REVIEW ARTICLE

Modulation of microRNAs in Tooth Root and Periodontal Tissue Development

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Abstract: Tooth root development begins after the completion of tooth crown development. Both the tooth root and crown undergo a series of interactions between the epithelium and adjacent mesenchymal cells. Although many studies have evaluated tooth crown formation, little is known about the regulatory mechanisms of tooth root development. MicroRNAs (miRNAs) are small noncoding RNAs that regulate protein expression through post-transcriptional mechanisms and participate in a broad range of biological processes, from development to tumorigenesis. In this review, we summarize current knowledge on the role of miRNAs in regulating the morphogenesis of root odontoblasts and periodontal tissues and regarding the interplay between miRNAs and signaling molecules during these processes.

Keywords: microRNA, root odontogenesis, periodontal tissue.

1. INTRODUCTION

Tooth regeneration is a promising approach based on a comprehensive understanding of reciprocal interactions between the epithelium and underlying mesenchymal cells [1]. Unlike crown development, tooth root development begins postnatally and is a consequence of sequential crosstalk between Hertwig's epithelial root sheath and mesenchymal cells. Dysregulation of these interactions leads to malformed tooth roots or abnormal periodontal structures [2]. A variety of mesenchymal molecules contribute to root odontoblast and cementoblast differentiation and periodontal tissue construction during tooth root development [3-7]. Previous studies have implied that signals from members of the Wnt family, transforming growth factor β (TGF-β) superfamily, and sonic hedgehog (SHH) pathway are involved in the epithelial-mesenchymal interactions required for regulating tooth root development [4, 7-9]. Transcription factors such as nuclear factor 1 C (NF1C), osterix, and runt-related transcription factor 2 (RUNX2) govern the expression of proteins in these signaling pathways and play important roles during tooth root development [4, 10-12].

MicroRNAs (miRNAs) are small, noncoding, single-stranded RNAs with approximately 18-22 nucleotides that regulate gene expression post-transcriptionally. miRNAs are transcribed from endogenous miRNA genes and generate primary (pri-) miRNAs [13, 14]. Pri-miRNAs are processed into single hairpins or precursor miRNAs (pre-miRNAs) by

the RNAase III enzyme in the nucleus. The pre-miRNAs undergo cleavage by a second RNA III enzyme, Dicer, after translocation of pre-miRNAs into the cytoplasm. Eventually, the mature miRNAs function in the form of an RNA-induced silencing complex and regulate the expression of multiple targets by binding to the 3′untranslated region (UTR) [15, 16]. One miRNA can target several genes, and several miRNAs can target a single gene [17, 18]. Thus, miRNAs act as potent molecular managers that can control multiple developmental and physiological processes [19, 20].

miRNAs plays an essential role in both bone and tooth formation and are upstream regulators of several signaling pathways and transcription factors, such as RUNX2 and osterix [21]. Recently, the important and unique role of miRNAs in tooth root formation has been gradually realized [22]. In this review, we summarize recent discoveries of the biological functions of miRNAs in root odontogenesis , the development of periodontal tissues and stem cells from dental or periodontal tissues, providing a better understanding of clinical tooth root malformation and potential therapeutic approaches.

2. TOOTH ROOT AND PERIODONTAL TISSUE DEVELOPMENT

Tooth crown development begins in the late embryonic period with epithelial invaginations from the alveolar mucosa, also known as the dental lamina. The dental lamina gives rise to enamel organs and subsequently induced the underlying mesenchymal cells to differentiate and form the structure of dental papilla and dental sac [1]. Crown development is completed through the interaction between the

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dental lamina and dental papilla, which occur via amelogenesis and crown odontogenesis [23]. With the establishment of conditional knockout mice, it is possible to manipulate specific genes in the dental epithelium or mesenchyme and observe *in vivo* functions of numerous genes in tooth morphogenesis. Members of various signaling pathways, such as the TGF -β superfamily, Wnt, Notch, and Shh, have indispensable roles in amelogenesis and crown odontogenesis [24-27].

