REVIEW ARTICLE

Specific microRNAs Regulate Dental Pulp Stem Cell Behavior

Promphakkon Kulthanaamondhita, DDS, MSc, Chatvadee Kornsuthisopon, DVM, MSc, PhD,* Suphalak Photichailert, BSc,* Jeeranan Manokawinchoke, BSc, MSc, PhD,* Phoonsuk Limraksasin, DDS, PhD,*† and Thanaphum Osathanon, DDS, PhD**

ABSTRACT

Introduction: MicroRNAs (miRNAs), small noncoding RNAs, control the translation of messenger RNAs into proteins. miRNAs have a crucial role in regulating the diverse biological processes of many physiological and pathological activities. The aim of this systematic review was to explore various functions of miRNAs in the regulation of dental pulp stem cell (DPSC) behavior. **Methods:** The articles were searched in PubMed, SCOPUS, and ISI Web of Science database using designated keywords. Full-length manuscripts published in English in peer-reviewed journals relevant to the role of miRNAs in DPSC functions were included and reviewed by 2 independent researchers. **Results:** The original search of the database generated 299 studies. A total of 102 duplicate studies were removed. After their exclusion, 48 studies were selected for review. miRNAs have shown to modulate the stemness and differentiation of various mesenchymal stem cells. The miRNAs expression profiles in DPSCs were differed compared with other cell types and have been demonstrated to regulate the levels of proteins crucial for promoting or inhibiting DPSC proliferation as well as differentiation. Further, miRNAs also modulate inflammatory processes in dental pulp. **Conclusion:** miRNAs have various functions on the regulation of DPSCs and understanding these roles of miRNAs is crucial for the development of new therapeutics in regenerative dental medicine. With the advancing technologies, the utilization of miRNA technology could revolutionarily change the future of regenerative endodontics. *(J Endod 2022;■:1–11.)*

KEY WORDS

Dental pulp stem cells; differentiation; inflammation; microRNAs; proliferation

SIGNIFICANCE

Understanding the functions of miRNAs on the dental pulp–derived cells is crucial for the development of new therapeutics in regenerative dental medicine. With the advancing technologies, miRNA technology could revolutionarily change the future of regenerative treatment in endodontics.

Dental pulp stem cells (DPSCs) are mesenchymal stem cells residing in dental pulp tissue. DPSCs are clonogenic and exhibit a high proliferative rate and express mesenchymal cell surface protein markers.[1,2] These cells have multipotential differentiation ability and can differentiate into many cell types.[3–7] Previous studies demonstrated that DPSCs were effective in regenerating bone tissue,[8] heart muscle,[9] hair follicles,[10] corneal epithelium, and neuronal cells.[11] Moreover, DPSCs have a role in inflammatory responses and immune-privilege and immunomodulatory effects.[12] These properties make DPSCs an appropriate candidate for regenerative medicine applications, and currently there are several applications that are in clinical trials.[13–15] One of the crucial processes in manipulating stem cells is the tight control of cell responses so that the desired function of the cells is obtained in the specific application. MicroRNAs (miRNAs) are one of the mechanisms regulating cellular responses.

miRNAs are small noncoding RNA molecules consisting of ~25 nucleotides. The miRNAs modulate expression of numerous genes in various biological processes, including development, healing, and regeneration, as well as the initiation and progression of diseases. miRNAs have important functions in the behavior of multicellular organisms by controlling key metabolic and homeostasis processes (e.g., proliferation, differentiation, gene expression, protein modification, and apoptosis).[16,17] miRNAs regulate cell responses by messenger RNA (mRNA) degradation, mRNA deadenylation, and translation repression.[18,19] Canonical miRNAs are transcribed from DNA sequences by RNAse pol II and processed by RNAse III Drosha activity, resulting in primary miRNAs (pri-miRNAs).[20] However, noncanonical miRNAs do not require processing by Drosha. The noncanonical miRNA processing requires the functions of spliceosome and debranching enzymes.[20] The precursor miRNAs (pre-miRNAs) have 65 nucleotides with a hairpin structure and are transported by exportin-5 from the nucleus to the cytoplasm.[21] In the cytoplasm, pre-miRNAs are processed into mature miRNAs by Dicer.[22,23]

Previous studies have demonstrated several roles of miRNAs in the regulation of numerous cellular responses, including cell proliferation, apoptosis, metabolism, and differentiation in various types of stem cells;[24–27] however, the roles of miRNAs in the DPSCs have not been extensively studied. The aim of this

From the *Dental Stem Cell Biology Research Unit and Department of Anatomy, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand; †Division of Molecular and Regenerative Prosthodontics, Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan

Address requests for reprints to Thanaphum Osathanon, Dental Stem Cell Biology Research Unit, Faculty of Dentistry, Chulalongkorn University, Bangkok, 10330 Thailand.
E-mail address: thanaphum.o@chula.ac.th

0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2022.02.012

study was to systematically review the relevant studies on the various functions of miRNAs in the regulation of DPSCs, including cell proliferation, differentiation, and inflammation.

METHODS

The articles were searched in PubMed (MEDLINE) and SCOPUS using Medical Subject Heading or Title/Abstract words ("microRNAs/miRNAs" and "dental pulp stem cells/DPSCs"). Only English-based literature and non-English studies with available English abstracts were evaluated. Duplicate studies were removed, and the relevant articles were selected based on the title and abstract screening. Conference abstracts, reviews, and studies without full articles were excluded. Full-length articles published in English in peer-reviewed journals that explored the role of miRNAs in relation to proliferation, differentiation, inflammation, and other potential DPSC functions were included.

