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Epigenetic regulation in dental pulp inflammation

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Abstract

Dental caries, trauma, and other possible factors could lead to injury of the dental pulp. Dental infection could result in immune and inflammatory responses mediated by molecular and cellular events and tissue breakdown. The inflammatory response of dental pulp could be regulated by genetic and epigenetic events. Epigenetic modifications play a fundamental role in gene expression. The epigenetic events might play critical roles in the inflammatory process of dental pulp injury. Major epigenetic events include methylation and acetylation of histones and regulatory factors, DNA methylation, and small non-coding RNAs. Infections and other environmental factors have profound effects on epigenetic modifications and trigger diseases. Despite growing evidences of literatures addressing the role of epigenetics in the field of medicine and biology, very little is known about the epigenetic pathways involved in dental pulp inflammation. This review summarized the current knowledge about epigenetic mechanisms during dental pulp inflammation. Progress in studies of epigenetic alterations during inflammatory response would provide opportunities for the development of efficient medications of epigenetic therapy for pulpitis.

Keywords

epigenetics; DNA methylation; histone modifications; microRNAs; dental pulp inflammation

Introduction

Pulpitis is a typical inflammatory disease of dental pulp. It has been characterized by the local accumulation of inflammatory mediators, including cytokines and chemokines, which participated actively in destructive and reparative processes in the pulp (Kokkas *et al*, 2011). Bacterial infection is usually described as the most important etiological factor in pulp

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Conflicts of interest

None.

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disease (Trope, 2008). Bacteria and their toxins are key candidates in the direct stimulation during pulpal inflammatory processes (Durand *et al*, 2006; Lai *et al*, 2014). The balance between the inflammation and reparative processes could determine the extent of pulp inflammation and the viability of the affected tooth, in which multiple signal pathways are involved (Magloire *et al*, 1992; Tziafas *et al*, 2000; Botero *et al*, 2006; Choi *et al*, 2009). Under pathological conditions, dentin matrix secreted by odontoblasts could be induced in response to an injurious challenge and then stimulated the reparative process (Murray *et al*, 2000). However, clinically it is very difficult to know the inflammatory status of the pulp, such as the mild/severe inflammation, according to the chief complaint of patients. In addition, the cytological state of the odontoblasts is difficult to explain pulp inflammatory status in patients because pulpal histology processed on dental pulp tissue (from patients or experimental animal models) does not necessarily correlate well with clinical manifestations. This makes it very difficult to design an effective treatment plan in such situations (Stephane Simon *et al*, 2012). Fortunately, regenerative endodontic therapies might be possible according to the recent literature (Galler, 2015).

In view of the fact that tissue regeneration is a balance between inflammation and repair (Cooper *et al*, 2010). The effect of dentin matrix proteins might serve the biological role of protecting the dental pulp by preventing pulp inflammation (Cooper *et al*, 2010). Infection control was reported to be the key factor for improving the clinical outcome (Mjor, 2002; Ward, 2002). Genetics can identify and determine the position of the specific genes within the genome that could cause a certain genetic disease, thereby opening the door for potential cure for several diseases (Manolio *et al*, 2009). However, the basic structure and function of DNA could not completely explain all of the underlying mechanisms of gene regulation and disease development (Poulsen *et al*, 2007). And the epigenetic events could partially complement the limitations (Poulsen *et al*, 2007; Xu *et al*, 2013; Zheng *et al*, 2014). We could suggest that environmental factors might play critical roles in pulp inflammation as bacterial infection has been usually described as the most important etiological factor in pulp inflammation (Trope, 2008).

Epigenetics has been introduced to play important roles in inflammatory diseases such as periodontitis and pulpitis (Lindroth and Park, 2013; Hui *et al*, 2014). It has been defined as the study of mitotically and meiotically heritable changes in gene function that were not dependent on DNA sequence (Feinberg, 2007). The molecular basis of epigenetic processes is complex and involves modifications of histones, methylation of DNA, positioning of histone variants, and regulation of non-coding RNAs. Epigenetic modifications are potentially reversible, and therefore, a thorough understanding of these changes may identify new therapeutic targets for inflammatory diseases (Bayarsaihan, 2011; Deng *et al*, 2015). Inflammation is a complex physiological response of an organism to harmful stimuli (Dunning, 2009). Transcription factors such as NF- κ B, FOXP3, IRF, and STAT families along with epigenetic phenomena, including DNA methylation and covalent histone modifications, have been shown to be critical in regulation of inflammatory genes (Medzhitov and Horng, 2009). In addition, several regulatory factors could be controlled by epigenetic mechanisms in T cells and monocytes (Lal *et al*, 2009; Wells, 2009; Wierda *et al*, 2010). The basic unit of chromatin consisted of a short segment of DNA wrapped around core histones made up of two copies of H2A, H2B, H3, and H4. This organization provided

