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Notch signaling pathway mediates alveolar bone resorption in apical periodontitis

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A B S T R A C T

Apical periodontitis represents a chronic inflammatory process within periapical tissues, mostly caused by etiological agents of endodontic origin. Progressive bone resorption in the periapical region represents the hallmark of apical periodontitis and occurs as the consequence of interplay between polymicrobial infections and host response. The Notch signaling pathway is an evolutionary conserved cell-signaling system that plays an important role in a variety of cell functions including proliferation, differentiation and apoptosis. In recent years its involvement in bone homeostasis has attracted a significant consideration. We hypothesized that Notch signaling pathway, which has a complex interplay with proinflammatory cytokines and bone resorption regulators, contributes to alveolar bone resorption via increased Notch receptors on immune cell surface and stimulates Notch receptor intracellular domain (NICD) translocation into the nucleus. The potential benefit of medications aimed to down-regulate these pathways in apical periodontitis treatment remains to be assessed.

Introduction

Apical periodontitis is a persistent inflammatory process within the periapical tissues of teeth devoid of vital pulp [1]. It is a consequence of a periapical tissue response to pulp necrosis, mostly caused by polymicrobial infection (e.g. bacterial, fungal and/or viral) of endodontic origin [2]. Infectious agents, their toxins and metabolic byproducts mediate a series of immunological responses in the dental pulp and periradicular tissues of the host. This is characterized by acute and/or chronic inflammation which involves the recruitment and activation of leukocytes of the innate and adaptive immune response, osteoclastogenesis and alveolar bone resorption at the apex of the tooth [3]. Progressive bone resorption in the periapical region represents the hallmark of apical periodontitis and it is a consequence of the interplay between a polymicrobial infection and the host response [1–3]. Development of apical periodontitis is initiated by an inflammatory cascade of events that includes activation of endothelial cells, polymorphonuclear leukocytes, macrophages, and osteoclasts leading to rapid bone destruction. Beside innate immune response, cellular components of adaptive response, including T – and B – lymphocytes, have been also implicated in alveolar bone resorption in apical periodontitis [4]. However, there is a significant overlap in the protective and destructive functions of these cellular elements and in the effects of inflammatory mediators produced by these cells [5].

Various mediators of inflammation, including arachidonic acid metabolites, metalloproteinases, oxygen-derived free radicals, cytokines, chemokines, bone resorption regulators, etc., have been associated with formation of apical periodontitis [5,6]. These molecules establish various network interrelationships in the inflamed periapical area, while the pattern and level of their gene expression differ between individuals and depend on the source of the stimulatory agent [5,6]. The balance between bone formation and resorption relies on dynamic interaction between osteoblasts, osteoclasts, their precursors and immunoregulatory mediators [7]. The involvement of bone resorption regulators (i.e. receptor activator of NF-κB ligand (RANKL), its cellular receptor – RANK and the decoy receptor osteoprotegerin (OPG)) is well established in the pathogenesis of alveolar bone loss [8]. Thus, increased RANKL, decreased OPG, or more generally an imbalanced ratio between RANKL and OPG, have been related to the progression of alveolar bone resorption in apical periodontitis [9–11]. In addition, previous studies confirmed that proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and IL-6 can either directly stimulate osteoclastogenesis and alveolar bone resorption, or indirectly via RANKL production by osteoblasts in periapical tissues [12–14].

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Notch signaling pathway and its link to bone homeostasis

The Notch signaling pathway is an evolutionary conserved cell-signaling system that allows cell communication trough molecular cell/cell interaction [15]. It was originally identified in *Drosophila*, in which a mutant allele gives rise to a notched wing [16]. Notch-mediated cell communication depends on the relationship between ligands and receptors on opposing cells. Four extracellular Notch receptors (Notch 1–4) and 5 membrane-bound ligands (Jagged 1 and −2, Delta 1, −3 and −4) have been identified in vertebrates [17]. The Notch receptors undergo several successive cleavages before allowing transcription of downstream targets. One of these cleavages is mediated by a furin-like convertase, leading to the cell surface presentation of the receptors. Thereafter, signaling is activated via the direct binding of the extracellular domain of Notch receptor (NECD) on one cell to the appropriate ligand present on a neighboring cell. Ligand – receptor interaction leads to proteolytic cleavage of the Notch receptor by a disintegrin and metalloprotease (ADAM) family member. Finally, the γ-secretase complex allows the cytoplasmic release of the Notch receptor intracellular domain (NICD). Dissociated NICD is then translocated to the nucleus where it binds to transcription factor such as Recombining binding protein suppressor of hairless (RBP-Jκ, also known as CLS) complex. This interaction ultimately initiates transcription of target genes, such as hairy and enhancer of split (Hes) and Hes-related protein (Hey), which in turn induce differentiation and proliferation of different cell types [15–18].

Notch signaling plays an important role in a variety of cell functions including cell proliferation, differentiation, and apoptosis [15]. Moreover, Notch signaling pathway is one of the key regulators of inflammation, and controls the differentiation and function of different cells (dendritic cells, natural killer cells, macrophages, B lymphocytes and various T cell types) included in innate and adaptive immune response [19]. It has been proposed as a mediator in the pathogenesis of different diseases, including ischemic heart disease, vitiligo, leprosy, cancer, etc. [20–24].

