Epigenetic regulation of odontogenic stem cells and their application in pulp and periodontal regeneration

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Abstract

Dental-derived stem cells, an important research target in tissue engineering, have excellent proliferation ability and multi-directional differentiation potential. In recent years, an increasing number of odontogenic stem cells have been discovered, including dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous tooth (SHEDs), apical papilla stem cells (SCAPs), dental follicle precursor cells (DFPCs) and periodontal ligament stem cells (PDLSCs). Due to the advantages of abundant sources, safe and effective, these stem cells have significant application prospects in tissue regeneration. The biological functions of odontogenic 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 stem cell fate. Existing studies have shown that odontogenic stem cells are also regulated by epigenetic modifications. Referring to replacing damaged pulp and periodontal tissue and restoring the tissue structure and function under normal physiological conditions, pulp regeneration and periodontal regeneration has better therapeutic effect than traditional treatments. This article reviews the recent research progress on the epigenetic regulation mechanism of odontogenic stem cells, and describes the potential applications of odontogenic stem cells-based epigenetic regulation in dental pulp and periodontal regeneration.

1. Introduction

Odontogenic stem cells, derived from dental papilla or dental follicle, can be isolated and cultured in teeth or periodontal soft tissue. Dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous tooth (SHEDs), and apical papilla stem cells (SCAPs) are all derived from dental papillae. Dental follicle precursor cells (DFPCs) and periodontal ligament stem cells (PDLSCs) are derived from dental follicle. In recent years, with the gradual discovery of odontogenic stem cells, their rich sources, 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, which plays an important role in the self-renewal and differentiation capacity of adult and embryonic stem cells[1]. Epigenetic regulation exists widely in natural organisms and participates in many biological processes, such as embryogenesis, germ cell formation, hematopoietic stem cell differentiation, tumor formation, etc [2–4]. More importantly, epigenetic modifications can not only regulate tooth formation, development and aging, but also affect the differentiation of odontogenic stem cells [5–7]. Epigenetic modifications have the advantage of not causing permanent DNA damage, off-target effects and deleterious mutations. Therefore, more and more studies have begun to focus on the role of epigenetic modifications in regulating the proliferation and differentiation of odontogenic stem cells.

Endodontic and periodontal diseases are common and frequently-occurring diseases in 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 can 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 odontogenic stem cells, pulp

and periodontal regeneration have gradually become the most potential way to treat these two diseases. With the gradual revealing of the mechanism of epigenetic modification of odontogenic stem cells, epigenetic regulation has gradually become a promising field in dental pulp and periodontal regeneration. This paper focuses on the epigenetic regulation of odontogenic stem cells, describes the application of epigenetic regulation based on odontogenic stem cells in dental pulp and periodontal regeneration, summarizes the shortcomings of existing research, and proposes possible future research directions.

2. Methods

The PubMed database for March 2022 was searched for relevant articles published in English using the keywords "odontogenic stem cells", "DNA methylation", "histone modifications" and "non-coding RNAs". Among the 482 articles obtained, we further read the titles, abstracts and texts of the articles, and finally excluded duplicates and out-of-scope articles. Non-research and non-review articles were not considered, including interim reports, letters, and short newsletters. The screening yielded 158 articles with high relevance to the keywords, based on which we summarized the epigenetic regulatory mechanisms of tooth-derived stem cells and their application in pulp regeneration and periodontal regeneration. All methods were carried out in accordance with relevant guidelines and regulations.

2.Properties of odontogenic stem cells

Stem cells have significant proliferative capacity and multi-directional differentiation potential. With the development of regenerative medicine, stem cells are widely used to repair damaged cells, tissues and organs which have low self-healing abilities, with excellent safety and efficacy. Odontogenic stem cells mediate the process of tooth regeneration by upregulating odontogenic and angiogenic capacity in the form of secreted exosomes (Exo) [8]. At present, odontogenic stem cells have been carried out in relevant clinical trials in the fields of periodontal tissue, maxillofacial bone tissue repair and apical pulp disease treatment [9-11]. At the same time, preclinical models of them for nerve regeneration, diabetes and autoimmune diseases have been preliminarily validated [12-14].

2.1 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 [15,16]. After dental pulp is damaged, dental pulp stem cells 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 [17-19].DPSCs can effectively promote pulp and periodontal regeneration. Guo et al. combined decellularized tooth matrix (DTM) with human dental pulp stem cells, and successfully achieved the regeneration of dental pulp and periodontal tissue [20].

2.2 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 [21]. SHEDs can express osteocalcin and RUNX-2 markers, resulting in the differentiation potential of osteoblast and odontoblast [22]. 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 [23]. SHEDs can also induce pulp and periodontal regeneration. Yang et al. combined SHEDs cell sheets and DFSCs cell sheets with dentin matrix (TDM) and implanted them into the orthotopic jawbone of nude mice [24]. The results indicated that SHED/TDM successfully achieved periodontal tissue regeneration with better migration ability and neurogenic differentiation potential [24].

2.3 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. Compared with other odontogenic stem cells, SCAPs are less resistant to immune cell-mediated toxicity, but can induce high levels of pro-inflammatory cytokine secretion [25]. 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 [26]. Wei et al. 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 [27]. This approach is promising in the further use in cell homing in dental pulp regeneration.

