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Epigenetic control of dental stem cells: progress and prospects in multidirectional differentiation

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Abstract
Dental stem cells, with their exceptional proliferative capacity and multidirectional differentiation potential, hold significant promise for dental and oral tissue regeneration. Epigenetic inheritance, which involves stable and heritable changes in gene expression and function without alterations to the DNA sequence, plays a critical role in numerous biological processes. Environmental factors are particularly influential in epigenetic inheritance, as variations in exposure can lead to changes in epigenetic modifications that subsequently impact gene expression. Epigenetic mechanisms are widely involved in processes such as bone homeostasis, embryogenesis, stem cell fate determination, and disease development. Recently, the epigenetic regulation of dental stem cells has attracted considerable research attention. This paper reviews studies focused on the epigenetic mechanisms governing the multidirectional differentiation of dental stem cells.

Keywords Dental stem cells, Epigenetic regulation, Stem cell differentiation

Introduction

Epigenetics involves heritable changes in gene function that do not alter the DNA sequence but ultimately result in phenotypic changes [1]. In eukaryotes, common epigenetic modifications include chromatin remodeling, DNA methylation (and demethylation), histone modifications (such as methylation and acetylation), and RNA modifications [2–4]. These modifications are reversible and can respond to specific cellular stimuli or epigenetic drugs, thereby influencing gene transcription [5]. The complex epigenetic landscape plays a crucial role in various biological processes, including cell proliferation, differentiation, and development, with its importance becoming increasingly recognized [6, 7]. Figure 1 shows that epigenetic mechanisms at different levels constitute a huge epigenetic regulatory network, which in turn affects multiple cellular processes.

Stem cells are defined as clone-forming, self-renewing progenitor cells capable of giving rise to one or more specialized cell types, with bone marrow being their primary source [8]. Characterized by high proliferative capacity, self-renewal potential, long lifespan, multispectral differentiation ability, trophic functions, migration/homing capacity, plasticity, and immunomodulatory as well as protective properties, mesenchymal stem cells (MSC) have garnered significant attention for their differentiation and regenerative potential [9–16]. Among the various stem cell types residing in specialized tissues, dental tissue-derived MSC-like populations have been isolated and characterized extensively in recent years. These

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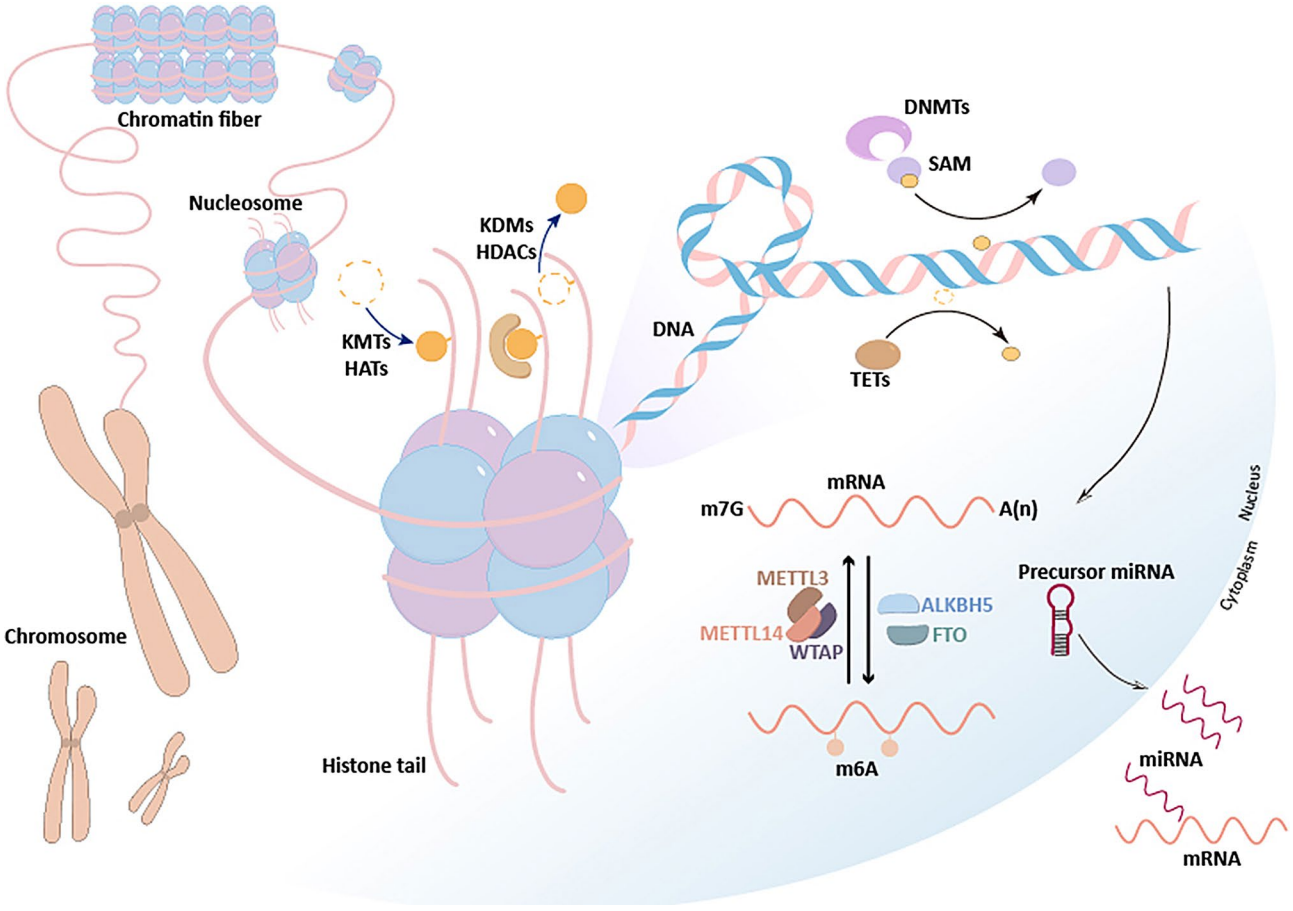

Fig. 1 DNA methylation, histone post-translational modifications, and non-coding RNAs (ncRNAs) are the most common epigenetic marks involved in chromatin remodeling. DNA methylation is achieved by DNA methyltransferases (DNMTs) using S-adenosylmethionine (SAM) as a methyl donor to add methyl groups to cytosine. DNA demethylation is achieved by members of the ten-eleven translocation (TET) family. Histone methyltransferases (KMTs) and histone acetyltransferases (HATs) regulate histone methylation and acetylation, respectively, while histone demethylation and deacetylation are catalyzed by histone lysine demethylases (KDMs) and histone deacetylases (HDACs). The N6-methyladenosine (m6A) mark is catalyzed by the formation of a complex of methyltransferases METTL3, METTL14, and WTAP, and can be catalyzed by demethylases FTO and ALKBH5. Non-coding RNAs, such as microRNAs (miRNAs), may interact with mRNA and affect cell processes

include dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAPs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicular precursor cells (DFPCs), and gingival mesenchymal stem cells (GMSCs) [17]. In vitro studies have demonstrated that dental stem cells (DSCs) positively express MSC-associated markers such as CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD146, and STRO-1, while lacking the expression of hematopoietic markers, including CD14, CD31, CD34, and CD45 [8, 18, 19]. Key pluripotency markers, such as Oct4, Sox2, and Nanog, are also expressed at varying levels in DSCs [20]. This evidence highlights the ability of DSCs to differentiate into multiple tissue types. Studies have shown that DSCs can differentiate towards odontogenic, myogenic, chondrogenic, adipogenic, osteogenic and neurogenic [18, 21–23]. Notably, DPSCs, owing to their neural crest origin, exhibit superior neurogenic potential compared to other MSCs [24]. Additionally, several studies have reported that DPSCs can differentiate into endothelial cells [25]. Figure 2 demonstrates the multilineage differentiation potential of DPSCs under epigenetic regulation.

DSCs share MSC-like properties [26], including high proliferation rates, pluripotency, self-renewal capacity [27], and immunomodulatory capabilities [28], making them promising tools for in vitro and in vivo differentiation studies, as well as for the treatment of immune-related diseases. In adult stem cells, the process of differentiation involves a gradual transition from a stem cell phenotype to a lineage- or tissue-specific specialized phenotype, driven by various regulatory mechanisms, including epigenetic modifications [29]. This review aims to highlight the critical role of epigenetic modifications in odontogenic stem cell differentiation, with certain