a rigid structure to chromatin (Campos and Reinberg, 2009). The covalent modifications of histones could be essential epigenetic mechanisms of gene regulation. These post-translational modifications (methylation of lysines and arginines, acetylation, phosphorylation, ubiquitination) occurred most frequently at the N-terminal tails of the core histones (Fuchs *et al*, 2006). In addition, we have indicated that epigenetic regulation has been involved in the process of pulp inflammation (Hui *et al*, 2014). Inflammatory reactions in infected pulp and periodontal tissue affected epigenetic modifications, which could cause changes of gene expression (Cardoso *et al*, 2010, 2014; Zhang *et al*, 2013). Researches about dental pulp inflammation indicated that epigenetic modifications might affect the behavior of dental pulp cells (Duncan *et al*, 2012, 2013).

The aim of this review was to describe the features of epigenetics such as DNA methylation, histone modifications, polycomb group (PcG) proteins, and miRNAs in the inflammatory response of dental pulp for indicating the future implications of epigenetics in the field of dentistry. We have prepared a table to summarize the functions and targets of DNA methylation, histone modification, and miRNAs in dental pulp inflammation (Table 1). And the detailed information about epigenetics in dental pulp inflammation has been represented in the review sections below.

DNA methylation in dental pulp inflammation

DNA methylation is the covalent transfer of a methyl group from S-adenosyl-L-methionine (SAM) to cytosines in CpG dinucleotides (Herman and Baylin, 2003; Weber and Schubeler, 2007). It typically occurs in CpG poor regions, and the promoter region of the gene is not methylated (Bock *et al*, 2006). DNA methyltransferases (DNMTs) establish and maintain DNA methylation patterns using SAM as the methyl donor (Barros and Offenbacher, 2009). The exposed methylation sites allow for interaction with methyl-binding proteins, such as the methyl-CpG-binding domain proteins (MBDs) (Loenen, 2006). DNA methylation patterns affect gene expression strongly (Seo *et al*, 2015). Unmethylated islands are related with transcriptionally active structure, whereas methylated DNA recruits methyl-binding proteins that promote chromatin compaction and prevent transcription (Jones *et al*, 1998; Egger *et al*, 2004). Abnormal methylation status can lead to disease development. According to recent studies in the field of dentistry, DNA methylation patterns could be altered by inflammation (Seo *et al*, 2015). Despite the small number of studies that evaluated CpG methylation in cytokine genes, initial findings have offered insights into the importance of methylation in the pathogenesis of inflammatory disease such as periodontitis (Offenbacher *et al*, 2008). Changes of DNA methylation patterns and expression of cytokine genes can be observed in chronic periodontitis (Zhang *et al*, 2010). The interaction between DNA methylation and inflammation may have relevance to pulpitis (Cardoso *et al*, 2010). Several cells and inflammatory mediators have been involved in the initial pulpal responses to caries, including IFN- γ cytokine. The IFN- γ cytokine may interfere the immune response during the process of pulp inflammation (Cardoso *et al*, 2010). CpG methylation in the IFN- γ promoter is considered a negative transcriptional regulator of IFN- γ production (White *et al*, 2002). A high prevalence of IFN- γ messenger RNA in inflamed pulps has been detected, being the initial stage of pulpal inflammatory response (Hahn *et al*, 2000). In addition, it has been reported that unmethylated sequences of genes could be correlated with high

messenger RNA levels of IFN- γ in mice mononuclear cells (Kersh *et al*, 2006). And the loss of DNA methylation has been detected in the dental pulp inflammation (Cardoso *et al*, 2010). This indicated that the presence of inflammation in human dental pulp could be associated with loss of DNA methylation in the promoter region of the IFN- γ . And inflammation in human dental pulp is characterized by a change from a totally methylated to a partially methylated status of the IFN- γ (Cardoso *et al*, 2010). These findings suggested that epigenetic events might be relevant to IFN- γ modulation in dental pulp.