2.4 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 [28]. DFPCs are derived from neural crest and are direct precursor cells of periodontal tissue [29]. DFPCs can promote pulp regeneration through the paracrine pathway. Hong et al. 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 [30] .

2.5 Properties and potential of PDLSCs

Periodontitis often leads to destructions of periodontal tissue and even tooth loss. After root canal treatment, the periodontal tissue is the only source of nutrition for root canals. Multiple preclinical demonstrated that it was effective to use PDLSCs to restore damaged periodontal tissue in periodontal regenerative therapy [31]. PDLSCs are mainly isolated from the periodontal tissue of permanent teeth and can be further differentiated into osteoblasts, chondrocytes and adipocytes under appropriate conditions [32]. Studies discovered that PDLSCs had the strongest osteogenic ability, followed by DPSCs and the weakest DFPCs [33].

3. Epigenetic Regulations Of Odontogenic 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 [34]. The way epigenetic mechanisms regulate gene expression which is related to environmental factors, plays an important role in the development of various diseases such as tumors and inflammation [35,36]. In addition, epigenetic regulatory mechanisms may also affect the shape and size of teeth [5]. The common epigenetic regulations mainly include DNA methylation, histone modification and non-coding RNA regulations. In Tables 1-3, we summarize the regulatory mechanisms and potential applications of DNA methylation, histone modifications and ncRNAs in odontogenic stem cells.

3.1DNA methylation

DNA methylation is one of the important epigenetic modifications and is widespread in most eukaryotes. The formation of 5-methylcytosine (5mC) from cytosine-phosphorothioate-guanine (CpG) dinucleotides by DNA methyltransferase (DNMT) leads to Silencing of gene expression [37].The level of methylated CpG is mainly regulated by DNMT[38] and DNA demethylase ten-eleven translocation(TET) [39].

DNA methylation levels are associated with stemness and differentiation potential of odontogenic stem cells. DNMT inhibitors, metabolic enzymes and Wnt signaling factors can all affect stemness and tooth/bone-derived differentiation by modulating DNA methylation levels of DPSCs. When DPSCs are treated with the DNMT inhibitor 5-Aza-2'-deoxycytidine (5-Aza-CdR), the expressions of RUNX2 (runt-related transcription factor 2), DLX5 (distal-less homeobox 5) and other genes are up-regulated, which promote the odontogenic growth and differentiation of DPSCs [40]. Decreased expression of the serine metabolism-related enzyme phosphoserine aminotransferase 1 (PSAT1) provides less methyl donor S-adenosylmethionine (SAM) for the aging marker p16 (CDNK2A) methylation, resulting in reduced stemness and osteogenic differentiation capacity of DPSCs [41]. Wnt can effectively regulate the epigenetic mechanism of DPSCs. Short-term activation of Wnt signaling by Wnt-3A can cause a decrease in the content of 5-methylcytosine (5-mC) in DPSCs, which in turn reduces the ability of DPSCs to differentiate into osteoblasts [42].

Different odontogenic stem cell genes have different 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 DNA methylation profiles. The transcription levels of osteogenic differentiation-related factors are higher in PDLSCs, together with their osteogenic differentiation potential is also higher[43] . 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 [44]. Additionally, Periostin (POSTN) can reduce the level of AGE receptors and DNA methylation of CALCA promoter, thereby attenuating the inhibitory effect of AGE induction [44].

3.2 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 [45] . The regulations of specific genes can also be achieved through the binding of specific proteins to sites. Among the modifications of histones, there are many studies on methylation and acetylation.

3.2.1 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) [46]. 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 both inhibit gene expression [47]. On the other hand, 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 [48].

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 [49,50]. 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 [51,52].

Besides, histone demethylases such as KDM6B, KDM1A and KDM2A also play a regulatory role in the gene expression of odontogenic stem cells . KDM6B catalyzes demethylation of histone H3K27me3 located near the promoter of bone morphogenetic protein-2 (BMP2), which activates BMP2 expression and promotes osteogenic and odontogenic growth of dental mesenchymal stem cells[53,54]. 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 [55].

KDM1A can cooperate with 2-oxoglutarate 5-dioxygenase 2 (PLOD2) to regulate the differentiation process of SACPs [56]. Knockdown of KDM1A or PLOD2 reduces ALP activity, promotes the expression of DSPP, DMP1 and RUNX2, and enhances bone/dentin production in SCAPs [56]. Homeobox C8 (homeobox, HOXC8) significantly inhibits the osteogenic differentiation ability of SCAPs by directly binding to the KDM1A promoter and enhancing its transcription [57].

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 [58]. On the other hand, attenuation of KDM2A prevents cell cycle progression in G1/S phase of SCAPs [58]. Apart from that, inflammation and hypoxia can cause upregulation of KDM2A expression and repress the secreted frizzled-related protein 2

(SFRP2) transcription by reducing histone methylation in the SFRP2 promoter [59]. SFRP2 can inhibit the Wnt/β-catenin signaling pathway and further inhibit target genes of the nuclear factor kappa B (NF-kB) signaling pathway, which enhances the bone/odontogenic differentiation capacity of SCAPs[59].Similarly, histone demethylase KDM3B is also capable of regulating the bone/dental differentiation, cell proliferation and migratory potential of SCAPs[60].

3.2.2 Histones 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 [61]. In addition, histone deacetylases cause chromatin condensation by deacetylating amino acids in histone tails, thereby repressing gene transcription [62].

