Epigenetic regulation of dental pulp stem cells and its potential in regenerative endodontics

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Abstract

Regenerative endodontics (RE) therapy means physiologically replacing damaged pulp tissue and regaining functional dentin–pulp complex. Current clinical RE procedures recruit endogenous stem cells from the apical papilla, periodontal tissue, bone marrow and peripheral blood, with or without application of scaffolds and growth factors in the root canal space, resulting in cementum-like and bone-like tissue formation. Without the involvement of dental pulp stem cells (DPSCs), it is unlikely that functional pulp regeneration can be achieved, even though acceptable repair can be acquired. DPSCs, due to their specific odontogenic potential, high proliferation, neurovascular property, and easy accessibility, are considered as the most eligible cell source for dentin–pulp regeneration. The regenerative potential of DPSCs has been demonstrated by recent clinical progress. DPSC transplantation following pulpectomy has successfully reconstructed neurovascularized pulp that simulates the physiological structure of natural pulp. The self-renewal, proliferation, and odontogenic differentiation of DPSCs are under the control of a cascade of transcription factors. Over recent decades, epigenetic modulations implicating histone modifications, DNA methylation, and noncoding (nc)RNAs have manifested as a new layer of gene regulation. These modulations exhibit a profound effect on the cellular activities of DPSCs. In this review, we offer an overview about epigenetic regulation of the fate of DPSCs; in particular, on the proliferation, odontogenic differentiation, angiogenesis, and neurogenesis. We emphasize recent discoveries of epigenetic molecules that can alter DPSC status

and promote pulp regeneration through manipulation over epigenetic profiles.

Key Words: Dental pulp stem cells; Regenerative endodontics; Epigenetic regulation; Noncoding RNAs; Histone deacetylase inhibitor; DNA methyltransferase inhibitor

Core Tip: We review the role of epigenetic modifications during fate determination of dental pulp stem cells, highlighting cellular processes implicating proliferation, odontogenesis, angiogenesis, and neurogenesis that are tightly correlated with regenerative endodontics (RE). We emphasize the potential of epigenetic manipulation through enzyme inhibitors in RE and provide insights for future development in regaining dental pulp function.

INTRODUCTION

Regenerative endodontics (RE) has been defined as "biologically-based procedures designed to physiologically replace damaged tooth structures, including dentin and root structures, as well as cells of the pulp–dentin complex"[1]. The goal of RE is the restoration of the natural function of the dental pulp, including sensing exogenous stimuli, activating defense reactions, and forming reparative dentin, which contributes to the long-term preservation of natural teeth and dentition. Up till now, clinical RE procedures without cell transplantation, such as revascularization and cell homing, are capable of eliminating apical periodontitis. Yet most studies have indicated that they are unlikely to achieve pulp–dentin regeneration. Hence, there is an urgent desire to achieve pulp regeneration to develop a novel RE procedure that will not only solve apical periodontitis but also restore organized pulp–dentin complex structure and function. To this end, three essential elements of tissue engineering are recommended for further study: scaffold, growth factors, and stem cells. It has been demonstrated that pulp–dentin regeneration *in vivo* is achieved through RE with dental pulp stem cells (DPSCs). DPSCs were first isolated from dental pulp tissue of permanent third molars, namely permanent DPSCs (pDPSCs)[2]. Later, DPSCs were collected from human exfoliated deciduous teeth, which are named stem cells from human exfoliated deciduous teeth (SHED)[3]. Considering the remarkable potential of odontogenesis, vasculogenesis and neurogenesis *in vivo* and *in vitro*, DPSCs have been prized in pulp–dentin complex regeneration[4-6]. The latest clinical trial has detected pulp–dentin regeneration with blood vessels, sensory nerves, and lining odontoblast layer by implanting autologous SHED into necrotic immature permanent incisors. The regenerated dental pulp tissue promotes root elongation and apical foramen closure [7]. DPSCs have shown potential in pulp–dentin complex regeneration and have important directive significance for RE clinically.

It has been documented that odontogenesis is controlled by an intricate regulatory network composed of exogenous signaling stimuli, endogenous signaling molecules, and epigenetic regulators[8,9]. The epigenetic regulation, without DNA sequence changing, is made up of post-translational modifications of histones, DNA methylation, and nuclear regulatory ncRNAs[10]. Epigenetic regulation plays a crucial role in odontogenesis, eventually yielding the entire variety of dental tissues comprising complex teeth. Global epigenomes are indispensable to our understanding of gene regulation, cell fate determination, tooth development, and regeneration[11, 12]. The levels of acetylated histone H3 Lysine 9 (H3K9ac) and H3K27ac increase during odontoblast differentiation of mouse dental papilla cells. These changes are coordinated by the upregulation of histone acetyltransferase (HAT) p300 and downregulation of histone deacetylase (HDAC) 3[13]. The limited odontoblast differ-

entiation of DPSCs is enhanced by overexpression of *p300*[14] or knockdown of *HDACs*[15]. The application of HDAC inhibitor (HDACi) in promoting odontogenesis, such as odontoblast differentiation, has been highlighted in several studies. Entinostat (MS-275), a selective HDACi targeting HDAC1 and HDAC3, could induce DPSC odontoblast differentiation even without mineralization medium[16]. The significant mineralized tissue regenerative potential of HDACis has been confirmed in several animal models[17,18], and their application in RE is anticipated.

Therefore, a thorough understanding of this epigenetic regulation is important for researchers and endodontists to maximize the odontogenesis potential of DPSCs and fully realize pulp–dentin regeneration and RE. In this review, we discuss epigenetic mechanisms, including ncRNAs, histone modifications, and DNA methylation, and research progress in modulating cell fate determination of DPSCs. It has become clear that the regulation of epigenetic layer plays an essential role in the pulp–dentin regeneration based on DPSCs, and has potential in RE, which is also discussed.

PDPSCS, SHED AND THEIR CHARACTERISTICS

Dental tissue involves postnatal mesenchymal stem cells (MSCs) with easy accessibility and regenerative potential. Currently identified dental-derived stem cells include pDPSCs, SHED, periodontal ligament stem cells (PDLSCs), dental follicle progenitor stem cells (DFSCs), and stem cells from apical papilla[19]. They all possess osteogenic, adipogenic, and chondrogenic differentiation potential, along with a peculiar ability to form mineralized tissue. The translational clinical application of dental-derived stem cells in regenerative medicine has been broadly exploited. One direction is through bioscaffolds/biomaterials loaded with growth factors[20]. Another approach is to investigate the bio-induction effects of natural compounds such as polydatin, beer polyphenols on dental-derived stem cells[21,22].