In recent years, its involvement in bone homeostasis has also attracted a major attention [25]. Previous studies reported that Notch regulates the differentiation and function of cells of the osteoblastic and osteoclastic lineages and plays a critical role in skeletal development, chondrogenesis, osteoblastogenesis and osteoclastogenesis. In that regard, it is important to emphasize that the effects of the Notch signaling pathway molecules are cell-context dependent. Namely, in immature osteoblasts Notch suppresses cell differentiation, while in mature osteoblast Notch depresses their differentiated function [25]. In addition, several studies reported the role of Notch 2 in the promotion of osteoclast differentiation [26,27]. In an *in vitro* model, Ashley et al. [26] confirmed that Notch signaling enhanced osteoclastogenesis of RANKL pre-stimulated osteoclast precursors and increased osteoclastic resorption. Additionally, Fukushima et al. [27] reported that induction of Notch signaling by Jagged 1 or by ectopic expression of intracellular Notch 2 promoted RANKL-induced osteoclastogenesis.

Despite the marked importance of Notch signaling in bone metabolism, only few *in vitro* studies have investigated its activation in alveolar bone resorption [28–32]. Nakao et al. [28] demonstrated that parathyroid-hormone-related protein induces Jagged 1 expression in periodontal ligament (PDL) cells, leading to osteo- and odontoclastogenesis, and likely promoting tooth and alveolar bone resorption. Kikuta et al. [29] also reported that the Notch signaling response to excessive orthodontic forces stimulates the process of root resorption via RANKL and IL-6 production from human PDL cells. Furthermore, another study demonstrated that Jagged 1 activates Notch signaling in human PDL cells and consequently decreases OPG expression [30]. The expression patterns of Notch proteins in the lining epithelium of human periapical cysts, a form of apical periodontitis lesions have been assessed in two studies only, by means of immunostaining [31,32].

Since the exact role of the Notch signaling pathway involvement in the periapical alveolar bone resorption has not been fully elucidated, this study aimed to propose a mechanism by which Notch signaling molecules in a complex cross-talk with proinflammatory cytokines and bone resorption regulators contribute to alveolar bone resorption.

The hypothesis

Due to stimuli from necrotic pulp at the site of inflammation in the periapical area different immune cells start to produce various mediators of inflammation including proinflammatory cytokines (TNL-α, IL-1β, and IL-6). Increased production of proinflammatory cytokines stimulates the higher expression of Notch ligands and receptors on immune cells at the site of inflammation, and promotes NICD nuclear translocation. This translocation is mediated by NF-κB and/or mitogen-activated protein kinases (MAPKs) pathways. As the consequence of NICD nuclear translocation and its interaction with RBP-Jκ complex, transcription of target genes (Hes and/or Hey) is initiated. These events induce differentiation and proliferation of osteoclasts which promote periapical alveolar bone resorption. Increased production of RANKL at the site of inflammation also activates the Notch signaling pathway leading to stimulation of osteoclastogenesis and bone resorption. Therefore, the interaction between proinflammatory cytokines, RANKL and Notch pathway molecules at the site of inflammation is responsible for alveolar bone resorption in apical periodontitis (Fig. 1).

Evaluation of the hypothesis

In order to evaluate this hypothesis our research group conducted an investigation on human apical periodontitis lesions [33]. Namely, in order to better understand the contribution of the Notch signaling pathway in alveolar bone resorption, we aimed to analyze the expression of Notch signaling molecules (Notch 2, Jagged 1 and Hey 1) and proinflammatory cytokines (TNF-α, IL-1β and IL-6) in human apical periodontitis lesions with different RANKL/OPG ratio, and to determine their potential correlation.

Fifty apical periodontitis samples were collected following standard apicoectomy procedure from fifty consecutive patients who required surgical apicoectomy due to the failure of conventional root canal treatment at the for Oral Surgery, School of Dental Medicine, University of Belgrade. Complete surgical protocol and processes of tissue harvesting and preparation for further laboratory use were thoroughly described elsewhere [34,35]. Reverse transcriptase – real-time polymerase chain reaction (RT-qPCR) method was used for relative gene expression analysis of the Notch signaling pathway molecules (Notch 2, Jagged 1 and Hey 1), bone resorption regulators (RANKL and OPG), and proinflammatory cytokines (TNF-α, IL-1β and IL-6) in all tissues samples. Details of primers and PCR conditions used in this study were