Histone acetylation regulates the stemness and differentiation process of odontogenic 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 the acetylation and promoting the expression of histone H3K9 within the promoter regions of DMP-1, DSPP and DSP [63]. At the same time, p300 can also maintain the stemness of DPSCs by regulating the expression of NANOG and SOX2 [64]. Furthermore, MORF and GCN5 are mainly involved in the osteogenic differentiation process of PDLSCs under inflammatory conditions, among which GCN5 regulates DKK1 expression through acetylation of H3K9 and H3K14 promoter regions [65]. 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 [66]. Methoxyparvacrol (Osthole) upregulates MORF in PDLSCs and catalyzes the acetylation of H3K9 and H3K14, which promotes the osteogenic differentiation of PDLSCs under inflammatory conditions [67].

In addition, silencing HDAC expression or using histone deacetylase inhibitor (HDACi) can regulate gene expression by inhibiting HDAC activity. 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, which cause the up-regulation of gene expression of odontogenic differentiation-related proteins in DPSC, including RUNX2, DMP1, ALP and DSPP[68]. Similarly, silencing of HDAC6 induce the expression of odontogenic marker genes such as OSX, OCN and OPN in DPSCs, while inhibiting osteoclast differentiation [69]. 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 $p27^{Kip1}$. Inhibition of HDAC6 promotes senescence in PDLSCs and attenuates their osteogenic differentiation and migration abilities [70]. HDAC9, 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 [71]. Finally, HDACi can regulate the differentiation process of odontogenic stem cells by inhibiting HDAC. Studies have shown that HDACi trichostatin A (TSA) promotes the osteogenesis of DPSCs by promoting the

expression of proliferating cell nuclear antigen (PCNA), DSPP and DMP1 [72]. Similarly, coculture of DPSCs with HDACi such as valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA) or LMK-235 can promote cell proliferation and odontogenic differentiation [73-76].

3.3 Noncoding RNA

Non-coding RNA (ncRNAs) refers to RNA that does not encode any protein, including ribosomal RNA (rRNA), transfer RNA (tRNA) and microRNA (miRNA),among which microRNA is the most studied regulation method in the field of epigenetics [77]. miRNA can lead to transcriptional degradation or post-translational inhibition of target mRNA by binding to the 3'-untranslated region, thereby regulating gene expression [77]. Endogenous competing RNAs(CeRNAs) mainly regulate gene expression by competitively binding to miRNA [78].CeRNAs mainly include long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs).

3.3.1 miRNA

miRNAs regulate odontogenic 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. 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 [79]. 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 [79]. Similarly, miR-218 regulated the mineralization and differentiation process of DPSCs and PDLSCs by acting on the RUNX2 gene [80].

Transforming growth factor beta (TGF-β)/ BMP signaling pathway plays an important role in the odontogenic/osteogenic differentiation of odontogenic 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 [81]. CD105 is a co-receptor for type I transmembrane glycoprotein and TGFβ-1, which is associated with osteogenic differentiation of cells. By comparing the mineralization degree of SHED matrix with low/high expression of CD105, Ishiy et al. found that high expression of CD105 reduced osteogenic potential, while miR-1287 was negatively correlated with CD105 [82]. miRNA can affect cell differentiation by regulating the expression of Smad gene, which is an essential transcription factor in the TGF-β/BMP signaling pathway. Besides, miR-135b can inhibit the expression of genes Smad4 and Smad5, which hinder the odontoblast-like differentiation of dental pulp cells [83]. 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 [84]. Smad ubiquitination regulator (Smurf) regulates TGF-β/BMP signaling through ubiquitination, causing degradation of signaling molecules and preventing overactivation of TGF-β/BMP signaling [85]. 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 [86] . Furthermore, the expression of miR-26a can be upregulated in exosomes secreted from SHED, and miR-26a improve angiogenesis in SHED by regulating TGF-β/SMAD2/3 signaling [87].

Wnt/β-catenin signaling can regulate proliferation, development and cell fate aspects of odontogenic stem cells. Overexpression of miR-140-5p represses the Wnt1 gene, which affects Wnt/β-catenin signaling and ultimately inhibits odontoblast differentiation of DPSCs [88]. 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 [89]. For example, in SHED, upregulated miR-221 activates the Wnt/β-catenin pathway by inhibiting CHD8, which promotes neurogenic differentiation of cells [89].

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 odontogenic stem cells. miR-143-5p can regulate the expression of MAPK pathway-related genes in DPSCs. To be specific, 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[90] . IGF-I can enhance the odontogenic/osteogenic differentiation ability of MSCs by activating the MAPK pathway, while the IGFBPs/IGF-I complex is regulated by matrix metalloproteinase 1(MMP1) [91]. In SCAPs, miRNA let-7b inhibits bone/odontogenic differentiation of SCAP by targeting MMP1 [91]. Notch1 is a transmembrane receptor, and downregulation of Notch signaling inhibits self-renewal of DPSCs and induces thier differentiation [92]. miR-146a-5p can inhibit the expression of Notch1 and regulate the osteogenic/odontogenic differentiation process of DPSCs[93].