RESULTS

The original search of the databases generated 299 studies. A total of 102 duplicate studies were removed. After their exclusion, 48 studies were selected for review. The flowchart of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) is shown in Supplementary Figure 1.

miRNAs Biosynthesis

miRNAs are small noncoding regions of RNAs with 21 to 25 nucleotides. miRNA biosynthesis starts from DNA being transcribed to RNA. The canonical miRNA pri-miRNAs are produced by RNA polymerase II, and then converted to pre-miRNAs by Drosha-DiGeorge Syndrome Critical Region 8 enzymes in the nucleus. This process results in a cleaved sequence of the pre-miRNAs, which consists of ~80 to 100 nucleotides that preserves the RNA hairpin structure.[28] The pre-miRNAs are exported from the nucleus into the cytoplasm via the exportin 5/RAN-GTPase pathway. In the cytoplasm, the pre-miRNAs are altered to become mature miRNAs using RNA polymerase III Dicer, which is related to the TAR RNA-binding protein in pre-miRNAs, and then cleaved into an miRNA/miRNA duplex.[29] In addition, Argonaute 1–4 converts the miRNA/miRNA duplex into mature miRNAs. Ultimately, the RNA-induced silencing complex is loaded onto the 3′ untranslated regions (UTRs) rather than the 5′ UTRs of the target transcripts, which controls the translation of the complementary mRNA.[30,31] The miRNAs modulate 3 mechanisms: (1) translation repression, (2) mRNA destabilization, and (3) mRNA degradation (Fig. 1)

miRNAs in Stem Cells

miRNAs regulate numerous cellular responses, including the control of stem cell functions, for example cell proliferation, apoptosis, metabolism, and differentiation.[24,25] In this section, the roles of miRNAs in regulating stem cell functions are described.

miRNAs *in Stemness Maintenance, Cell Proliferation, and Apoptosis*

miRNAs play an important role in preserving the stem cell properties in embryonic stem cells.[26,27] During the undifferentiated stage of human embryonic stem cells, miR-145 levels are low;[32] however, the miR-145 levels increase corresponding with differentiation.[32] miR-145 targets several pluripotent marker genes (*OCT4*, *SOX2*, and *KLF4*).[32] Therefore, the regulation of miR-145 expression modulates the stemness maintenance and differentiation in human embryonic stem cells. In mouse embryonic stem cells, miR-134, miR-296, and miR-470 control stemness maintenance because the upregulation of these miRNAs is observed after differentiation induction.[33] The coding sequence targets of miRNA on pluripotent related genes (*Nanog*, *Oct4*, and *Sox2*) are different between species.[33]

miRNAs in Stem Cell Differentiation

The roles of miRNAs in osteogenic differentiation have been extensively investigated in various cell types to understand how they regulate bone formation and regeneration. During the osteogenic differentiation of human adipose stem cells, miR-149-3p expression increases in a time-dependent manner.[34] miR-149-3p targets v-akt murine thymoma viral oncogene homolog 1 (AKT1).[34] Overexpression of miR-149-3p mimics enhances alkaline phosphatase enzymatic activity, mineral deposition, and osteogenic marker gene expression (*BMP2*, *OPN*, *OCN*, *OSX*, *RUNX2*, and *COL1A1*) in human adipose stem cells after osteogenic induction.[34]

miR-138, targeting the 3′ translated region of EID-1, overexpression in human adipose tissue-derived mesenchymal stem cells attenuates intracellular lipid droplets accumulation and suppresses the mRNA expression of key adipogenic transcription factors and adipogenic marker genes.[35] miR-143 is involved in several stages of adipogenic differentiation. miR-143 suppresses MAP2K5 in the adipose stem cell clonogenic stage, which inhibits adipogenic differentiation.[36] A study in mice demonstrated that miR-143 upregulation is associated with increased body weight and mesenteric fat weight.[37]

A microarray study revealed that miR-140 is significantly differentially expressed between mesenchymal stem cells and mature chondrocytes, suggesting the involvement of this miRNA in chondrogenic differentiation.[38] Furthermore, exosomes containing miR-140 promote chondrogenic differentiation in bone marrow mesenchymal stem cells *in vitro*.[39] miR-140 targets the small GTPase RALA, which regulates the chondrogenic transcription factor, SOX9.[40]

miRNAs and DPSCs

Different sources of stem cells also demonstrated differences in miRNA expression profiles. Microarray analysis has shown that miR-146a and miR-155 were highly expressed in gingival fibroblasts, whereas the expression and regulation of these miRNAs are less pronounced in dental pulp cells.[41] Furthermore, an investigation of differentially expressed miRNAs in normal and inflamed dental pulp found that miR-150, miR-584, and miR-766 were upregulated in an inflamed dental pulp compared with normal pulp.[42] Hence, investigations regarding the role of specific miRNAs in specific cell types and conditions are needed.

miRNAs have been shown to control numerous cellular processes in dental pulp cells. For example, aging, a physiologic process, also influences the senescence of human dental pulp cells (hDPs). miR-433 is an miRNA that negatively regulates growth factor receptor-bound protein 2 (GRB2) and the RAS–mitogen-activated protein kinase (MAPK) signaling pathway, resulting in decreased hDP proliferation and mineralization, while increasing apoptosis.[43]

During odontogenic differentiation, several miRNAs, such as miR-17 and miR-338-5p, are significantly upregulated, while miR-542-5p and miR-1224-5p are significantly downregulated. These miRNAs were predicted to target several signaling pathways, including the Wnt and MAPK pathways.[44] However, the exact function of these miRNAs on DPSCs has not been explored.

miRNA Expression Profile in Dental Pulp Cells and Tissues

Specific cell types express specific miRNAs. A study using donor matched cells demonstrated that 9 miRNAs are expressed in hDPs, but not in human gingival fibroblasts and human periodontal ligament fibroblasts.[41] The miRNAs expressed in hDPs only are miR-24, miR-605, miR-488, miR181b, miR-589,