The tooth root development process begins with the extension of Hertwig's epithelial root sheath (HERS), a bilayered structure formed by inner and outer enamel epithelium. HERS acts as an inducer, expressing signaling molecules to promote differentiation of apical papilla into root odontoblasts, which later will produce root dentin. With the formation of root dentin, HERS breaks into nests and cords, leaving space for the outer mesenchymal cells to contact the newly formed root dentin and posteriorly differentiate into cementoblasts and periodontal tissues [28] (Fig. **1**). The crown is formed during early embryonic development, whereas the root is formed from the late embryonic stage to the postnatal stage (up to postnatal day 30.5 in mice) [29]. Few animal models can survive after birth, particularly those with deficiencies in critical genes; thus, tooth root development has not been sufficiently studied.

3. FUNCTION OF MIRNAS IN TOOTH ROOT AND PERIODONTAL TISSUE FORMATION

3.1. Function of miRNAs in Root Odontoblasts

Crown and root odontogenesis were previously thought to undergo similar mechanisms. However, in NF1C-null mice, root dentin development was blocked, while crown dentin formation was unaffected [30]. In the recent years, with the emergence of *Cre* transgenic lines that effectively ablate genes within tooth root odontoblasts or HERS, signaling pathways involved in manipulating the formation and

development of root dentin have been elucidated [7, 31, 32]. Signaling molecules of the TGF-β superfamily and Wnt family as well as transcription factors such as RUNX2, osterix, and NF1C are also involved in root odontoblast differentiation. Thus, miRNAs targeting these proteins may be vital for dentin formation.

Bone morphogenic protein (BMP) signaling, from both the apical papilla and HERS, has been shown to be critical in tooth root development [3, 31]. Moreover, miRNAs have been reported to regulate odontogenesis by modulating the BMP signaling pathway [33, 34]. In cultured human dental papilla cells, BMP7 protein is downregulated after transfection with an miR-34a mimic, but was upregulated after transfection with an miR-34a inhibitor. A previous study concluded that miR-34 may inhibit the expression of alkaline phosphatase (ALP) and upregulate dentine sialophosphoprotein (DSPP) by targeting BMP7 [35]. A recent study also showed that miR-135b binds to the 3′-UTR of *Smad5* and *Smad4* mRNAs and suppresses their expression, consequently blocking the differentiation of human dental pulp cells [36]. Our previous data suggested that Wnt/β-catenin signaling is functionally significant for root odontogenesis [7]. Notably, both miR-663 and miR-27 have binding sites in the 3′ UTR of the adenomatous polyposis coli (APC) gene. Binding of either miR-663 or miR-27 with APC activates Wnt/β-catenin signaling in MDPC-23 cells, promoting the differentiation of odontoblasts [37, 38]. RUNX2 is of vital importance in odontoblast differentiation, it shows higher expression in differentiating odontoblasts and decreased expression in differentiated odontoblast [39-41]. In RUNX2 deficient mice, the peripheral layer of cells in the dental papilla lost polarization and odontoblastic differentiation was blocked [42]. By overexpression Runx2 under the control of *Col1a1* promoter, the terminal differentiation of odontoblasts was severely disturbed by showing flat or cuboid shape with reduced differentiation markers *(Col1a1*, *DSPP*, nestin) [43]. According to a previous study, overexpression of miR-338-
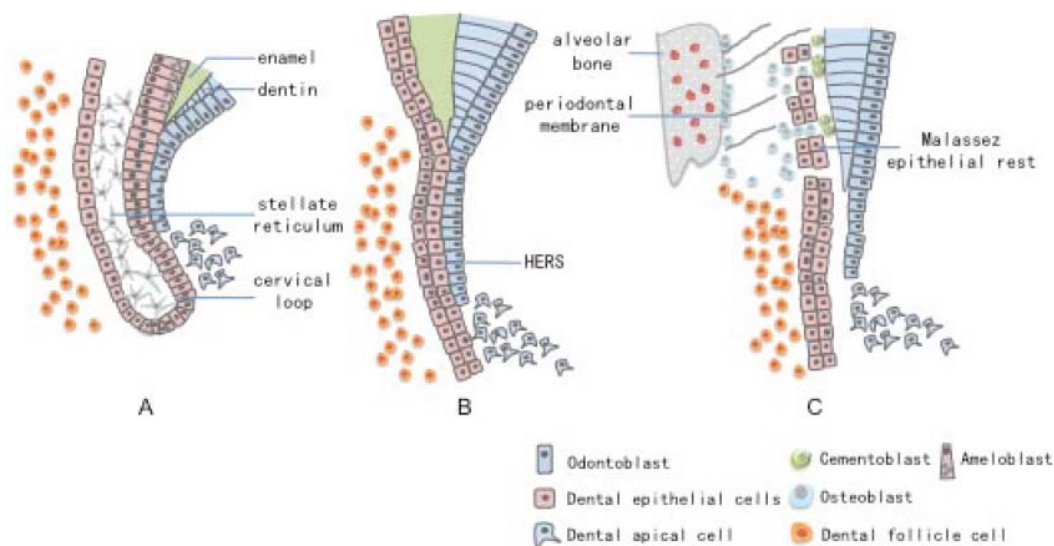