3.3.2CeRNA

3.3.2.1LncRNA

LncRNA can regulate the differentiation of odontogenic 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 p38 MAPK/JNK signaling pathway, which enhances the osteogenic differentiation of PDLSCs[94]. LncRNA H19 inhibits DNMT3B-mediated methylation of DLX3 gene through S-adenosyl-L-homocysteine hydrolase (SAHH), which regulates odontoblast differentiation of DPSCs[95]. And direct interaction of lncRNA SNHG1 with EZH2 regulates KLF2 promoter H3K27me3 methylation and inhibits differentiation of PDLSCs to osteoblasts [96].

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[97]. 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 [98]. FoxO1 promotes bone formation in PDLSCs by competing with TCF-4 for β-catenin and inhibiting the Wnt pathway [99]. And lncRNA-POIR can inhibit the expression of miR-182 target gene FoxO1 and affect the osteogenic differentiation process of PDLSCs [99]. 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 miRNA-mediated degradation of SPAG9, thereby increasing the phosphorylation levels of p38 and JNK, which promotes the bone/odontogenic differentiation of SCAPs [100]. In SHEDs, lncRNA C21 or f121 can compete with BMP2 for binding to miR-140-5p and promote neurogenic differentiation of SHEDs by

upregulating BMP2 expression [101]. Eventually, lncRNA-CCAT1 combined with miR-218 and lncRNA G043225 combined with miR-588 can both promote the odontogenic differentiation of DPSCs [102,103].

3.3.2.2 CircRNA

In PDLSCs, circRNAs can indirectly regulate the osteogenic differentiation by binding to miRNAs [104]. CircRNA cerebellar degeneration-related protein 1 transcript (CDR1as) and miR-7 can regulate the osteogenic differentiation and stemness of PDLSCs. CDR1as can promote the upregulation of GDF5 and phosphorylation of Smad1/5/8 and p38 MAPK by inhibiting the expression of miR-7, inducing 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 [105]. During the osteogenic differentiation of SCAPs, the expression profiles of circRNAs are significantly altered, and circRNAs mainly function as ceRNAs [106]. 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 [107].

3.4 Epigenetic regulatory network

In the epigenetic regulation of odontogenic 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 [95]. Similarly, miR-675 can also promote the odontogenic differentiation of DPSCs by inhibiting DNMT3B [108]. The lncRNA HOTAIRM1 can not only inhibit the expression and enrichment of DNMT1 on the HOXA2 promoter, but also mechanically bind to the CpG island in the HOXA2 promoter region, leading to hypomethylation and induction of HOXA2 and DFSC differentiation into osteoblasts [109].

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 attenuated osteogenic differentiation of PDLSCs [110]. miRNAs are involved in the aging and differentiation process of odontogenic 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 [111]. The upregulation of miRNA-383-5p can promote the down-regulation of HDAC9 mRNA level, which further leads to increased alkaline phosphatase activity, mineral node formation and the expressions of RUNX2, osteocalcin and Smad4 in PDLSCs and other osteogenic markers [112]. Similarly, miR-22 can inhibit HDAC6 expression and promote osteogenic differentiation of PDLSCs [113].

Table 1 Regulation of DNA methylation in odontogenic 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	Pulp regeneration	41
DNA demethylation	DPSCs	Wnt	WNT-3A activates Wnt signaling by diminishing their 5mC content.	NNMT	Pulp regeneration	42
DNA demethylation	DPSCs	OSX、DLX5、RUNX2	5-Aza-CdR induced the expression of OSX、DLX5 and RUNX2 by decreasing DNA methylation.	DSPP、DMP1	Pulp regeneration	40
DNA methylation	DPSCs	KDM6B	Alcohol suppressed KDM6B through dysregulating DNA methylation	ALP、BMP2、BMP4、DLX2、OCN、OPN	Pulp regeneration	53
DNA methylation	PDLSCs	MIR31HG	Mechanical force downregulates MIR31HG through DNA methylation.	IL-6	Periodontal ligament regeneration	114
DNA demethylation	PDLSCs	CALAL	POSTN attenuated the AGE-induced CALAL methylation	RUNX2、OSX、OPEN、RANGE	Periodontal bone regeneration	44
DNA methylation	PDLSCs	DKK-1	Downregulation of Tet1 and Tet2 leads to hypermethylation of DKK-1 promoter, activating WNT pathway.	FasL	Periodontal regeneration	115
DNA methylation	PDLSCs	TNFR-1 gene	High-glucose upregulates TNFR-1 via CpG island hypomethylation.	TNFR-1 protein	Periodontal regeneration	116
DNA methylation	SHEDs	IGF2	IGF2 was induced via DNA methylation and RXR/RAR pathways activation.	RUNX2、ALP、BGLAP、DLX5	Pulp regeneration	117
DNA demethylation	DFSCs	HOXA2	HOTAIRM1 induced HOXA2 via DNA hypomethylation	DSPP、DMP1	Periodontal tissue regeneration	109

PSAT1、phosphoserine aminotransferase 1、PHGDH、phosphoglycerate、5 mC、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、ysine specific demethylase 6B、IL-6、interleukin-6、ALP、alkaline phosphatase.

