Non-Coding RNAs in Endodontic Disease

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Abstract:

The immunocompetence and regeneration potential of the dental pulp and its surrounding apical tissues have been investigated extensively in the field of Endodontics. While research on the role of non-coding RNAs in these tissues is still in its infancy, it is envisioned that improved understanding of the regulatory function of ncRNAs in pulpal and periapical immune response will help prevent or treat endodontic disease. Of particular importance is the role of these RNAs in regenerating the dentin-pulp complex. In this review, we highlight recent progress on the role of non-coding RNAs in the immune response to endodontic infection as well as the repair and regenerative response to injury.

Key Words- Epigenetic, Non-Coding RNA, MicroRNA, siRNAs, Dental

1. Introduction

Endodontics is the branch of dentistry concerned with the morphology, physiology and pathology of the human dental pulp and periradicular tissues (the tissues surrounding the root of the tooth). The etiology for endodontic disease (i.e. pulpal and periapical disease) includes microbial infections from a carious lesions, inadequate (leaky) restorations, fractures and trauma. Dental caries is a highly prevalent disease. According to the Center for Disease Control the prevalence of dental caries in youth aged 2-19 years is 45.8% and the prevalence of untreated caries in the same age group is 13% [1, 2]. Even when the caries lesion is incipient and limited to the enamel, it evokes an immune response in the pulp [3]. As the carious infection progresses towards the pulp the immune/inflammatory response intensifies and can result in pulpal necrosis and periapical (aka periradicular) disease.

The cellular response to pulpal and periapical infection is mediated by several resident cells such as odontoblasts, fibroblasts and endothelial cells as well as immune cells which are recruited to the site of infection- macrophages, dendritic cells, neutrophils and lymphocytes [4]. The effector molecules released by these cells include cytokines, chemokines and proteases, among other pro-inflammatory mediators [5, 6]. The outcome of pulpal infection depends upon the extent of inflammation and may be regeneration and repair, or it may result in necrosis. The outcome of periapical infection is inflammation and bone resorption. A large number of studies have evaluated the role of the cellular and immune mediators in diseased dental pulps and periapical tissues [5, 7-11]. However, only a limited number of studies have examined the role of non-coding RNAs in endodontic disease.

Noncoding RNAs (ncRNAs) are RNAs that do not code for proteins. They can be classified based on subcellular localization (cytoplasmic versus nuclear), biological functions (housekeeping versus regulatory) or length (shorter or longer than 200nt) [12]. The putative role of ncRNAs in the pulp and periapical tissues include odontogenic differentiation, regeneration, the

immune response and bone resorption [13-16]. Given the limited information on the role of ncRNAs in endodontic disease, this review focusses on only those ncRNAs on which substantial research has already been conducted.

2. miRNA Regulation of Endodontic Diseases

2.1 MicroRNAs (miRNAs) in the pulpal response to infection

The role of miRNAs in the pulpal response to infection has been examined using both clinical biopsies and in vitro studies. The earliest report was from clinical biopsies of normal and inflamed pulps [17]. Thirty-three microRNAs were downregulated and only 3 were upregulated in the inflamed pulps as compared to normal pulps [17]. Given that miRNAs are negative regulators, decreased expression of a miRNA results in an increased stability or translation of their target messenger RNA. The miRNAs downregulated in biopsies of inflamed pulps included several members of the miR181 family [17]. miR-181a regulates expression of toll-like receptor 4 (TLR-4), which is known to play an important role in pathogen recognition and activation of the pulp's immune response. A subsequent *in vitro* study examined the role of miR-181a in the TLR agonist-induced response in the pulp [18]. Primary cultures of human pulp fibroblasts were incubated with LPS derived from *Porphyromonas gingivalis* (*Pg*), a known oral pathogen. This resulted in a downregulation of miR-181a and an increase in the expression of the chemoattractant IL-8. The regulatory role of miR-181a on IL-8 was further confirmed by *in-silico* analysis and dual-luciferase assays, which identified a functional miR-181a binding site on the 3'UTR of IL-8 . This data demonstrates that in response to *Pg* LPS, human pulp fibroblasts downregulate expression of miR-181a, which subsequently increases expression of IL-8. Another similar study used a combination of *in vivo* and *in vitro* approaches to examine the role of miR-30b in pulpitis [19]. This study reported lower levels of miR-30b and increased levels of IL-6 protein and mRNA in inflamed human pulps as compared to normal controls. The interaction between, miR-30b and IL-6 was