The existence of stem cells in dental pulp was confirmed by pulp healing potential after injury and maintenance of tissue homeostasis. pDPSCs were initially identified in 2000[2]. Primary cultures of pDPSCs express endothelial (vascular cell adhesion molecule 1 and CD146), osteogenic [alkaline phosphatase (ALP), type I collagen, osteonectin, osteopontin (OPN), and osteocalcin (OCN)], and fibroblastic [type III collagen, and fibroblast growth factor (FGF)-2] markers. The bone matrix protein bone sialoprotein (BSP) and odontoblast-specific marker like dentin sialophosphoprotein (DSPP) are absent in pDPSC cultures, which confirms its undifferentiation status[2].

pDPSCs can be induced to form mineralized nodules when subjected to osteogenic medium[5]. Under adipogenic induction *in vitro*, pDPSCs form oil-red-O-positive lipid clusters and express high levels of nestin and glial fibrillary acid protein, suggesting that pDPSCs possess both adipogenic and neurogenic potential[23]. *Ex vivo* cultured pDPSCs acquire a neuronal morphology, and express neuron-specific markers under neuronal media conditions. When adding basic FGF (bFGF) and epidermal growth factor (EGF) to culture medium, pDPSCs transform to neural precursor cells that express the specific marker nestin[6]. When xenotransplanted into the chicken embryo and exposed to the endogenous neuronal microenvironment, pDPSCs show a neuronal morphology and migrate into facial structures and the central nervous system within the developing avian embryo[24]. When transplanted with hydroxyapatite/tricalcium phosphate (HA/TCP) powder into immunocompromised mice, the pDPSC transplants generate a dentin-like structure with highly organized collagenous matrix deposited around the odontoblast-like layer but do not indicate any hematopoiesis or initiate adipocyte formation. However, well-established vascularity is seen when transplanting tooth fragments containing pDPSC coated with synthetic scaffolds subcutaneously into nude mice[25,26]. When applying thermoresponsive hydrogels instead of polymer scaffolds, blood components are also produced in pulp-like tissues[27]. Another way to improve neovascularization for pulp regeneration is through fractionating CD31-/CD146- cells from dental pulp; it turns out that CD31-/CD146- canine DPSCs have greater angiogenesis potential when applied to the sectioned pulp of dogs[28]. A clinical experiment conducted on patients with irreversible pulpitis showed that pDPSCs transplantation in pulpectomized teeth induced positive response of electric pulp test and functional dentin formation as tested by cone beam computed tomography[29]. This study confirmed the safety and efficacy of DPSC-based RE.

Songtao Shi firstly documented the discovery and identification of SHED in 2003[3]. Histologically, SHED appear at the 6th week of embryonic development, and consist of MSCs with multi-differentiating potential of adipocytes, chondrocytes, and

osteoblasts. SHED show even more extensive clonogenic, osteogenic potential than pDPSCs. Cotransplantation of SHED and HA/TCP material subcutaneously into immunocompromised mice produces similar dentin pulp-like tissues in accordance with pDPSCs. Other teams utilized human root canals to accommodate SHED that were coated with peptide hydrogel or collagen scaffold. They implanted this prepared root canal into immunocompromised mice, and new dentin was formed throughout the root canal and vascularization of pulp-like tissues was also identified[30]. SHED responded in a similar manner to pDPSCs during both *in vitro* neuronal induction and xenotransplantation into chicken embryos[6,24]. The breakthrough of DPSC-based pulp regeneration is associated with a clinical study in patients with pulp necrosis due to traumatic dental injuries. SHED implantation into injured incisor teeth regenerated dental pulp that recovered the formation of sensory nerves and blood vessels and ensured root elongation and closure of the apical foramen[7].

Besides the above multilineage differentiation potential, the expression of surface antigens provides another identification of DPSCs. DPSCs express MSC-specific markers such as STRO-1, CD90, CD44, CD73, CD90, CD105 and CD271[31]. These markers fail to distinguish DPSCs from other MSCs. Hematopoietic lineage markers such as CD34 and CD117, neurovascular markers such as glia 2 are also expressed in DPSCs[32]. Although these markers still lack specificity in distinguishing DPSCs, they provide further evidence for the regenerative potential of DPSCs.

DPSCs were collected from neonatal baby teeth (nDPSCs)[33] and dental bud (DBSCs)[5], and they exhibited more attractive stemness properties and higher proliferate rate, as indicated by more intensified expression of pluripotent markers such as v-myc avian myelocytomatosis viral oncogene homolog (c-Myc) and SRY-box transcription factor 2 (SOX2) compared to pDPSCs. When DBSCs underwent osteogenic induction, they expressed higher levels of c-Myc, SOX2, octamer-binding transcription factor 4, and homeobox transcription factor Nanog than pDPSCs expressed, suggesting that naive DPSCs hold functional advantages over pDPSCs[34].

Taken together, pDPSCs and SHED are the most widely studied DPSCs and have easier availability. They have neurovascular properties and unique odontoblastic and dentinogenic potential, which render them the most eligible stem cell source for pulp regeneration.

EPIGENETIC REGULATION OF DPSCS

The fate of DPSCs is strictly regulated on two levels: genetic control that involves signaling pathways and transcriptional factors; and epigenetic modulation that includes DNA methylation, histone modifications, and ncRNAs (Table 1 offers a summary of epigenetic enzymes in the fate determination of DPSCs). A thorough understanding of epigenetic modulation on DPSCs offers insights to manipulate DPSC fate towards pulp–dentin regeneration.

DNA methylation

DNA methylation refers to the covalent addition of a methyl group at the 5′ carbon of the cytosine by DNA methyltransferases (DNMTs, including DNMT1, DNMT3A, DNMT3B and DNMT3L). DNA methylation of promoters and enhancers leads to gene silencing by interfering with the binding of transcriptional factors or by chromatin structure remodeling[35]. The ten–eleven translocation (TET) family proteins (including TET1, TET2 and TET3) are responsible for the removal of the methyl group [36]. Gene expression is stringently controlled by the balance of methylation and demethylation.

