with exogenous SA-Exo could restores this process. The results of qRT-PCR confirmed that the expression of miR-26a was significantly increased in SA-Exo, and the inhibition of miR-26a in SA-Exo could not cause the endothelial differentiation of SHED and HUVECs. Western blot analysis revealed that the overexpression of miR-26a upregulates TGF-β/SMAD2/3 signaling, and the inhibition of these two pathways led to reduced endothelial differentiation in SHEDs and HUVECs (*Wu et al., 2021*). These studies confirm that miR-26a in SA-Exo promote angiogenesis in SHEDs through the TGF-β/SMAD2/3 signaling pathway.

ncRNAs have a strong ability to regulate endothelial cell migration, proliferation, and differentiation. miR-30a-3p targets the epigenetic factor methyl-CpG-binding protein 2 (MeCP2), and the overexpression of MeCP2 damages important genes involved in the regulation of endothelial function such as sirtuin1 (*Volkmann et al., 2013*). *Volkmann et al. (2013)* transfected endothelial cells with miR-30a-3p precursors, which significantly reduced MeCP2 protein levels and increased the migratory ability of endothelial cells. This suggests that miR-30a-3p has the ability to regulate endothelial cells. In addition, lncRNAs also have a role in regulating endothelial cells. *Neumann et al. (2018)* discovered that lncRNA GATA6 could inhibit the action of the epigenetic regulator, LOXL2, reduce the endothelial-mesenchymal transition *in vitro*, and promote the formation of blood vessels in mice. These two studies demonstrated the potential of ncRNAs in promoting angiogenesis. Future studies may reveal whether they can regulate dental-derived stem cells for angiogenesis.

Osteogenic differentiation

Different dental-derived stem cells have a diverse range of DNA methylation levels and unique osteogenic differentiation potentials. Compared with DPSCs and DFPCs, PDLSCs have lower methylation levels of genes related to osteogenesis, higher expression levels of factors such as SMAD3, ALP, OCN, and RUNX2, and a higher osteogenic differentiation potential (*Ai et al., 2018*). After culturing PDLSCs, DFPCs, and DPSCs14 in osteoinductive medium, *Ai et al. (2018)* used Alizarin Red S positive staining and found that the relative

intensity of staining in PDLSCs was significantly higher than that in DPFCs and DPSCs. The simultaneous subcutaneous transplantation of cell deposits mixed with hydroxyapatite onto the dorsal surface of immunocompromised male mice found that PDLSCs formed more osteoid. This study demonstrated that DNA methylation can regulate the osteogenic differentiation potential of dental-derived stem cells by affecting the expression of related genes.

Insulin-like growth factor (IGF) and its binding proteins play an important role in promoting bone formation (*Nguyen et al., 2013*). KDM6B can promote IGFBP5 transcription by reducing histone K27 methylation (*Han et al., 2017*). *Han et al. (2017)* found that by administering a local injection of rhIGFBP5 into the periodontitis area of a piglet model, they could significantly promote the regeneration of periodontal tissues such as alveolar bone and gingiva after 12 weeks.

The Wnt/β-catenin pathway can promote/inhibit the osteogenic differentiation of cells under various conditions (*Wagner et al., 2011*). HAT GCN5 inhibits the Wnt/β-catenin signaling pathway by increasing the levels of H3K9ac and H3K14ac in the DKK1 promoter region (*Li et al., 2016*). *Li et al. (2016)* found that more active osteogenic differentiation was presented in cell populations with higher GCN5 expression, and GCN5 downregulation may lead to defective osteogenic differentiation of PDLSCs. GCN5 knockdown resulted in increased expression of β-catenin and decreased expression of genes and proteins related to osteogenic differentiation, such as RUNX2 and ALP. ChIP assays indicated that GCN5 binds to the promoter region of DKK1. Alveolar bone loss in the first and second maxillary molars was significantly reduced with increased GCN5 expression in periodontital rats (*Li et al., 2016*). Therefore, HAT GCN5 can promote the osteogenic differentiation of PDLSCs to regenerate alveolar bone by inhibiting the Wnt/β-catenin signaling pathway.

Both the MAPK and TGF-β/Smad signaling pathways may be involved in BMP-mediated osteogenesis (*Kim, Park & Choung, 2018*; *Zhu et al., 2018*). CDR1as is an inhibitor of miR-7 that can cause the upregulation of TGF-β family member GDF5, and the phosphorylation of p38 MAPK (*Li et al., 2018c*). In PDLSCs, the knockdown of CDR1as or the overexpression of miR-7 significantly suppressed the mRNA and protein levels of GDF5. In contrast, a lower expression of GDF5 resulted in a decrease in the osteogenic markers ALP and RUNX2, as well as phosphorylated p38 MAPK. *Li et al. (2018c)* loaded CDR1as siRNA and negative control siRNA-treated PDLSCs onto scaffold material and implanted this into the calvarial defect area of nude mice. The results showed that the CDR1as knockdown group had less bone formation and significantly lower new bone formation than the control group. In the control group, bone tissue was generated at the edges of the bone defects, but in the CDR1as knockout group, little new bone was observed (*Li et al., 2018c*). This study strongly demonstrates that CDR1as can promote the osteogenic differentiation of PDLSCs.

CONCLUSIONS

Dental-derived stem cells, as mesenchymal stem cells, play an essential role in pulp and periodontal regeneration. Epigenetic regulation can adjust gene expression independent

of DNA sequence changes, which can affect the proliferation, differentiation, and function of dental-derived stem cells. DNA methylation, histone modifications, and ncRNAs constitute a grand epigenetic regulatory network that can function independently or coherently. Pulp regeneration and periodontal regeneration can be well achieved through epigenetic regulation.

Considering the various types of epigenetic modifications and different mechanisms, the epigenetic research on dental-derived stem cells is still lacking at this stage. Current research has focused on classical epigenetic modifications and modification sites, while some potential modifications such as DNA 6mA modification, mRNA m6A modification, and modification on tRNA still need more experimental verifications. Epigenetic modifications are widespread in eukaryotes; some modification mechanisms in mesenchymal stem cells can be further studied in dental-derived stem cells. As new functions of epigenetic modification are revealed, we can also focus on their regulatory roles in dental-derived stem cells.

Current research has focused on the regulatory role of specific epigenetic modification mechanisms in dental-derived stem cells. However, less attention has been paid as to whether there are interactions between epigenetic modifications, which limits our further exploration of epigenetic regulatory networks.

Studies on the epigenetic regulation of dental-derived stem cells have also been influenced by the cells themselves. The special odontogenic potential of DPSCs and the excellent osteogenic potential of PDLSCs make them the best choice for pulp regeneration and periodontal regeneration. Therefore, the epigenetic mechanisms of DPSCs and PDLSCs are currently the most studied. SCAPs and SHEDs, which are obtained from tooth roots and exfoliated deciduous teeth respectively, have also been widely studied due to their abundant sources and low immunogenicity. In contrast, the sources of DFPCs are more limited, leading to fewer studies.