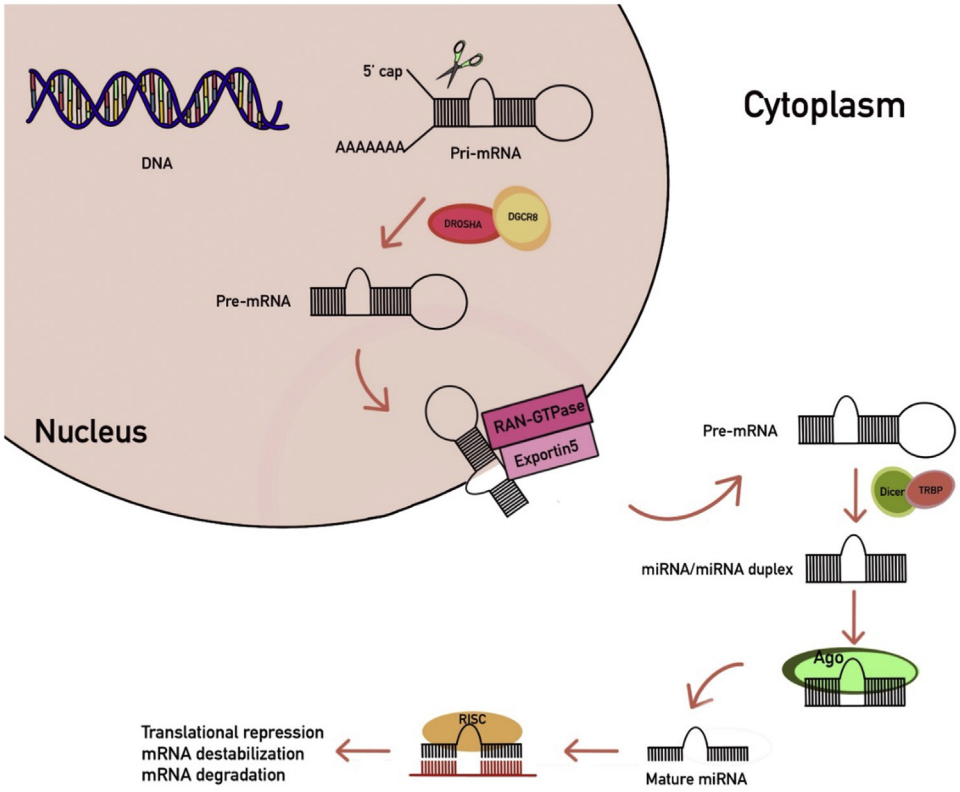

FIGURE 1 – Biogenesis of microRNAs (miRNAs).

miR-32, miR-212, and let-7e.[41] Compared with bone marrow–derived mesenchymal stem cells, 19 and 29 miRNAs are upregulated and downregulated, respectively, in DPSCs.[45] miR-516a-3p and miR-7-5p are the most upregulated miRNA in DPSCs and these two miRNAs target WNT5A and the epidermal growth factor receptor, respectively.[45] Distinct miRNA expression profiles are also observed between DPSCs and stem cells isolated from the apical papilla.[46] Comparing undifferentiated and odontogenic differentiated DPSCs, 22 miRNAs are differentially expressed in differentiated DPSCs.[44] These miRNAs target genes in the MAPK signaling pathway, adherens junction, insulin signaling pathway, and Wnt signaling pathway.[44] These specific miRNA expression profiles reflect the complex regulation of cell responses in different tissues and microenvironments.

hDPs isolated from young and older individuals exhibit 27 differentially expressed miRNAs; 16 and 11 miRNAs were up- and downregulated, respectively, in older hDPs compared with young hDPs.[43] miR-433 exhibited the highest fold change compared with the other upregulated miRNAs in older hDPs.[43] The predicted target genes of significantly expressed miRNA are in several KEGG pathways, including the MAPK signaling pathway, Wnt signaling pathway, PI3K-Akt signaling pathway, Insulin signaling pathway, focal adhesion, and chemokine signaling pathway.[43]

miRNAs and DPSC Proliferation

DPSC proliferation is crucial for tooth development and repairing damaged tissue. Previous studies have revealed the important role of miRNAs in promoting or inhibiting DPSC proliferation. Human dental pulp stem cells (hDPSCs) isolated from older patients exhibit higher miR-584 expression compared with those isolated from young patients.[47] miR-584 expression is decreased in a time-dependent manner during cell growth and overexpression of miR-584 results in decreased cell proliferation and migration as well as G0/G1 cell cycle arrest.[47] miR-584 targets transcriptional co-activator with a PDZ-binding motif (TAZ). TAZ expression is also decreased in hDPSCs isolated from older patients compared with those from young patients.[47] TAZ knockdown significantly decreased cell proliferation and migration. TAZ overexpression rescues the effects of miR-584 on cell proliferation, migration, and cell cycle progression.[47] Similarly, downregulation or inhibition of miR-224-5p promotes DPSC proliferation, colony-forming unit number, and migration.[48,49] miR-224-5p also regulates DPSC apoptosis because miR-224-5p inhibition promotes cell apoptosis as confirmed by Tunel and AnnexinV/PI staining.[48] Rac1 is targeted by miR-224-5p and further regulates cell apoptosis.[48] Another study reports that miR-433 overexpression induces cell proliferation and apoptosis in both young and older hDPs via regulating GRB2 and the RAS/MAPK pathway.[43]

Other miRNAs are involved in regulating DPSC proliferation. miR-146a-5p overexpressing STRO-1^+ hDPSCs exhibit a lower proliferation rate compared with the control cells.[50] miR-146a-5p binds to the 3′ UTR of NOTCH1, leading to decreased NOTCH1 and HES1 levels.[50] Knockdown of NOTCH1 in hDPSCs using small interfering RNA reduces cell proliferation.[50] Hence, miR-146a-5p could affect hDPSC proliferation by regulating Notch signaling. miR-152 upregulation is observed in senescent hDPSCs.[51] miR-152 overexpressing hDPSCs exhibit a decreased percentage of Ki67 positive cells and an increased percentage of SA-β-gal staining.[51] These effects occur via SIRT7 because overexpression of SIRT7 rescues miR-152-suppressed cell proliferation and miR-152-induced cell senescence.

There are also miRNAs that increase proliferation. miR-720 overexpression increases hDP proliferation as shown by

increased Ki67-positive cells; however, the pluripotent stem cell marker, NANOG, is decreased at both the mRNA and protein levels.[52] Overexpression of miR-140-5p enhances hDPSC proliferation as confirmed by an MTT assay and an increased S phase population as confirmed by a cell cycle assay.[53] These miR-140-5p effects occur via TLR4 because miR-140-5p overexpression reduced TLR4 expression and a TLR4 inhibitor rescues the effect of miR-140-5p inhibition on cell proliferation.[53] Further, miR-139-59 also promotes DPSC proliferation and reduces apoptosis via Wnt/β-catenin signaling.[54] The functions of miRNAs in regulating DPSC proliferation are summarized in Table 1.