DNA methylation states of pDPSCs, PDLSCs and DFSCs significantly differ, especially for surface antigens like CD109, and other factors implicating osteogenic pathways. PDLSCs express higher levels of osteogenic-related factors, a higher osteogenic potential *in vitro* and an enhanced mineralization capacity *in vivo*. Thus, the methylation profile is thought to be tightly correlated with differentiation potential [37]. When DNA methylation status is suppressed *via* pretreatment with 5-Aza-2′-deoxycytidine (5-Aza-CdR; a DNMT suppressor), pDPSCs exhibit receded proliferation and intensified mineralization and ALP activity under odontogenic induction [38]. Kruppel-like factor (KLF) 4 is an important regulator of cytodifferentiation and proliferation that promotes the odontoblastic differentiation and inhibits proliferation of pDPSCs[39]. The promoter region of *KLF4* is demethylated during odontoblastic differentiation, so as to facilitate the effective binding and transcriptional regulation of SP1[40]. The mRNA and protein expression level of TET1 increases during

Table 1 Enzymes related to epigenetic modifications of dental pulp stem cells activities

Proteins and their function	Epigenetic Targets	Gene manipulation of each epigenetic marker	Downstream targets	Biological process implicated	Reference
DNA methylation					
TET1, demethylation		Gene activation	*FAM20C*	(↑) proliferation, (↑) odontoblast differentiation	[41-43]
Histone methylation					
EHMT1, methylation	H3K9me2	Gene repression	*Runx2*	(↓) odontoblast differentiation	[51]
KMT2A, methylation	H3K4me3	Gene activation	*WNT5A*, *RUNX2*, *MSX2*, *DLX5*	(↑) odontoblast differentiation	[12,52]
EZH2, demethylation	H3K27me2/me3	Gene repression	Wnt/β-Catenin pathway, *IL-6*, *IL-8*, *CCL2*	(↑) Inflammation, (↓) odontoblast differentiation	[54-56]
KDM6B/JMJD3, demethylation	H3K27me3	Gene repression	*WNT5A*, *BMP2*	(↑) odontoblast differentiation	[52,58]
KDM5A, demethylation	H3K4me3/me2	Gene activation	*DMP1*, *DSPP*, *OSX*, *OCN*	(↓) odontoblast differentiation	[60]
Histone acetylation					
p300, acetylation	H3K9ac		*OCN*, *NANOG*, *SOX2*, *DSPP*, *Dmp1*, *Osx*	(↑) pluripotency, (↑) proliferation, (↑) odontoblast differentiation	[14,64]
HDAC3, deacetylation	H3K27ac		*Dmp1*, *Osx*	(↓) odontoblast differentiation	[13,66]
HDAC6, deacetylation				(↓) odontoblast differentiation	[15]

TET1: Ten-eleven translocation 1; EHMT1: Euchromatic histone lysine methyltransferase 1; KMT2A: Lysine methyltransferase 2A; KDM6B: Lysine demethylase 6B; KDM5A: Lysine demethylase 5A; p300: E1A binding protein p300; HDAC3: Histone deacetylase 3; HDAC6: Histone deacetylase 6; H3K9me2: Dimethylated histone H3 lysine 9; H3K4me3/me2: Tri-/di-methylated histone H3 lysine 4; H3K27me2/me3: Di-/tri-methylated histone H3 lysine 27; H3K9ac: Acetylated histone H3 lysine 9; H3K27ac: Acetylated histone H3 lysine 27; FAM20C: FAM20C golgi associated secretory pathway kinase; Runx2: RUNX family transcription factor 2; WNT5A: Wnt family member 5A; MSX2: Msh homeobox 2; DLX5: Distal-less homeobox 5; CCL2: C-C motif chemokine ligand 2; IL-6/-8: Interleukin 6/8; BMP2: Bone morphogenetic protein 2; DMP1: Dentin matrix acidic phosphoprotein 1; DSPP: Dentin sialophosphoprotein; OSX: Osterix; OCN: Osteocalcin; NANOG: Nanog homeobox; SOX2: SRY-box transcription factor 2.

odontogenic differentiation[41]. Knockdown of *TET1* inhibits pDPSC proliferation and impairs ALP activity, mineralized nodule formation, and decreases expression levels of DSPP and dentin matrix protein (DMP) 1 during odontogenic differentiation[42,43]. DNA methylation is also in charge of gene expression related to myogenic differentiation. Increased expression of myogenin, Myod1, and Pax7 is detected, along with myotube formation and myosin heavy chain expression after treating pDPSCs with 5-Aza-CdR. 5-Aza-CdR-mediated DNA demethylation induces skeletal myogenic differentiation of murine DPSCs *in vitro*[44]. Local injection of 5-Aza-CdR-pretreated pDPSCs into mice with cardiotoxin-induced muscle injury shows enhanced muscle regeneration[45].

Reparative dentin formation results from the delicate balance of inflammation and odontogenic differentiation. DNA methylation is involved in the inflammatory reaction of the human dental pulp as well. Administration of 5-Aza-CdR increases expression of inflammatory indicators interleukin (IL)-6 and IL-8 in lipopolysaccharide (LPS)-induced pDPSC inflammation. miRNA expression profile is altered by 5-Aza-CdR application. Among those differentially expressed miRNAs, miR-146a-5p is affected by DNA methylation[46]. In LPS-induced pDPSC inflammation, application of 5-Aza-CdR upregulates nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) signaling activity and stimulates inflammatory cytokine expression *via* demethylation of the promoter of an intracellular signal transducer, TNF-receptor-associated factor (TRAF) 6[47]. In lipoteichoic acid-treated pDPSCs, similar results are achieved with knockdown of *DNMT1* expression. Signal transducer MyD88 and

TRAF6 are both upregulated, but only the promoter of MyD88 is demethylated[48].

Histone modification

The tightly coiled DNA and histone cores (mainly H2A, H2B, H3 and H4) constitute a nucleosome, which functions as the fundamental subunit of chromatin. Different chemical modifications imparted on the histones result in alterations of chromatin architecture. To be specific, N termini of histone tails modified by methylation, acetylation, ubiquitination, phosphorylation, and other modifications of lysine and arginine residues can change the interaction among histones themselves or between histones and DNA[49].