Table 2、Regulation of histone modifications in odontogenic stem cells

Modification	Stem cell	Locus	Pathway mechanism	Target protein	Potential applications	Ref
Histone acetylation	DPSCs	HAT/KAT8	WNT-3A activates Wnt by induces HAT expression and increased H3AC.	ACLY	Pulp regeneration	42
Histone acetylation and methylation	DPSCs	WNT3A、DVL3	Ferutinin regulates Wnt/β-catenin pathway by H3K9 acetylation and H3K4 trimethylation.	Osteocalcin、collagen 1A1	Periodontal bone regeneration	118
Histone demethylation	DPSCs	BMP2、HOX	KDM6B catalyzes the demethylation of H3K27me3 and activates BMP2 and HOX	ALP、BMP2、BMP4、DLX2、OCN、OPN	Pulp regeneration	53
Histone demethylation	DFSCs	Wnt	Downregulated MEG3/EZH2 activated Wnt/β-catenin signaling pathway via demethylation on H3K27	β-catenin and Wnt5a protein	Periodontal tissue regeneration	52
Histone methylation	DFSCs	PTH1R	CHD7 activates PTH/PTH1R signaling pathway and interaction with H3K4me	RUNX2、SP7、BGLAP、DLX5、BMP2、COL1A1	Periodontal bone regeneration	119
Histone methylation	DFSCs	RUNX2、MSX2、DLX5、OSX	Mineralization induction promote the expression of neurogenesis genes by histone modification	DSPP、DMP1	Pulp regeneration	49
Histone methylation	DFSCs	SFRP1	WAY-316606 inhibits SFRP1 via histone H3K4me3 and activates Wnt pathway	β-catenin、RUNX2、ALP、osteocalcin, collagen I	Periodontal tissue regeneration	120
Histone methylation	PDLSCs	COL1A1、RUNX2、IL-1β、CCL5	LPS downregulated COL1A1, COL3A1、RUNX2 by H3K4me3 and upregulated CCL5、DEFA4、 IL-	COL1A1、COL3A1、RUNX2、CCL5、DEFA4、IL-1β	Periodontal lineage regeneration	121

			1β gene expression by H3K27me3			
Histone methylation	PDLSCs	RUNX2、MSX2、DLX5	The H3K4me3 active methyl mark globally switch to the H3K27me3 repressive mark under osteogenic induction conditions.	DSPP、DMP1	Periodontal regeneration	50
Histone demethylation	PDLSCs	IGFBP5	BCOR inhibits IGFBP5 through histone K27 methylation.	ALP	Periodontal tissue regeneration	55
Histone methylation	SCAPs	$p15^{INK4B}$;$p27^{Kip1}$	KDM2A increased H3K4 trimethylation at loci p15 and p27	cyclin-CDK	Pulp regeneration	58

H3AC：acetylated-Histone 3；H3K4me3：the 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；$p15^{INK4B}$：cyclin-dependent kinase inhibitor 2B；$p27^{Kip1}$：cyclin-dependent kinase inhibitor 1B.

Table 3：The regulatory role of ncRNAs in odontogenic 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	Pulp regeneration	122
miRNA	DPSCs	Rac1	miR-224-5p targets the 3'-untranslated region of Rac1 gene and downregulates Rac1.	MAPK8、caspase-3、caspase-9、Fas ligand	Pulp regeneration	123
miRNA	DPSCs	TGFBR1	miR-24-3p and LEF1-AS1 sponged to regulate TGFBR1 expression.	RUNX2、OSX、ALP	Pulp regeneration	124
miRNA	DPSCs	CAB39	miR-34a-3p activates AMPK/mTOR signaling pathway by downregulating CAB39	AMPK、mTOR	Pulp regeneration	125
miRNA	DPSCs	Foxq1	miR-320b mediated Foxq1 upregulation after calcium hydroxide stimulation.	cyclin E1、cyclin D1	Pulp regeneration	126
miRNA	DFSCs	Runx2, ALP and SPARC	miR-204 negatively targets the gene of Runx2、ALP and SPARC.	Runx2, ALP and SPARC	Pulp regeneration	127
miRNA	PDLSCs	IL-17、IL-35	Overexpression of miRNA-146a downregulates IL-17 and IL-35 expression under periodontitis	IL-17、IL-35	Periodontal lineage regeneration	128
miRNA	PDLSCs	PTEN	miR-181b-5p regulates PTEN/AKT pathway and promotes BMP2/ Runx2	PKB、BMP2、Runx2	Pulp regeneration	129
miRNA	PDLSCs	Satb2	miR-31 promotes Satb2 siRNA and inhibits osteogenic differentiation	Runx2	Periodontal bone regeneration	130
miRNA	PDLSCs	Spry1	Upregulating miR-21 repressing Spry1 and inhibits TNF-α	Spry1、TNF-α	Periodontal regeneration	131
miRNA	PDLSCs	Notch2	miR-758 regulated Notch2 and interacts with lncRNA-ANCR	Notch2	Periodontal bone regeneration	98
circRNA	DPSCs	SATB2、RUNX2、OCN	Exosome circLPAR1 induced osteogenic differentiation via downregulation of hsa-miR-31.	SATB2、RUNX2、and OCN	Pulp regeneration	132

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	Periodontal bone regeneration	133
circRNA	SCAPs	ALPL	CircSIPA1L1 is sponge for miR-204-5p，which upregulates ALPL.	ALPL	Pulp regeneration	107
circRNA	PDLSCs	SMAD5	circFAT1 inhibits miR-4781-3p targeting SMAD5.	SMAD5	Periodontal bone regeneration	134
circRNA	PDLSCs	ERK	CDR1as functioned as an miR-7 sponge to activate the ERK signal pathway.	ERK	Periodontitis regeneration	135
lncRNA	SHEDs	BMP2	lncRNA C21orf121 competes with BMP2 binding to miR-140-5p, upregulates BMP2 expression.	BMP2、Nestin、βIII-tubulin、MAP2、NSE	Pulp regeneration	101
lncRNA	SCAPs	ALP、RUNX2	LncRNA-H19 bound to miR-141, elevating phosphorylated levels of p38 and JNK.	SPAG9	Pulp regeneration	100

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，ofneuron-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.