Also, it has been shown that promoter hypomethylation of the Toll-like receptor 2 (TLR2) gene is associated with increased pro-inflammatory response (Shuto *et al*, 2006). Many studies have found an increase in the expression level of TLRs in inflamed pulp tissues (Mutoh *et al*, 2007). TLRs could influence the osteogenesis/odontogenesis, which is very critical in dental pulp repair (Muthukuru and Darveau, 2014). However, there is no significant difference of promoter methylation of TLR2 in the inflamed pulp tissue, which could indicate that an unmethylated profile in the TLR2 genes is a usual feature in human dental pulp.

In summary, the investigation of methylation profile could help us better understanding the molecular mechanisms which are related to development of immune response in inflammatory diseases.

The histone modifications in dental pulp inflammation

The modifications of histones take place mostly at the N-terminal tails of the protein (Cheng and Blumenthal, 2010). It is important to note that DNA methylation and histone methylation are tightly related events. For example, histone H3 N-terminal tail with an unmethylated lysine 4 (H3K4) is required for DNA methylation (Hu *et al*, 2009). Tri-methylation of histone H3 on lysine 9 (H3K9me3) participates in DNA methylation (Rottach *et al*, 2010). It was reported recently that several PcG targets were subject to aberrant DNA methylation following chronic inflammation (Hahn *et al*, 2008). Thus, histone modifications might have relevance in dental pulp inflammation process. However, there will be different effects on gene expression of histone modifications (Kouzarides, 2007). For example, histone methylation can either result in an activated or a repressed chromatin state. Tri-methylation of histone H3 on lysine residues 4 and 36 could promote an open chromatin structure, leading to active transcription (Fischle *et al*, 2003). While histone methylation on lysine residues 9 and 27 could lead to condensation of the chromatin and thus, gene silencing (Cao and Zhang, 2004). At present, it has been reported that histone methylation on H3K27 might have involved in dental pulp inflammation and reparative processes (Xu *et al*, 2013; Hui *et al*, 2014). It has been demonstrated that H3K27me3 was decreased in inflamed pulp tissue and pulp cells, which might induce the regeneration processes of dental pulp (Hui *et al*, 2014). In addition, Jmjd3, a member of the Jumonji family, is an enzyme that erases histone H3K27 marks (De Santa *et al*, 2007). Jmjd3 could be induced in macrophages exposed to bacterial products and inflammatory cytokines, where it bound the PcG target genes and regulated the expression and transcriptional activity of H3K27me3 (De Santa *et al*, 2007). It was recently found to control differentiation and cell identity in macrophages. Therefore, it might provide a link between inflammation and reprogramming of the

epigenome (Ishii *et al*, 2009). In addition, the methyltransferase of H3K27, enhancer of zeste homolog 2(EZH2), has been introduced in dental pulp inflammation (Hui *et al*, 2014). EZH2 involved in the mechanism of histone modifications might act as an epigenetic marker (Hake *et al*, 2004). We have identified the different expression of Jmjd3 and EZH2 in normal and inflamed dental pulp. And we proposed that EZH2 could be regarded as a regulator of dental pulp inflammation (Hui *et al*, 2014). It might be applied to dental pulp regeneration through further studies about precise mechanisms.