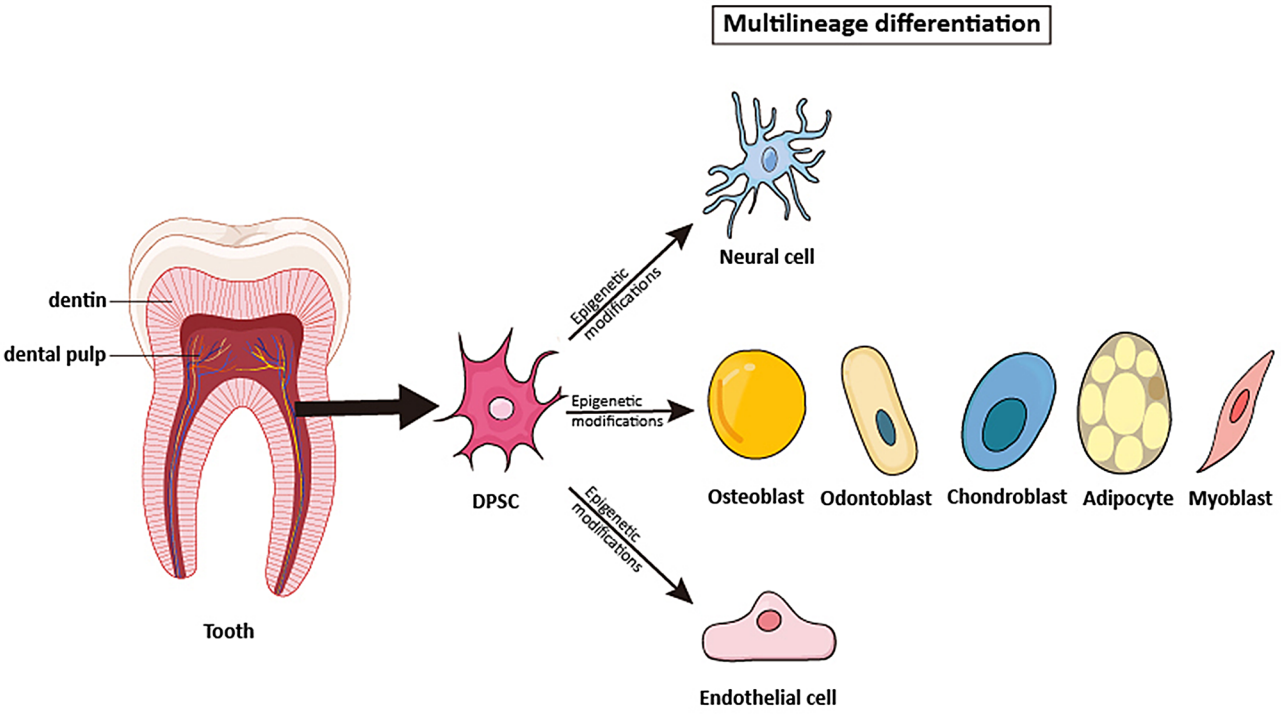

Fig. 2 Multilineage differentiation potential of human dental pulp stem cells (DPSCs). DPSCs have the capacity to differentiate under epigenetic modification into different somatic cell, and finally contribute to regeneration of pulp or periodontal tissue

modification sites potentially providing a theoretical foundation for tooth and periodontal tissue regeneration.

DNA methylation

DNA methylation

DNA methylation is a crucial process catalyzed by DNA methyltransferases (DNMTs), which use S-adenosylmethionine (SAM) as the methyl donor to add a methyl group selectively to the cytosine of CG dinucleotides in DNA, forming 5-methylcytosine (5mC) [30]. CpG sequences exist in two forms: scattered CpGs and CpG islands. DNA methylation primarily occurs in CpG islands, which are cytosine- and guanine-rich sequences predominantly found in promoter regions and exon 1 of genes [31]. Typically, CpG islands are hypomethylated and play a crucial role in regulating gene expression [32]. DNA methylation represses gene expression through two main mechanisms: directly inhibiting transcription factor binding to their target sequences, or through the recruitment of transcriptional repression cofactors by methyl CpG-binding proteins (MBDs), which recognize methylated CpG dinucleotides. These cofactors include chromatin remodelers, histone deacetylases, and histone methyltransferases [33].

The regulation of DNA methylation is carried out by several DNMTs, with DNMT1, DNMT3A, and DNMT3B being the primary enzymes responsible for establishing and maintaining DNA methylation in cells, while DNMT3L, despite lacking enzymatic activity, plays a supportive role in the process [34]. In addition, the ten-eleven translocation (TET) family, which includes TET1, TET2, and TET3, is essential for DNA demethylation [35]. The demethylation process begins with the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) by TET dioxygenases, followed by the replacement of 5hmC with cytosine via the thymine-DNA glycosylase (TDG) and base excision repair (BER) pathways [36, 37].

DNA methylation in DSCs differentiation

The significant role of DNA methylation in the multidirectional differentiation of dental stem cells has been widely documented in numerous studies. For example, Li et al. demonstrated that demethylation of the KLF4 promoter region facilitates the differentiation of DPSCs into dentinogenic cells while concurrently inhibiting their proliferation [38]. Similarly, Sun et al. found that inhibiting DNMTs with the compound RG108 led to demethylation of the SP1/KLF4 binding motif and upregulated the expression of genes associated with dentin differentiation in DPSCs [39]. Another DNMT inhibitor, 5-Aza-CdR, reduces DNA methylation by forming a covalent complex with DNMTs and 5-Aza-CdR-substituted DNA [40]. Zhang et al. reported that treating DPSCs with 5-Aza-CdR suppressed their proliferation, upregulated the expression of DSPP, DMP-1, OSX, RUNX2, and DLX5,

increased ALP activity, and accelerated the formation of calcified nodules [41].

The methyl CpG-binding protein MeCP2 is also involved in this process; melatonin has been shown to promote dentinogenic differentiation of DPSCs by inhibiting DNMT1 and MeCP2, thereby affecting DNA methylation levels [42]. In another study, Li et al. used lentivirus-mediated shRNA to knock down TET1, which inhibited its binding to the FAM20C promoter, leading to reduced FAM20C hydroxymethylation, transcription, and expression. This suppression of TET1 consequently inhibited the dentinogenic differentiation and proliferation of DPSCs [43, 44].

DNA methylation also plays a role in the lipogenic differentiation of DPSCs. WNT-3 A signaling has been shown to induce comprehensive epigenetic reprogramming in DPSCs, steering their differentiation toward either osteogenic or adipogenic lineages [45]. The activation of Wnt/Notch signaling through transient pre-treatment has been found to enhance DPSC differentiation into mature osteoblasts and adipocytes [46]. However, DPSCs have limited lipogenic potential, and under lipogenic conditions, they overexpress genes that inhibit adipogenesis. Notably, increased expression of DNMT1 and DNMT3A leads to the downregulation of adipogenesis-associated genes such as KLF4, c-myc, and PPARγ, thereby promoting osteogenic differentiation [47].

Nakatsuka et al. explored the myogenic differentiation potential of mouse DPSCs using 5-Aza-CdR. They found that 5-Aza-CdR-induced DNA demethylation upregulated muscle-specific transcription factors like myoblastogenin and Pax7 [48–50]. Additionally, Diomede et al. discovered that 5-Aza-CdR enhances the reprogramming efficiency of GMSCs [51]. Differences in DNA methylation profiles have been proposed as predictors of osteogenic potential among various odontogenic cell types. PDLSCs, in particular, exhibit higher levels of osteogenesis-related transcripts, superior in vitro osteogenic potential, and enhanced in vivo bone formation compared to DPSCs and DFPCs [52]. Furthermore, Liu et al. observed that PDLSCs exposed to high glucose conditions showed increased DNA methylation and decreased osteogenic differentiation, with downregulated expression of key osteogenesis-related genes such as ALP, OCN, OPN, and OSX [53].

Histone modification types

Eukaryotic chromatin, composed of DNA and proteins, exists in two forms: euchromatin, which is loosely packed and promotes gene transcription, and heterochromatin, which is tightly packed and leads to gene repression [54]. The fundamental unit of chromatin is the nucleosome, consisting of DNA wrapped around octamers of core histones H2A, H2B, H3, and H4. Each histone octamer has a positively charged N-terminal tail that protrudes from the nucleosome core and can undergo various post-translational modifications, including methylation, acetylation, ubiquitination, and phosphorylation [55–57]. These modifications play a crucial role in altering chromatin structure and activity, thereby regulating gene transcription [58]. Among these, histone methylation and acetylation are the most extensively studied modifications.

Histone methylation

Histone methylation

Histone methylation is a process facilitated by histone methyltransferases (KMTs) and histone lysine demethylases (KDMs), which involves the transfer of methyl groups from S-adenosyl methionine (SAM) to specific arginine or lysine residues on histone proteins [59]. Depending on the lysine residue targeted, histone methylation can either activate or repress gene expression [60]. This regulatory mechanism plays a critical role in modulating terminal cell differentiation by dynamically adjusting gene expression as needed, either promoting or inhibiting transcription [61].

Histone H3 is particularly notable for its prevalent modifications around the transcription start site (TSS). Specifically, the trimethylation of lysines 4, 36, and 79 (H3K4, H3K36, and H3K79) on histone H3 is associated with open chromatin structures and transcriptional activation. In contrast, the trimethylation of lysines 9 and 27 (H3K9 and H3K27) leads to chromatin compaction and is linked to transcriptional repression [62, 63].

Histone methylation in DSCs differentiation

EZH2 is the sole histone methyltransferase responsible for H3K27 methylation. As a major subunit of Polycomb Repressive Complex 2 (PRC2), EZH2 represses gene expression by catalyzing the trimethylation of histone H3K27 [64, 65]. Wang et al. reported that EZH2 plays a crucial role in regulating osteogenic differentiation of PDLSCs within an inflammatory microenvironment via the TLR4/MyD88/NF-κB signaling pathway [66]. Similarly, Cheng et al. discovered that downregulation of EZH2 enhances osteogenic differentiation of PDLSCs under inflammatory conditions by activating the Wnt/β-catenin pathway [67].