Histone methylation: Histone methylation refers to the methylation of lysine or arginine residues of histone tails, which is regulated by histone methylases and demethylases[50]. It is the most widely studied histone modifications so far. pDPSCs and DFSCs respond differentially under mineralization induction. pDPSCs express higher levels of pluripotency-related genes and exhibit a faster rate of mineralization. Part of the explanation for this difference relies on different histone methylation profiles. Both cell types exhibit H3K4me3 (trimethylated histone H3 Lysine 4) active marks on early mineralization genes [runt related transcription factor (*RUNX*) 2, msh homeobox (*MSX*) 2, distal-less homeobox (*DLX*) 5], H3K9me3 or H3K27me3 on late mineralization markers [osterix (*OSX*), *BSP* and *OCN*], but H3K27me3 on odontogenic genes *DSPP* and *DMP1* are only seen in DFSCs[12]. CBFA2T2 (core-binding factor, runt domain, a subunit 2, translocated to 2) is upregulated during bone morphogenetic protein (BMP) 2-induced osteogenic differentiation of pDPSCs. CBFA2T2 is required for mineralization since it can inhibit euchromatic histone methyltransferase 1-mediated H3K9me2 on *RUNX2* promoter[51]. The bivalent histone domains of H3K4me3 and H3K27me3 on *WNT5A* promoter make the activation of *WNT5A* by the removal of H3K27me3 mark and increase of H3K4me3 mark on the promoter[52]. Ferutinin, a phytoestrogen extracted from *Ferula* species, has been used as an antibacterial, antioxidant, anti-inflammatory, and apoptosis-inducing agent. Pretreatment of ferutinin significantly increases H3K9ac and H3K4me3 in the promoter sites of the *WNT3A* and *DVL3* genes in pDPSCs and promotes osteogenic differentiation[53].

Enhancer of zeste homolog (EZH) 2 is specifically in charge of methylation of H3K27me3. EZH2 has been proved to participate in pulp tissue inflammation and regeneration[54]. Suppression of EZH2 function during TNF-α stimulation results in downregulation of proinflammatory factors and intensified osteogenic differentiation potential of pDPSCs[55]. EZH2-mediated H3K27me3 attenuates odontogenic differentiation of pDPSCs through modifying the β-catenin promoter and thus impairing the Wnt/β-catenin pathway[56]. The Jumonji domain-containing protein (JMJ) D3, also known as lysine-specific demethylase (KDM) 6B, removes the methyl marker of H3K27me2/3 specifically[57]. Overexpression of *JMJD3* promotes odontogenic commitment through combining with *BMP2* promoter site, removing H3K27me3 marker, leading to activation of genes associated with odontogenic differentiation[58]. When alcohol is added to mineralization-inducing media, the osteogenic potential of pDPSCs is inhibited *via* suppression of JMJD3[59]. H3K4me3 is another epigenetic mark related to odontogenic differentiation. Knockdown of *KDM5A*, an exclusive demethylase for H3K4me3, pDPSCs exhibited more intense ALP activity and more mineral deposition formation through the increment of H3K4me3 enrichment on odontogenic markers such as *DMP1*, *DSPP*, *OSX*, and *OCN*[60].

Histone acetylation: Histone acetylation is controlled by HATs and HDACs[61]. Eighteen human HDAC isoforms can be classified into three categories: class I (HDACs 1–3 and 8); class II (Zn-dependent enzymes, HDACs 4–7 and 9–11), and class III (sirtuins 1–7)[62]. Histone acetylation renders chromatin structure more favorable for transcriptional activation. Histone H3 acetylation is upregulated during odontogenic induction of pDPSCs[63]. The histone acetyltransferase p300 can activate *NANOG* and *SOX2* promoters and help maintain pDPSCs stemness. When pDPSCs are cultured in a normal medium, upregulation of p300 suppresses the expression of *DMP1*, *DSPP*, *DSP*, *OPN* and *OCN*. However, when they undergo odontoblastic differentiation, overexpression of *p300* leads to increased odontoblastic marker expression. p300 assembles at the promoter of *OCN* and *DSPP* and increases H3K9ac mark on *OCN* and *DSPP*[14]. Knockdown of *p300*, however, impairs ALP activity and mineralized nodule formation of pDPSCs during odontogenic differentiation[64]. Immediately after photo-biomodulation therapy on pDPSCs *in vitro*, H3K9ac is upregulated, which explains the improved viability and migration[65]. Another facet related to histone acetylation level is HDACs. When *HDAC6* is knocked down, the

ALP activity and mineralization potential of pDPSCs are increased[15]. When subjecting murine dental papilla mesenchymal cells to odontoblast induction, increased expression level of p300 and reduced HDAC3 expression are detected, leading to upregulated enrichment of H3K9ac and H3K27ac. HATs and HDACs modulate the process of dentinogenesis and odontogenic differentiation in a coordinated way[13]. Furthermore, p300 and HDAC3 modulate odontogenic differentiation in a time-specific manner through interacting with KLF4. At the differentiation initiation stage, HDAC3 acts on KLF4; thus DMP1 and OSX remain at a limited level. As the differentiation proceeds, HDAC3 translocates to the cytoplasm and KLF4 is able to bind with p300, transactivates *Dmp1* and *Osx*, ultimately enhances odontoblast differentiation[66].

ncRNAs

ncRNAs do not code for proteins, which can be categorized into small noncoding (snc) RNAs (< 200 nt) and long noncoding (lnc) RNAs (> 200 nt). sncRNAs can be further classified into miRNAs, PIWI-interacting (pi) RNAs, and siRNAs. miRNA and lncRNAs are two of the most-studied ncRNAs[67].

miRNAs: The processing of primary miRNA transcripts is initially tailored by two enzymes in the nucleus, known as Drosha and DGCR8, generating precursor (pre-) miRNAs. Subsequently, pre-miRNAs are exported to the cytoplasm and converted to mature miRNA duplexes by RNase III, namely Dicer. Mature miRNAs are combined into RNA-induced silencing complexes (RISCs). The incorporation of RISCs and the 3′ untranslated region (UTR) of specific mRNAs targets leads to gene repression by undermining mRNA stability or reducing translation[68,69].

Downregulation of miR-320b during calcium hydroxide stimulation can ease the inhibitory effect on the proliferation-related transcription factor Foxq1, leading to upregulation of Foxq1 and promoting the proliferation of pDPSCs[70]. miR-584 is another ncRNA that represses pDPSC growth, and it exerts this effect by targeting the 3′ UTR of PDZ-binding motif (TAZ)[71]. Sirtuin (*SIRT*) *7* is the downstream target of miR-152-mediated pDPSC senescence. Inhibition of miR-152 upregulates SIRT7 and represses pDPSC senescence[72]. Inhibition of miR-224 induces amplified MAPK8, caspase-3, caspase-9, and Fas ligand expression in pDPSCs, which is a sign of apoptosis, suggesting that miR-224 is essential for maintaining pDPSC viability[73]. Downregulation of miR-224 enhances pDPSC migration and proliferation[74].