The other major histone modification is histone acetylation (Strahl and Allis, 2000). The acetylation process is homeostatically balanced by two groups of cellular enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Shahbazian and Grunstein, 2007). It has been suggested that histone deacetylase inhibitors (HDACis) could epigenetically promote reparative events in dental pulp cells by reducing proliferation and increasing mineralization (Duncan *et al*, 2011). Histone acetylation by HATs could activate inflammatory genes, whereas increased HDAC activity could result in inflammatory gene repression (Kleff *et al*, 1995; Leoni *et al*, 2002; Barnes, 2009). Promoters of several pro-inflammatory cytokines (IL-1, IL-2, IL-8, and IL-12) are rapidly acetylated by CBP/p300, which is a well-known HAT, leading to transcriptional activation (Villagra *et al*, 2010). Moreover, p300 knockdown could restrain the proliferation and odontogenic differentiation potential of dental pulp cells (Liu *et al*, 2014). This indicated that HAT might have important roles in pulp inflammatory processes, although the accurate mechanisms need further studies. Acetylation of histone H3 at the promoters of several cytokines and chemokines after inflammation could result in the increased recruitment of NF- κ B to these regions, and HDAC2 could reverse this process inhibiting NF- κ B-dependent inflammatory genes (Barnes, 2009). In addition, it has been shown that HDACis epigenetically promoted reparative events in dental pulp cells by reducing proliferation and increasing mineralization (Duncan *et al*, 2011).

According to the researches above, it has been proposed that modulation of histone acetylation could be applied in clinical practice including inflammation control, repair, and regeneration in the field of restorative dentistry (Duncan *et al*, 2011).

MicroRNAs (miRNAs) in dental pulp inflammation

MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate the expression of target genes at the post-transcriptional level (Zhong *et al*, 2012). miRNAs are transcribed as long preliminary transcripts and translocate to the cytoplasm to be processed by dicer into 18- to 24-bp miRNA duplexes. The RNA-induced silencing complex (RISC) incorporates these short RNA duplexes and binds to the 3' untranslated region (3' UTR) of specific messenger RNAs for subsequent degradation or translational repression (Bayarsaihan, 2011). Multiple miRNAs might be involved in the inflammatory response of oral inflammatory diseases such as pulpitis (Kong *et al*, 2014). Thirty-four miRNAs were downregulated in inflamed pulps as compared with normal, among which miR-221 was predicted to be associated with a variety of biological functions such as immune response in Toll-like signaling pathways (Kong *et al*, 2014). Three miRNAs, miR-150, miR-584, and miR-766, were significantly upregulated in inflamed pulps as compared with normal pulps

(Zhong *et al*, 2012). Growing evidence suggested that miRNAs, like miR-155 and miR-147, might play important roles in regulating immunity and the inflammatory response (O'Connell *et al*, 2007; Liu *et al*, 2009). The exposure of cultured macrophages to lipopolysaccharide (LPS) could lead to upregulation of miR-155 implicated in the regulation of pro-inflammatory cytokines during macrophage activation (Worm *et al*, 2009). MiR-155-deficient dendritic cells are unable to induce efficient T-cell activation in response to antigens (Tili *et al*, 2007). In contrast, miR-125b is downregulated on lipopolysaccharide (LPS) stimulation in macrophages. This suggested that miR-125b is required to ensure a proper inflammatory response by macrophages in response to microbial stimuli (Tili *et al*, 2007). TLR stimulation induces miR-147 and requires activation of both NF- κ B and IRF3. Furthermore, miR-147 attenuates the TLR-induced inflammatory response in macrophages (Lu *et al*, 2010). MiR-146a limits Toll-like receptor signaling by blocking the signaling molecule TRAF6 (Taganov *et al*, 2006). In another study, miR-105 was shown to modulate TLR-2 translation (Benakanakere *et al*, 2009). It was recently suggested that the TLR4-dependent reprogramming of inflammatory genes could be mediated by miRNAs (miR-221, miR-579, and miR-125b) (El Gazzar and McCall, 2010). MiR-152, a negative regulator of the innate response and antigen presenting capacity of dendritic cells, inhibits the production of cytokines including IL-12, IL-6, and TNF- α . MiR-148, a negative regulator of the innate response, similar to miR-152, also functions to regulate the antigen presenting capacity of dendritic cells (Zhong *et al*, 2012). Several of the miRNA 181 family members were downregulated in dental pulp inflammation. These members include miR-181a, which is known to regulate IL-6 (Pichiorri *et al*, 2008); miR-181b, which is known to regulate CCL8 (Dave and Khalili, 2010); miR-181c, which regulates IL-2 (Xue *et al*, 2011); and miR-181d, which regulates MMP9 expression (Wang *et al*, 2010). These findings highlighted the specific roles of miRNAs in inflammation and immunity and key aspects of pulpal pathology. Importantly, deregulated miRNA expression has been reported in autoimmune and inflammatory conditions such as rheumatoid arthritis and osteoarthritis (Niimoto *et al*, 2010). It has been reported that miR-146a participated in the IL-17 expression, which might be involved in the dental pulp inflammatory process (Niimoto *et al*, 2010; Xiong *et al*, 2014). It suggested that miRNAs might also play a contributory role in endodontic pathogenesis.