Fig. (1). The illustration of tooth root development. (**A**) Initial stage. The inner and outer epitheliums constitute a structure named cervical loop, and began to extend into the apical direction. (**B**) Intermediate stage. The extension of HERS induces the odontoblastic differentiation of apical papilla cells. (**C**) Late stage. HERS cells break into nests and cords allowing the dental follicle cells to contact the newly-formed dentin and then form the periodontal structures.

3p decreases the expression of RUNX2, while miR-338-3p inhibitors increase the expression of RUNX2, indicating that miR-338-3p activates odontoblast differentiation by targeting RUNX2 [44]. In odontoblast differentiation, Kruppel-like factor 4 (KLF4) represses miR-143, which promotes the expression of miR-145; miR-145 is then able to repress KLF4, consequently forming a feedback loop in the regulation of odontoblast differentiation [45]. In addition, miR-665 targets *Dlx3* mRNA and blocks the expression of *Dlx3* as well as *Runx2, Osteorix*, and *Dspp* in mouse odontoblasts [46]. Despite the important role of NF1C in root odontoblast differentiation, the regulatory mechanisms of miRNAs and NF1C in odontoblasts have not been reported to date.

3.2. Function of miRNAs in HERS

HERS plays an indispensable role in regulating tooth root development; any malformation of the HERS leads to disturbance in root number, structure, length and abnormalities in periodontal structures [5, 31, 47-49]. By depleting Alk3 from the dental epithelia, miR-200a and miR-200b were downregulated, both of which are important for maintaining the epithelial state and prohibiting the epithelial-mesenchymal transition (EMT) by functioning with pro-EMT factors [50]. Consequently, the mutant incisor dental epithelial stem cells differentiated into the root lineage and cementoblast-like cells [51]. Up till now, several cell lines of HERS have been established and several *Cre-LoxP* systems can mediate gene inactivation from HERS cells, however, the functional importance of miRNAs in HERS cells remains largely unknown [2, 31, 52-56].

3.3. Function of miRNAs in Periodontal Tissues

Periodontal tissues comprise alveolar bone, periodontal ligaments, gingival, and cementum. The periodontal ligament, alveolar bone, and cementoblasts are derived from dental follicles, a loose connective tissue derived from the ectomesenchyme, which surrounds the developing tooth germ prior to eruption, whereas the gingival tissues are originated from both the oral mucosa and the developing tooth germ [57]. miRNAs play an indispensable role in the homeostasis and pathogenesis of periodontal tissues.