The expression profiles of miRNAs in differentiated and undifferentiated DPSCs illustrate 22 differentially expressed miRNAs[75]. These miRNAs affect DPSC differentiation through various signaling pathways. Most identified miRNAs exert an inhibitory effect on odontogenic differentiation. Upregulation of miR-143 or miR-143-5p can attenuate osteogenic differentiation of pDPSCs, downstream inactivated pathways containing the NF-κB signaling pathway[76], osteoprotegerin receptor activator of the NF-κB ligand signaling pathway[77], and MAPK signaling pathway[78]. Disparate miRNAs can result in downregulation of the same signaling pathway, although their targets might be different. miR-488 and miRNA *let-7c* modulate the p38 MAPK signaling pathway; the former impacts MAPK1[79], and the latter downregulates insulin-like growth factor 1 receptor expression[80]. miR-215 and miR-219a-1-3p are both responsible for the cell-passage-related reduction of heat-shock protein B8 expression[81]. This reduction leads to weakened osteogenic differentiation capability of murine DPSCs[82]. Moreover, there are miRNAs that participate in the fate choice of pDPSCs in a multifaceted way. Among these miRNAs, miR-720 impacts the stemness of pDPSCs by inhibiting translation and stability of *NANOG* transcripts and repression of *DNMT3A* and *DNMT3B*. miR-720 mimics enhance osteogenic differentiation with intensified ALP activity, alizarin red staining, and increased expression of *ALP* and *OPN* and promotes proliferation of pDPSCs with an increased number of ki67-positive cells[83]. The modulation of miRNA in odontogenic differentiation is complicated, with multiple miRNAs, diverse signaling mechanisms, and disparate cellular processes. It provides both opportunities and challenges for precise miRNA-based regulation of dentinogenesis.

miRNAs participate in the regulation of angiogenesis and neovascularization under both physiological and pathological conditions[84]. When cultured in a medium supplemented with bFGF and vascular EGF (VEGF)-165, pDPSCs are induced toward endothelial differentiation, during which miR-424 is downregulated gradually, resulting in alleviation of the inhibitory effect on VEGF and kinase insert domain receptor expression[85]. 5-Aza-CdR can prompt myogenic differentiation of pDPSCs with a remarkable decrease of miR-135, and miR-143 expression. pDPSCs cotrans-

fected with miR-135 and miR-143 inhibitors acquire apparent myocyte properties even without administration of 5-Aza-CdR[86].

miRNAs are indispensable for the immunomodulation of dental pulp inflammation. The miRNA expression profile differs in healthy and inflamed dental pulp[87]. LPS or TNF-α *per se* can promote the odontoblastic differentiation of pDPSCs[88,89]. In most circumstances, the protective effects of miRNAs are realized through attenuating inflammatory reactions or promoting odontogenic differentiation. miR-223-3p is one of the markedly upregulated miRNAs in inflamed dental pulp as detected in clinically derived pulp tissues. Overexpression of miR-223-3p promotes odontoblastic differentiation of pDPSCs *in vitro*[90]. miR-506 and *let-7c-5p* confer a protective effect on LPS-induced inflammation of pDPSCs through decreasing expression of pro-inflammatory cytokines[91]. *In vivo* experiments have confirmed that *let-7c-5p* agomir decreases LPS-induced pulpitis in Sprague–Dawley rats[92]. Moreover, *let-7c-5p* possesses additional pro-osteogenesis potential in inflamed pDPSCs[93]. Knockdown of miR-140-5p increases odontoblastic differentiation and inhibits proliferation of pDPSCs under LPS stimulation. Toll-like receptor-4 is involved in the miR-140-5p-mediated effects on pDPSCs[94]. The expression of Fyn, a Src-family kinase associated with various types of inflammation, is upregulated in the microenvironment of deep caries. miR-125a-3p has been detected as the upstream factor of *Fyn* and identified as a positive factor regulating the odontoblastic differentiation of pDPSCs under TNF-α stimulation[95].

lncRNAs: lncRNAs can hardly be classified due to their diverse distribution in the genome and wide range of sizes. lncRNAs regulate gene expression at multiple levels, including transcriptional and post-transcriptional.

There are 139 differentially expressed genes between induced and undifferentiated human pDPSCs, with downstream pathways implicating cell cycle, extracellular matrix receptor interaction, and transforming growth factor (TGF)-β signaling pathways[96]. lncRNAs undergo transitional alterations during TNF-α-mediated osteogenic differentiation of pDPSCs, since lncRNA expression patterns differ after 7 and 14 d of treatment with TNF-α. These alterations in lncRNAs expression are predicted to be associated with mRNA alterations at day 7 and 14 posts TNF-α induction[97]. lncRNA DANCR declines with time during odontoblast-like differentiation of pDPSCs. The inhibitory effect of DANCR on odontogenic differentiation is realized through the inactivation of the Wnt/β-catenin signaling pathway. Downregulation of lncRNA DANCR has little impact on pDPSC proliferation but promotes the osteogenic, adipogenic and neurogenic differentiation of pDPSCs[98]. lncRNAs play a vital role in the angiogenesis of dental pulp and may be modulators of dental pulp angiogenesis. pDPSCs with normal culture and vascular induction show differential expression profiles of lncRNAs, which have been validated by microarray analysis [99]. Several proangiogenic factors including angiotensin, placental growth factor, FGF and EGF, are enriched in vascular differentiation, and they might serve as potential regulatory sites for lncRNAs.

It is worth noting that RNA methylation has emerged as an important post-translational modification mechanism on the fate determination of pDPSCs. Its discovery has brought a novel perspective of gene regulation. N6-methyladenosine (m6A), is the most prevalent internal modification of mRNA. The addition and removal of methyl groups are mediated by methyltransferases and demethylases, and this structural alteration dynamically regulates various aspects of RNA metabolism, including changes in RNA folding, marking mRNA for decay, and facilitating the processing, maturation and translation of mRNA[100]. Downregulation of m6A *via* depletion of methyltransferase 3 in pDPSCs significantly undermines the proliferation, migration and odontogenic differentiation of pDPSCs *in vitro*[101]. Upregulation of total m6A content and methyltransferase 3 expression is observed in pDPSCs treated with LPS. When knocking down methyltransferase 3, LPS-induced NF-κB and MAPK signaling pathway activation is inhibited, along with decreased expression of proinflammatory cytokines[102], suggesting that RNA methylation is a promising target in the regulation of differentiation and immunomodulation of DPSCs.