Finally, the application of the epigenetic regulation of dental-derived stem cells is influenced by its mechanism of action. Tissue engineering can be accomplished through stem cells, scaffolds and signaling molecules, all of which are applicable. However, epigenetic regulation needs to act on specific modification sites, and most of the current experiments are done in the form of virus transfection, greatly limiting the application of epigenetic regulation in tissue regeneration. In addition, dental-derived stem cells interfere with the proliferation and differentiation of surrounding cells by secreting exosomes. Therefore, further research on exosomes will be beneficial to the application of epigenetic regulation in dental-derived stem cells.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by the National Natural Science Foundation of China (No. 81800929 and No. 82170921), the Chengdu Science and Technology Program (No.2019-YF05-00441-SN) and the Research and Develop Program, West China Hospital of

Stomatology Sichuan University (No. LCYJ2019-24). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
National Natural Science Foundation of China: 81800929, 82170921.
Chengdu Science and Technology Program: 2019-YF05-00441-SN.
Research and Develop Program, West China Hospital of Stomatology Sichuan University: LCYJ2019-24.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Yuyang Chen conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Xiayi Wang performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Zhuoxuan Wu performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Shiyu Jia analyzed the data, prepared figures and/or tables, and approved the final draft.
- Mian Wan conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

This is a literature review and there is no raw data.

REFERENCES

Ai T, Zhang J, Wang X, Zheng X, Qin X, Zhang Q, Li W, Hu W, Lin J, Chen F. 2018. DNA methylation profile is associated with the osteogenic potential of three distinct human odontogenic stem cells. *Signal Transduction and Targeted Therapy* **3**:1 DOI 10.1038/s41392-017-0001-6.

Barros M, Martins J, Maria D, Wenceslau C, De Souza D, Kerkis A, Câmara N, Balieiro J, Kerkis I. 2015. Immature dental pulp stem cells showed renotropic and pericyte-like properties in acute renal failure in rats. *Cell Medicine* **7**:95–108 DOI 10.3727/215517914x680038.

Blanc R, Richard S. 2017. Arginine methylation: the coming of age. *Molecular Cell* **65**:8–24 DOI 10.1016/j.molcel.2016.11.003.

Canovas S, Ross P, Kelsey G, Coy P. 2017. DNA methylation in embryo development: epigenetic impact of ART (Assisted Reproductive Technologies). *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* **39**:1700106 DOI 10.1002/bies.201700106.

Chang K, Chen RS, Chang FH, Chen MH. 2019. Promoting dentinogenesis of DPSCs through inhibiting microRNA-218 by using magnetic nanocarrier delivery. *Journal of the Formosan Medical Association* **118**:1005–1013 DOI 10.1016/j.jfma.2018.10.018.

Chen F, Zhang J, Zhang M, An Y, Chen F, Wu Z. 2010. A review on endogenous regenerative technology in periodontal regenerative medicine. *Biomaterials* **31**:7892–7927 DOI 10.1016/j.biomaterials.2010.07.019.

Chen Q, Yan W, Duan E. 2016. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nature Reviews Genetics* **17**:733–743 DOI 10.1038/nrg.2016.106.

Chen Y, Hong T, Wang S, Mo J, Tian T, Zhou X. 2017. Epigenetic modification of nucleic acids: from basic studies to medical applications. *Chemical Society Reviews* **46**:2844–2872 DOI 10.1039/c6cs00599c.

Chen Z, Zhang K, Qiu W, Luo Y, Pan Y, Li J, Yang Y, Wu B, Fang F. 2020a. Genome-wide identification of long noncoding RNAs and their competing endogenous RNA networks involved in the odontogenic differentiation of human dental pulp stem cells. *Stem Cell Research & Therapy* **11**:114 DOI 10.1186/s13287-020-01622-w.

Chen Z, Zheng J, Hong H, Chen D, Deng L, Zhang X, Ling J, Wu L. 2020b. lncRNA HOTAIRM1 promotes osteogenesis of hDFSCs by epigenetically regulating HOXA2 via DNMT1 in vitro. *Journal of Cellular Physiology* **235**:8507–8519 DOI 10.1002/jcp.29695.

Deng C, Sun Y, Liu H, Wang W, Wang J, Zhang F. 2018a. Selective adipogenic differentiation of human periodontal ligament stem cells stimulated with high doses of glucose. *PLOS ONE* **13**:e0199603 DOI 10.1371/journal.pone.0199603.

Deng L, Hong H, Zhang X, Chen D, Chen Z, Ling J, Wu L. 2018b. Down-regulated lncRNA MEG3 promotes osteogenic differentiation of human dental follicle stem cells by epigenetically regulating Wnt pathway. *Biochemical and Biophysical Research Communications* **503**:2061–2067 DOI 10.1016/j.bbrc.2018.07.160.

Dernowsek J, Pereira M, Fornari T, Macedo C, Assis A, Donate P, Bombonato-Prado K, Passos-Bueno M, Passos G. 2017. Posttranscriptional interaction between miR-450a-5p and miR-28-5p and STAT1 mRNA triggers osteoblastic differentiation of human mesenchymal stem cells. *Journal of Cellular Biochemistry* **118**:4045–4062 DOI 10.1002/jcb.26060.

Duncan H, Smith A, Fleming G, Reid C, Smith G, Cooper P. 2017. Release of bio-active dentine extracellular matrix components by histone deacetylase inhibitors (HDACi). *International Endodontic Journal* **50**:24–38 DOI 10.1111/iej.12588.

Fanganiello RD, Andre Ishiy FA, Kobayashi GS, Alvizi L, Sunaga DY, Passos-Bueno MR. 2015. Increased in vitro osteopotential in SHED associated with higher IGF2 expression when compared with hASCs. *Stem Cell Reviews and Reports* **11**:635–644 DOI 10.1007/s12015-015-9592-x.

Feng F, Akiyama K, Liu Y, Yamaza T, Wang T, Chen J, Wang B, Huang G, Wang S, Shi S. 2010. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. *Oral Diseases* **16**:20–28 DOI 10.1111/j.1601-0825.2009.01593.x.

Fernández A, Veloso P, Astorga J, Rodríguez C, Torres VA, Valdés M, Garrido M, Gebicke-Haerter PJ, Hernández M. 2020. Epigenetic regulation of TLR2-mediated periapical inflammation. *International Endodontic Journal* **53**:1229–37 DOI 10.1111/iej.13329.

Francis M, Gopinathan G, Foyle D, Fallah P, Gonzalez M, Luan X, Diekwisch T. 2020. Histone methylation: achilles heel and powerful mediator of periodontal homeostasis. *Journal of Dental Research* **99**:1332–1340 DOI 10.1177/0022034520932491.

Francis M, Pandya M, Gopinathan G, Lyu H, Ma W, Foyle D, Nares S, Luan X. 2019. Histone methylation mechanisms modulate the inflammatory response of periodontal ligament progenitors. *Stem Cells and Development* **28**:1015–1025 DOI 10.1089/scd.2019.0125.

Galvani A, Thiriet C. 2015. Nucleosome dancing at the tempo of histone tail acetylation. *Genes* **6**:607–621 DOI 10.3390/genes6030607.

Gandia C, Armiñan A, García-Verdugo J, Lledó E, Ruiz A, Miñana M, Sanchez-Torrijos J, Payá R, Mirabet V, Carbonell-Uberos F, Llop M, Montero J, Sepúlveda P. 2008. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. *Stem Cells* **26**:638–645 DOI 10.1634/stemcells.2007-0484.