Key demethylases involved in reversing repressive H3K27 methylation marks to activate gene expression include KDM6A, KDM6B, and KDM7A. KDM6A, also known as UTX, specifically demethylates trimethylated H3K27 to initiate transcriptional activation and can also promote H3K4 methylation [68, 69]. Knockdown of KDM6A in PDLSCs increases H3K27 methylation at the SOX9 promoter, reducing the expression of chondrogenic markers such as SOX9, Col2a1, and ACAN [68]. Jiang et al. found that inhibiting miR-153-3p led to

increased KDM6A expression, and promoted the osteogenic di erentiation of PDLSCs, as demonstrated by increases in ALP activity, matrix mineralization, and ALP, Runx2, and OPN expression [69]. KDM6B, or JMJD3, demethylates both trimethylated and dimethylated H3K27 [70, 71]. Yang et al. showed that silencing Jmjd3 resulted in downregulation of osteogenic transcription factors Runx2 and OSX, as well as osteogenic markers OPN, BSP, and OCN, inhibiting osteoblast differentiation and mineralization [72]. In DSCs, KDM6B knockdown led to reduced ALP activity, mineralized nodule formation, and lower mRNA expression of dentinogenic markers SP7 (OSX), BGLAP (OCN), and SPP1 (OPN). Mechanistically, KDM6B removes H3K27 methylation from the BMP2 promoter, activating the gene [73]. Additionally, Hoang et al. found that alcohol-induced DPSCs with downregulated KDM6B exhibited increased methylation, decreased mineralization, and reduced osteogenic differentiation [74].

KDM5A, also known as JARID1A/RBP2, is a histone demethylase that targets H3K4 trimethylation and dimethylation [75]. Li et al. utilized lentivirus to knock down KDM5A in DPSCs, resulting in increased ALP activity, enhanced mineral deposit formation, and upregulation of dentinogenic markers [76]. Another study showed that silencing KDM5A in LPS-treated PDLSCs led to increased miR-495-3p methylation and expression, which upregulated HOXC8 and subsequently enhanced ALP activity, mineralized nodule formation, and expression of Runx2, OCN, and OPN [77]. FBXL11, the first identified JmjC domain-containing protein with histone demethylase activity, also known as JHDM1A, selectively demethylates H3K36, with a preference for the dimethylated form [78]. FBXL11 downregulates EREG transcription in SCAPs by demethylating H3K36me2 and H3K4me3 through binding to BCOR, thus inhibiting their osteogenic and odontogenic differentiation potential [79]. Additionally, KDM3B has been found to promote osteogenic differentiation in SCAPs [80].

Histone acetylation

Histone acetylation

Histone acetylation has been extensively studied in the context of odontogenic stem cells. This process involves the addition of acetyl groups to lysine residues on histone proteins, which neutralizes their positive charge and reduces the electrostatic interactions between DNA and histones [81, 82]. This reduction in charge leads to a more relaxed chromatin structure, facilitating the formation of open, transcriptionally active chromatin. Conversely, deacetylation tightens the DNA-histone interaction, leading to chromatin compaction and gene repression [83].

The dynamics of histone acetylation are regulated by two main types of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs) [84]. HATs are classified into several subfamilies based on sequence conservation within their catalytic domains. These include HAT1, GCN5/PCAF, MYST, CBP/p300, and Rtt109 [85]. According to Allis et al.'s nomenclature, these correspond to KAT1, KAT2A/KAT2B, KAT3B/KAT3A, and KAT11 [86].

The HDAC enzyme family comprises at least 18 genes, which are categorized into four classes: Class I (HDAC1, HDAC2, HDAC3, HDAC8), Class II (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9, HDAC10), and Class IV (HDAC11) are Zn2+-dependent, while Class III HDACs (Sirtuins1-7) are identified based on their similarity to the yeast transcriptional repressor Sir2 [87]. Notably, Class I HDACs are broadly expressed across various tissues, whereas Class II HDACs exhibit more tissue-specific expression and cellular localization [88].

Histone acetylation in DSCs differentiation

Li et al. found that GCN5 regulates DKK1 expression in PDLSCs by acetylating histones H3K9 and H3K14, which affects the expression of osteogenic differentiation factors through modulation of the Wnt/β-catenin pathway [89]. Sun et al. observed that Osthole enhances osteogenic differentiation of PDLSCs by stimulating MOZ or MORF-specific acetylation of H3K9 and H3K14 [90]. Rolph D. N. et al. used ferutinin to activate DPSCs, resulting in significant increases in H3K9 acetylation and H3K4 trimethylation at the promoter regions of WNT3A and DVL3 genes. This activation subsequently triggered the Wnt/β-catenin signaling pathway, leading to upregulation of collagen 1A1 and osteocalcin expression, thereby promoting osteogenic differentiation of DPSCs [91]. Wang et al. found that DPSCs overexpressing p300 showed increased dentinogenic markers in dentinogenic induction medium, suggesting that p300 enhances DPSC differentiation into dentinogenic cells. Interestingly, p300 mutants lacking the HAT domain displayed similar enhancements, implying that p300 might function as a co-activator in regulating dentinogenic gene expression [92]. Feng et al. demonstrated that SIRT1, also known as Sir2, promotes osteogenic differentiation of DPSCs in an inflammatory microenvironment through Wnt/β-catenin signaling, with resveratrol, an activator of SIRT1, facilitating this process [93].

Numerous studies highlight the critical role of HDAC inhibitors (HDACis) in dental stem cell differentiation by disrupting enzyme homeostasis and leading to the accumulation of acetylated proteins, which in turn regulate cellular processes [94]. Tricostatin A (TSA), an inhibitor specific to Class I and Class II HDACs, significantly enhances acetylation of histone H3K9 and H3K14, facilitating osteogenic differentiation of PDLSCs. Interestingly, TSA treatment also leads to downregulation of HDAC3

expression during osteogenic differentiation, indicating that histone deacetylation may regulate HDAC3 expression [95]. Additionally, TSA induces hyperacetylation of RUNX2, which may play a crucial role in its enhancement of PDLSC osteogenic differentiation [96].

Inhibition of HDAC6 with specific inhibitors such as Rocilinostat and Tubastatin A has been shown to accelerate senescence in PDLSCs, reducing their osteogenic differentiation and migratory capacity [97]. Liu et al. reported increased mRNA and protein levels of ALP, DSPP, RUNX2, and OCN in DPSCs cultured with low concentrations of LMK-235, a specific inhibitor of HDAC4 and HDAC5, without affecting cell proliferation. This suggests that LMK-235 promotes dentinogenesis in DPSCs by decreasing HDAC4 activity and activating the VEGF/Akt/mTOR pathway [98]. Valproic acid (VPA), another HDAC inhibitor, was found to promote BSP and OPN expression while reducing OC expression in DPSCs, aligning with the effects observed from silencing HDAC2. This indicates that VPA promotes osteogenesis through selective inhibition of HDAC2 [83]. Moreover, Lee et al. discovered that MS-275 inhibits HDAC activity by replacing zinc ions in the HDAC structure. In the presence of MS-275, selective inhibition of HDAC1 and HDAC3 resulted in increased expression of dentin-associated proteins in DPSCs, suggesting that HDAC inhibitors can directly enter the nucleus to regulate gene expression without requiring external signaling [99] (Table. 1).

Table 1 The role of HDACis in the differentiation of DSCs

Types of HDACis	Inhibited HDAC	Effects on DSCs differentiation	Other Content
TSA95, 96	Class I and Class II HDACs	Promote osteogenic differentiation of PDLSCs	The presence of TSA leads to downregulation of HDAC3 expression and induces hyperacetylation of RUNX2
Rocilinostat97 Tubastatin A97	HDAC6	Reduced osteogenic differentiation of PDLSCs	Accelerate the senescence of PDLSCs and weaken their migration ability
LMK-23598	HDAC4 and HDAC5	Promoting dentinogenesis in DPSCs by reducing HDAC4 activity and activating the VEGF/Akt/mTOR pathway	—
VPA83	HDAC2	Promote osteogenic differentiation of DPSCs	—
MS-27599	HDAC1 and HDAC3	Resulting in increased expression of dentin-related proteins in DPSCs.	HDAC inhibitors can directly enter the nucleus to regulate gene expression without the need for external signaling

RNA modifications

RNA modifications play a crucial role in stem cell differentiation, with over 170 known natural RNA modifications [100, 101]. These include N6-methyladenosine (m6A), N6,2'-O-dimethyladenosine (m6Am), N1-methyladenosine (m1A), 5-methylcytosine (m5C), 5-hydroxymethylcytosine (hm5C), N4-acetylcytidine (ac4C), pseudouridine (Ψ), and N7-methylguanosine (m7G), among others [102, 103]. Most of these modifications are commonly found in abundant RNA types such as messenger RNAs (mRNAs), ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and other non-coding RNAs (ncRNAs) [104–106]. They are typically located at highly conserved sites and can influence critical processes like ribosome maturation and tRNA stability [107]. Among these modifications, m6A and certain ncRNAs have garnered significant research interest, particularly concerning their roles in the differentiation of odontogenic stem cells.

RNA methylation

RNA methylation

m6A was first identified by Desrosiers et al. in 1974 as a prevalent methylated modification occurring at the 6-position nitrogen atom of adenine [108]. It is the most abundant and widespread post-transcriptional modification in mRNAs [109, 110]. This reversible modification dynamically regulates nearly every step of RNA metabolism, including mRNA stability, translation, non-coding RNA processing, and alternative splicing. m6A is involved in critical biological processes such as cellular differentiation, angiogenesis, immune and inflammatory responses, and carcinogenesis [111]. Notably, m6A is also present in various mammalian and bacterial non-coding RNAs, including 18 S rRNAs [112]. For instance, Ignatova et al. identified that the RNA methyltransferase METTL5 catalyzes m6A modification at the A1832 position of 18 S rRNA [113], and METTL5 deletion impairs differentiation in mouse embryonic stem cells (mESCs) [114].