EPIGENETIC REGULATORY NETWORKS IN THE FATE DETERMINATION OF DPSCS

Multiple direct and indirect connections exist between histone modifications, DNA methylation and ncRNAs. For instance, silencing of *MYT1* gene expression requires both EZH2 and DNMTs. EZH2 assists the binding of DNA methyltransferases and

facilitates CpG methylation of EZH2-target promoters[103]. Specific protein–RNA interactions with lncRNA are responsible for the initiation of deposition of polycomb-repression-complex-2-mediated H3K27me3[104].

During the fate determination of pDPSCs, complicated epigenetic networks regarding lncRNAs, miRNAs and DNA methylation have been revealed in recent studies. lncRNA G043225 promotes odontogenic differentiation of pDPSCs *via* directly binding to miR-588 and fibrillin 1[105]. lncRNA H19 can repress the activity of DNMT3B, reduce the methylation level of *DLX3*, and thus lead to the promotion of odontogenic differentiation of pDPSCs[106]. Similar mechanisms can be applied to miR-675, which is capable of inhibiting DNMT3B-mediated methylation of *DLX3* to promote odontogenic differentiation of human DPSCs[107]. lncRNA CCAT1 is upregulated in pDPSCs and promotes cell proliferation and differentiation by repressing the expression of miR-218, an antiodontogenic factor[108].

THERAPEUTIC APPLICATION OF EPIGENETIC MODIFICATION IN RE

RE is a biological process that aims to regain both structure and function of the dentin–pulp complex. There have been extensive searches for novel bio-inductive approaches for the regeneration of damaged dental tissues over recent years. The process of RE requires a microenvironment conducive to repair, agents with anti-inflammatory properties, induction of mineralization, angiogenesis and neurogenesis, and recruitment and differentiation of DPSCs. The discovery of novel factors, which manipulate epigenetic modulation and contribute to inducing DPSCs toward odontogenic differentiation, angiogenesis and neurogenesis would accelerate research in RE.

Kuang *et al*[26] performed RE on first molars of rats by implantation of hypoxia-primed pDPSCs blended with a synthetic polymer and found pulp-like tissues histologically, and vascularization were generated in this *in situ* model. Another team performed autogenous transplantation of the BMP2-treated DPSCs culture onto the amputated canine pulp of dogs. The BMP2 pretreated group produced odontoblast-like cells with long processes attached to the osteodentin and formed tubular dentin [109]. There are subsets of progenitor cells derived from dental pulp that exhibited greater angiogenic and neurogenic potential; for instance, CD105+ DPSCs. CD105+ DPSCs were fractionated by flow cytometry and further transplanted in canine teeth after pulpectomy with the addition of stromal cell-derived factor-1 and collagen mixtures. Regenerated pulp including nerves and vasculature was produced, followed by new dentin formation along the dentinal wall[110]. The granulocyte-colony stimulating factor (G-CSF) is capable of sorting out CD105+ DPSCs. Ectopic tooth root transplantation of DPSCs subset mobilized by G-CSF in immunodeficient mice exhibited larger fibrous matrix formation and larger neovascularization compared with unsorted DPSCs[111]. These results suggest that preconditioned pDPSCs during the process of RE might guide differentiation specifically and ensure optimal functional pulp regeneration. Despite the inspiring and cheerful outcomes of clinical experiments carried out by Xuan *et al*[7], long-term follow-up of autologous SHED-based RE is required. Besides, self-derived DPSCs sources are limited. The efficacy and safety of allogenic DPSC transplantation need to be explored. Preconditioning of DPSCs with epigenetic molecules to optimize pulp regeneration might offer solutions to those problems[112].

Histone acetylation and HDACis in RE

HDACis have received intensive focus as potential agents for the treatment of cancer [113], inflammatory disease[114], and neurodegenerative disorders[115]. HDACs play a crucial part in the modulation of dental pulp development and repair. HDACis, as small molecules, have been put forward as an agent for pulp–dentin regeneration (Table 2). There are basically two types of HDACis: pan-HDACis and isoform-specific HDACis. Valproic acid (VPA), suberoylanilide hydroxamic acid (vorinostat, SAHA) and trichostatin A (TSA) are pan-HDACis that have been extensively studied to promote mineralization and differentiation at low concentrations[116,117]. Exposure of pDPSCs to 1 mmol/L VPA or 20 nmol/L TSA promotes cell proliferation, migration and adhesion[118]. VPA (0.125–5 mmol/L) and TSA (12.5–400 nmol/L) significantly increases mineralization in a dose-dependent manner[116,119]. Low concentration of VPA promotes matrix mineralization through selective inhibition of HDAC2 over HDAC1. To mimic 3D tissue formation, Paino *et al*[117] exploited Gingistat collagen sponges to grow pDPSCs. The seeded scaffolds were bathed in osteogenic medium

Table 2 Epigenetic molecules promoting odontogenic differentiation of dental pulp stem cells

	Targets	Cell population	Medium	Upregulated odontogenic genes	Downregulated odontogenic genes	Reference
HDACis						
TSA	HDAC 1, 2, 3 (Class I); HDAC 4, 5, 6 (Class II)	pDPSCs	MM	*DMP1, DSPP, BSP*	*OCN*	[124]
		Murine MDPC23	MM	*Dmp1*		[119]
		Rodent primary dental pulp cells	MM	*Bmp4, Dspp, Bmp2, Opn*		[116]
		Rodent MDPC23	GM	*Bmp4, Ocn, Dmp1, Runx2*		[16]
SAHA	classes I and II	Murine MDPC23	MM	*Nfic, Dspp, Alp, Dmp1, nestin*		[122]
VPA	HDAC 1, 2, 3 (class I)	pDPSCs	GM, MM	*BSP, OPN*	*OCN*	[117]
		Murine MDPC23	MM	*Dmp1, Bmp4, Tgfβ1*		[119]
		rodent MDPC23	GM	*Bmp2/4, Ocn, Runx2*		[16]
		Rodent primary DPSCs	MM	*Dmp1, Bmp2, Bmp4, Dspp, Opn*		[116]
MS-275	HDAC 1, 3 (class I)	pDPSCs	GM	*RUNX2, DMP1, ALP, DSPP*		[126]
		rodent MDPC23	GM	*Bmp2/4, Col1α1, Ocn, Dmp1, Dspp, Runx2, Klf5, Msx1*		[16]
LMK-235	HDAC 4, 5 (class II)	pDPSCs	GM	*ALP, DSPP*		[127]
			MM	*ALP, DSPP, RUNX2*		[127]
DNMTis						
5-Aza-CdR		pDPSCs	MM	*DSPP, DMP1, OSX, DLX5, RUNX2*		[38]
RG-108		Murine mDPC6T	MM	*Klf4, Dspp, Dmp1*		[40]