4. Therapeutic Application Of Odontogenic Stem Cells In Dental Pulp Regeneration

In 1971, Nygaard-Östby and Hjortdal proposed the concept of pulp tissue regeneration [136]. 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 apexoplasty may result in 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 fail 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 [137]. The key elements of tissue regeneration are stem cells, scaffolds and signaling molecules. The biological behavior of stem cells is regulated by epigenetics. With development of the research, an increasing number of new factors will be discovered. These factors regulate the development of stem cells towards odontogenic differentiation, angiogenesis, neurogenesis and osteogenic differentiation through epigenetic mechanisms, and will thereby facilitate the application of pulp regeneration and periodontal regeneration. In Figure 1, we demonstrate the epigenetic regulation of odontogenic stem cell differentiation and its application in pulp regeneration and periodontal regeneration.

4.1 Odontogenic differentiation

Transcription factor RUNX2, mainly mediates osteogenic/odontogenic differentiation, can effectively promote the expression of dentin matrix proteins or induce the transdifferentiation of cells into osteoblasts [138]. HDACi can affect the expression of RUNX2 gene in stem cells by acting on HDAC. MS-275 can inhibit the expression of HDAC1 and HDAC3 and induce the up-regulation of odontogenic related proteins in DPSCs, including RUNX2, DMP1, ALP and DSPP, which promotes odontogenic differentiation of DPSCs [68]. The study by Sultana et al. showed that without 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 [139]. In conclusion, 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 [140]. The HDACi inhibitor TSA can significantly upregulate the levels of Smad and NFI-C in DPSCs by inhibiting HDAC3. Jin et al. treated DPSCs with TSA and found that the expression of BSP, DMP1, DSPP was significantly increased compared with the control group, and 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 [72]. In addition to regulating gene expression in DPSCs, Duncan et al. demonstrated that TSA could also promote the release of dentin matrix components from dentin [141]. These two studies show that HDACi can promote not only the differentiation of DPSCs into odontoblasts, but also the release of dentin matrix, which is very beneficial to the repair of dental pulp-dentin complex.

The Wnt/β-catenin signaling pathway can 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 [142]. miR-140-5p can repress Wnt1 gene and affect Wnt/β-catenin signaling process [88].Lu et al. collected impacted third molars from patients aged 14-22 years and divided the extracted DPSCs into miR-140-5p inhibition group, negative control group (NC) and blank control group. After 14 days of induction of cells to differentiate into odontoblasts, Alizarin Red S staining showed that the mineralized matrix deposition was the most in the inhibitor group and the 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 [88]. 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[143]. The activation of MAPK signaling pathway is also associated with osteogenic/odontogenic differentiation of DPSCs[144]. LncRNA-H19 can competitively bind to miR-141 and upregulate the phosphorylation levels of p38 and JNK. Li et al. induced transfected SCAPs in 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 stably expressing 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 [100]. This indicates that LncRNA-H19 can promote the odontoblast differentiation process of SCAPs by activating p38 and JNK signaling pathways.

4.2 Nerve regeneration

CHD8 can affect neural progenitor cells and neurons, and also plays a role in maintaining active transcription of neural-specific genes [145]. Meanwhile, CHD8 can also alter neurogenesis and cortical development by regulating the Wnt/β-catenin signaling pathway [146]. In SHEDs, upregulated miR-221 can bind to CHD8 and activate the Wnt/β-catenin pathway [89]. Wen et al. divided the SHEDs in the third-generation logarithmic growth phase into 6 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 exhibited 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 [89]. 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, while 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 [147]. In SHEDs, lncRNA C21 or f121 competitively binds to miR-140-5p and upregulates BMP2 expression [101]. The results of bioinformatics analysis conducted by Liu et al. showed that there was a targeting relationship between the second spliceosome of lncRNA C21orf121 and miR-140-5p, the same as miR-140-5p and BMP2. This suggested that lncRNA C21 or f121 competed with BMP2 for binding to miR-140-5p. Liu et al. grouped and experimented with SHEDs in the third-generation logarithmic growth phase. The results showed that compared with the NC

group (transfected with lncRNA C21 or f121 negative sequence), BMP2 and MAP2 in the si-C21orf121 group, the miR-140-5p group and the si-C21orf121+miR-140-5p group, The protein expressions of both Nestin and βIII-tubulin were decreased, but increased in the transfected miR-140-5p inhibitor group. Further exper iments exhibited that up-regulation of lncRNA C21orf121 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 [101].All the above 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, the study by Cui et al. found that the total number of remyelinated nerve fibers and renervated muscle fibers was significantly increased in VPA-treated rats compared with untreated VPA-treated rats, along with faster recovery of motor function [148]. Another research exhibited the application of VPA at progenitor stages strongly inhibited cell proliferation and induced neuronal differentiation, while VPA also inhibited glial differentiation of hippocampal neural progenitor cells through acetylation of histone H4 associated with pro-neural genes [149]. The above studies demonstrate the role of HDACi in inducing neurogenic differentiation, and it is hoped that further research will reveal whether it can induce odontogenic stem cells to differentiate into neural cells.