The expression of miR-101 is increased during the osteogenic differentiation of dental follicle cells. When transfected with an miR-101 mimic, SP7 and ALP were increased, indicating that miR-101 sustained the osteogenic differentiation of dental follicle cells [58]. miRNAs have been shown to have functions in the process of periodontitis [59]. The main clinical manifestation of periodontitis is alveolar bone resorption, which involves osteoclasts. miRNAs can regulate the differentiation and function of osteoclasts in periodontal disease [60]. In one study, the authors screened mature miRNA expression in cells treated with tumor necrosis factor-α (TNF-α), a cytokine that promotes alveolar bone resorption, and/or receptor activator for nuclear factor-κ B ligand (RANKL), which is expressed in stromal cells, osteoblasts, and T cells and is essential for osteoclast differentiation. During osteoclast formation, the expression of 44 mature miRNAs differed between untreated cells and cells treated with TNF-α plus RANKL; additionally, the expression of 52 mature miRNAs differed upon treatment with RANKL alone, suggesting that

miRNAs may be involved in osteoclast differentiation during the pathogenesis of periodontitis [61]. In a mouse model of periodontitis, miR-146a and several inflammatory cytokines were upregulated compared with that in controls, indicating that miR-146a may directly or indirectly modulate the pathogenesis of periodontitis [62]. The role of miRNAs in osteoclast differentiation and function during alveolar bone loss in periodontitis is critical and has been discussed in several review articles [63, 64].

miRNAs can stimulate the differentiation of periodontal ligament cells (PDLCs). In immortalized PDLC (I-PDLC) lines, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling was believed to regulate bone differentiation. I-PDLCs with exogenous miR-146a expression showed attenuation of NF-κB activity and exhibited higher differentiation relative to the controls. Exogenous NF-κB expression decreased the expression of differentiation markers, while the inactivation of endogenous NF-κB increased ALP in I-PDL cells. Additionally, miR-146a was found to promote the differentiation in PDL cells through inhibition of NF-κB signaling [65]. Additionally, in another study, miR-146a was found to play a functional role in regulating a negative feedback loop by decreasing the secretion of pro-inflammatory cytokines and disrupting the Toll-like receptor (TLR) signaling pathway in PDLCs stimulated with *Porphyromonasgingivalis* lipopolysaccharide [66]. In mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis, miR-17 expression was inhibited, and exogenous miR-17 partly reversed the differentiation potential of periodontal ligament tissue-derived mesenchymal stem cells (PDLSCs) in the inflammatory environment. Additionally, the inflammatory microenvironment enhanced the expression of Smad ubiquitin regulatory factor 1 (Smurf1), an important negative regulator of mesenchymal stem cell osteogenic differentiation. Western blotting and 3′-UTR reporter assays identified Smurf1 as a direct target of miR-17 in PDLSCs; thus, inflammatory cytokines caused Smurf1 activation and decreased miR-17 expression, thereby increasing degradation of Smurf1-mediated osteoblast-specific factors [67]. Periodontal ligament-associated protein-1 (PLAP-1/asporin) regulated the osteogenic differentiation of PDLCs, and miR-101 and miR-21 were shown to target PLAP-1, regulating its expression during PDLC differentiation [68]. A recent study showed that miR195-5p could regulate osteogenic differentiation of PDLCs by directly targeting WNT3A, fibroblast growth factor (FGF) 2 and bone morphogenetic protein receptor (BMPR)-1A, which are important for osteogenic activity and stability under mechanical loading [69].

Previous studies described the occurrence of miRNAs in healthy and inflamed gingival tissues from patients with periodontitis, indicating a role of miRNA in gingival tissues and their pathogenesis [70-72]. With the stimulation of Porphyromonas gingivalis (Pg) to human gingival epithelial cells, expressions of several miRNAs (miR-584, miR-203, miR-200, miR-126, miR-155, and miR-210) were altered by targeting different molecules relating to immunity, inflammation and EMT [73-75]. In gingival fibroblasts, deubiquitinase cezanne is a direct target of miR-218 in regulating myofibroblast differentiation in fibroblasts via TGF-β [76]. In one study, the author compared the expression of miR-146a and miR-155 in dental pulp (DP), gingival (G) and

Table 1. miRNAs in tooth root and periodontal tissues.