Gao R, Dong R, Du J, Ma P, Wang S, Fan Z. 2013. Depletion of histone demethylase KDM2A inhibited cell proliferation of stem cells from apical papilla by de-repression of p15INK4B and p27Kip1. *Molecular and Cellular Biochemistry* **379**:115–122 DOI 10.1007/s11010-013-1633-7.

Giuliani A, Manescu A, Langer M, Rustichelli F, Desiderio V, Paino F, De Rosa A, Laino L, d'Aquino R, Tirino V, Papaccio G. 2013. Three years after transplants in human mandibles, histological and in-line holotomography revealed that stem cells regenerated a compact rather than a spongy bone: biological and clinical implications. *Stem Cells Translational Medicine* **2**:316–324 DOI 10.5966/sctm.2012-0136.

Gopinathan G, Foyle D, Luan X, Diekwisch TGH. 2019. The Wnt antagonist SFRP1: a key regulator of periodontal mineral homeostasis. *Stem Cells and Development* **28**:1004–1014 DOI 10.1089/scd.2019.0124.

Gopinathan G, Kolokythas A, Luan X, Diekwisch T. 2013. Epigenetic marks define the lineage and differentiation potential of two distinct neural crest-derived intermediate odontogenic progenitor populations. *Stem Cells and Development* **22**:1763–1778 DOI 10.1089/scd.2012.0711.

Greenblatt M, Kim J, Oh H, Park K, Choo M, Sano Y, Tye C, Skobe Z, Davis R, Park J, Bei M, Glimcher L, Shim J. 2015. p38 α MAPK is required for tooth morphogenesis and enamel secretion. *The Journal of Biological Chemistry* **290**:284–295 DOI 10.1074/jbc.M114.599274.

Gu S, Ran S, Liu B, Liang J. 2016. miR-152 induces human dental pulp stem cell senescence by inhibiting SIRT7 expression. *FEBS Letters* **590**:1123–1131 DOI 10.1002/1873-3468.12138.

Gu X, Li M, Jin Y, Liu D, Wei F. 2017. Identification and integrated analysis of differentially expressed lncRNAs and circRNAs reveal the potential ceRNA

networks during PDLSC osteogenic differentiation. *BMC Genetics* **18**:100 DOI 10.1186/s12863-017-0569-4.

Gu X, Li X, Jin Y, Zhang Z, Li M, Liu D, Wei F. 2021. CDR1as regulated by hnRNPM maintains stemness of periodontal ligament stem cells via miR-7/KLF4. *Journal of Cellular and Molecular Medicine* **25**:4501–4515 DOI 10.1111/jcmm.16541.

Guo H, Li B, Wu M, Zhao W, He X, Sui B, Dong Z, Wang L, Shi S, Huang X, Liu X, Li Z, Guo X, Xuan K, Jin Y. 2021. Odontogenesis-related developmental microenvironment facilitates deciduous dental pulp stem cell aggregates to revitalize an avulsed tooth. *Biomaterials* **279**:121223 DOI 10.1016/j.biomaterials.2021.121223.

Han N, Zhang F, Li G, Zhang X, Lin X, Yang H, Wang L, Cao Y, Du J, Fan Z. 2017. Local application of IGFBP5 protein enhanced periodontal tissue regeneration via increasing the migration, cell proliferation and osteo/dentinogenic differentiation of mesenchymal stem cells in an inflammatory niche. *Stem Cell Research & Therapy* **8**:210 DOI 10.1186/s13287-017-0663-6.

Han Y, Yang Q, Huang Y, Li X, Zhu Y, Jia L, Zheng Y, Li W. 2021. Mechanical force inhibited hPDLSCs proliferation with the downregulation of MIR31HG via DNA methylation. *Oral Diseases* **27**:1268–1282 DOI 10.1111/odi.13637.

Hegarty S, Sullivan A, O'Keeffe G. 2013. BMP2 and GDF5 induce neuronal differentiation through a Smad dependant pathway in a model of human midbrain dopaminergic neurons. *Molecular and Cellular Neurosciences* **56**:263–271 DOI 10.1016/j.mcn.2013.06.006.

Hoang M, Kim J, Kim Y, Tong E, Trammell B, Liu Y, Shi S, Lee C, Hong C, Wang C, Kim Y. 2016. Alcohol-induced suppression of KDM6B dysregulates the mineralization potential in dental pulp stem cells. *Stem Cell Research* **17**:111–121 DOI 10.1016/j.scr.2016.05.021.

Hodjat M, Khan F, Saadat K. 2020. Epigenetic alterations in aging tooth and the reprogramming potential. *Ageing Research Reviews* **63**:101140 DOI 10.1016/j.arr.2020.101140.

Hombach S, Kretz M. 2016. Non-coding RNAs: classification, biology and functioning. *Advances in Experimental Medicine and Biology* **937**:3–17 DOI 10.1007/978-3-319-42059-2_1.

Hong H, Chen X, Li K, Wang N, Li M, Yang B, Yu X, Wei X. 2020. Dental follicle stem cells rescue the regenerative capacity of inflamed rat dental pulp through a paracrine pathway. *Stem Cell Research & Therapy* **11**:333 DOI 10.1186/s13287-020-01841-1.

Hunter D, Bardet C, Mouraret S, Liu B, Singh G, Sadoine J, Dhamdhere G, Smith A, Tran X, Joy A, Rooker S, Suzuki S, Vuorinen A, Miettinen S, Chaussain C, Helms J. 2015. Wnt acts as a prosurvival signal to enhance dentin regeneration. *Journal of Bone and Mineral Research: the Official Journal Of the American Society for Bone and Mineral Research* **30**:1150–1159 DOI 10.1002/jbmr.2444.

Hussain A, Tebyaniyan H, Khayatan D. 2022. The role of epigenetic in dental and oral regenerative medicine by different types of dental stem cells: a comprehensive overview. *Stem Cells International* **2022**:5304860 DOI 10.1155/2022/5304860.

Ishiy F, Fanganiello R, Kobayashi G, Kague E, Kuriki P, Passos-Bueno M. 2018. CD105 is regulated by hsa-miR-1287 and its expression is inversely correlated with osteopotential in SHED. *Bone* **106**:112–120 DOI 10.1016/j.bone.2017.10.014.

Ito K, Tomoki R, Ogura N, Takahashi K, Eda T, Yamazaki F, Kato Y, Goss A, Kondoh T. 2020. MicroRNA-204 regulates osteogenic induction in dental follicle cells. *Journal of Dental Sciences* **15**:457–465 DOI 10.1016/j.jds.2019.11.004.

Jang S, sukho P, Hyong-Ho C, Ung Y, Maru K, Jong-Seong P, Park S, Han-Seong J. 2019. Effect of histone deacetylase inhibitors on differentiation of human bone marrow-derived stem cells into neuron-like cells. *Journal of the Chosun Natural Science* **12**:133–141 DOI 10.13160/ricns.2019.12.4.133.

Ji F, Zhu L, Pan J, Shen Z, Yang Z, Wang J, Bai X, Lin Y, Tao J. 2020. hsa_circ_0026827 promotes osteoblast differentiation of human dental pulp stem cells through the beclin1 and RUNX1 signaling pathways by sponging miR-188-3p. *Frontiers in Cell and Developmental Biology* **8**:470 DOI 10.3389/fcell.2020.00470.