4.3 Angiogenesis

TGF-β/SMAD2 signaling can promote secretion of vascular endothelial growth factor and angiogenesis [150]. Wu et al. 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 [87]. After being treated with SA-Exo, the expression of angiogenesis-related proteins (VEGF, angiopoietin 2 and PDGF) of SHEDs was up-regulated, and the endothelial differentiation potential was increased. Meanwhile, SA-Exo treatment also increased the expression levels of angiogenesis-related proteins in HUVECs. Wu et al. implanted SHED aggregates into immunodeficient mice and performed histological analysis, which showed 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, and the expression level of the angiogenic marker CD31 was also higher. While inhibition of SA-Exo inhibited dentin-pulp complex regeneration, supplementation with exogenous SA-Exo could rescue 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 endothelial differentiation of SHED and HUVECs. Eventually, by using western blot analysis, they found that overexpression of miR-26a upregulates TGF-β/SMAD2/3 signaling, and inhibition of these two pathways also led to reduced endothelial differentiation in SHEDs and HUVECs [87]. These studies confirm that miR-26a in SA-Exo promote angiogenesis in SHEDs through 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 overexpression of MeCP2 directly damage important genes involved in the regulation of endothelial function such as sirtuin1 [151]. Olkmann et al. transfected endothelial cells with miR-30a-3p precursors significantly reduced MeCP2 protein levels and

increased migration ability of endothelial cell migration ability [151]. 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. found that the lncRNA GATA6-could inhibit the action of the epigenetic regulator LOXL2, reduced the endothelial-mesenchymal transition in vitro and promoted the formation of blood vessels in mice [152]. These two studies demonstrate the potential of ncRNAs in promoting angiogenesis, and it is hoped that further studies in the future will reveal whether they can regulate odontogenic stem cells for angiogenesis.

4.4 Osteogenic differentiation

Different odontogenic stem cells have different DNA methylation levels and different 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 higher osteogenic differentiation potential [43]. After culturing PDLSCs, DFPCs and DPSCs14 in osteoinductive medium, Ai et al. 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. Simultaneous subcutaneous transplantation of cell deposits mixed with hydroxyapatite onto the dorsal surface of immunocompromised male mice found that PDLSCs formed more osteoid [43]. This study demonstrate that DNA methylation can regulate the osteogenic differentiation potential of odontogenic 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 [153]. KDM6B can promote IGFBP5 transcription by reducing histone K27 methylation[55,154]. Experiments conducted by Liu et al. targeting KDM6B by short hairpin RNA (shRNA) demonstrated the effect of KDM6B on IGFBP5 [154]. Knockdown of IGFBP5 significantly inhibited the proliferation, osteogenic differentiation and mineralization of PDLSCs. While using TNFα to simulate inflammation, the use of rhIGFBP5 (recombinant human IGFBP5) significantly promoted cell proliferation and mineralization. By local injection of rhIGFBP5 into the periodontitis area of the piglet model, Han et al. found that this could significantly promote the regeneration of periodontal tissues such as alveolar bone and gingiva after 12 weeks [55]. The above studies show that KDM6B can effectively promote the regeneration of periodontal support tissues by up-regulating the expression of IGFBP5.

The Wnt/β-catenin pathway can promote/inhibit the osteogenic differentiation of cells under different conditions [155]. HAT GCN5 inhibits the Wnt/β-catenin signaling pathway by increasing the levels of H3K9ac and H3K14ac in the DKK1 promoter region [65]. The study carried out by Li et al. found that more active osteogenic differentiation was presented in cell populations with higher GCN5 expression, and GCN5 downregulation may led 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 bound to the promoter region of DKK1. Alveolar bone loss in first and second maxillary molars was significantly reduced with increased GCN5 expression in periodontitis rats [65]. Therefore, HAT GCN5 can promote the osteogenic differentiation of PDLSCs to regenerate alveolar bone by inhibiting the Wnt/β-catenin signaling pathway.

Both MAPK and TGF-β/Smad signaling pathways can be involved in BMP-mediated osteogenesis[156,157]. CDR1as is an inhibitor of miR-7 that can cause upregulation of TGF-β family member GDF5 and phosphorylation of p38 MAPK [158]. In PDLSCs, either knockdown of CDR1as or overexpression of miR-7 significantly suppresses the mRNA and protein levels of GDF5. In contrast, decreased GDF5 expression results in decreased osteogenic markers ALP and RUNX2, as well as phosphorylated p38 MAPK. Li et al. loaded CDR1as siRNA and negative control siRNA-treated PDLSCs onto scaffold material and implanted 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[158]. This study strongly demonstrates that CDR1as can promote the osteogenic differentiation of PDLSCs.

5. Retrospect And Outlook

Considering the various types of epigenetic modifications and different mechanisms, the epigenetic research on odontogenic stem cells is still lacking at this stage. Current research focuses 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, and some modification mechanisms demonstrated in mesenchymal stem cells are expected to be further studied in odontogenic stem cells. At the same time, as new functions of epigenetic modification are revealed, we can also focus on their regulatory roles in odontogenic stem cells.