Location	MicroRNAs	Target	Origin	Function	Ref.
Dental follicle	miR-98; miR-193 miR-203, miR-27560	(-)	mice	expression	Oommen S., *et al.*, 2012
	miR101	(-)	human	osteogenic diff. ↑	Klingelhöffer *et al.*, 2016
Cervical loop	miR-23b, miR-98 miR-148a, miR-193 miR-203, miR-205 miR-218, miR-363 miR-378, miR-27560	(-)	mice	expression	Oommen S., *et al.*, 2012
DEpSC(P7)	miR200a, miR200b	ZEB1, ZEB2	mice	EMT↓	Yang *et al.*, 2013
DPaC	miR-34a	NOTCH, TGF-β signaling	human	DPaC diff.↑	Wan, *et al.*, 2012
	miR-145	KLF4, Osterix	mice	od. diff.↓	Liu, *et al.*, 2013
DPaSC	miR-34a	NOTCH2, HES1	human	odontogenic, osteogenic diff. ↑	Sun *et al.*, 2014
DPCs (third molar)	miR-135a	Smad4, Smad5	human	DPC diff. ↓	Song, *et al.*, 2016
DPSC	miR-720	NANOG	human	stem cell diff. ↑	Hara *et al.*, 2013
	miR-143, miR-135	MEF2C	human	myogenicdiff.↓	Li *et al.*, 2015
	miR-424	VEGF, KDR	human	endothelial diff. ↓	Liu *et al.*, 2014
	miR-152	Sirtuin 7	human	senescence↑, prolif. ↓	Gu *et al.*, 2016
MDPC-23	miR-27	APC (Wnt/ β-catenin)	mice	od. diff. ↑	Park, *et al.*, 2014
	miR-663				Kim, *et al.*, 2014
MDPC6T	miR-338-3p	Runx2	mice	od. diff. ↑	Sun, *et al.*, 2013
MDPC-23, OD-21, M06-G3	miR-665	Kat6a/Dlx3	mice	od. diff. and maturation↓	Heair *et al.*, 2015
IPDLC	miR-146a	IRAK1, TRAF6	human	PDL cell diff. ↑	Hung *et al.*, 2010
P-PDLSC	miR-17	Smurf-1	human	osteogenic diff.: normal condition↓, infla.↑	Liu *et al.*, 2011
PDLC	miR-21, miR-101	PLAP-1	human	osteogenic diff. ↓	Li *et al.*, 2012
PDLC (under loading)	miR-195-5p	WNT3A, FGF2, BMPR1A	human	osteogenic diff. ↓	Chang *et al.*, 2016
PDLSC, DPSC, GSC	miR-218	RUNX2	human	osteogenic diff. ↓	Gay *et al.*, 2014
PDL progenitor cell	miR-138	osteocalcin	human	cell diff. (under infla.)	Zhou *et al.*, 2016
PDL fibroblasts	miR-200c	IL-6, IL-8, CCL-5	human	osteogenic diff. ↓	Hong *et al.*, 2016
PDLSC	miR-21	ACVR2B	human	osteogenic diff. (under stress) ↑	Wei *et al.*, 2015
gingival epithelial cell (with Pg)	miR-584	LfR	human	anti-infla. ↓	Ouhara *et al.*, 2014
	miR-203	SOCS3	human	host cell response ↑	Moffatt *et al.*, 2011
	miR-126, miR-155, miR-210	NFκB, IKKε	human	inflammatory response ↑	Chen *et al.*, 2016
gingival fibroblast	miR-218	deubiquitinase cezanne	human	myofibroblast diff. ↑	Guo *et al.*, 2014

* diff.: differentiation; DEpSC: dentalepithelial stem cell; DPaC: dental papilla cell; DPC: dental pulp cell; Od.: odontoblast; Proli.: proliferation; EMT: epithelial mesenchymal transition;MEF2C: myocyte-specific enhancer factor 2C; KLF4: Krüppel-like factor 4; VEGF: vascular endothelial growth factor; KDR kinase insert domain receptor; APC: adenomatous polyposis coli; IPDLC: Immortalized periodontal ligamentcell; P-PDLSC: periodontitis-affected periodontal ligament stem cell; infla.: inflammation; LfR: lactoferrin receptor; SOCS3: suppressor of cytokine signaling 3; IKKε: inhibitor-κB kinase ε.

periodontal ligament fibroblasts (PLF) and found that expression of miR-146a and miR-155 are more pronounced in GF than in DPF and PLF, indicating a more prominent role of miR-146a and miR-155 in the regulation GF biological behaviors [77].