miRNAs and DPSC Differentiation

Understanding the molecular mechanisms underlying stem cell, particularly DPSC, differentiation is important for designing the optimum dental regenerative strategies. Numerous studies have demonstrated that miRNAs are an important factor in promoting or suppressing DPSC differentiation. During murine pre-odontoblast cell differentiation, miR-3065-5p expression is upregulated in a time-dependent manner.[55] Overexpression of miR-3065-5p enhances odontoblastic differentiation by binding to bone morphogenetic protein receptor type II (BMPR2) in the terminal differentiation stage.[55] Moreover, miR-675 expression facilitates the odontogenic differentiation process by epigenetically regulating distal-less homeobox expression.[56] Expression of miR-21 concurrent with the signal transducer and activator of transcription 3 is found to facilitate DPSC odontoblast differentiation as indicated by increased odontoblast marker expression.[57] miR-27a-5p promotes DPSC odontogenic differentiation possibly via the upregulation of transforming growth factor (TGF)β1, TGFR1, p-Smad2/3, and Smad4.[58] miR-588 is also shown to participate in DPSC odontogenic differentiation.[59] miR-146a-5p overexpression upregulates RUNX2, OSX, alkaline phosphatase (ALP), and DSPP mRNA expression and also increases alkaline phosphatase enzymatic activity in STRO-1$^+$ DPSCs, potentially by targeting NOTCH1.[50]

In inflamed dental pulp, overexpression of miR-223-3p increased dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1) expression, while the Smad3 protein level was significantly decreased, indicating that miR-223-3p regulates odontoblast differentiation.[60] Likewise, decreased miR-125a-3p expression during chronic pulp inflammation also upregulated Fyn, a member of the protein tyrosine kinase Src family. Fyn forms a complex with Neuropilin-1 that inhibits odontoblastic differentiation and prolonged inflammation through nuclear factor-κB (NF-κB) signal pathways.[61]

In addition to promoting DPSC differentiation, several miRNAs can suppress differentiation by targeting different genes or proteins. Increased expression of miR295-5p and miR-218 reduced osteogenic differentiation through an unclear mechanism.[62,63] Overexpression of several miRNAs, such as miR-135b, suppresses DPSC differentiation by attenuating the expression of Smad5 and Smad4.[64] miR-218 targets RUNX2 and decreases its expression, reducing DPSC osteogenic differentiation.[65] Another target pathway is Wnt signaling. miR-140-5p decreases odontoblastic differentiation by suppressing the expression of Wnt1.[66] Upregulation of miR-508-5p inhibits odontogenesis corresponding with reduced DMP-1, DSPP, and OCN expression in odontoblasts.[67]

Researchers have found that the downregulation of several miRNAs promotes DPSC differentiation. Downregulated miR-143-5p or miR-488 leads to the increased expression of genes related to the p38 MAPK pathway and the increased expression of odontoblastic differentiation markers.[68,69] miR-488 targets MAPK1.[69] Moreover, the downregulation of miR-145 and miR-143 promotes odontogenic differentiation as shown by increased DSPP and DMP-1 expression.[70] miR-145-5p inhibition increases alkaline phosphatase enzymatic activity and mineralization in hDPSCs and these effects can be attenuated by a TLR-4 inhibitor, implying that miR-145-5p targets TLR4.[53] miR-143 also targets TNF-α and miR-143 suppression promotes osteogenic marker expression via the NF-κB pathway.[71] The functions of miRNAs in regulating DPSC osteogenic and odontogenic differentiation are summarized in Table 2.

miRNAs and Dental Pulp Inflammation

Pulp inflammation is an oral problem that frequently occurs due to dental caries and dental pulp exposure. The differential miRNA expression profiles of healthy and inflamed human pulp tissues have been investigated and many correlations have been found between each miRNA and inflammation-inducing conditions.[42,72] The functions of miRNAs in dental pulp inflammation are summarized in Table 3.

Lipopolysaccharide (LPS) is the primary bacterial component that has a crucial role in bacterial pathogenicity to the human body.[73] LPS treatment promotes miR-146a expression in hDPs, concomitant with increased cell migration.[74] miR-146a overexpression significantly increased hDP migration via decreased IRAK1 and TRAF6 expression.[74] In contrast, miR-146a delivery rescued the LPS-attenuated cell proliferation, DMP1 expression, and mineral deposition in hDPs.[75] Thus, the conflicting effects of miR-146 in LPS-induced inflammation require further investigation.

LPS-treated hDPs upregulated miR-21-5p expression in a similar pattern to those of the inflammatory cytokines *IL-1A*, *IL-1B*, *IL-6*, and *TNFA*.[76] This inductive effect was inhibited by an NF-κB signaling inhibitor, that this gene

TABLE 1 - miRNAs That Regulate DPSC or DP Cell Proliferation

Function	miRNA	Target	Effect of miRNA mimic treatment	Reference
Promotion of cell proliferation	miR-140-5p	TLR4	Increased cell proliferation Increased S phase population	Song et al, 2017
Inhibition of cell proliferation	miR-720	DNMT3A, DNMT3B	Increased Ki-67 positive cells	Hara et al, 2013
	miR-146a-5p	NOTCH1	Decreased cell proliferation	Qiu et al, 2019
	miR-152	SIRT7	Decreased Ki-67 positive cells	Gu et al, 2016
	miR-224-5p	n/a	Decreased cell proliferation	Ke et al, 2019
	miR-584	TAZ	Decreased cell proliferation Increased G0/G1 phase population Decreased S phase population Decreased CDK4, Cyclin D, and CTGF expression	Tian et al, 2020

DP, dental pulp cell; DPSC, dental pulp stem cell; miRNA, micro RNA; n/a, not available.