This article focuses on m6A modifications in mRNAs. The addition of m6A is regulated by RNA methyltransferases, including METTL3, METTL14, and WTAP, which form a complex with catalytic functions. This complex facilitates the modification of adenosine to form m6A, which can be reversed by demethylases such as FTO and ALKBH5 [115]. METTL3 is the first identified methyltransferase capable of catalyzing m6A modifications and is the only catalytically active subunit in this complex

[116]. It plays a key role in reading and modulating m6A marks near the 3' untranslated region (3'UTR) of mRNA, enhancing translation [117]. The positioning of m6A modifications affects mRNA differently: enrichment in the 3'UTR influences RNA stability, localization, and translation, while modifications in the 5' untranslated region (5'UTR) impact translation in a cap-independent manner [118, 119]. METTL14, although not catalytically active, is crucial for stabilizing METTL3's binding to target mRNAs [120]. Recent studies have also identified less common methyltransferases such as METTL5, METTL16, ZC3H13, and ZCCHC4 [121].

The dynamic modifications of m6A are recognized and interpreted by "reader" proteins, including those from the YT521-B homology (YTH) domain family (YTHDF1/2/3, YTHDC1/2) and the insulin-like growth factor 2 binding protein family (IGF2BP1/2/3) [122, 123].

mRNA methylation in DSCs differentiation

Luo et al. observed that during the mineralization of DPSCs, the m6A level in NOG increases while its expression decreases. This process regulates osteogenic differentiation of DPSCs by influencing the downstream Smad1/5 signaling pathway [124]. Another study found that METTL3 enhances the mRNA stability of GDF6 and STC1 by adding m6A modifications, thereby promoting dentinogenic differentiation in DPSCs [125].

Cai et al. showed that METTL3 regulates the methylation levels of ATP citrate lyase (ACLY) and mitochondrial citrate transporter protein (SLC25A1) through the m6A readers IGF2BP2 and IGF2BP3, impacting glycolysis. Given that ACLY-derived acetyl coenzyme A is crucial for osteogenic differentiation, it is hypothesized that IGF2BP2 and IGF2BP3 may promote the degradation of ACLY and SLC25A1, thereby inhibiting the proliferation and differentiation of DPSCs [126].

FOXO1, a transcription factor, activates METTL3, leading to the activation of the PI3K/AKT pathway and subsequently promoting osteogenic differentiation in PDLSCs [127]. Zhang et al. found that overexpressing METTL3 increases the stability and expression of lncRNA4114, promoting osteogenic differentiation in PDLSCs derived from periodontitis. This underscores the regulatory role of METTL3-mediated m6A-lncRNA signaling in PDLSC osteogenesis [128].

Gu et al. demonstrated that METTL3-mediated methylation of pre-miR-665/DLX3 promotes odontogenic and osteogenic differentiation of SCAPs. This process is linked to the accelerated degradation of pre-miR-665 by YTHDF2, highlighting METTL3's crucial role in dental stem cell differentiation [129].

Regulation of non-coding RNAs

Non-coding RNAs (ncRNAs) are categorized by their length into short ncRNAs, which are fewer than 30 nucleotides, and long ncRNAs, which are more than 200 nucleotides. Key examples of ncRNAs include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) [130, 131].

ncRNAs play crucial roles in regulating gene expression through various mechanisms, such as histone modification, gene silencing, and targeted DNA methylation [132]. Despite lacking the capacity to encode proteins, ncRNAs can modulate the expression of other genes by interacting with various molecular pathways [133]. They contribute to the overall balance and activity of the gene network through complex regulatory interactions among lncRNAs, miRNAs, and mRNAs [18].

miRNAs in DSCs differentiation

miRNAs are a class of non-coding RNAs, ranging from 17 to 25 nucleotides in length, that regulate gene expression post-transcriptionally. They typically bind to the 3'UTR of target mRNAs, leading to the inhibition of protein translation or destabilization of the mRNA [134, 135]. Bioinformatics predictions suggest that miRNAs may regulate at least one-third of all mRNAs [136].

Studies have identified several miRNAs associated with dentinogenic differentiation in DPSCs, including miR-21, miR-675, and miR-140-5p [137–139]. For instance, Mi et al. discovered that in PDLSCs, CUL4B epigenetically represses miR-320c and miR-371-373 by promoting mono-ubiquitination of H2AK119 and binding of EZH2. This repression affects RUNX2 levels and activates osteogenic marker genes such as ALP and OPN [140]. Hao et al. performed a microarray analysis that identified 116 miRNAs with altered expression following osteogenic induction in PDLSCs, including 30 up-regulated and 86 down-regulated miRNAs. Among these, 31 were found to target osteogenesis-related genes. Notable miRNAs with altered expression included miR-654-3p, miR-4288, and miR-34c-5p (up-regulated), and miR-218-5p, miR-663a, and miR-874-3p (down-regulated). These miRNAs are believed to influence osteogenic differentiation in PDLSCs by targeting relevant genes [141].

Additionally, miR-101 and miR-21 have been shown to regulate osteogenic differentiation in PDLSCs [65]. Furthermore, let-7, one of the earliest identified miRNAs, has been reported to inhibit the odontogenic/osteogenic differentiation potential of SCAPs by targeting MMP1 [142].

lncRNAs in DSCs differentiation

lncRNAs are RNA molecules longer than 200 nucleotides that do not encode proteins [143]. They are classified based on their cellular localization and function.

Intranuclear lncRNAs can influence various physiological and pathophysiological processes by interacting with chromatin-modifying factors, such as PRC2, or by mediating relevant signaling pathways [144–146]. Cytoplasmic lncRNAs often function as competing endogenous RNAs (ceRNAs), interacting with miRNAs to regulate their stability and thereby affecting the expression of downstream target genes related to bone formation [147].

Research has highlighted the critical roles of various lncRNAs in the differentiation of dental stem cells. For example, Liu et al. found that knockdown of lncRNA SNHG7 reduced mineralization in DPSCs, possibly through the upregulation of miR-1226-3p and miR-210-5p [148]. Zeng's study demonstrated that lncRNA H19 affects SAHH activity, influences DLX3 methylation and expression via DNMT3B, and regulates dentinogenic differentiation in DPSCs [149]. HOTAIRM1, another lncRNA, promotes osteogenic differentiation of DFSCs by epigenetically regulating HOXA2 through DNMT1 [150].

The lncRNA ANCR/miR-758/Notch2 axis is involved in osteogenic differentiation of PDLSCs. Overexpression of lncRNA ANCR has been shown to inhibit bone formation in these cells [151]. SNHG8, a mechanically force-sensitive lncRNA, downregulates EZH2, thereby promoting osteogenic differentiation in PDLSCs [152]. Conversely, SNHG1 inhibits osteogenic differentiation by silencing the KLF2 gene through EZH2-mediated methylation of the KLF2 promoter at H3K27 [153]. Another study found that knockdown of lncRNA MEG3 in DFSCs enhanced osteogenic differentiation, suggesting that MEG3 contributes to PRC2 recruitment and gene silencing [154].

Additionally, comparisons between PDLSCs and GMSCs revealed differences in differentiation capacities. GMSCs exhibit higher proliferative, clone-forming, and lipogenic differentiation capacities but lower osteogenic differentiation capabilities compared to PDLSCs [155]. Jia et al. utilized gene microarray technology to identify 2,162 differentially expressed lncRNAs and 1,347 mRNAs between PDLSCs and GMSCs, suggesting these molecules' potential regulatory roles in dental stem cell differentiation processes [156].

circRNAs in DSCs differentiation

circRNAs are a diverse family of non-coding RNAs characterized by their covalently closed loop structures, which make them more stable and less prone to degradation compared to linear RNAs [157, 158]. They are highly conserved across species and exhibit tissue-specific expression profiles [159]. One of their key functions is acting as ceRNAs, where they can sponge or sequester miRNAs, thus modulating miRNA availability and affecting gene expression through intricate circRNA-miRNA-mRNA networks [160, 161].

In the context of dental stem cell differentiation, several studies have highlighted the regulatory roles of circRNAs. Chen et al. identified 43 up-regulated and 144 down-regulated circRNAs during the dentinogenic differentiation of DPSCs. Notably, hsa_circRNA_104101 was found to significantly promote dentinogenic differentiation in DPSCs [162]. In another study, Li et al. reported a total of 333 up-regulated and 317 down-regulated circRNAs during the osteogenic differentiation of SCAPs. They proposed that circRNA circNTATC1 might enhance osteogenic differentiation by interacting with miR-4483 [163].

Furthermore, research by Yang et al. demonstrated that circRNA CDR1as functions as a competitive inhibitor of miR-7, which in turn leads to the upregulation of GDF5. This mechanism activates the Smad1/5/8 and p38 MAPK pathways, thereby promoting osteogenic differentiation in PDLSCs [164]. These findings collectively illustrate the crucial roles circRNAs play in regulating the differentiation of various types of dental stem cells, providing insights into their potential as therapeutic targets in regenerative medicine.