3.4. Function of miRNAs in Stem Cells from Dental or Periodontal Tissues

Stem cells from dental or periodontal tissues are mainly dental pulp stem cells (DPSC), periodontal ligament stem

cell (PDLSC), stem cells from the apical papilla (SCAP) and gingival stem cell (GSC) [78-81]. In a study conducted in 2014, the author identified that expression of miR-218 was decreased with the differentiation and mineralization of all four stem cell types (bone mesenchymal stem cell, DPSC, GSC and PDLSC) by interacting with Runx2, revealing a regulated role of miRNAs in the differentiation of dental stem cells [82]. Apart from miR-218, several other miRNAs (miR-720, miR-135, miR-143, miR-424 and miR-151) also have different roles in inducing the differentiation of DPSCs, indicating different applications of miRNAs and their inhibitors for therapeutic purposes [83-86]. As for PDLSC, it shares some similarities with osteoblasts and can differentiate into osteoblasts or cementoblasts under certain conditions, it has been selected as potential seeds for tissue regeneration [87]. A previous study found ibandronate, a nitrogen-containing bisphosphonate, could promote the osteogenic differentiation of PDLSCs by regulating the expression of miRNAs (miR125b, miR130a, miR18a, miR19a etc.), which are involved in bone metabolism by regulating the expression of different target genes. Similar investigations on the role of miRNAs in osteogenic differentiation were reported in several papers not only in normal condition but also in inflammation or tissue regeneration [88-92]. Given mechanical force, PDLSCs showed different expression of more than 50 miRNAs (miR21, miR1246 and miR5096 etc.) compared with controls according to the microarray data, and most of the potential target genes are enriched in MAPK and Wnt signalings [93, 94]. Furthermore, in PDLSCs, miRNAs and their inhibitors have potential applications like promoting orthodontic tooth movement, accelerating the healing process of adult stem cell regeneration in smoking people or inducing transdifferentiation into retinal ganglion-like cells for retinal repair purpose [95-97]. In human SCAPs, Notch signaling plays an important role in maintaining of cell differentiation, and miR-34a interacts with Notch signaling to promote both odontogenic and osteogenic differentiation of SCAPs [98].

No studies have yet evaluated the role of miRNAs in the differentiation of cementoblasts. Cementoblasts possess many genetic properties similar to those of osteoblasts and express the same key markers, such as osteopontin, osteorix, and bone sialoprotein [7]. It may be possible to apply the knowledge of osteoblasts to cementoblasts due to their similar biological behaviors. However, further studies are required to confirm this hypothesis.

CONCLUSION

Tooth root development is a sophisticated process involving interactions between HERS and the adjacent mesenchymal cells, associated with numerous signaling pathways and crosstalk among these pathways. Compared with our knowledge of crown development, little is known about the regulatory mechanisms of tooth root development. To date, the functions of numerous miRNAs have been determined; however, few miRNAs have been shown to participate in root dentin formation, homeostasis, and pathogenesis in periodontal tissues. Moreover, most research explaining miRNA functions have used *in vitro* rather than *in vivo* experiments; accordingly, *in vivo* mouse models may be needed to elucidate the details of the interactions among these cell types.

Further studies are also needed to determine how miRNAs function temporally and interact with other targets, particularly *in vivo*. Besides, deeper understanding of the role of miRNAs in tissue development and pathogenesis will greatly facilitate tooth root regeneration and the use of miRNA target drugs.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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