Conclusion

The field of epigenetics is taking center stage in pursuit of better understanding the genome and ultimate gene expression (Seo *et al*, 2015). Historically, epigenetics was used to describe cases that could not be explained by genetic principles (Morange, 2002). This review demonstrated the major epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNAs, could regulate gene expression and affect progression of inflammatory diseases. Although studies focusing on epigenetics are being actively investigated in the field of medicine and biology, epigenetics in dental research is still at the early stages. In addition, there are few researches in the dental pulp inflammation mediated by other epigenetic mechanisms such as histone phosphorylation and ubiquitination.

In this review, epigenetic mechanisms, such as DNA methylation and histone modifications, PcG proteins, and microRNAs are introduced in the process of dental pulp inflammation. DNA methylation and histone modifications play important roles in the establishment of the

epigenetic landscape (Sullivan *et al*, 2007). Environmental factors including bacterial infection were shown to contribute to the epigenetic status of the genome (Barros and Offenbacher, 2009). In addition, it has been reported that non-coding RNAs may affect odontoblast differentiation and oral diseases. And non-coding RNAs could be affected by the inflammatory status in dental pulp (O'Connell *et al*, 2007; Pichiorri *et al*, 2008; Liu *et al*, 2009; Zhong *et al*, 2012). It has been reported that an ambiguous relationship exists in dental pulp: inflammation may impair or support pulp regeneration. And the equilibrium between inflammation and regeneration determines the outcome of pulpitis (Hui *et al*, 2014). Studies on epigenetics in dentistry deserve attention because epigenetic mechanisms play important roles in gene expression during tooth development and may affect oral diseases.

However, the other pulp thematic has not been explored by knowledge of epigenetics such as angiogenesis, which is recognized as an important step to get wound healing in dental pulp injury (Zheng *et al*, 2009). There are some researches demonstrated that epigenetics have been involved in angiogenesis (Fork *et al*, 2015; Safe, 2015). In addition, another pulp thematic, pain control, has still not been researched by epigenetics. Approximately 90% of dental emergency visits with pain is the chief complaint in endodontic (Hasselgren and Calev, 1994). It has been proposed that the epigenetic modification may have a role in inflammatory pain (Bai *et al*, 2010; Geranton and Tochiki, 2015). We suggested that exploring the pulp thematic by knowledge of epigenetics would be a further potential action from which dental treatment may benefit.

Future researches are required for better understanding of the effect of epigenetics on the dental pulp disease for developing new therapeutic methods in dental pulp restoration.

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Table 1

Summary of the functions and targets of DNA methylation, histone modification, and miRNAs in dental pulp inflammation

	Function	Target genes	References
DNA methylation	Unmethylated: transcription activation; Methylated: transcription inactivation	IFN- γ	Cardoso <i>et al</i> (2010)
Histone modification	Altering chromatin states Histone methylation: either result in an activated or a repressed chromatin state; Histone acetylation: elevating transcriptional activity; Histone deacetylation: silences gene expression	IFN- γ TNF- α IL-1 IL-6 IL-8 IL-10	Hui <i>et al</i> (2014); Kleff <i>et al</i> (1995); Leoni <i>et al</i> (2002); Villagra <i>et al</i> (2010)
MiRNAs	Regulate gene expression via post-transcriptional repression; Evoking messenger RNA (mRNA) degradation or translational repression	TLR2; TLR4; CCL8; MMP9; IL-1; IL-6; IL-10; IL-17; TNF- α ; MAPK 8	Benakanakere <i>et al</i> , (2009); Dave and Khalili (2010); Zhong <i>et al</i> (2012); Pichiorri <i>et al</i> (2008); Xue <i>et al</i> (2011); Wang <i>et al</i> (2010); Niimoto <i>et al</i> (2010)