Microenvironment, epigenetic modifications, and the migration and differentiation of DSCs

DSCs can produce and secrete a broad range of bioactive factors that promote tissue growth and regeneration [165]. In addition, DSCs exhibit a strong migratory capacity, enabling them to migrate to sites of tissue damage and inflammation, making them highly effective for tissue regeneration [166]. Numerous studies have highlighted the significant impact of epigenetic modifications on the migration and mobilization of tooth-derived stem cells. For instance, lncRNA H19 inhibits LATS1 expression by enhancing EZH2-induced trimethylation of histone H3 at lysine 27, which subsequently promotes the differentiation, proliferation, and migration of DPSCs [167]. Zhang et al. found that KDM3B enhances the proliferation, migration, chemotaxis, and osteogenic/odontogenic differentiation of SCAPs [168]. Furthermore, the HDAC pan-inhibitor SAHA has been shown to promote mineralization and migration by increasing the expression of matrix metalloproteinase 13 (MMP-13) in rat DPSCs [169]. It is important to note that the differentiation potential of DSCs is not only influenced by their intrinsic epigenetic state but is also closely tied to the complex signaling network of the microenvironment in which they reside.

Epigenetic modifications and the in vivo microenvironment work together through a multidimensional regulatory mechanism to drive the precise differentiation of odontogenic stem cells. When MSCs are exposed to an

inflammatory microenvironment, inflammatory factors such as TNF-α, LPS, and the IL family activate MSCs, promoting their proliferation, migration, and differentiation [170]. Evidence suggests that EZH2 plays a crucial role in the inflammatory response within periodontal tissues. Knockdown of EZH2 prevents the LPS-induced upregulation of TNF-α, IL-1β, and IL-6, while reversing the LPS-induced inhibition of proliferation and migration in PDLSCs, and promoting osteogenesis in PDLSCs [66]. Additionally, metabolites in the in vivo environment, such as acetyl coenzyme A and SAM, directly participate in epigenetic modifications. These metabolites provide chemical groups necessary for histone acetylation and DNA methylation, respectively, thereby influencing stem cell gene expression at the molecular level [171, 172]. Furthermore, hypoxia has been shown to induce the expression of ncRNAs, which are involved in the regulation of migration, proliferation, apoptosis, senescence and other viability of different MSCs. Hypoxia also alters histone modification patterns, affecting the osteogenic differentiation of stem cells [173].

Specific biomaterials can bind biomolecules to guide the migration of odontogenic stem cells to designated areas, promoting the regeneration of damaged tissues. These biomaterials facilitate stem cell differentiation, migration, and survival by mimicking the interaction between DSCs and the natural extracellular matrix (ECM) [166]. For instance, Wei et al. enhanced the migration and proliferation of SCAPs using a filipin-RGD-stem cell factor scaffold (where RGD refers to an arginine-glycine-aspartic acid peptide) [174]. Similarly, A. Khayat et al. demonstrated that PAMAM dendritic polymers functionalized with cyclic RGD peptides increased the tooth-forming potential of pulp cells [175]. Overall, the differentiation of odontogenic stem cells is not only influenced by their intrinsic epigenetic state but is also tightly regulated by external microenvironmental factors, including inflammatory mediators, metabolites, and hypoxia. A deeper understanding of these regulatory mechanisms could lead to the development of more precise intervention strategies, advancing the applications of tissue engineering and regenerative medicine.

Conclusion

Dental stem cells (DSCs), including dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAPs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicle precursor cells (DFPCs), and gingival mesenchymal stem cells (GMSCs), play crucial roles in dental pulp repair, tooth regeneration, and bone tissue engineering. Epigenetic regulation significantly influences these cells by affecting key signaling pathways without altering the DNA sequence. This regulation includes mechanisms such as DNA methylation, histone modification, and RNA modification, which together form a complex network that governs stem cell migration, self-renewal, and differentiation.

In addition to intrinsic epigenetic states, stem cell migration and differentiation are also influenced by various environmental factors. For instance, inflammatory factors, oxygen levels, and metabolites can alter the stem cell microenvironment, thereby modulating their behavior. Understanding the role of epigenetics in oral diseases is crucial for the development of regenerative medicine. By studying the interplay between environmental factors and epigenetic modifications, new theoretical frameworks and potential therapeutic targets for stem cell-based treatments can be developed. Furthermore, specific epigenetic modification sites and environmental regulatory factors are expected to become key research directions in the field of regenerative medicine, providing more precise intervention strategies for clinical applications.

Abbreviations

MSCs	Mesenchymal stem cells
DPSCs	Dental pulp stem cells
SCAPs	Stem cells from the apical papilla
SHEDs	Stem cells from deciduous teeth
PDLSCs	Periodontal ligament stem cells
DFSCs	Dental follicular precursor cells
GMSCs	Gingival mesenchymal stem cells
DSCs	Dental stem cells
mESCs	Mouse embryonic stem cells
DNMTs	DNA methyltransferases
SAM	S-adenosylmethionine
5mC	5-methylcytosine
5hmC	5-hydroxymethylcytosine
TET	Ten-eleven translocation
KMTs	Histone methyltransferases
HATs	Histone acetyltransferases
KDMs	Histone lysine demethylases
HDACs	Histone deacetylases
TSS	Transcription start site
PRC2	Polycomb repressive complex 2
HDACis	HDAC inhibitors
m6A	N6-methyladenosine
YTH	YT521-B homology
3'UTR	3' untranslated region
5UTR	5' untranslated region
ncRNAs	Non-coding RNAs
miRNAs	MicroRNAs
lncRNAs	Long non-coding RNAs
circRNAs	Circular RNAs
ceRNAs	Competing endogenous RNAs