TABLE 2 - miRNAs That Regulate DPSC or DP Osteogenic and Odontogenic Differentiation

Function	miRNA	Target	Effect of miRNA mimic treatment	Reference
Promoting differentiation	miR-27a-5p	LTBP1	Increased DSP, DMP-1, ALP, and RUNX2 mRNA expression	Hu et al, 2019
	miR-125a-3p	Fyn	Increased ALP activity Increased DSPP and DMP-1 mRNA and protein expression Increased mineralization	Wang et al, 2020
	miR-146a-5p	NOTCH1	Increased ALP activity Increased RUNX2, OSX, ALP, DSPP mRNA expression	Qiu et al, 2019
	miR-223	Smad3	Increased ALP activity Increased DSPP and DMP-1 protein expression Increased mineralization	Huang et al, 2019
	miR-675	DLX3	Increased ALP activity Increased DSPP, DMP-1, ALP, Nes, and DLX5 mRNA expression Increased DSPP and DMP-1 protein expression Increased mineralization	Zeng et al, 2018
	miR-720	DNMT3A, DNMT3B	Increased ALP activity Increased ALP, OPN mRNA expression Increased mineralization	Hara et al, 2013
	miR-3065	Bmpr2	Increased Alp activity Increased Dspp, Dmp1, Alp mRNA expression Increased Dmp1 and Dsp protein expression Increased mineralization	Lin et al, 2018
Inhibiting differentiation	miR-135b	Smad4, Smad5	Decreased DSPP and DMP-1 protein expression	Song et al, 2017
	miR-140-5p	TLR4	Decreased ALP activity Decreased mineralization	Sun et al, 2017
		WNT1	Decreased DSPP and DMP-1 protein expression Decreased mineralization	Lu et al, 2019
	miR-143	TNF-α	Decreased ALP activity Decreased BMP2, ALP, RUNX2, and COL1 mRNA and protein expression	Zhang et al, 2018
	miR-143-5p	MAPK1	Decreased ALP activity Decreased DSPP, ALP, and OCN mRNA expression Decreased mineralization	Wang et al, 2019
	miR-215	HspB8	Decreased BSP and OCN, mRNA expression Decreased mineralization	Yao et al, 2019
	miR-219a-1-3p	HspB8	Decreased ALP, BSP, OCN, and RUNX2 mRNA expression Decreased mineralization	Yao et al, 2019
	miR-295-5p	HspB8	Decreased mineralization	Yao et al, 2019
	miR-488	MAPK1	Decreased ALP activity Decreased DSPP, ALP, and OCN mRNA and protein expression Decreased mineralization	Yu et al, 2019
	miR-508-5p	GPNMB	Decreased ALP activity Decreased ALP, DMP-1, DSPP, and OCN mRNA expression Decreased DMP-1, DSPP, and OCN protein expression Decreased mineralization	Liu et al, 2019

ALP, alkaline phosphatase; DMP, dentin matrix protein; DPSC, dental pulp stem cell; DSPP, dentin sialophosphoprotein; MPK, mitogen-activated protein kinase; miRNA, micro RNA; mRNA, messenger RNA; TLR, Toll-like receptor; TNF, tumor necrosis factor.

TABLE 3 - Other Potential Function of miRNAs in DPSCs

miRNA	Target	Function	Reference
miR-21-5p	TRAF6, PDCD4	Upregulation during dental pulp inflammation, prevention of excessive inflammation	Nara et al, 2013
miR-140	MMP14	Downregulation during dental pulp inflammation	Brozikowska et al, 2019
miR-181a miR-181b	3′UTR of IL-8	Reduce dental pulp inflammation	Galicia et al, 2014
miR-506	DIMP1, SIRT1	Dental pulp protection	Yuan et al, 2018 Wang et al, 2019
miR-150 miR-584 miR-766	n/a	Upregulation during dental pulp inflammation	Zhong et al, 2012
miR-183 miR-101a miR-101b miR-181c miR-29a miR-29b miR-29c miR-30e	n/a	Involve in insulin-producing cell differentiation of DPSC	Nozaki et al, 2014
miR-242	VEGF, KDR	Involve in endothelial cell differentiation of DPSC	Liu et al, 2014
miR-135 miR-143 miR-1 miR-206	n/a	Involve in myogenic differentiation of DPSC	Li et al, 2014
miR-139-5p	Wnt/β catenin	Involve in skeletal myogenic differentiation of DPSC	Xie et al, 2018

DPSC, dental pulp stem cell; IL, interleukin; miRNA, micro RNA; n/a, not available; UTR, untranslated region; VEGF, vascular endothelial growth factor.

expression pattern occurred via the NF-κB pathway.[76] LPS-induced miR-21-5p negatively regulates NF-κB signaling by decreasing TRAF6 and PDCD4 expression.[76] This loop can prevent an excessive inflammatory response in the dental pulp. In contrast, miR-21 downregulation was observed in LPS-treated hDPs.[77] miR-21 targets KBTBD7 and is involved in the berberine-attenuated effects of LPS on cytokine expression and cell proliferation.[77] This regulation also involves the inhibition of NF-κB signaling.[77]

A study has demonstrated that miR-410 expression is significantly lower in inflamed pulp tissue compared with healthy tissue. This observation corresponds with increased MMP14, the target of miR-410, in inflamed dental pulp tissue.[78] This evidence suggests that inflammation affects the regulation of miRNA in dental pulp tissue and is involved in the pathology of pulp tissue inflammation.[78]

Apart from increasing inflammation, several miRNAs also play a protective role in suppressing inflammation. During inflammation, the level of the proinflammatory cytokine, interleukin-8 (IL-8) is negatively correlated with the miR-181a level, and miR-181b is observed in hDPs. Further investigation found that miR-181a directly binds to the 3′UTR of IL-8 and modulates its levels, implicating miR-181a in reducing inflammation.[79] Other studies demonstrated that miR-506 and let-7c-5p had a pulp protecting effect by inhibiting the DMP1-mediated NF-κB pathway and by inhibiting the sirtuin 1 (SIRT1)-mediated TLR4 pathway, respectively.[80,81]

Comparing miRNA expression profiles between normal and inflamed pulp tissues, 36 miRNAs are differentially expressed. Only 3 miRNAs (miR-150, miR-584, and miR766) are upregulated in inflamed pulp tissues.[42] miR-584 and miR-664 exhibit the highest upregulated and downregulated fold change, respectively.[42] The target genes of these differentially expressed miRNAs in inflamed pulp are related to microbial recognition, chemotaxis, proteolysis, pro- and anti-inflammatory cytokines, and signal transduction.[42]

miRNAs and Their Other Potential Functions in Dental Pulp

In addition to the miRNA functions previously discussed, DPSC multipotency can be induced by miRNAs and transform into different cell lineage rather than pulp forming cells. This mulitpotent potential makes it possible for DPSCs to be used in various medical and dental therapeutics. The summary of those studies is shown in Table 3.