Ji H, Li Y, Jiang F, Wang X, Zhang J, Shen J, Yang X. 2014. Inhibition of transforming growth factor beta/SMAD signal by MiR-155 is involved in arsenic trioxide-induced anti-angiogenesis in prostate cancer. *Cancer Science* **105**:1541–1549 DOI 10.1111/cas.12548.

Jiang H, Jia P. 2021. MiR-153-3p inhibits osteogenic differentiation of periodontal ligament stem cells through KDM6A-induced demethylation of H3K27me3. *Journal of Periodontal Research* **56**:379–387 DOI 10.1111/jre.12830.

Jin H, Park J, Choi H, Choung P. 2013. HDAC inhibitor trichostatin A promotes proliferation and odontoblast differentiation of human dental pulp stem cells. *Tissue Engineering Part A* **19**:613–624 DOI 10.1089/ten.TEA.2012.0163.

Kanafi M, Rajeshwari Y, Gupta S, Dadheech N, Nair P, Gupta P, Bhonde R. 2013. Transplantation of islet-like cell clusters derived from human dental pulp stem cells restores normoglycemia in diabetic mice. *Cytotherapy* **15**:1228–1236 DOI 10.1016/j.jcyt.2013.05.008.

Kim H, Park S, Choung S. 2018. Enhancing effects of myricetin on the osteogenic differentiation of human periodontal ligament stem cells via BMP-2/Smad and ERK/JNK/p38 mitogen-activated protein kinase signaling pathway. *European Journal of Pharmacology* **834**:84–91 DOI 10.1016/j.ejphar.2018.07.012.

Koyama N, Okubo Y, Nakao K, Bessho K. 2009. Evaluation of pluripotency in human dental pulp cells. *Journal of Oral and Maxillofacial Surgery: Official Journal of the American Association of Oral and Maxillofacial Surgeons* **67**:501–506 DOI 10.1016/j.joms.2008.09.011.

Kushioka J, Kaito T, Okada R, Ishiguro H, Bal Z, Kodama J, Chijimatsu R, Pye M, Narimatsu M, Wrana J, Inoue Y, Ninomiya H, Yamamoto S, Saitou T, Yoshikawa H, Imamura T. 2020. A novel negative regulatory mechanism of Smurf2 in BMP/Smad signaling in bone. *Bone Research* **8**:41 DOI 10.1038/s41413-020-00115-z.

Lai L, Wang Z, Ge Y, Qiu W, Wu B, Fang F, Xu H, Chen Z. 2022. Comprehensive analysis of the long noncoding RNA-associated competitive endogenous RNA

network in the osteogenic differentiation of periodontal ligament stem cells. *BMC Genomics* **23**:1 DOI 10.1186/s12864-021-08243-4.

Lee E, Kim Y, Lim H, Ki G, Seo Y. 2020. The histone deacetylase inhibitor (MS-275) promotes differentiation of human dental pulp stem cells into odontoblast-like cells independent of the MAPK signaling system. *International Journal of Molecular Sciences* **21**:5771 DOI 10.3390/ijms21165771.

Li B, Sun J, Dong Z, Xue P, He X, Liao L, Yuan L, Jin Y. 2016. GCN5 modulates osteogenic differentiation of periodontal ligament stem cells through DKK1 acetylation in inflammatory microenvironment. *Scientific Reports* **6**:26542 DOI 10.1038/srep26542.

Li B, Yu F, Wu F, Hui T, P A, Liao X, Yin B, Wang C, Ye L. 2018a. EZH2 impairs human dental pulp cell mineralization via the Wnt/β-catenin pathway. *Journal of Dental Research* **97**:571–579 DOI 10.1177/0022034517746987.

Li D, Guo B, Wu H, Tan L, Lu Q. 2015. TET family of dioxygenases: crucial roles and underlying mechanisms. *Cytogenetic and Genome Research* **146**:171–180 DOI 10.1159/000438853.

Li L, Ge J. 2022. Exosome-derived lncRNA-Ankrd26 promotes dental pulp restoration by regulating miR-150-TLR4 signaling. *Molecular Medicine Reports* **25**: DOI 10.3892/mmr.2022.12668.

Li L, Liu W, Wang H, Yang Q, Zhang L, Jin F, Jin Y. 2018b. Mutual inhibition between HDAC9 and miR-17 regulates osteogenesis of human periodontal ligament stem cells in inflammatory conditions. *Cell Death & Disease* **9**:480 DOI 10.1038/s41419-018-0480-6.

Li Q, Ma Y, Zhu Y, Zhang T, Zhou Y. 2017. Declined expression of histone deacetylase 6 contributes to periodontal ligament stem cell aging. *Journal of Periodontology* **88**:e12–e23 DOI 10.1902/jop.2016.160338.

Li Q, Yang G, Li J, Ding M, Zhou N, Dong H, Mou Y. 2020a. Stem cell therapies for periodontal tissue regeneration: a network meta-analysis of preclinical studies. *Stem Cell Research & Therapy* **11**:427 DOI 10.1186/s13287-020-01938-7.

Li S, Kong H, Yao N, Yu Q, Wang P, Lin Y, Wang J, Kuang R, Zhao X, Xu J, Zhu Q, Ni L. 2011. The role of runt-related transcription factor 2 (Runx2) in the late stage of odontoblast differentiation and dentin formation. *Biochemical and Biophysical Research Communications* **410**:698–704 DOI 10.1016/j.bbrc.2011.06.065.

Li X, Feng L, Zhang C, Wang J, Wang S, Hu L. 2022. Insulin-like growth factor binding proteins 7 prevents dental pulp-derived mesenchymal stem cell senescence via metabolic downregulation of p21, Science China. *Life Sciences* DOI 10.1007/s11427-021-2096-0.

Li X, Zheng Y, Zheng Y, Huang Y, Zhang Y, Jia L, Li W. 2018c. Circular RNA CDR1as regulates osteoblastic differentiation of periodontal ligament stem cells via the miR-7/GDF5/SMAD and p38 MAPK signaling pathway. *Stem Cell Research & Therapy* **9**:232 DOI 10.1186/s13287-018-0976-0.

Li Y, Bian M, Zhou Z, Wu X, Ge X, Xiao T, Yu J. 2020b. Circular RNA SIPA1L1 regulates osteoblastic differentiation of stem cells from apical papilla via miR-204-5p/ALPL pathway. *Stem Cell Research & Therapy* **11**:461 DOI 10.1186/s13287-020-01970-7.

Li Z, Guo X, Wu S. 2020c. Epigenetic silencing of KLF2 by long non-coding RNA SNHG1 inhibits periodontal ligament stem cell osteogenesis differentiation. *Stem Cell Research & Therapy* **11**:435 DOI 10.1186/s13287-020-01953-8.

Li Z, Li N, Ge X, Pan Y, Lu J, Gobin R, Yan M, Yu J. 2019a. Differential circular RNA expression profiling during osteogenic differentiation of stem cells from apical papilla. *Epigenomics* **11**:1057–1073 DOI 10.2217/epi-2018-0184.

Li Z, Yan M, Yu Y, Wang Y, Lei G, Pan Y, Li N, Gobin R, Yu J. 2019b. LncRNA H19 promotes the committed differentiation of stem cells from apical papilla via miR-141/SPAG9 pathway. *Cell Death & Disease* **10**:130 DOI 10.1038/s41419-019-1337-3.