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References

1. Zhang L, Lu Q, Chang C. Epigenetics in Health and Disease. Adv Exp Med Biol. 2020;1253:3–55.
2. Sun H, Li K, Liu C, Yi C. Regulation and functions of non-m6A mRNA modifications. Nat Rev Mol Cell Biol. 2023. https://doi.org/10.1038/s41580-023-00622-x.
3. Trixl L, Lusser A. The dynamic RNA modification 5-methylcytosine and its emerging role as an epitranscriptomic mark. Wiley Interdiscip Rev RNA. 2019;10:e1510.
4. Gopinathan G, Kolokythas A, Luan X, Diekwisch TG. H. epigenetic marks define the lineage and differentiation potential of two distinct neural crest-derived intermediate odontogenic progenitor populations. Stem Cells Dev. 2013;22:1763–78.
5. Assis RIF, et al. Osteogenic commitment of Human Periodontal Ligament cells is predetermined by methylation, chromatin accessibility and expression of key transcription factors. Cells. 2022;11:1126.
6. Fan Z, et al. BCOR regulates mesenchymal stem cell function by epigenetic mechanisms. Nat Cell Biol. 2009;11:1002–9.
7. Ye L, et al. Histone demethylases KDM4B and KDM6B promotes osteogenic differentiation of human MSCs. Cell Stem Cell. 2012;11:50–61.
8. Rodríguez-Lozano FJ, et al. Mesenchymal stem cells derived from dental tissues. Int Endod J. 2011;44:800–6.
9. Estrada JC, et al. Culture of human mesenchymal stem cells at low oxygen tension improves growth and genetic stability by activating glycolysis. Cell Death Differ. 2012;19:743–55.
10. Thiagarajan L, Abu-Awwad HA-DM, Dixon JE. Osteogenic Programming of Human Mesenchymal Stem Cells with highly efficient intracellular delivery of RUNX2. Stem Cells Transl Med. 2017;6:2146–59.
11. Pollock K, et al. Human mesenchymal stem cells genetically Engineered to overexpress brain-derived Neurotrophic Factor Improve Outcomes in Huntington's Disease Mouse models. Mol Ther. 2016;24:965–77.
12. Chelluboina B, et al. Mesenchymal stem cell treatment prevents Post-stroke Dysregulation of Matrix metalloproteinases and tissue inhibitors of Metalloproteinases. Cell Physiol Biochem. 2017;44:1360–9.
13. Vono R, Jover Garcia E, Spinetti G, Madeddu P. Oxidative stress in mesenchymal stem cell senescence: regulation by coding and noncoding RNAs. Antioxid Redox Signal. 2018;29:864–79.
14. Espagnolle N, Balguerie A, Arnaud E, Sensebé L, Varin A. CD54-Mediated Interaction with pro-inflammatory macrophages increases the immunosuppressive function of human mesenchymal stromal cells. Stem Cell Rep. 2017;8:961–76.
15. Bar JK, Lis-Nawara A, Grelewski PG. Dental Pulp Stem Cell-Derived Secretome and its regenerative potential. Int J Mol Sci. 2021;22:12018.
16. Naji A, et al. Biological functions of mesenchymal stem cells and clinical implications. Cell Mol Life Sci. 2019;76:3323–48.
17. Shi X, Mao J, Liu Y. Pulp stem cells derived from human permanent and deciduous teeth: biological characteristics and therapeutic applications. Stem Cells Transl Med. 2020;9:445–64.
18. Chen J et al. Key Markers and Epigenetic Modifications of Dental-Derived Mesenchymal Stromal Cells. *Stem Cells Int* 2021, 5521715 (2021).
19. Yan X, Yan F, Mohammed HAG, Liu O. Maxillofacial-Derived Mesenchymal Stem Cells: Characteristics and Progress in Tissue Regeneration. *Stem Cells Int* 2021, 5516521 (2021).
20. Hu L, et al. Regeneration characteristics of different dental derived stem cell sheets. J Oral Rehabil. 2020;47(Suppl 1):66–72.
21. Chang J, et al. Noncanonical Wnt-4 signaling enhances bone regeneration of mesenchymal stem cells in craniofacial defects through activation of p38 MAPK. J Biol Chem. 2007;282:30938–48.
22. Kunimatsu R, et al. Comparative characterization of stem cells from human exfoliated deciduous teeth, dental pulp, and bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun. 2018;501:193–8.
23. Yalvac ME, et al. Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neovascularization, osteo-, adipo- and neurogenesis. Pharmacogenomics J. 2010;10:105–13.
24. Pagella P, et al. Human dental pulp stem cells exhibit enhanced properties in comparison to human bone marrow stem cells on neurites outgrowth. FASEB J. 2020;34:5499–511.
25. Hilkens P et al. The Angiogenic Potential of DPSCs and SCAPs in an In Vivo Model of Dental Pulp Regeneration. *Stem Cells Int* 2017, 2582080 (2017).
26. Huang GT-J, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res. 2009;88:792–806.
27. Naz S, et al. Comparative analysis of dental pulp stem cells and stem cells from human exfoliated teeth in terms of growth kinetics, immunophenotype, self-renewal and multi lineage differentiation potential for future perspective of calcified tissue regeneration. Pak J Med Sci. 2022;38:1228–37.
28. Smojver I, et al. Mesenchymal stem cells based treatment in Dental Medicine: a narrative review. Int J Mol Sci. 2022;23:1662.
29. Raik S, et al. Temporal modulation of DNA methylation and gene expression in Monolayer and 3D spheroids of Dental Pulp Stem cells during osteogenic differentiation: a comparative study. Tissue Eng Regen Med. 2022;19:1267–82.
30. Meng R, Li D, Feng Z, Xu Q. MyD88 hypermethylation mediated by DNMT1 is associated with LTA-induced inflammatory response in human odontoblast-like cells. Cell Tissue Res. 2019;376:413–23.
31. Meng H, et al. DNA methylation, its mediators and genome integrity. Int J Biol Sci. 2015;11:604–17.
32. Shi L, et al. Roles of DNA methylation in influencing the functions of dental-derived mesenchymal stem cells. Oral Dis. 2023. https://doi.org/10.1111/odi.14770.
33. Kearney M, Cooper PR, Smith AJ, Duncan HF. Epigenetic approaches to the Treatment of Dental Pulp Inflammation and repair: opportunities and obstacles. Front Genet. 2018;9:311.
34. Wu H, Zhang Y, Reversing. DNA methylation: mechanisms, genomics, and biological functions. Cell. 2014;156:45–68.
35. Wang X, Feng Z, Li Q, Yi B, Xu Q. DNA methylcytosine dioxygenase ten-eleven translocation 2 enhances lipopolysaccharide-induced cytokine expression in human dental pulp cells by regulating MyD88 hydroxymethylation. Cell Tissue Res. 2018;373:477–85.
36. Weber AR, et al. Biochemical reconstitution of TET1-TDG-BER-dependent active DNA demethylation reveals a highly coordinated mechanism. Nat Commun. 2016;7:10806.
37. Shen L, et al. Genome-wide analysis reveals TET- and TDG-dependent 5-methylcytosine oxidation dynamics. Cell. 2013;153:692–706.
38. Lin H, et al. KLF4 promotes the odontoblastic differentiation of human dental pulp cells. J Endod. 2011;37:948–54.
39. Sun Z, Yu S, Chen S, Liu H, Chen Z. SP1 regulates KLF4 via SP1 binding motif governed by DNA methylation during odontoblastic differentiation of human dental pulp cells. J Cell Biochem. 2019;120:14688–99.
40. Feng Z, Zhan M, Meng R, Wang X, Xu Q. 5-Aza-2'-deoxycytidine enhances lipopolysaccharide-induced inflammatory cytokine expression in human dental pulp cells by regulating TRAF6 methylation. Bioengineered. 2019;10:197–206.
41. Zhang D, Li Q, Rao L, Yi B, Xu Q. Effect of 5-Aza-2'-deoxycytidine on odontogenic differentiation of human dental pulp cells. J Endod. 2015;41:640–5.
42. Li J, et al. Melatonin-induced suppression of DNA methylation promotes odontogenic differentiation in human dental pulp cells. Bioengineered. 2020;11:829–40.
43. Li Q, et al. FAM20C could be targeted by TET1 to promote odontoblastic differentiation potential of human dental pulp cells. Cell Prolif. 2018;51:e12426.
44. Rao L-J, Yi B-C, Li Q-M, Xu Q. TET1 knockdown inhibits the odontogenic differentiation potential of human dental pulp cells. Int J Oral Sci. 2016;8:110–6.