An *in vitro* study demonstrated that dental pulp cells can be directly reprogrammed to insulin-producing cells. miRNA microarray analysis identified 8 microRNAs that are differentially expressed pre- and post-induction. Only miR-183 was found to be downregulated, whereas miR-101a, miR-101b, miR-181c, miR-183, miR-29a, miR-29b, miR-29c, and miR-30e were upregulated, implying that these miRNAs are involved in insulin-producing cell differentiation by DPSCs.[82] However, the targets of these miRNAs in regulating insulin-producing cell differentiation by DPSCs remain unknown.

Angiogenesis, the formation of new blood vessels, is crucial during regenerative therapy. Liu et al.[83] investigated the role of miRNAs in the differentiation of endothelial cells from hDPSCs. miR-424 decreased after endothelial induction. Knockdown of miR-424 enhanced, while miR-424 overexpression attenuated, vessel formation by hDPSCs in Matrigel.[83] miR-424 inhibited vascular endothelial growth factor (VEGF) and KDR expression, implying the involvement of VEGF and KDR in the miR-424-controlled endothelial cell differentiation from hDPSCs.[83]

Myogenesis is also an important aspect in tissue regeneration, especially for treating muscle degenerative diseases. During the myogenic differentiation of hDPSCs, miR-135 and miR-143 are significantly downregulated, whereas miR-1 and miR-206 are significantly increased.[84] Correspondingly, inhibiting miR-135 or miR-143 individually or in combination

increases myogenic marker expression and myotube formation.[84] These data indicate the participation of miR-135 and miR-143 in myogenic differentiation from DPSCs. Further, it has been found that miR-139-5p induces skeletal myogenic differentiation as demonstrated by the expression myogenic markers in cells overexpressing miR-139-5p.[54] This regulation by miR-139-5p occurs through the activation of the Wnt/β catenin signaling pathway.[54]

DISCUSSION

Technical developments have allowed for the biological functions of miRNAs in cells to be better understood. Previous investigations have demonstrated the role of miRNAs in DPSCs, including cell development, proliferation, apoptosis, and differentiation. However, the functions of many DPSC-associated miRNAs remain unclear, and further studies are required to assess the involvement of these miRNAs in DPSCs.

As previously mentioned, miRNAs have important therapeutic implications. The upregulation or downregulation of specific miRNAs may be beneficial when treating pulp diseases. With the advances in regenerative medicine, miRNAs also could be used for dental tissue regeneration, particularly tissues that originate from the pulp. Furthermore, due to the multipotency of DPSCs, stimulating these cells with the appropriate miRNAs may allow their use in nonodontogenic tissue regeneration.

In conclusion, several miRNAs play an important role in promotion or inhibition of cell proliferation as well as promoting or inhibiting DPSC osteogenic and odontogenic differentiation by targeting various genes and pathways. Understanding these roles of miRNAs is crucial for the development of new therapeutics in regenerative dental medicine. With the advancing technologies, the utilization of miRNA technology could revolutionarily change the future of regenerative endodontics.

ACKNOWLEDGMENTS

This project was funded by the National Research Council of Thailand (N41A640135) and the Faculty Research Fund, Faculty of Dentistry, the Second Century Fund (C2F), Chulalongkorn University. The authors thank Miss Wajathip Bulawichit for her assistance in data gathering. The authors thank Dr. Kevin Tompkins for language editing. The authors deny any conflicts of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found in the online version at www.jendodon.com (https://doi.org/10.1016/j.joen.2022.02.012).

REFERENCES

1. Shi S, Bartold PM, Miura M, et al. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 2005;8:191–9.
2. Gronthos S, Mankani M, Brahim J, et al. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 2000;97:13625–30.
3. Sawangmake C, Nowwarote N, Pavasant P, et al. A feasibility study of an *in vitro* differentiation potential toward insulin-producing cells by dental tissue-derived mesenchymal stem cells. Biochem Biophys Res Commun 2014;452:581–7.
4. Osathanon T, Nowwarote N, Pavasant P. Basic fibroblast growth factor inhibits mineralization but induces neuronal differentiation by human dental pulp stem cells through a FGFR and PLCgamma signaling pathway. J Cell Biochem 2011;112:1807–16.
5. Osathanon T, Sawangmake C, Nowwarote N, Pavasant P. Neurogenic differentiation of human dental pulp stem cells using different induction protocols. Oral Dis 2014;20:352–8.
6. Gronthos S, Brahim J, Li W, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531–5.
7. d'Aquino R, Graziano A, Sampaolesi M, et al. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 2007;14:1162–71.
8. de Mendonça Costa A, Bueno DF, Martins MT, et al. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. J Craniofac Surg 2008;19:204–10.
9. Gandia C, Armiñan A, García-Verdugo JM, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 2008;26:638–45.
10. Reynolds AJ, Jahoda CA. Cultured human and rat tooth papilla cells induce hair follicle regeneration and fiber growth. Differentiation 2004;72:566–75.
11. Kadar K, Kiraly M, Porcsalmy B, et al. Differentiation potential of stem cells from human dental origin–promise for tissue engineering. J Physiol Pharmacol 2009;60(Suppl 7):167–75.
12. Andrukhov O, Behm C, Blufstein A, Rausch-Fan X. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: implication in disease and tissue regeneration. World J Stem Cells 2019;11:604–17.

13. Ye Q, Wang H, Xia X, et al. Safety and efficacy assessment of allogeneic human dental pulp stem cells to treat patients with severe COVID-19: structured summary of a study protocol for a randomized controlled trial (Phase I/II). Trials 2020;21:520.

14. Meza G, Urrejola D, Saint Jean N, et al. Personalized cell therapy for pulpitis using autologous dental pulp stem cells and leukocyte platelet-rich fibrin: a case report. J Endod 2019;45:144–9.

15. Potdar PD, Jethmalani YD. Human dental pulp stem cells: applications in future regenerative medicine. World J Stem Cells 2015;7:839–51.

16. Waddington RJ, Youde SJ, Lee CP, Sloan AJ. Isolation of distinct progenitor stem cell populations from dental pulp. Cells Tissues Organs 2009;189:268–74.

17. Ambros V, Bartel B, Bartel DP, et al. A uniform system for microRNA annotation. RNA 2003;9:277–9.

18. Goff LA, Boucher S, Ricupero CL, et al. Differentiating human multipotent mesenchymal stromal cells regulate microRNAs: prediction of microRNA regulation by PDGF during osteogenesis. Exp Hematol 2008;36:1354–69.