subsequently confirmed using dual luciferase assays. Whether suppression of miR-181a and miR-30b occur at transcriptional or post-transcriptional levels remain to be dissected. Nonetheless, downregulation of miRNAs by oral pathogens appears a central theme in endodontal inflammatory infections.

Macrophages are considered to be the predominant cells in the innate immune response of the pulp. An *in vitro* study examined the role of macrophages miRNAs in response to LPS. This study showed that the microRNA expression pattern in CD14+ human macrophages challenged with *E.Coli* LPS changes in a time and dose –dependent manner [20]. The miRNAs which changed in response to LPS challenge target key signaling and pathogen recognition pathways including PIK3-Akt, MAPK, ErbB, Wnt and TGF-β. Given that macrophages play a key role in the innate immune response of the pulp and, that *E.coli* is detected in infected root canal systems, this study sheds light on the miRNA response to infections of the root canal systems [21].

Dental pulp stem cells (DPSCs) play an important role in the reparative and regenerative response of the pulp to trauma and injury [22]. DPSC miRNAs may regulate the proliferation and differentiation of these cells. Under hypoxia (3% O_2) seven miRNAs are upregulated and an equal number are downregulated in DPSCs as compared to normoxia (20% O_2) [22]. Aging pulps, which are thought to have a lower potential for repair and regeneration, express higher levels of miR-584 as compared to younger pulps [23]. This miRNA potentially plays an important role in the proliferation of DPSCs via regulation of transcriptional co-activator with PDZ-binding motif via AKT signaling [23]. Other miRNAs which play a role in DPSCs differentiation and function include miR-218 and miR-488 [24, 25]. The former is a negative regulator of dentinogenesis as inhibition of miR-218 results in increased calcium deposits in DPSCs. Downregulation of miR-488 promotes odontoblastic differentiation of DPSCs via the p38 MAPK signaling pathway. Improved understanding of the role of miRNAs in DPSC biology is likely to result in better treatment outcomes for vital pulp therapy.

While the studies described above focus on host microRNAs, viral miRNAs may also play a role in the pathogenesis of pulpal disease. Viruses encode miRNAs in order to regulate their life cycle inside the host [26]. Host proteins recognize and process the viral transcripts generated in the nucleus. To date, more than 200 different microRNAs of viral origin have been identified. They regulate both viral and/or host transcripts and have the potential to significantly influence the host transcriptome [27, 28]. An observational study comparing the viral encoded microRNA profiles in human dental pulps found 4 viral microRNAs- HSV1-miR-H1, HCMV-miR-US4, HCMV-miR-UL70-3p, and KSHV-miR-K12-3 to be expressed at higher levels in diseased human dental pulps [29]. *In silico* target prediction of the above-mentioned viral microRNAs identified the potential host target genes to be key mediators involved in the detection of microbial ligands (TLR-1), proteolysis (MMP24), chemotaxis (Chemokine ligand 25), pro- and anti-inflammatory cytokines (IL8, IL-10) and, signal transduction molecules (Inducible T cell co-stimulator). The role of viral miRNAs in the response to endodontic infection needs to be explored further.