45. Uribe-Etxebarria V, et al. Wnt-3a induces epigenetic remodeling in Human Dental Pulp Stem cells. Cells. 2020;9:652.
46. Uribe-Etxebarria V, et al. Notch/Wnt cross-signalling regulates stemness of dental pulp stem cells through expression of neural crest and core pluripotency factors. Eur Cell Mater. 2017;34:249–70.
47. Argaez-Sosa AA, et al. Higher expression of DNA (de)methylation-Related genes reduces adipogenicity in Dental Pulp Stem cells. Front Cell Dev Biol. 2022;10:791667.
48. Zeng B, Liu G, Huang JDNA. Methylation and histone modification in Dental-derived mesenchymal stem cells. Stem Cell Rev Rep. 2022;18:2797–816.
49. Liu Y, et al. Epigenetic regulation of dental pulp stem cells and its potential in regenerative endodontics. World J Stem Cells. 2021;13:1647–66.
50. Nakatsuka R, et al. 5-Aza-2'-deoxycytidine treatment induces skeletal myogenic differentiation of mouse dental pulp stem cells. Arch Oral Biol. 2010;55:350–7.
51. Diomede F, et al. 5-Aza exposure improves reprogramming process through embryoid body formation in human gingival stem cells. Front Genet. 2018;9:419.
52. Ai T, et al. DNA methylation profile is associated with the osteogenic potential of three distinct human odontogenic stem cells. Signal Transduct Target Ther. 2018;3:1.
53. Liu Z, et al. DNA demethylation rescues the impaired osteogenic differentiation ability of Human Periodontal Ligament Stem cells in high glucose. Sci Rep. 2016;6:27447.
54. Teven CM et al. Epigenetic regulation of mesenchymal stem cells: a focus on osteogenic and adipogenic differentiation. *Stem Cells Int* 2011, 201371 (2011).
55. Duncan HF, Smith AJ, Fleming GJP, Cooper PR. Epigenetic modulation of dental pulp stem cells: implications for regenerative endodontics. Int Endod J. 2016;49:431–46.
56. Cao J, et al. Epigenetic regulation of osteogenic differentiation of periodontal ligament stem cells in periodontitis. Oral Dis. 2023;29:2529–37.
57. Millán-Zambrano G, Burton A, Bannister AJ, Schneider R. Histone post-translational modifications - cause and consequence of genome function. Nat Rev Genet. 2022;23:563–80.
58. Biswas M, Voltz K, Smith JC, Langowski J. Role of histone tails in structural stability of the nucleosome. PLoS Comput Biol. 2011;7:e1002279.
59. Chen Y, Wang X, Wu Z, Jia S, Wan M. Epigenetic regulation of dental-derived stem cells and their application in pulp and periodontal regeneration. PeerJ. 2023;11:e14550.
60. Martin C, Zhang Y. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol. 2005;6:838–49.
61. Hui T, et al. EZH2, a potential regulator of dental pulp inflammation and regeneration. J Endod. 2014;40:1132–8.
62. Hui T, et al. Epigenetic regulation in dental pulp inflammation. Oral Dis. 2017;23:22–8.
63. Bei Y, et al. ASH1L suppresses Matrix Metalloproteinase through Mitogen-activated protein kinase signaling pathway in Pulpitis. J Endod. 2017;43:306–e3142.
64. Ma H, et al. Enhancer of zeste homolog 2 enhances the migration and chemotaxis of dental mesenchymal stem cells. J Int Med Res. 2020;48:300060519882149.
65. Hui T, et al. EZH2 regulates dental pulp inflammation by direct effect on inflammatory factors. Arch Oral Biol. 2018;85:16–22.
66. Wang P, Tian H, Zhang Z, Wang Z. EZH2 Regulates Lipopolysaccharide-Induced Periodontal Ligament Stem Cell Proliferation and Osteogenesis through TLR4/MyD88/NF-κB Pathway. *Stem Cells Int* 2021, 7625134 (2021).
67. Cheng M, Zhou Q. Targeting EZH2 ameliorates the LPS-Inhibited PDLSC osteogenesis via Wnt/β-Catenin pathway. Cells Tissues Organs. 2020;209:227–35.
68. Wang P, et al. KDM6A promotes chondrogenic differentiation of periodontal ligament stem cells by demethylation of SOX9. Cell Prolif. 2018;51:e12413.
69. Jiang H, Jia P. MiR-153-3p inhibits osteogenic differentiation of periodontal ligament stem cells through KDM6A-induced demethylation of H3K27me3. J Periodontal Res. 2021;56:379–87.
70. Salminen A, Kaarniranta K, Hiltunen M, Kauppinen A. Histone demethylase Jumonji D3 (JMJD3/KDM6B) at the nexus of epigenetic regulation of inflammation and the aging process. J Mol Med (Berl). 2014;92:1035–43.
71. Huszar JM, Payne CJ. MIR146A inhibits JMJD3 expression and osteogenic differentiation in human mesenchymal stem cells. FEBS Lett. 2014;588:1850–6.
72. Yang D, Okamura H, Nakashima Y, Haneji T. Histone demethylase Jmjd3 regulates osteoblast differentiation via transcription factors Runx2 and osterix. J Biol Chem. 2013;288:33530–41.
73. Xu J, Yu B, Hong C, Wang C-Y. KDM6B epigenetically regulates odontogenic differentiation of dental mesenchymal stem cells. Int J Oral Sci. 2013;5:200–5.
74. Hoang M, et al. Alcohol-induced suppression of KDM6B dysregulates the mineralization potential in dental pulp stem cells. Stem Cell Res. 2016;17:111–21.
75. Torres IO, et al. Histone demethylase KDM5A is regulated by its reader domain through a positive-feedback mechanism. Nat Commun. 2015;6:6204.
76. Li Q-M, Li J-L, Feng Z-H, Lin H-C, Xu Q. Effect of histone demethylase KDM5A on the odontogenic differentiation of human dental pulp cells. Bioengineered. 2020;11:449–62.
77. Niu F, Xu J, Yan Y. Histone demethylase KDM5A regulates the functions of human periodontal ligament stem cells during periodontitis via the miR-495-3p/HOXC8 axis. Regen Ther. 2022;20:95–106.
78. Tsukada Y, et al. Histone demethylation by a family of JmjC domain-containing proteins. Nature. 2006;439:811–6.
79. Du J, Ma Y, Ma P, Wang S, Fan Z. Demethylation of epiregulin gene by histone demethylase FBXL11 and BCL6 corepressor inhibits osteo/dentinogenic differentiation. Stem Cells. 2013;31:126–36.
80. Hussain A, Tebyaniyan H, Khayatan D. The Role of Epigenetic in Dental and Oral Regenerative Medicine by Different Types of Dental Stem Cells: A Comprehensive Overview. *Stem Cells Int* 2022, 5304860 (2022).
81. Audia JE, Campbell RM. Histone modifications and Cancer. Cold Spring Harb Perspect Biol. 2016;8:a019521.
82. Chakraborty R, et al. Histone acetyltransferases p300 and CBP Coordinate Distinct Chromatin Remodeling Programs in vascular smooth muscle plasticity. Circulation. 2022;145:1720–37.
83. Paino F, et al. Histone deacetylase inhibition with valproic acid downregulates osteocalcin gene expression in human dental pulp stem cells and osteoblasts: evidence for HDAC2 involvement. Stem Cells. 2014;32:279–89.
84. Legube G, Trouche D. Regulating histone acetyltransferases and deacetylases. EMBO Rep. 2003;4:944–7.
85. Chen H, Huang Z, Chen C. The role of histone acetylation modification in Dental tissue-derived mesenchymal stem cells and Odontogenesis. Cell Reprogram. 2023;25:11–9.
86. Allis CD, et al. New nomenclature for chromatin-modifying enzymes. Cell. 2007;131:633–6.
87. Seto E, Yoshida M. Erasers of histone acetylation: the histone deacetylase enzymes. Cold Spring Harb Perspect Biol. 2014;6:a018713.
88. Yamauchi Y, et al. Histone acetylation as a regenerative target in the dentine-pulp complex. Front Genet. 2020;11:1.
89. Li B, et al. GCN5 modulates osteogenic differentiation of periodontal ligament stem cells through DKK1 acetylation in inflammatory microenvironment. Sci Rep. 2016;6:26542.
90. Sun J, et al. Osthole improves function of periodontitis periodontal ligament stem cells via epigenetic modification in cell sheets engineering. Sci Rep. 2017;7:5254.
91. Rolph DN, et al. Ferutinin directs dental pulp-derived stem cells towards the osteogenic lineage by epigenetically regulating canonical wnt signaling. Biochim Biophys Acta Mol Basis Dis. 2020;1866:165314.
92. Wang T, Liu H, Ning Y, Xu Q. The histone acetyltransferase p300 regulates the expression of pluripotency factors and odontogenic differentiation of human dental pulp cells. PLoS ONE. 2014;9:e102117.
93. Feng G, et al. SIRT1 was involved in TNF-α-promoted osteogenic differentiation of human DPSCs through Wnt/β-catenin signal. Vitro Cell Dev Biol Anim. 2016;52:1001–11.
94. Duncan HF, Smith AJ, Fleming GJP, Cooper PR. HDACi: cellular effects, opportunities for restorative dentistry. J Dent Res. 2011;90:1377–88.
95. Huynh NC-N, Everts V, Pavasant P, Ampornaramveth RS. Inhibition of histone deacetylases enhances the osteogenic differentiation of Human Periodontal Ligament cells. J Cell Biochem. 2016;117:1384–95.
96. Nc H, V, E., A, N., P, P., Rs A. Histone deacetylase inhibition enhances in-vivo bone regeneration induced by human periodontal ligament cells. Bone 95, (2017).
97. Q L, Y, M., Y, Z., T, Z., Y Z. Declined expression of Histone Deacetylase 6 contributes to Periodontal Ligament Stem Cell Aging. J Periodontol 88, (2017).
98. Z L et al. HDAC inhibitor LMK–235 promotes the odontoblast differentiation of dental pulp cells. Mol Med Rep 17, (2018).
99. Lee E-C, Kim Y-M, Lim H-M, Ki G-E, Seo Y-K. The histone deacetylase inhibitor (MS-275) promotes differentiation of Human Dental Pulp Stem cells into Odontoblast-Like cells Independent of the MAPK signaling system. Int J Mol Sci. 2020;21:5771.

