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Involvement of the Notch signaling system in alveolar bone resorption

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ABSTRACT

The Notch pathway is an evolutionarily preserved signaling pathway involved in a variety of vital cell functions. Additionally, it is one of the key regulators of inflammation, and controls the differentiation and function of different cells. Moreover, it was found to be involved in skeletal development and bone remodeling process. This review provides an overview of the involvement of the Notch signaling pathway in the pathogenesis of alveolar bone resorption in different forms of pathological conditions such as apical periodontitis, periodontal disease, and peri-implantitis. *In vitro* and in vivo evidence have confirmed the involvement of Notch signaling in alveolar bone homeostasis. Nonetheless, Notch signaling system, along with complex network of different biomolecules are involved in pathological process of bone resorption in apical periodontitis, periodontitis, and peri-implantitis. In this regard, there is a substantial interest to control the activity of this pathway in the treatment of disorders associated with its dysregulation. This review provides knowledge on Notch signaling and outlines its functions in alveolar bone homeostasis and alveolar bone resorption. Further investigations are needed to determine whether inhibition of the Notch signaling pathways might be beneficial and safe as a novel approach in the treatment of these pathological conditions.

Contents

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1. Introduction

Alveolar bone is a unique osseous tissue in the human body structurally dependent on the development, eruption and maintenance of the teeth. Besides its primary function in protecting the roots of the teeth and supporting the mastication, it is a reservoir of hematopoietic and mesenchymal stem cells, as well as various electrolytes (e.g., calcium, phosphorus and magnesium) [1]. Alveolar bone undergoes constant remodeling through skeletal renewal process relying on a fine-tuned and balanced activities of hematopoietic derived osteoclasts that demineralize and resorb bone, and mesenchymal osteoblasts that produce and mineralize bone matrix. Alveolar bone remodeling is controlled by the endocrine system (e.g., parathyroid hormone, Vitamin D, fibroblast grow factors, etc.), signaling interactions between bone and immune cells, and mechanical loading forces [2]. Therefore, alveolar bone homeostasis relies on hormonally balanced activity of bone-resorbing osteoclasts, bone-producing osteoblasts, their precursors and immunoregulatory mediators. The imbalance between physiological bone resorption and deposition results in pathological resorption and consequent loss of function. These pathological events are primarily caused by an interplay between different microorganisms and immune system of the host. Progressive alveolar bone resorption is mostly attributed to the interaction between bone resorption regulators (i.e., receptor activator of NF-κB ligand (RANKL), its cellular receptor – RANK, and the decoy receptor osteoprotegerin (OPG)), different proinflammatory cytokines (i.e., tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-6, interferon-gamma (IFN-γ)), as well as different immune cells and signaling pathways [3]. Consequently, acute and/or chronic inflammation occurs, thus triggering the development of different forms of apical periodontitis, periodontal diseases or peri-implant lesions.

The Notch pathway is an evolutionarily preserved signaling pathway involved in a variety of vital cell functions, such as proliferation, differentiation, and apoptosis [4]. Moreover, it 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 [5]. Also, it was found to be involved in skeletal development and bone remodeling process [6], with multiple studies demonstrating a variety of regulatory functions, both stimulatory and inhibitory, of the Notch pathway in the osteoblastic cell lineage [7]. In addition, it is important to stress that Notch effects on the skeleton are cell-context-dependent [8], suggesting its role in suppression of bone resorption in cancellous bone, and enhancement of bone formation in cortical bone [9]. Several studies have reported that the Notch signaling pathway enhanced osteoclastogenesis of RANKL pre-stimulated osteoclast precursors and boosted osteoclastic resorption [10,11], yet Bai and coworkers have reported that signaling via Notch 1 may lead to the suppression of osteoclast differentiation [12]. Keeping in mind the significance of the Notch signaling pathway in bone homeostasis, this review provides an overview of the literature data (Table 1) regarding the involvement of the Notch signaling pathway in the pathogenesis of alveolar bone resorption in different forms of pathological conditions such as apical periodontitis, periodontal disease, and peri-implantitis.

2. Methods

The following electronic databases were used for the search: Clarivate Analytics' Web of Science, including Web of Science Core Collection - WoS, Korean Journal Database - KJD, Russian Science Citation Index - RSCI, SciELO Citation Index - SCIELO, Scopus, and PubMed (including MEDLINE). Furthermore, to identify relevant unpublished manuscripts, conference papers, doctoral dissertations, and other grey literature, OpenGrey (http://www.opengrey.eu), Google Scholar (first 100 returns), and other available digital repositories (e.g., Networked Digital Library of Theses and Dissertations (http://www.ndltd.org), Open Access Theses and Dissertations (https://oatd.org), DART-Europe E-theses Portal – DEEP (https://www.dart-europe.org/basic-search.php), Open access to UK theses – EThOS (https://ethos.bl.uk) were explored. The following search terms were used: "Notch", "Jagged", "Hes", "Hey", "alveolar bone", "resorption", "apical periodontitis", "periapical lesions", "periapical granuloma", "radicular cysts", "periodontitis", "marginal periodontitis", "aggressive periodontitis", "chronic periodontitis", "peri implant mucositis" and "peri-implantitis" in different combinations using the Boolean operators (AND, OR), and truncation (*, $) during the electronic search process. Articles evaluating the role of the Notch signaling pathway in alveolar bone resorption published in English were gained for further narrative review.