2.2 miRNAs in periapical disease

Periapical disease is essentially an inflammatory response to a chronic polymicrobial infection from the root canal system. As with pulpal disease, most studies on periapical disease have focused on the nature of the infection and on the cellular and molecular response to it. Very few studies have examined the role of miRNAs in this disease. An observational study comparing diseased human periapical tissues to normal controls reported that 24 microRNAs were downregulated as compared to normal controls. These included some of the same microRNAs downregulated in pulpitis such as the miR-181 family and miR-155. In contrast, a similar study on periapical lesions found that miR-155 was upregulated [30]. This microRNA targets semaphorin 3a, known to play a role in bone remodeling and macrophage apoptosis. Another microRNA reported to have a potential role in the pathogenesis of apical periodontitis is miR-335-5p, a promoter of RANKL [31]. This microRNA promotes RANKL in human periodontal ligament fibroblasts. It acts as

a positive mediator of inflammation in these cells by targeting the urokinase-type plasminogen activator receptor.

3. Long noncoding RNA in endodontic Infections

In addition to microRNAs, the Human Genome Organization (HUGO) lists several other ncRNAs, namely: long non-coding RNAs, transfer RNAs, small nucleolar RNAs, ribosomal RNAs, small nuclear RNAs, piwi-interacting clusters, vault RNAs, Ro60-associated Y and small NF90 (ILF3) associated RNAs [28, 32]. Except for long non-coding RNAs, the role of these other ncRNAs in the pathogenesis of endodontic disease has not been elucidated as yet.

Long non-coding RNAs (lncRNAs) are non-protein coding transcripts with lengths of over 200 nucleotides [33, 34]. The current knowledge about lncRNAs is still in its early stages; however, their role in various biological processes, particularly in regulating gene expression at many levels, has been reported. They regulate transcription, cellular localization, mRNA stability, translation, and other post-transcriptional events, influencing a broad range of activity like hematopoesis, immune response, aging, cell growth and development [35-37]. In addition, their deregulation has been implicated in cancer metastasis, angiogenesis and drug resistance [38-40].

In a microarray study in DPSCs, 47 lncRNAs (20 upregulated and 27 downregulated) were differentially expressed in hypoxia group compared with the normoxia group. The depletion of the lncRNA streptolydigin, a potent inhibitor of RNA polymerase, inhibited the osteo/odontogenic differentiation potentials of DPSCs [40]. This result could further improve the potential targets in enhancing DPSC function in regenerative endodontics and may lead to a better understanding of the mechanisms of hypoxia's effects on DPSCs.

To determine the regulatory mechanism of lncRNAs in the odontogenic differentiation, human DPSCs were induced to differentiate into odontoblasts *in vitro*. Next, the expression profiles and functional analyses of lncRNAs, miRNAs, and mRNAs in differentiated and undifferentiated cells were investigated [41]. 132 lncRNAs, 114 miRNAs, and 172 mRNAs were

differentially expressed. Among these, 41 lncRNAs were upregulated and 91 were downregulated.

4. Circular RNA in Endodontal Diseases

In another DPSC microarray study, the global expression of circular RNAs (circRNAs) was analyzed to elucidate its role during odontogenesis [42]. In this study, human DPSCs were cultured for 14 days before RNA was extracted for microarray analysis and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) validation. A total of 187 circRNAs were differentially expressed in DPSCs during odontogenic differentiation. Furthermore, bioinformatic analysis of the expression data suggested that circRNA-miRNA-mRNA cooperation might act as a crucial mechanism for the odontogenic differentiation of DPSCs. Odontogenic differentiation plays a fundamental role in the response to injury and the outcome of vital pulp therapy. Similar to the lncRNAs study in DPSCs, the role of circRNAS in pulpal regeneration warrant further investigation.

5. Conclusion

The role of ncRNAs in biological processes remains to be clarified as knowledge in this field is still in its infancy; however, their potential use in dentistry, particularly in the field of endodontics, can be envisioned already. With the current impetus in preserving pulp tissue [43] and in maintaining the tooth in the oral cavity [44], understanding the mechanisms governing the health and disease of the pulp and the periapical tissues cannot be more timely. The putative regulatory function of ncRNAs in pulpal and periapical immune response and regeneration will continue to be elucidated as research methods and interest in this field continue to evolve.

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