Lin Y, Zheng L, Fan L, Kuang W, Guo R, Lin J, Wu J, Tan J. 2018. The epigenetic regulation in tooth development and regeneration. *Current Stem Cell Research & Therapy* **13(1)**:4–15 DOI 10.2174/1574888x11666161129142525.

Liu C, Li Q, Xiao Q, Gong P, Kang N. 2020a. CHD7 regulates osteogenic differentiation of human dental follicle cells via PTH1R signaling. *Stem Cells International* **2020**:8882857 DOI 10.1155/2020/8882857.

Liu H, Wang T, Li Q, Guan X, Xu Q. 2015b. Knock-down of p300 decreases the proliferation and odontogenic differentiation potentiality of HDPCs. *International Endodontic Journal* **48**:976–985 DOI 10.1111/iej.12392.

Liu J, Wang X, Song M, Du J, Yu J, Zheng W, Zhang C, Wang Y. 2020b. Smurf2MiR-497-5p regulates osteo/odontogenic differentiation of stem cells from apical papilla via the smad signaling pathway by targeting. *Frontiers in Genetics* **11**:582366 DOI 10.3389/fgene.2020.582366.

Liu J, Zhang Z, Yu H, Yang A, Hu P, Liu Z, Wang M. 2018a. Long noncoding RNA C21orf121/bone morphogenetic protein 2/microRNA-140-5p gene network promotes directed differentiation of stem cells from human exfoliated deciduous teeth to neuronal cells. *Journal of Cellular Biochemistry* **120**:1464–1476 DOI 10.1002/jcb.27313.

Liu Y, Gan L, Cui D, Yu S, Pan Y, Zheng L, Wan M. 2021. Epigenetic regulation of dental pulp stem cells and its potential in regenerative endodontics. *World Journal of Stem Cells* **13**:1647–1666 DOI 10.4252/wjsc.v13.i11.1647.

Liu Z, Lee HL, Suh JS, Deng P, Lee CR, Bezouglaia O, Mirnia M, Chen V, Zhou M, Cui ZK, Kim RH, Lee M, Aghaloo T, Hong C, Wang CY. 2022. The ER α/KDM6B regulatory axis modulates osteogenic differentiation in human mesenchymal stem cells. *Bone Research* **10**:3 DOI 10.1038/s41413-021-00171-z.

Lu X, Chen X, Xing J, Lian M, Huang D, Lu Y, Feng G, Feng X. 2019. miR-140-5p regulates the odontoblastic differentiation of dental pulp stem cells via the Wnt1/β-catenin signaling pathway. *Stem Cell Research & Therapy* **10**:226 DOI 10.1186/s13287-019-1344-4.

Luo H, Zhu W, Mo W, Liang M. 2020. High-glucose concentration aggravates TNF-alpha-induced cell viability reduction in human CD146-positive periodontal ligament cells via TNFR-1 gene demethylation. *Cell Biology International* **44**:2383–2394 DOI 10.1002/cbin.11445.

Luo L, Avery S, Waddington R. 2021. Exploring a chemotactic role for EVs from progenitor cell populations of human exfoliated deciduous teeth for promoting migration of Naïve BMSCs in bone repair process. *Stem Cells International* **2021**:6681771 DOI 10.1155/2021/6681771.

Luo Z, Wang Z, He X, Liu N, Liu B, Sun L, Wang J, Ma F, Duncan H, He W, Cooper P. 2018. Effects of histone deacetylase inhibitors on regenerative cell responses in human dental pulp cells. *International Endodontic Journal* **51**:767–78 DOI 10.1111/iej.12779.

Lv P, Gao P, Tian G, Yang Y, Mo F, Wang Z, Sun L, Kuang M, Wang Y. 2020. Osteocyte-derived exosomes induced by mechanical strain promote human periodontal ligament stem cell proliferation and osteogenic differentiation via the miR-181b-5p/PTEN/AKT signaling pathway. *Stem Cell Research & Therapy* **11**:295 DOI 10.1186/s13287-020-01815-3.

Ma L, Hu J, Cao Y, Xie Y, Wang H, Fan Z, Zhang C, Wang J, Wu C, Wang S. 2019. Maintained properties of aged dental pulp stem cells for superior periodontal tissue regeneration. *Aging and Disease* **10**:793–806 DOI 10.14336/ad.2018.0729.

Ma L, Wu D. 2021. MicroRNA-383-5p regulates osteogenic differentiation of human periodontal ligament stem cells by targeting histone deacetylase 9. *Archives of Oral Biology* **129**:105166 DOI 10.1016/j.archoralbio.2021.105166.

Mai Z, Chen H, Ye Y, Hu Z, Sun W, Cui L, Zhao X. 2021. Translational and clinical applications of dental stem cell-derived exosomes. *Frontiers in Genetics* **12**:750990 DOI 10.3389/fgene.2021.750990.

Mead B, Logan A, Berry M, Leadbeater W, Scheven B. 2017. Concise review: dental pulp stem cells: a novel cell therapy for retinal and central nervous system repair. *Stem Cells* **35**:61–67 DOI 10.1002/stem.2398.

Meier K, Brehm A. 2014. Chromatin regulation: how complex does it get? *Epigenetics* **9**:1485–1495 DOI 10.4161/15592294.2014.971580.

Miura M, Gronthos S, Zhao M, Lu B, Fisher L, Robey P, Shi S. 2003. SHED: stem cells from human exfoliated deciduous teeth. *Proceedings of the National Academy of Sciences of the United States of America* **100**:5807–5812 DOI 10.1073/pnas.0937635100.

Mohammad H, Barbash O, Creasy C. 2019. Targeting epigenetic modifications in cancer therapy: erasing the roadmap to cancer. *Nature Medicine* **25**:403–418 DOI 10.1038/s41591-019-0376-8.

Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, Hoffmann K. 2005. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biology: Journal of the International Society for Matrix Biology* **24**:155–165 DOI 10.1016/j.matbio.2004.12.004.

Mortada I, Mortada R. 2018. Dental pulp stem cells and osteogenesis: an update. *Cytotechnology* **70**:1479–1486 DOI 10.1007/s10616-018-0225-5.

Nakashima M, Iohara K. 2014. Mobilized dental pulp stem cells for pulp regeneration: initiation of clinical trial. *Journal of Endodontics* **40**:S26–S32 DOI 10.1016/j.joen.2014.01.020.

Neumann P, Jaé N, Knau A, Glaser S, Fouani Y, Rossbach O, Krüger M, John D, Bindereif A, Grote P, Boon R, Dimmeler S. 2018. The lncRNA GATA6-AS epigenetically regulates endothelial gene expression via interaction with LOXL2. *Nature Communications* **9**:237 DOI 10.1038/s41467-017-02431-1.

Nguyen D, Calzi SLi, Shaw L, Kielczewski J, Korah H, Grant M. 2013. An ocular view of the IGF-IGFBP system. *Growth Hormone & IGF Research: Official Journal of the Growth Hormone Research Society and the International IGF Research Society* **23**:45–52 DOI 10.1016/j.ghir.2013.03.001.