HDACis: Histone deacetylase inhibitors; TSA: Trichostatin A; SAHA: Suberoylanilide hydroxamic acid; VPA: Valproic acid; MS-275: Entinostat; DNMTis: DNA methyltransferases inhibitors; 5-Aza-CdR: 5-Aza-2'-deoxycytidine; HDAC: Histone deacetylase; pDPSCs: Dental pulp stem cells from permanent teeth; MDPC23: Murine odontoblast-like cell line; mDPC6T: Murine preodontoblast cell line; MM: Mineralized medium; GM: Growth medium; DMP1: Dentin matrix acidic phosphoprotein 1; DSPP: Dentin sialophosphoprotein; BSP: Bone sialoprotein; BMP4: Bone morphogenetic protein 4; BMP2: Bone morphogenetic protein 2; OPN: Osteopontin; Runx2: RUNX Family transcription factor 2; Nfic: Nuclear factor I C; ALP: Alkaline phosphatase; Tgfβ1: Transforming growth factor beta 1; OCN: Osteocalcin; Col1α1: Collagen type I alpha 1; Klf5: Kruppel like factor 5; MSX1: Msh homeobox 1.

supplemented with VPA for 30 d. More intense calcium deposits were observed in this system. pDPSCs preconditioned with HDACi (VPA, TSA or SAHA) and 15-d osteogenic induction were transplanted subcutaneously into immunodeficient mice. VPA treatment produced a well-organized lamellar bone tissue although a decrease of OCN expression was observed[120]. SAHA, an FDA-approved drug for treatment of lymphoma, mainly acts on class I and II HDACs[121]. Addition of SAHA to culture medium enhances matrix mineralization and expression levels of odontoblast marker genes during odontoblast differentiation of MDPC23 cells, which is an odontoblast-like cell line[122]. Similar results were found during mineralization induction of murine DPSCs. Moreover, short-term SAHA treatment promotes mineralization without loss of cell viability, while long-term SAHA inhibits differentiation. Low dose (1 μmol/L) SAHA even promotes cell migration[123]. TSA enhances pDPSCs proliferation *via* activation of the JNK/c-Jun pathway and promotes DPSC differentiation and increased expression of *DSPP, DMP1, BSP* and *OCN in vitro* through affecting Smad2/3- and NFI-C-related signaling pathways. TSA can promote odontoblast differentiation and dentin formation *in vivo*. Neonatal mice with maternal exposure to TSA exhibited thicker dentin, larger dentin areas, and higher odontoblast numbers in their postnatal molars with stronger DSP expression[124]. Apart from regulation of DPSC

gene expression, VPA, SAHA and TSA can promote pulp–dentin repair through facilitating the release of dentinal matrix components from dentin. Although they are not as effective as EDTA treatment, each of them shows different extraction profiles [125].

MS-275 is a selective HDACi that targets HDAC1 and HDAC3. Administration of MS-275 to pDPSCs under normal culture can induce upregulation of odontogenesis-associated proteins expression, including RUNX2, DMP1, ALP, and DSPP. Cytotoxicity can be avoided at a concentration of 20 nmol/L. The MAPK signaling system was barely activated under MS-275 stimulation, suggesting that MS-275 induces odontogenesis independent of MAPK signaling[126]. The pro-odontogenic potential of MS-275 was also tested on a murine odontoblast-like cell line, MDPC-23. Without the induction of mineralization medium, MS-275 alone was capable of increasing expression of *Bmp2*, *Bmp4*, *Col1α1*, *Ocn*, *Dmp1*, *Dspp*, *Runx2*, *Klf5*, and *Msx1*, with elevated ALP activity and intensified calcified nodule formation[16]. Isoform-specific agents like LMK-235 selectively inhibit HDAC4 and HDAC5. LMK-235 at 100 nmol/L barely affected the proliferation of pDPSCs, but possessed pro-odontogenic potential. Odontoblast markers (ALP, DSPP, and RUNX2) were downregulated when the concentration increased. Expression of OCN was not affected by LMK-235 administration, indicating that LMK-235 might act on early stages of odontogenic differentiation. LMK-235 combined with mineralization induction medium enhances odontoblastic marker expression of pDPSCs[127]. As topical agents for pulp repair, HDACis generally exhibit low toxicity since non-cancer cells are resistant to HDACi-mediated apoptosis compared to cancerous cells[128]. Adverse effects such as fatigue, nausea, and hypocalcemia due to high-dose systemic administration of HDACi can also be avoided.

The presence of either 5-Aza-CdR or TSA can increase expression of the endothelial marker genes in bone-marrow-derived multipotent adult progenitor cells in basal differentiation medium, which indicates the possibility of HDACi-induced angiogenesis[129]. VPA treatment can enhance sciatic nerve regeneration and recovery of motor function in adult rats[130]. VPA tends to induce neuronal differentiation and inhibit glial differentiation of adult hippocampal neural progenitors *via* acetylation of histone H4 associated with proneural genes[131]. HDACis have emerged as potent contenders in the treatment of chronic immune and inflammatory disorders, including rheumatoid arthritis, psoriasis, inflammatory bowel disease, and multiple sclerosis [132]. The underlying mechanism is still controversial but possibly relies on reduced inflammatory cytokines and nitric oxide production and inhibition of NF-κB transcriptional activity[133]. The angiogenesis, neurogenesis, and immunomodulatory potential of HDACis on inflamed dental pulp remain to be explored, but these characteristics cater to the requirements of pulp regeneration.