100. Thapar R, et al. RNA modifications: reversal mechanisms and Cancer. Biochemistry. 2019;58:312–29.
101. Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA modifications in Gene expression regulation. Cell. 2017;169:1187–200.
102. Morena F, Argentati C, Bazzucchi M, Emiliani C, Martino S. Above the Epitranscriptome: RNA modifications and stem cell identity. Genes (Basel). 2018;9:329.
103. Zhao L-Y, Song J, Liu Y, Song C-X, Yi C. Mapping the epigenetic modifications of DNA and RNA. Protein Cell. 2020;11:792–808.
104. Teng P-C, et al. RNA modifications and epigenetics in modulation of Lung Cancer and Pulmonary diseases. Int J Mol Sci. 2021;22:10592.
105. Cayir A. RNA modifications as emerging therapeutic targets. Wiley Interdiscip Rev RNA. 2022;13:e1702.
106. Barbieri I, Kouzarides T. Role of RNA modifications in cancer. Nat Rev Cancer. 2020;20:303–22.
107. Thomas JM, Bryson KM, Meier JL. Nucleotide resolution sequencing of N4-acetylcytidine in RNA. Methods Enzymol. 2019;621:31–51.
108. Desrosiers R, Friderici K, Rottman F. Identification of methylated nucleosides in messenger RNA from Novikoff hepatoma cells. Proc Natl Acad Sci U S A. 1974;71:3971–5.
109. Wei J, et al. Differential m6A, m6Am, and m1A demethylation mediated by FTO in the cell nucleus and cytoplasm. Mol Cell. 2018;71:973–e9855.
110. Ren W, et al. Structure and regulation of ZCCHC4 in m6A-methylation of 28S rRNA. Nat Commun. 2019;10:5042.
111. Li L, et al. METTL3-mediated long non-coding RNA MIR99AHG methylation targets miR-4660 to promote bone marrow mesenchymal stem cell osteogenic differentiation. Cell Cycle. 2023;22:476–93.
112. Oerum S, Meynier V, Catala M, Tisné C. A comprehensive review of m6A/m6Am RNA methyltransferase structures. Nucleic Acids Res. 2021;49:7239–55.
113. Ignatova VV, et al. The rRNA m6A methyltransferase METTL5 is involved in pluripotency and developmental programs. Genes Dev. 2020;34:715–29.
114. Xing M, et al. The 18S rRNA m6 a methyltransferase METTL5 promotes mouse embryonic stem cell differentiation. EMBO Rep. 2020;21:e49863.
115. Du Y, et al. SUMOylation of the m6A-RNA methyltransferase METTL3 modulates its function. Nucleic Acids Res. 2018;46:5195–208.
116. Luo H, et al. METTL3-mediated m6A modification regulates cell cycle progression of dental pulp stem cells. Stem Cell Res Ther. 2021;12:159.
117. Wang X, et al. N6-methyladenosine modulates Messenger RNA translation efficiency. Cell. 2015;161:1388–99.
118. Zhou J, et al. N6-Methyladenosine guides mRNA alternative translation during Integrated stress response. Mol Cell. 2018;69:636–e6477.
119. Sun T, Wu R, Ming L. The role of m6A RNA methylation in cancer. Biomed Pharmacother. 2019;112:108613.
120. An Y, Duan H. The role of m6A RNA methylation in cancer metabolism. Mol Cancer. 2022;21:14.
121. Ma H, et al. N6-Methyladenosine methyltransferase ZCCHC4 mediates ribosomal RNA methylation. Nat Chem Biol. 2019;15:88–94.
122. Ma S, et al. The interplay between m6A RNA methylation and noncoding RNA in cancer. J Hematol Oncol. 2019;12:121.
123. Zhang C, Liu N. N6-methyladenosine (m6A) modification in gynecological malignancies. J Cell Physiol. 2022;237:3465–79.
124. Luo H, et al. Stage-specific requirement for METTL3-dependent m6A modification during dental pulp stem cell differentiation. J Transl Med. 2022;20:605.
125. Pan Y, et al. METTL3 enhances dentinogenesis differentiation of dental pulp stem cells via increasing GDF6 and STC1 mRNA stability. BMC Oral Health. 2023;23:209.
126. Cai W, et al. METTL3-Dependent glycolysis regulates Dental Pulp Stem Cell differentiation. J Dent Res. 2022;101:580–9.
127. Wang Q, Shi W, Lin S, Wang H. FOXO1 regulates osteogenic differentiation of periodontal ligament stem cells through the METTL3 signaling pathway. J Orthop Surg Res. 2023;18:637.
128. Zhang X, et al. N6-methyladenosine promotes osteogenic differentiation of PDLSCs from periodontitis patients. Oral Dis. 2022. https://doi.org/10.1111/odi.14467.
129. Gu T, et al. METTL3-mediated pre-miR-665/DLX3 m6A methylation facilitates the committed differentiation of stem cells from apical papilla. Exp Mol Med. 2024. https://doi.org/10.1038/s12276-024-01245-8.
130. Sufianov A, et al. The role of noncoding RNAs in the osteogenic differentiation of human periodontal ligament-derived cells. Noncoding RNA Res. 2023;8:89–95.
131. Bakinowska E, et al. Non-coding RNA as biomarkers and their role in the Pathogenesis of gastric Cancer-A narrative review. Int J Mol Sci. 2024;25:5144.
132. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet. 2011;12:861–74.
133. Matsui M, Corey DR. Non-coding RNAs as drug targets. Nat Rev Drug Discov. 2017;16:167–79.
134. Mo Z, Li Q, Cai L, Zhan M, Xu Q. The effect of DNA methylation on the miRNA expression pattern in lipopolysaccharide-induced inflammatory responses in human dental pulp cells. Mol Immunol. 2019;111:11–8.
135. Yan H, Bu P. Non-coding RNA in cancer. Essays Biochem. 2021;65:625–39.
136. Zhong S, Zhang S, Bair E, Nares S, Khan AA. Differential expression of microRNAs in normal and inflamed human pulps. J Endod. 2012;38:746–52.
137. Sun D-G, et al. Mir-140-5p-mediated regulation of the proliferation and differentiation of human dental pulp stem cells occurs through the lipopolysaccharide/toll-like receptor 4 signaling pathway. Eur J Oral Sci. 2017;125:419–25.
138. Zeng L, et al. miR-675 promotes odontogenic differentiation of human dental pulp cells by epigenetic regulation of DLX3. Exp Cell Res. 2018;367:104–11.
139. Xu K, et al. MiR-21/STAT3 Signal is involved in Odontoblast differentiation of Human Dental Pulp Stem cells mediated by TNF-α. Cell Reprogram. 2018;20:107–16.
140. Mi J, et al. CUL4B Upregulates RUNX2 to promote the osteogenic differentiation of Human Periodontal Ligament Stem cells by Epigenetically repressing the expression of miR-320c and miR-372/373-3p. Front Cell Dev Biol. 2022;10:921663.
141. Hao Y, et al. Identification of MicroRNAs by Microarray Analysis and prediction of target genes involved in osteogenic differentiation of Human Periodontal ligament stem cells. J Periodontol. 2017;88:1105–13.
142. Wang Y, et al. MicroRNA hsa-let-7b suppresses the odonto/osteogenic differentiation capacity of stem cells from apical papilla by targeting MMP1. J Cell Biochem. 2018;119:6545–54.
143. X T, S, L., G, C., J, W., S X. Approaches for modes of Action Study of Long noncoding RNAs: from single Verification to genome-wide determination. Int J Mol Sci 24, (2023).
144. Mattick JS, et al. Long non-coding RNAs: definitions, functions, challenges and recommendations. Nat Rev Mol Cell Biol. 2023;24:430–47.
145. Quinodoz S, Guttman M. Long noncoding RNAs: an emerging link between gene regulation and nuclear organization. Trends Cell Biol. 2014;24:651–63.
146. Rutenberg-Schoenberg M, Sexton AN, Simon MD. The properties of long noncoding RNAs that regulate chromatin. Annu Rev Genomics Hum Genet. 2016;17:69–94.
147. Thomson DW, Dinger ME. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet. 2016;17:272–83.
148. Liu Z, Xu S, Dao J, Gan Z, Zeng X. Differential expression of lncRNA/miRNA/mRNA and their related functional networks during the osteogenic/odontogenic differentiation of dental pulp stem cells. J Cell Physiol. 2020;235:3350–61.
149. Zeng L, et al. Long non-coding RNA H19/SAHH axis epigenetically regulates odontogenic differentiation of human dental pulp stem cells. Cell Signal. 2018;52:65–73.
150. Chen Z, et al. lncRNA HOTAIRM1 promotes osteogenesis of hDFSCs by epigenetically regulating HOXA2 via DNMT1 in vitro. J Cell Physiol. 2020;235:8507–19.
151. Peng W, et al. Long noncoding RNA ANCR suppresses bone formation of periodontal ligament stem cells via sponging miRNA-758. Biochem Biophys Res Commun. 2018;503:815–21.
152. Zhang Z, et al. Mechanical force-sensitive lncRNA SNHG8 inhibits osteogenic differentiation by regulating EZH2 in hPDLSCs. Cell Signal. 2022;93:110285.
153. Li Z, Guo X, Wu S. Epigenetic silencing of KLF2 by long non-coding RNA SNHG1 inhibits periodontal ligament stem cell osteogenesis differentiation. Stem Cell Res Ther. 2020;11:435.
154. Deng L, et al. Down-regulated lncRNA MEG3 promotes osteogenic differentiation of human dental follicle stem cells by epigenetically regulating wnt pathway. Biochem Biophys Res Commun. 2018;503:2061–7.
155. Gao Y, et al. Isolation and multiple differentiation potential assessment of human gingival mesenchymal stem cells. Int J Mol Sci. 2014;15:20982–96.
156. Jia L, et al. Comparative analysis of lncRNA and mRNA expression profiles between periodontal ligament stem cells and gingival mesenchymal stem cells. Gene. 2019;699:155–64.
157. Dong J, et al. Challenges and opportunities for circRNA identification and delivery. Crit Rev Biochem Mol Biol. 2023;58:19–35.
158. Kristensen LS, Jakobsen T, Hager H, Kjems J. The emerging roles of circRNAs in cancer and oncology. Nat Rev Clin Oncol. 2022;19:188–206.
159. Qu S, et al. Circular RNA: a new star of noncoding RNAs. Cancer Lett. 2015;365:141–8.

160. Zhou W-Y, et al. Circular RNA: metabolism, functions and interactions with proteins. Mol Cancer. 2020;19:172.
161. Chen L, et al. The bioinformatics toolbox for circRNA discovery and analysis. Brief Bioinform. 2021;22:1706–28.
162. Chen M, Yang Y, Zeng J, Deng Z, Wu B. circRNA Expression Profile in Dental Pulp Stem Cells during Odontogenic Differentiation. *Stem Cells Int* 2020, 5405931 (2020).
163. Li Z, et al. Differential circular RNA expression profiling during osteogenic differentiation of stem cells from apical papilla. Epigenomics. 2019;11:1057–73.
164. Li X, et al. Circular RNA CDR1as regulates osteoblastic differentiation of periodontal ligament stem cells via the miR-7/GDF5/SMAD and p38 MAPK signaling pathway. Stem Cell Res Ther. 2018;9:232.
165. Hung C-N, et al. A comparison between adipose tissue and dental pulp as sources of MSCs for tooth regeneration. Biomaterials. 2011;32:6995–7005.
166. Yuan W, Ferreira L, de Yu AQ, Ansari B, S., Moshaverinia A. Dental-derived stem cells in tissue engineering: the role of biomaterials and host response. Regen Biomater. 2024;11:rbad100.
167. Du Z, Shi X, Guan A. lncRNA H19 facilitates the proliferation and differentiation of human dental pulp stem cells via EZH2-dependent LATS1 methylation. Mol Ther Nucleic Acids. 2021;25:116–26.
168. Zhang C et al. The Histone Demethylase KDM3B Promotes Osteo-/Odontogenic Differentiation, Cell Proliferation, and Migration Potential of Stem Cells from the Apical Papilla. *Stem Cells Int* 2020, 8881021 (2020).
169. Duncan HF, et al. The histone-deacetylase-inhibitor Suberoylanilide Hydroxamic Acid promotes Dental Pulp Repair mechanisms through Modulation of Matrix Metalloproteinase-13 activity. J Cell Physiol. 2016;231:798–816.
170. Li C, Li G, Liu M, Zhou T, Zhou H. Paracrine effect of inflammatory cytokine-activated bone marrow mesenchymal stem cells and its role in osteoblast function. J Biosci Bioeng. 2016;121:213–9.
171. Shvedunova M, Akhtar A. Modulation of cellular processes by histone and non-histone protein acetylation. Nat Rev Mol Cell Biol. 2022;23:329–49.
172. Chiang PK, et al. S-Adenosylmethionine and methylation. FASEB J. 1996;10:471–80.
173. Yoon D-K, et al. The involvement of histone methylation in osteoblastic differentiation of human periosteum-derived cells cultured in vitro under hypoxic conditions. Cell Biochem Funct. 2017;35:441–52.
174. Wei J, Sun X-Q, Hou B-X. Evaluation of Silk Fibroin-RGD-Stem Cell Factor Scaffold Effect on Adhesion, Migration, and Proliferation of Stem Cells of Apical Papilla. *Stem Cells Int* 2021, 6612324 (2021).
175. Kim JK, et al. Differentiating dental pulp cells via RGD-dendrimer conjugates. J Dent Res. 2010;89:1433–8.

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