Nygaard-Ostby B, Hjortdal O. 1971. Tissue formation in the root canal following pulp removal. *Scandinavian Journal of Dental Research* **79**:333–349 DOI 10.1111/j.1600-0722.1971.tb02019.x.

Okubo T, Hayashi D, Yaguchi T, Fujita Y, Sakaue M, Suzuki T, Tsukamoto A, Murayama O, Lynch J, Miyazaki Y, Tanaka K, Takizawa T. 2016. Differentiation of rat adipose tissue-derived stem cells into neuron-like cells by valproic acid, a histone deacetylase inhibitor. *Experimental Animals* **65**:45–51 DOI 10.1538/expanim.15-0038.

Omi M, Kulkarni A, Raichur A, Fox M, Uptergrove A, Zhang H, Mishina Y. 2020. BMP-smad signaling regulates postnatal crown dentinogenesis in mouse molar. *JBMR Plus* **4**:e10249 DOI 10.1002/jbm4.10249.

Pedersen M, Helin K. 2010. Histone demethylases in development and disease. *Trends in Cell Biology* **20**:662–671 DOI 10.1016/j.tcb.2010.08.011.

Peng W, Deng W, Zhang J, Pei G, Rong Q, Zhu S. 2018. Long noncoding RNA ANCR suppresses bone formation of periodontal ligament stem cells via sponging miRNA-758. *Biochemical and Biophysical Research Communications* **503**:815–821 DOI 10.1016/j.bbrc.2018.06.081.

Platt R, Zhou Y, Slaymaker I, Shetty A, Weisbach N, Kim J, Sharma J, Desai M, Sood S, Kempton H, Crabtree G, Feng G, Zhang F. 2017. Chd8 mutation leads to autistic-like behaviors and impaired striatal circuits. *Cell Reports* **19**:335–350 DOI 10.1016/j.celrep.2017.03.052.

Qi X, Zhang D, Wu N, Xiao J, Wang X, Ma W. 2015. ceRNA in cancer: possible functions and clinical implications. *Journal of Medical Genetics* **52**:710–718 DOI 10.1136/jmedgenet-2015-103334.

Qiao W, Li D, Shi Q, Wang H, Wang H, Guo J. 2020. miR-224-5p protects dental pulp stem cells from apoptosis by targeting Rac1. *Experimental and Therapeutic Medicine* **19**:9–18 DOI 10.3892/etm.2019.8213.

Qiu Z, Lin S, Hu X, Zeng J, Xiao T, Ke Z, Lv H. 2019. Involvement of miR-146a-5p/neurogenic locus notch homolog protein 1 in the proliferation and differentiation of STRO-1 human dental pulp stem cells. *European Journal of Oral Sciences* **127**:294–303 DOI 10.1111/eos.12624.

Qu G, Li Y, Chen L, Chen Q, Zou D, Yang C, Zhou Q. 2021. Comparison of osteogenic differentiation potential of human dental-derived stem cells isolated from dental pulp, periodontal ligament, dental follicle, and alveolar bone. *Stem Cells International* **2021**:6631905 DOI 10.1155/2021/6631905.

Radhakrishnan R, Kabekkodu S, Satyamoorthy K. 2011. DNA hypermethylation as an epigenetic mark for oral cancer diagnosis. *Journal of Oral Pathology & Medicine: Official Publication Of the International Association of Oral Pathologists and the American Academy of Oral Pathology* **40**:665–676 DOI 10.1111/j.1600-0714.2011.01055.

Ren H, Wang Q. 2021. Non-coding RNA and diabetic kidney disease. *DNA and Cell Biology* **40**:553–567 DOI 10.1089/dna.2020.5973.

Ren W, Gao L, Song J. 2018. Structural basis of DNMT1 and DNMT3A-mediated DNA methylation. *Genes* **9**:620 DOI 10.3390/genes9120620.

Rolph DN, Deb M, Kanji S, Greene CJ, Das M, Joseph M, Aggarwal R, Leblebicioglu B, Das H. 2020. Ferutinin directs dental pulp-derived stem cells towards the osteogenic lineage by epigenetically regulating canonical Wnt signaling. *Biochim Biophys Acta Molecular Basis of Disease* **1866**:165314 DOI 10.1016/j.bbadis.2018.10.032.

Rombouts C, Giraud T, Jeanneau C, About I. 2017. Pulp vascularization during tooth development, regeneration, and therapy. *Journal of Dental Research* **96**:137–144 DOI 10.1177/0022034516671688.

Saito M, Silvério K, Casati M, Sallum E, Nociti F. 2015. Tooth-derived stem cells: update and perspectives. *World Journal of Stem Cells* **7**:399–407 DOI 10.4252/wjsc.v7.i2.399.

Shen WC, Lai YC, Li LH, Liao K, Lai HC, Kao SY, Wang J, Chuong CM, Hung SC. 2019. Methylation and PTEN activation in dental pulp mesenchymal stem cells promotes osteogenesis and reduces oncogenesis. *Nature Communications* **10**:2226 DOI 10.1038/s41467-019-10197-x.

Shimojima C, Takeuchi H, Jin S, Parajuli B, Hattori H, Suzumura A, Hibi H, Ueda M, Yamamoto A. 2016. Conditioned medium from the stem cells of human exfoliated deciduous teeth ameliorates experimental autoimmune encephalomyelitis. *Journal of Immunology* **196**:4164–4171 DOI 10.4049/jimmunol.1501457.

Song Z, Chen L, Wang R, Qin W, Huang S, Guo J, Lin Z, Tian Y. 2017. MicroRNA-135b inhibits odontoblast-like differentiation of human dental pulp cells by regulating Smad5 and Smad4. *International Endodontic Journal* **50**:685–693 DOI 10.1111/iej.12678.

Strahl B, Allis C. 2000. The language of covalent histone modifications. *Nature* **403**:41–45 DOI 10.1038/47412.

Sultana S, Uehara O, Yoshida K, Saito T, Abiko Y. 2021. The histone deacetylase inhibitor, entinostat (MS-275), induces the odontogenic differentiation of an odontoblast-like cell line in the absence of an osteoblast mineralization medium. *Odontology* **109**:661–671 DOI 10.1007/s10266-020-00588-8.

Sun DG, Xin BC, Wu D, Zhou L, Wu HB, Gong W, Lv J. 2017a. miR-140-5p-mediated regulation of the proliferation and differentiation of human dental pulp stem cells occurs through the lipopolysaccharide/toll-like receptor 4 signaling pathway. *European Journal of Oral Sciences* **125**:419–425 DOI 10.1111/eos.12384.

Sun J, Dong Z, Zhang Y, He X, Fei D, Jin F, Yuan L, Li B, Jin Y. 2017b. Osthole improves function of periodontitis periodontal ligament stem cells via epigenetic modification in cell sheets engineering. *Scientific Reports* **7**:5254 DOI 10.1038/s41598-017-05762-7.

Surani M, Hayashi K, Hajkova P. 2007. Genetic and epigenetic regulators of pluripotency. *Cell* **128**:747–762 DOI 10.1016/j.cell.2007.02.010.

Townsend G, Hughes T, Luciano M, Bockmann M, Brook A. 2009. Genetic and environmental influences on human dental variation: a critical evaluation of studies involving twins. *Archives of Oral Biology* **54(1)**:S45–S51 DOI 10.1016/j.archoralbio.2008.06.009.