The mineralized tissue regenerative potential of HDACis has been tested on several animal models. Huynh *et al*[134] conducted an experiment on a murine model with calvarial defects. They seeded human periodontal ligament cells, which were preincubated with TSA in growth medium, onto a scaffold to induce repair. Apparent bone formation was detected 4 wk after implantation[134]. Topical application of MS-275 to calvarial defects of Sprague–Dawley rats stimulated bone formation. Collagen sponges loaded with MS-275 were applied to the injury site, in which significant bone healing was observed in the MS-275-treated groups in a dose-dependent manner. The pro-osteogenic capacity of MS-275 was demonstrated in an osteoporosis mouse model induced by soluble receptor activator of NF-κB ligand, as consecutive injection of MS-275 recovered bone volume, thickness, and separation[17] . Promising outcomes of MS-275 in bone regeneration promote the analysis of its potential in RE.

DNA methylation and DNMT inhibitors in RE

Inhibition of DNMTs has been demonstrated to enhance odontoblast differentiation. There are two DNMT inhibitors that affect the differentiation potential of pDPSCs, 5-Aza-CdR and RG108 (Table 2). They were initially identified as antitumor agents and were used for the treatment of leukemia and myelodysplastic syndromes[135]. When administered in combination with odontogenic medium, 5-Aza-CdR intensifies the expression of odontogenic markers and promotes ALP activity and mineral nodule formation[38]. The pro-odontogenic effect of RG108 was tested in a murine preodontoblast cell line, mDPC6T. RG108 is effective in suppressing DNMT activity and promotes odontogenic differentiation[40]. Melatonin, N-acetyl-5-methoxytryptamine, an endogenous hormone mainly in charge of circadian rhythms, decreases DNMT expression. Melatonin can cause global DNA hypomethylation and promote odontogenic differentiation of pDPSCs *in vitro*[136]. Although DNMT inhibitors show angiostatic activity when utilized as antitumor agents[137], they may show an

opposite effect on stem cells. 5-Aza-CdR downregulates protein expression of the pluripotency gene *Oct4* and upregulates protein expression of endothelial cell marker genes in differentiated mouse embryonic stem cells (ESCs). With the involvement of 5-Aza-CdR, differentiated ESCs form capillary-like structures when plated on Matrigel [138]. When treating human bone-marrow-derived MSCs with 5-Aza-CdR, reactivation of endothelial cells specification occurs and arterial marker gene expression level is elevated, accompanied by tube-like structure formation[139]. These small molecules have been indicated applicable in pulp tissue regeneration, but animal studies are necessary to determine whether DNMT inhibitors can be utilized as regenerative agents.

ncRNA-based RE

Numerous miRNAs and lncRNAs are actively involved in dentin formation and pulp mineralization processes during tooth development and pulp repair, and are crucial for inflammation control and immunomodulation. Emerging evidence has shown that ncRNAs are critical for angiogenesis and neovascularization, both in health and disease contexts[84]. miRNAs have the ability to regulate the migration, proliferation, and differentiation of endothelial cells[140,141]. lncRNAs such as MANTIS and GATA6-AS can promote angiogenic sprouting[142,143]. Consequently, miRNAs and lncRNAs have therapeutic potential in RE. However, their susceptibility to nucleases and poor penetration into cell membranes largely restrict their clinical application [144]. Effective delivery of therapeutic miRNAs has aroused much interest over recent decades. With the development of biotechnology and pharmaceutical progress, substantial approaches are being invented to deliver miRNAs: virus-based delivery, nonviral delivery (lipid nanocarriers, biomaterials, or chemical modifications), and exosome-based delivery systems[144,145]. It can be expected that an increasing number of ncRNA-targeting therapeutics will progress through clinical development in the upcoming years.

When it comes to ncRNA-targeting therapeutics in RE, ncRNA delivery systems highlight topical instead of the systemic application. This is a nascent topic. A team has developed a serum-endurable magnetic GCC-Fe_3O_4 nanocarrier and tested it on cultured pDPSCs, and found that this carrier has delivered miR-218 mimics/inhibitors into cells efficiently with low cytotoxicity[146]. Although further *in vivo* experiments are needed to confirm its efficacy, this could be a promising start. More effort needs to be made in ncRNA-targeting RE until it is clinically feasible.

CHALLENGES OF EPIGENETIC APPROACHES IN PULP REGENERATION

Although epigenetic agents are capable of modulating multiple biological processes implicating proliferation, multi-lineage differentiation, migration, and immunoregulation, which is promising in the process of tissue regeneration, several regulatory obstacles and translational challenges need to be resolved before clinical application. Firstly, the off-target effects cannot be ignored. The majority of HDACis like VPA, SAHA and TSA are pan-inhibitors without specific selectivity, which leads to general upregulation of histone acetylation. Single miRNAs can silence multiple target genes; for instance, during the process of immunomodulation, one miRNA can overexpress anti-inflammatory factors and at the same time upregulate proinflammatory cytokines [147]. Secondly, there are concerns about neoplastic transformation during the process of regeneration. The targets of epigenetic-modulating agents need to be screened and investigated thoroughly so as to minimize unwanted effects before the clinical application of epigenetic therapeutics. Lastly, it requires both financial and technical support to bank DPSCs and reserve this "biological insurance". Standardized and optimized manufacturing protocols need to be established to manage the procedures of collection, isolation, expansion, and cryopreservation and to ensure the quality of cell sources[148].

CONCLUSION

The process of dentin-pulp regeneration relies on stem cells with proliferation and pluripotency capacity, signaling molecules that can regulate cellular fate and scaffold which offers a favorable microenvironment. Complicated regulatory networks of histone modifications, DNA methylation, and ncRNAs are involved in guiding dentin-

pulp regeneration. A thorough understanding of epigenetic regulation in the orchestration of DPSC fate will facilitate the self-renewal, migration, and multi-differentiation of DPSCs during pulp tissue regeneration. Cheerful results of HDACis, such as TSA and MS-275, on bone repair have been achieved in animal models, and thus *in vivo* pulp-dentin regeneration of HDACis can be anticipated. The regenerative potential of DNMTis and ncRNAs is still absent *in vivo* studies. However, risks concerning the delivery system, off-targets, and neoplastic transformation are vigorous research fields and need to be tackled before epigenetic strategies applied in optimizing dentin-pulp regeneration. The epigenetic manipulation of DPSCs towards differentiation and regeneration with small molecules will be a hopeful direction in the search for approaches of functional pulp reconstruction.

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