DNA methylation, histone modifications, and ncRNAs can all participate in the epigenetic regulation of odontogenic stem cells in an independent or interactive manner, thereby affecting proliferation, differentiation, and mobility. Current research focuses more on the regulatory role of a certain epigenetic modification mechanism in odontogenic stem cells, but pay less attention to explore whether there are interactions between epigenetic modifications, which limits our further exploration of epigenetic regulatory networks.

Studies on the epigenetic regulation of odontogenic 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. Apart from that, SCAPs and SHEDs, which are obtained from tooth roots and exfoliated deciduous teeth respectively, have also been widely studied due to the abandant sources and low immunogenicity. In contrast, the sources of DFPC are more limited, leading to fewer studies.

Finally, the application of epigenetic regulation of odontogenic stem cells is influenced by the way it works. Tissue engineering can be accomplished through stem cells, scaffolds and signaling molecules, which are all 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, both of which greatly limits the application of epigenetic regulation in tissue regeneration. Studies have shown that odontogenic stem cells regulate the

growth process of cells by secreting exosomes, therefore, further research on exosomes will be beneficial to the better application of epigenetic regulation in odontogenic stem cells.

6. Conclusion

Odontogenic stem cells, as mesenchymal stem cells, play an essential role in pulp regeneration and periodontal regeneration. Epigenetic regulation can regulate gene expression independent of DNA sequence changes, which can affect the proliferation, differentiation and driving function of odontogenic stem cells. DNA methylation, histone modifications, and ncRNAs constitute a grand epigenetic regulatory network that can function independently or interact with each other. Pulp regeneration and periodontal regeneration can be well achieved through epigenetic regulation. However, current research on epigenetic regulation in odontogenic stem cells is still in the early stage with further research still needed.

Declarations

Conflicts of Interest

The authors declare that they have no competing interest.

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Author contributions

W.Z. wrote the main manuscript text and W.X., C.Y. and J.S. prepared figures 1 and tables 1-3. W.M. supervised and wrote manuscript. All authors reviewed the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

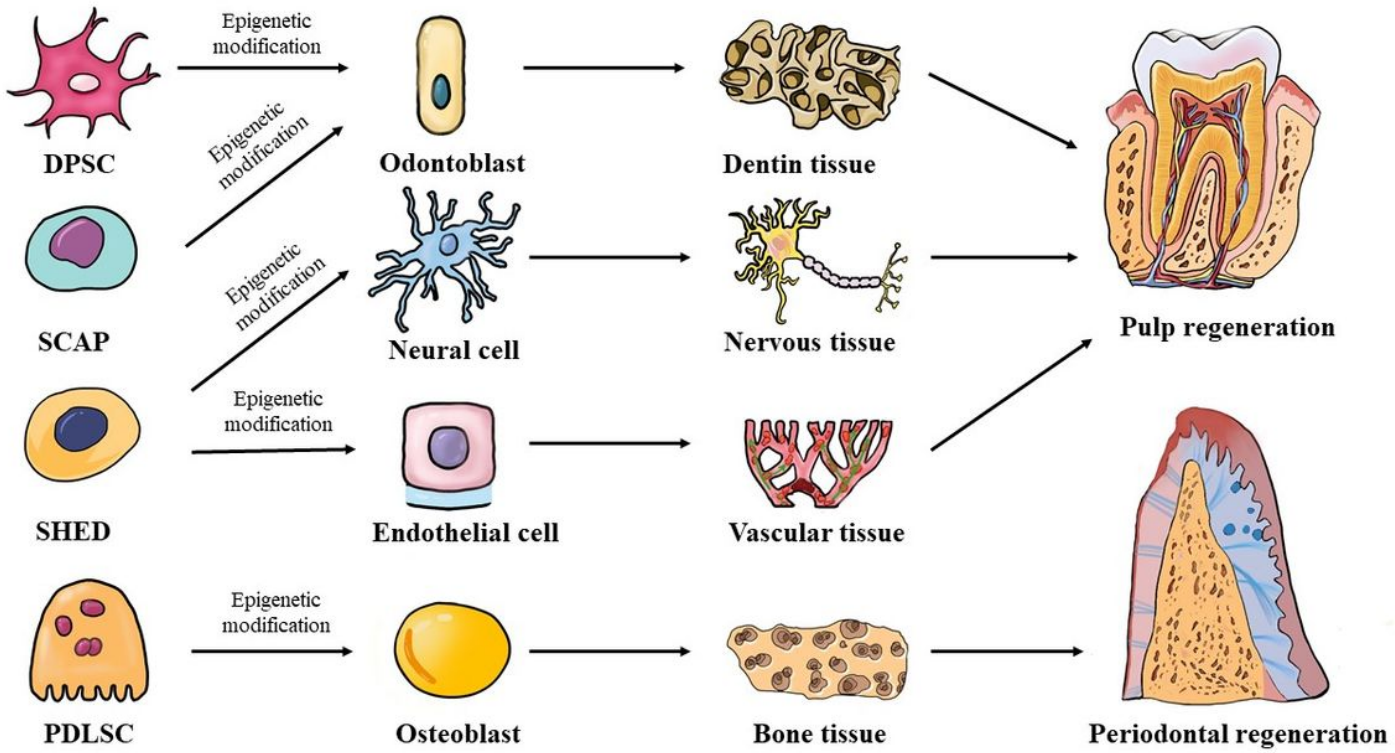

Figure 1

Multilineage potential of human odontogenic stem cells. Four kinds of human odontogenic 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.