19. Sen CK, Ghatak S. miRNA control of tissue repair and regeneration. Am J Pathol 2015;185:2629–40.

20. Divisato G, Piscitelli S, Elia M, et al. MicroRNAs and stem-like properties: the complex regulation underlying stemness maintenance and cancer development. Biomolecules 2021;11:1074.

21. Shalgi R, Lieber D, Oren M, Pilpel Y. Global and local architecture of the mammalian microRNA-transcription factor regulatory network. PLoS Comput Biol 2007;3:e131.

22. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004;303:83–6.

23. Zhao Y, Srivastava D. A developmental view of microRNA function. Trends Biochem Sci 2007;32:189–97.

24. Mattick JS, Makunin IV. Small regulatory RNAs in mammals. Hum Mol Genet 2005;14 Spec No 1:R121–32.

25. Li N, Long B, Han W, et al. microRNAs: important regulators of stem cells. Stem Cell Res Ther 2017;8:110.

26. Stadler B, Ivanovska I, Mehta K, et al. Characterization of microRNAs involved in embryonic stem cell states. Stem Cells Dev 2010;19:935–50.

27. Qi J, Yu JY, Shcherbata HR, et al. microRNAs regulate human embryonic stem cell division. Cell Cycle 2009;8:3729–41.

28. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 2009;10:126–39.

29. Zeng Y, Cullen BR. Sequence requirements for micro RNA processing and function in human cells. RNA 2003;9:112–23.

30. Kwak PB, Iwasaki S, Tomari Y. The microRNA pathway and cancer. Cancer Sci 2010;101:2309–15.

31. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science 2001;294:858–62.

32. Xu N, Papagiannakopoulos T, Pan G, et al. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 2009;137:647–58.

33. Tay Y, Zhang J, Thomson AM, et al. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 2008;455:1124–8.

34. Lai AN, Zhou R, Chen B, et al. MiR-149-3p can improve the osteogenic differentiation of human adipose-derived stem cells via targeting AKT1. Kaohsiung J Med Sci 2021;37:1077–88.

35. Yang Z, Bian C, Zhou H, et al. MicroRNA hsa-miR-138 inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells through adenovirus EID-1. Stem Cells Dev 2011;20:259–67.

36. Chen L, Hou J, Ye L, et al. MicroRNA-143 regulates adipogenesis by modulating the MAP2K5-ERK5 signaling. Sci Rep 2014;4:3819.

37. Takanabe R, Ono K, Abe Y, et al. Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet. Biochem Biophys Res Commun 2008;376:728–32.

38. Miyaki S, Nakasa T, Otsuki S, et al. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. Arthritis Rheum 2009;60:2723–30.

39. Won Lee G, Thangavelu M, Joung Choi M, et al. Exosome mediated transfer of miRNA-140 promotes enhanced chondrogenic differentiation of bone marrow stem cells for enhanced cartilage repair and regeneration. J Cell Biochem 2020;121:3642–52.

40. Karlsen TA, Jakobsen RB, Mikkelsen TS, Brinchmann JE. microRNA-140 targets RALA and regulates chondrogenic differentiation of human mesenchymal stem cells by translational enhancement of SOX9 and ACAN. Stem Cells Dev 2014;23:290–304.

41. Sipert CR, Morandini AC, Dionísio TJ, et al. MicroRNA-146a and microRNA-155 show tissue-dependent expression in dental pulp, gingival and periodontal ligament fibroblasts *in vitro*. J Oral Sci 2014;56:157–64.

42. Zhong S, Zhang S, Bair E, et al. Differential expression of microRNAs in normal and inflamed human pulps. J Endod 2012;38:746–52.

43. Wang K, Li L, Wu J, et al. The different expression profiles of microRNAs in elderly and young human dental pulp and the role of miR-433 in human dental pulp cells. Mech Ageing Dev 2015;146-148:1–11.

44. Gong Q, Wang R, Jiang H, et al. Alteration of microRNA expression of human dental pulp cells during odontogenic differentiation. J Endod 2012;38:1348–54.

45. Vasanthan P, Govindasamy V, Gnanasegaran N, et al. Differential expression of basal microRNAs' patterns in human dental pulp stem cells. J Cell Mol Med 2015;19:566–80.

46. Tan X, Dai Q. Characterization of microRNAs expression profiles in human dental-derived pluripotent stem cells. PLoS One 2017;12(5):e0177832.

47. Tian S, Liu Y, Dong F, et al. Knockdown of microRNA-584 promotes dental pulp stem cells proliferation by targeting TAZ. Cell Cycle 2020;19:1048–58.

48. Qiao W, Li D, Shi Q, et al. miR-224-5p protects dental pulp stem cells from apoptosis by targeting Rac1. Exp Ther Med 2020;19:9–18.

49. Ke Z, Qiu Z, Xiao T, et al. Downregulation of miR-224-5p promotes migration and proliferation in human dental pulp stem cells. Biomed Res Int 2019;2019:4759060.

50. Qiu Z, Lin S, Hu X, et al. Involvement of miR-146a-5p/neurogenic locus notch homolog protein 1 in the proliferation and differentiation of STRO-1(+) human dental pulp stem cells. Eur J Oral Sci 2019;127:294–303.

51. Gu S, Ran S, Liu B, Liang J. miR-152 induces human dental pulp stem cell senescence by inhibiting SIRT7 expression. FEBS Lett 2016;590:1123–31.

52. Hara ES, Ono M, Eguchi T, et al. miRNA-720 controls stem cell phenotype, proliferation and differentiation of human dental pulp cells. PLoS One 2014;8:e83545.

53. Sun DG, Xin BC, Wu D, 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.

54. Xie Y, Shen G. MicroRNA-139-5p elevates skeletal myogenic differentiation of human adult dental pulp stem cells through Wnt/β-catenin signaling pathway. Exp Ther Med 2018;16:2835–42.

55. Lin C, Zhang Q, Yu S, et al. miR-3065-5p regulates mouse odontoblastic differentiation partially through bone morphogenetic protein receptor type II. Biochem Biophys Res Commun 2018;495:493–8.

56. Zeng L, Zhao N, Li F, et al. miR-675 promotes odontogenic differentiation of human dental pulp cells by epigenetic regulation of DLX3. Exp Cell Res 2018;367:104–11.