Tu S, Zheng J, Gao X, Guan C, Cai B, Xiang L. 2018. The role of Foxq1 in proliferation of human dental pulp stem cell. *Biochemical and Biophysical Research Communications* **497**:543–549 DOI 10.1016/j.bbrc.2018.02.077.

Uribe-Etxebarria V, García-Gallastegui P, Pérez-Garrastachu M, Casado-Andrés M, Irastorza I, Unda F, Ibarretxe G, Subirán N. 2020. Wnt-3a induces epigenetic remodeling in human dental pulp stem cells. *Cells* **9**:652 DOI 10.3390/cells9030652.

Volkmann I, Kumarswamy R, Pfaff N, Fiedler J, Dangwal S, Holzmann A, Batkai S, Geffers R, Lother A, Hein L, Thum T. 2013. MicroRNA-mediated epigenetic silencing of sirtuin1 contributes to impaired angiogenic responses. *Circulation Research* **113**:997–1003 DOI 10.1161/circresaha.113.301702.

Wagner E, Zhu G, Zhang B, Luo Q, Shi Q, Huang E, Gao Y, Gao J, Kim S, Rastegar F, Yang K, He B, Chen L, Zuo G, Bi Y, Su Y, Luo J, Luo X, Huang J, Deng Z, Reid R, Luu H, Haydon R, He T. 2011. The therapeutic potential of the Wnt signaling pathway in bone disorders. *Current Molecular Pharmacology* **4**:14–25 DOI 10.2174/1874467211104010014.

Wang B, Wang Z, Nan X, Zhang Q, Liu W. 2019a. Downregulation of microRNA-143-5p is required for the promotion of odontoblasts differentiation of human dental pulp stem cells through the activation of the mitogen-activated protein kinases 14-dependent p38 mitogen-activated protein kinases signaling pathway. *Journal of Cellular Physiology* **234**:4840–4850 DOI 10.1002/jcp.27282.

Wang F, Chen X, Han Y, Xi S, Wu G. 2019b. circRNA CDR1as regulated the proliferation of human periodontal ligament stem cells under a lipopolysaccharide-induced inflammatory condition. *Mediators of Inflammation* **2019**:1625381 DOI 10.1155/2019/1625381.

Wang L, Wu F, Song Y, Li X, Wu Q, Duan Y, Jin Z. 2016. Long noncoding RNA related to periodontitis interacts with miR-182 to upregulate osteogenic differentiation in periodontal mesenchymal stem cells of periodontitis patients. *Cell Death & Disease* **7**:e2327 DOI 10.1038/cddis.2016.125.

Wang L, Yang H, Lin X, Cao Y, Gao P, Zheng Y, Fan Z. 2018a. KDM1A regulated the osteo/dentinogenic differentiation process of the stem cells of the apical papilla via binding with PLOD2. *Cell Proliferation* **51**:e12459 DOI 10.1111/cpr.12459.

Wang Q, Yan Y, Zhang X, Lv J, Nie H, Wu J, Wu D, Yuan S, Tang C. 2022. Rescuing effects of periostin in advanced glycation end-products (AGEs) caused

osteogenic and oxidative damage through AGE receptor mediation and DNA methylation of the CALCA promoter. *Chemico-Biological Interactions* **354**:109835 DOI 10.1016/j.cbi.2022.109835.

Wang X, He F, Tan Y, Tian W, Qiu S. 2011. Inhibition of Delta1 promotes differentiation of odontoblasts and inhibits proliferation of human dental pulp stem cell in vitro. *Archives of Oral Biology* **56**:837–845 DOI 10.1016/j.archoralbio.2011.02.006.

Wang Y, Jia S. 2009. Degrees make all the difference: the multifunctionality of histone H4 lysine 20 methylation. *Epigenetics* **4**:273–276 DOI 10.4161/epi.4.5.9212.

Wang Y, Pang X, Wu J, Jin L, Yu Y, Gobin R, Yu J. 2018b. MicroRNA hsa-let-7b suppresses the odonto/osteogenic differentiation capacity of stem cells from apical papilla by targeting MMP1. *Journal of Cellular Biochemistry* **119**:6545–6554 DOI 10.1002/jcb.26737.

Wang Y, Shi Z, Feng J, Cao J. 2018c. HDAC6 regulates dental mesenchymal stem cells and osteoclast differentiation. *BMC Oral Health* **18**:190 DOI 10.1186/s12903-018-0624-1.

Wei J, Sun X, Hou B. 2021. Evaluation of silk fibroin-RGD-stem cell factor scaffold effect on adhesion, migration, and proliferation of stem cells of apical papilla. *Stem Cells International* **2021**:6612324 DOI 10.1155/2021/6612324.

Wen B, He C, Zhang Q, Zhang F, Li N, Pan Y, Deng M, Wang Y, Li J, Qiu J. 2020. Overexpression of microRNA-221 promotes the differentiation of stem cells from human exfoliated deciduous teeth to neurons through activation of Wnt/β-catenin pathway via inhibition of CHD8. *Cell Cycle* **19**:3231–3248 DOI 10.1080/15384101.2020.1816308.

Whiting D, Chung W, Johnson J, Paranjpe A. 2018. Characterization of the cellular responses of dental mesenchymal stem cells to the immune system. *Journal of Endodontics* **44**:1126–1131 DOI 10.1016/j.joen.2018.03.018.

Wilkinson B, Grepo N, Thompson B, Kim J, Wang K, Evgrafov O, Lu W, Knowles J, Campbell D. 2015. The autism-associated gene chromodomain helicase DNA-binding protein 8 (CHD8) regulates noncoding RNAs and autism-related genes. *Translational Psychiatry* **5**:e568 DOI 10.1038/tp.2015.62.

Wu J, Li N, Fan Y, Wang Y, Gu Y, Li Z, Pan Y, Romila G, Zhou Z, Yu J. 2019. The conditioned medium of calcined tooth powder promotes the osteogenic and odontogenic differentiation of human dental pulp stem cells via MAPK signaling pathways. *Stem Cells International* **2019**:4793518 DOI 10.1155/2019/4793518.

Wu M, Liu X, Li Z, Huang X, Guo H, Guo X, Yang X, Li B, Xuan K, Jin Y. 2021. SHED aggregate exosomes shuttled miR-26a promote angiogenesis in pulp regeneration via TGF-β/SMAD2/3 signalling. *Cell Proliferation* **54**:e13074 DOI 10.1111/cpr.13074.

Wu Y, Lian K, Sun C. 2020. LncRNA LEF1-AS1 promotes osteogenic differentiation of dental pulp stem cells via sponging miR-24-3p. *Molecular and Cellular Biochemistry* **475**:161–169 DOI 10.1007/s11010-020-03868-7.

Xie L, Guan Z, Zhang M, Lyu S, Thuaksuban N, Kamolmattayakul S, Nuntanaranont T. 2020. Exosomal circLPAR1 promoted osteogenic differentiation of homotypic

dental pulp stem cells by competitively binding to hsa-miR-31. *Biomed Research International* **2020**:6319395 DOI 10.1155/2020/6319395.

Xu Y, Ren C, Zhao X, Wang W, Zhang N. 2019. microRNA-132 inhibits osteogenic differentiation of periodontal ligament stem cells via GDF5 and the NF- κB signaling pathway. *Pathology, Research and Practice* **215**:152722 DOI 10.1016/j.prp.2019.152722.