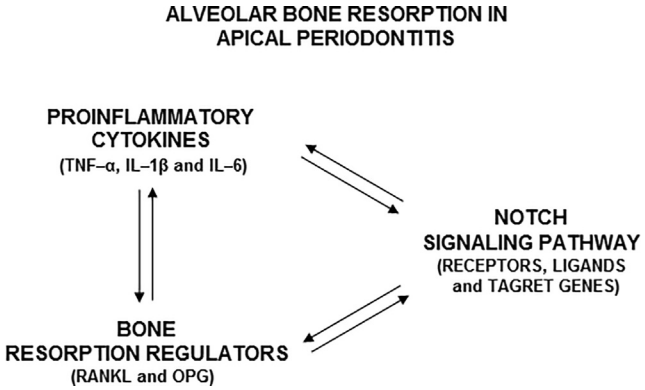

Fig. 1. Mechanism of alveolar bone resorption mediated by interrelationship network between Notch signaling pathway molecules, RANKL, and proinflammatory cytokines.

also thoroughly describer elsewhere [33]. As previously addressed, increased RANKL, decreased OPG and an imbalanced ratio between RANKL and OPG are related to the progression of bone resorption in apical periodontitis [9,35,36]. Therefore, based on the values of relative gene expression of RANKL and OPG, all apical periodontitis samples in our study were either RANKL predominant (RANKL > OPG; n = 33) or OPG predominant (RANKL < OPG; n = 17). We showed that Notch 2, Jagged 1, Hey 1 and TNF-α expression levels were significantly higher in apical periodontitis lesions with predominant RANKL compared to lesions with predominant OPG. On the other hand, relative gene expression of IL-6 was higher in apical periodontitis lesions with OPG predominance. We also reported significant positive correlation between gene expression levels of RANKL and Notch 2, RANKL and Jagged 1, Jagged 1 and Notch 2, Jagged 1 and TNF-α., Jagged 1 and Hey, and TNF-α and IL-1β. Additionally, Jagged 1 levels significantly correlated with the expression of Notch 2 and TNF-α. There was also a significant negative correlation between Notch 2 and OPG, and Jagged 1 and OPG.

These results showed that RANKL predominant apical periodontitis lesions exhibited higher relative gene expression of the Notch signaling pathway molecules and proinflammatory cytokines compared to OPG predominant lesions. Additionally observed significant positive and negative correlations between investigated molecules corroborate the interrelationship between these molecules and their potential involvement in alveolar bone resorption in apical periodontitis.

Consequences of the hypothesis and discussion

Apical periodontitis represents a remarkably widespread problem. Pak et al. [37] reported very high prevalence of periapical radiolucency in a meta-analysis performed on 300.861 analyzed teeth (e.g. 5% of sample or broadly equivalent to 1 radiolucency per patient). The prevalence of apical periodontitis increased with age and this finding was confirmed by Hamedy et al. [38] who reported the increase of the prevalence of periapical radiolucency in individuals over 65 years compared to general adult population. Posterior teeth and those with poor quality root filling represent risk factors for the development of apical periodontitis [39]. Moreover, the presence of apical periodontitis has been associated with several systemic diseases including diabetes, cardiovascular disease, liver pathologies, etc. [40–42]. The seriousness of the problem does not seem to have been fully appreciated, and apical periodontitis has not attracted the attention needed by such a common disease. Pathophysiological mechanisms of apical periodontitis development are important for understanding the function of the complex relationship between different stimuli and host's immune response.

The Notch signaling pathway has been investigated in various skeletal diseases with loss-of- and gain-of-Notch function (i.e. Adams Olivier syndrome, Alagille syndrome, Hejdu-Cheney syndrome, brachydactyly, etc.) [25]. Only few *in vitro* studies have investigated its potential involvement in alveolar bone resorption [28–32]. Meliou et al. [31] reported that Notch signaling has been downstream activated in the lining epithelium of periapical cysts, while Nikolic et al. [33] showed higher relative gene expression of Notch 2, Jagged 1 and TNF-α in RANKL predominant apical periodontitis lesions compared to those with OPG predominance. All these findings corroborate the link between the Notch signaling pathway, proinflammatory cytokines and RANKL in periapical bone resorption.

From the clinician's point of view, there is a considerable interest to control the activity of these pathways in the treatment of disorders associated with their dysregulation. In this regard, Kuritani et al. [43] reported that intraperitoneal administration of anti-RANKL antibodies significantly inhibited alveolar bone destruction and tooth root exposure in the mice periodontitis model. Although the approaches to down-regulate Notch signaling are diverse (the use of biochemical inhibitors of Notch activation, antibodies to Notch receptors or their ligands, etc.), they have not yet been investigated in either animal or human (*in vitro* or *in vivo*) models of alveolar bone resorption. It is important to emphasize that the generalized inhibition of Notch signaling is correlated with serious unwanted events, including vascular neoplasms and severe liver pathology [25]. Also, Aghaloo et al. [44] reported that RANKL inhibitors are capable of inducing osteonecrosis of the jaw in the mice model of periapical disease. Therefore, these drugs could be used as an additional or alternative endodontic treatment, but great caution is necessary.

In summary, previous studies have shown that Notch signaling pathway is implicated in bone resorption. These findings led us to formulate the hypothesis of alveolar bone resorption in apical periodontitis mediated by a multifaceted relationship between Notch signaling, RANKL and proinflammatory cytokines. Further studies are needed to determine whether inhibition of these pathways might be beneficial and safe as a novel approach in apical periodontitis treatment.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.02.018.

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