57. Xu K, Xiao J, Zheng 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.

58. Hu X, Zhong Y, Kong Y, et al. Lineage-specific exosomes promote the odontogenic differentiation of human dental pulp stem cells (DPSCs) through TGFβ1/smads signaling pathway via transfer of microRNAs. Stem Cell Res Ther 2019;10:170.

59. Chen Z, Zhang K, Qiu W, et al. Genome-wide identification of long noncoding RNAs and their competing endogenous RNA networks involved in the odontogenic differentiation of human dental pulp stem cells. Stem Cell Res Ther 2020;11:114.

60. Huang X, Liu F, Hou J, Chen K. Inflammation-induced overexpression of microRNA-223-3p regulates odontoblastic differentiation of human dental pulp stem cells by targeting SMAD3. Int Endod J 2019;52:491–503.

61. Wang J, Zheng Y, Bai B, et al. MicroRNA-125a-3p participates in odontoblastic differentiation of dental pulp stem cells by targeting Fyn. Cytotechnology 2020;72:69–79.

62. Yao S, Li C, Budenski AM, et al. Expression of microRNAs targeting heat shock protein B8 during *in vitro* expansion of dental pulp stem cells in regulating osteogenic differentiation. Arch Oral Biol 2019;107:104485.

63. Chang K, Chen RS, Chang FH, Chen MH. Promoting dentinogenesis of DPSCs through inhibiting microRNA-218 by using magnetic nanocarrier delivery. J Formos Med Assoc 2019;118:1005–13.

64. Song Z, Chen LL, Wang RF, et al. MicroRNA-135b inhibits odontoblast-like differentiation of human dental pulp cells by regulating Smad5 and Smad4. Int Endod J 2017;50:685–93.

65. Gay I, Cavender A, Peto D, et al. Differentiation of human dental stem cells reveals a role for microRNA-218. J Periodontal Res 2014;49:110–20.

66. Lu X, Chen X, Xing J, et al. miR-140-5p regulates the odontoblastic differentiation of dental pulp stem cells via the Wnt1/β-catenin signaling pathway. Stem Cell Res Ther 2019;10:226.

67. Liu F, Wang X, Yang Y, et al. The suppressive effects of miR-508-5p on the odontogenic differentiation of human dental pulp stem cells by targeting glycoprotein non-metastatic melanomal protein B. Stem Cell Res Ther 2019;10:35.

68. Wang BL, Wang Z, Nan X, et al. Downregulation of microRNA-143-5p is required for the promotion of odontoblasts differentiation of human dental pulp stem cells through the activation of the mitogen-activated protein kinases 14-dependent p38 mitogen-activated protein kinases signaling pathway. J Cell Physiol 2019;234:4840–50.

69. Yu D, Zhao X, Cheng JZ, et al. Downregulated microRNA-488 enhances odontoblast differentiation of human dental pulp stem cells via activation of the p38 MAPK signaling pathway. J Cell Physiol 2019;234:1442–51.

70. Liu H, Lin H, Zhang L, et al. miR-145 and miR-143 regulate odontoblast differentiation through targeting Klf4 and Osx genes in a feedback loop. J Biol Chem 2013;288:9261–71.

71. Zhang P, Yang W, Wang G, Li Y. miR-143 suppresses the osteogenic differentiation of dental pulp stem cells by inactivation of NF-kappaB signaling pathway via targeting TNF-alpha. Arch Oral Biol 2018;87:172–9.

72. Lei F, Zhang H, Xie X. Comprehensive analysis of an lncRNA-miRNA-mRNA competing endogenous RNA network in pulpitis. PeerJ 2019;7:e7135.

73. Bertani B, Ruiz N. Function and biogenesis of lipopolysaccharides. EcoSal Plus 2018;8(1). https://doi.org/10.1128/ecosalplus.ESP-0001-2018.

74. Wang MC, Hung PS, Tu HF, et al. Lipopolysaccharide induces the migration of human dental pulp cells by up-regulating miR-146a. J Endod 2012;38(12):1598–603.

75. Liu L, Shu S, Cheung GS, Wei X. Effect of miR-146a/bFGF/PEG-PEI nanoparticles on inflammation response and tissue regeneration of human dental pulp cells. Biomed Res Int 2016;2016:3892685.

76. Nara K, Kawashima N, Noda S, et al. Anti-inflammatory roles of microRNA 21 in lipopolysaccharide-stimulated human dental pulp cells. J Cell Physiol 2019;234:21331–41.

77. Song J, Wu Q, Jiang J, et al. Berberine reduces inflammation of human dental pulp fibroblast via miR-21/KBTBD7 axis. Arch Oral Biol 2020;110:104630.

78. Brodzikowska A, Gondek A, Rak B, et al. Metalloproteinase 14 (MMP-14) and hsa-miR-410-3p expression in human inflamed dental pulp and odontoblasts. Histochem Cell Biol 2019;152:345–53.

79. Galicia JC, Naqvi AR, Ko CC, et al. MiRNA-181a regulates Toll-like receptor agonist-induced inflammatory response in human fibroblasts. Genes Immun 2014;15:333–7.

80. Yuan H, Zhang H, Hong L, et al. MicroRNA let-7c-5p suppressed lipopolysaccharide-induced dental pulp inflammation by inhibiting dentin matrix protein-1-mediated nuclear factor kappa B (NF-κB) pathway *in vitro* and *in vivo*. Med Sci Monit 2018;24:6656–65.

81. Wang J, Du Y, Deng J, et al. MicroRNA-506 is involved in regulation of the occurrence of lipopolysaccharides (LPS)-induced pulpitis by Sirtuin 1 (SIRT1). Med Sci Monit 2019;25:10008–15.

82. Nozaki T, Ohura K. Regulation of miRNA during direct reprogramming of dental pulp cells to insulin-producing cells. Biochem Biophys Res Commun 2014;444(2):195–8.

83. Liu W, Gong Q, Ling J, et al. Role of MiR-424 on angiogenic potential in human dental pulp cells. J Endod 2014;40(1):76–82.

84. Li D, Deng T, Li H, Li Y. MiR-143 and miR-135 inhibitors treatment induces skeletal myogenic differentiation of human adult dental pulp stem cells. Arch Oral Biol 2015;60(11):1613–7.