Xue P, Li B, An Y, Sun J, He X, Hou R, Dong G, Fei D, Jin F, Wang Q, Jin Y. 2016. Decreased MORF leads to prolonged endoplasmic reticulum stress in periodontitis-associated chronic inflammation. *Cell Death and Differentiation* **23**:1862–1872 DOI 10.1038/cdd.2016.74.

Yan G, Wang X, Yang F, Yang M, Zhang G, Wang G, Zhou Q. 2017. MicroRNA-22 promoted osteogenic differentiation of human periodontal ligament stem cells by targeting HDAC6. *Journal of Cellular Biochemistry* **118**:1653–1658 DOI 10.1002/jcb.25931.

Yang H, Li G, Han N, Zhang X, Cao Y, Cao Y, Fan Z. 2020a. Secreted frizzled-related protein 2 promotes the osteo/odontogenic differentiation and paracrine potentials of stem cells from apical papilla under inflammation and hypoxia conditions. *Cell Proliferation* **53**:e12694 DOI 10.1111/cpr.12694.

Yang H, Liang Y, Cao Y, Cao Y, Fan Z. 2020b. Homeobox C8 inhibited the osteo-/dentinogenic differentiation and migration ability of stem cells of the apical papilla via activating KDM1A. *Journal of Cellular Physiology* **235**:8432–8445 DOI 10.1002/jcp.29687.

Yang N, Li Y, Wang G, Ding Y, Jin Y, Xu Y. 2017. Tumor necrosis factor-alpha suppresses adipogenic and osteogenic differentiation of human periodontal ligament stem cell by inhibiting miR-21/Spry1 functional axis. *Differentiation* **97**:33–43 DOI 10.1016/j.diff.2017.08.004.

Yang Q, Han Y, Liu P, Huang Y, Li X, Jia L, Zheng Y, Li W. 2020c. Long noncoding RNA GAS5 promotes osteogenic differentiation of human periodontal ligament stem cells by regulating GDF5 and p38/JNK signaling pathway. *Frontiers in Pharmacology* **11**:701 DOI 10.3389/fphar.2020.00701.

Yang R, Huang H, Han C, Cui S, Zhou Y, Zhou Y. 2021. Serine metabolism controls dental pulp stem cell aging by regulating the DNA methylation of p16. *Journal of Dental Research* **100**:90–97 DOI 10.1177/0022034520958374.

Yang X, Ma Y, Guo W, Yang B, Tian W. 2019. Stem cells from human exfoliated deciduous teeth as an alternative cell source in bio-root regeneration. *Theranostics* **9**:2694–2711 DOI 10.7150/thno.31801.

Ye Y, Ke Y, Liu L, Xiao T, Yu J. 2021. CircRNA FAT1 regulates osteoblastic differentiation of periodontal ligament stem cells via miR-4781-3p/SMAD5 pathway. *Stem Cells International* **2021**:5177488 DOI 10.1155/2021/5177488.

Yu T, Liu D, Zhang T, Zhou Y, Shi S, Yang R. 2019. Inhibition of Tet1-and Tet2-mediated DNA demethylation promotes immunomodulation of periodontal ligament stem cells. *Cell Death & Disease* **10**:780 DOI 10.1038/s41419-019-2025-z.

Yuan X, Dong Z, Shen S. 2022. GACAT3LncRNA: a promising biomarker and therapeutic target in human cancers. *Frontiers in Cell and Developmental Biology* **10**:785030 DOI 10.3389/fcell.2022.785030.

Zarzour A, Kim H, Weintraub N. 2019. Epigenetic regulation of vascular diseases. *Arteriosclerosis, Thrombosis, and Vascular Biology* **39**:984–990 DOI 10.1161/atvbaha.119.312193.

Zeng L, Sun S, Han D, Liu Y, Liu H, Feng H, Wang Y. 2018a. Long non-coding RNA H19/SAHH axis epigenetically regulates odontogenic differentiation of human dental pulp stem cells. *Cellular Signalling* **52**:65–73 DOI 10.1016/j.cellsig.2018.08.015.

Zeng L, Zhao N, Li F, Han D, Liu Y, Liu H, Sun S, Wang Y, Feng H. 2018b. miR-675 promotes odontogenic differentiation of human dental pulp cells by epigenetic regulation of DLX3. *Experimental Cell Research* **367**:104–111 DOI 10.1016/j.yexcr.2018.03.035.

Zhang C, Han X, Liang Y, Liu H, Fan Z, Zhang J. 2020. The histone demethylase KDM3B promotes osteo-/odontogenic differentiation, cell proliferation, and migration potential of stem cells from the Apical Papilla. *Stem Cells International* **2020**:8881021 DOI 10.1155/2020/8881021.

Zhang J, Ding H, Liu X, Sheng Y, Liu X, Jiang C. 2019a. Dental follicle stem cells: tissue engineering and immunomodulation. *Stem Cells and Development* **28**:986–994 DOI 10.1089/scd.2019.0012.

Zhang D, Li Q, Rao L, Yi B, Xu Q. 2015. Effect of 5-Aza-2'-deoxycytidine on odontogenic differentiation of human dental pulp cells. *Journal of Endodontics* **41**:640–645 DOI 10.1016/j.joen.2014.12.006.

Zhang S, Zhang R, Qiao P, Ma X, Lu R, Wang F, Li C, L E, Liu H. 2021. Metformin-induced MicroRNA-34a-3p downregulation alleviates senescence in human dental pulp stem cells by targeting CAB39 through the AMPK/mTOR signaling pathway. *Stem Cells International* **2021**:6616240 DOI 10.1155/2021/6616240.

Zhang Y, Li S, Yuan S, Zhang H, Liu J. 2019b. MicroRNA-23a inhibits osteogenesis of periodontal mesenchymal stem cells by targeting bone morphogenetic protein signaling. *Archives of Oral Biology* **102**:93–100 DOI 10.1016/j.archoralbio.2019.04.001.

Zhao S, Cheng Y, Kim J. 2019. microRNA-146a downregulates IL-17 and IL-35 and inhibits proliferation of human periodontal ligament stem cells. *Journal of Cellular Biochemistry* **120**:13861–13866 DOI 10.1002/jcb.28659.

Zhen L, Jiang X, Chen Y, Fan D. 2017. MiR-31 is involved in the high glucose-suppressed osteogenic differentiation of human periodontal ligament stem cells by targeting Satb2. *American Journal of Translational Research* **9**:2384–2393.

Zhong Y, Li W, Liao L, Liang L. 2019. LncRNA CCAT1 promotes cell proliferation and differentiation via negative modulation of miRNA-218 in human DPSCs. *European Review for Medical and Pharmacological Sciences* **23**:3575–3583 DOI 10.26355/eurrev_201905_17779.

Zhu J, Liao Y, Li F, Hu Y, Li Q, Ma Y, Wang H, Zhou Y, He B, Su Y. 2018. Wnt11 promotes BMP9-induced osteogenic differentiation through BMPs/Smads and p38 MAPK in mesenchymal stem cells. *Journal of Cellular Biochemistry* **119**:9462–9473 DOI 10.1002/jcb.27262.