3. The Notch signaling pathway

The highly conserved Notch pathway represents a core signaling system during embryonic development [4]. Ligand-induced Notch signaling is active in adults as well in stem cell maintenance, but also affects the context dependent differentiation, proliferation, survival, and apoptosis of a variety of cell types, thus maintaining the tissue homeostasis [13]. Notch plays a critical role in many fundamental processes in a broad range of tissues, consequently it is no wonder that aberrant gain or loss of Notch signaling elements has been found in multiple human developmental syndromes, such as Alagille syndrome, syndactyly, spondylocostal dysostosis, familial aortic valve disease [14,15], adult-onset diseases such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy [16], and cancer.

The Notch signal transmission most often relies on a direct cell-to-cell interaction, via canonical membrane-bound DSL (Delta, Serrate, LAG-2) ligands that bind and activate the Notch receptors (Fig. 1). There are four Notch receptors (Fig. 1) and five DSL ligands, that are termed Jagged (Jag)1 and Jag2 and Delta-like (Dll)1, Dll3, and Dll4 [17] (Fig. 1). Ligand binding initiates a series of proteolytic cleavages by the γ-secretase complex, releasing the Notch intracellular domain (NICD) from the membrane, thus enabling its downstream signal transduction [18]. NICD translocates to the nucleus, where it interacts with CSL (CBF1 in mammals, Su(H) in Drosophila, and LAG-1 in Caenorhabditis elegans) DNA-binding protein to recruit transcriptional co-activators and turn on the expression of target genes such as hairy and enhancer of split (Hes)1, Hes5, Hes6, and Hes7, and HES-related with YRPW motif (Hey)1, Hey2, and Hey-like (HeyL). Additionally, NICD can associate with other intracellular proteins in loosely defined interactions termed the non-canonical Notch signaling pathway [19] (Fig. 1).

In addition to these well-characterized cell-to-cell, or so-called trans-interactions that activate Notch signaling, DSL ligands can also act as Notch signaling antagonists through intracellular interactions

Table 1
Summarized effects of the Notch signaling pathway molecules in investigated human diseases.

Author (year)	Investigated sample/method of analysis	Main results	Conclusions
Mitsiadis et al. (2003) [63]	Permanent intact and carious teeth/ Immunohistochemistry	Notch2 immunoreactivity was completely absent in adult intact teeth, but staining was observed in adult carious teeth (in odontoblasts situated beneath the carious front and in cells of the blood vessels).	Properly regulated activation of the Notch pathway is important for controlling cell fate choices during development.
Meliou et al. (2011) [65]	Periapical cysts/Immunohistochemistry, Western Blotting	Notch 1 and Notch 2 were expressed mainly in the cytoplasm, whereas HES 1, HES 5, and Delta 1 were expressed in both the cytoplasm and the cell membrane of epithelial cells of periapical cysts. Considering staining intensity, Notch 2 demonstrated the most strong staining (52.83 %), followed by Notch 1 (35.85 %), HES 5 (17.31 %), and HES 1 (9.09 %).	The Notch pathway is activated in the lining epithelium of periapical cysts, suggesting that this pathway might be associated with cell differentiation, proliferation, and apoptosis.
Gonçalves et al. (2012) [66]	Radicular cysts/Immunohistochemistry	All cases were positive for Notch-1. High expression for Notch-1 was noted in 28 cases (82.35 %) and 6 cases (17.65 %) showed low expression. Seventeen cases (50 %) showed strong staining intensity and 17 presented weak expression intensity.	The identification of Notch-1 in odontogenic cystic lesions, suggests that the Notch system participates in maintaining the integrity of the cystic epithelium, possibly contributing to the persistence of the lesion.
Nikolic et al. (2019) [67]	Periapical granulomas and radicular cysts/Nested and reverse transcriptase real-time polymerase chain reaction	Notch2, Jagged1, and Hey1 expression levels were significantly higher in apical periodontitis lesions with predominant RANKL compared with lesions with predominant OPG. Significant positive and negative correlations were observed between the expression levels of Notch signaling pathway molecules, bone resorption regulators, and proinflammatory cytokines in periapical lesions.	A significantly higher messenger RNA expression level of Notch signaling molecules, bone resorption regulators, and proinflammatory cytokines in RANKL predominant compared with OPG predominant periapical lesions
Jakovljevic et al. (2020) [68]	Periapical granulomas and radicular cysts/Reverse transcriptase real-time polymerase chain reaction	Significantly higher Notch2, and Jagged1mRNA levels were found in EBV positive lesions compared to EBV negative lesions. Significant positive correlation was present between Notch2 and Jagged1, and Jagged1 and RANKL in EBV positive periapical lesions.	There is a possibility that active EBV infection indirectly induces bone resorption in apical periodontitis via excessive oxidative stress and proinflammatory cytokines production and activation of RANKL-Notch bi-directional signaling cascade.
Djinic Krasavcevic et al. (2021) [90]	Patients suffering from periodontitis (aggressive periodontitis (AP) and chronic periodontitis (CP). Clinical parameters and reverse transcriptase-real-time polymerase chain reaction.	Significantly higher values of PPD in AP compared to CP AP: Negative correlations between OPG and CAL, and OPG and PI, while Hey 1 and PI had a positive correlation. OPG and Notch 2 were predictors of CAL in AP group. TNF-α and IL-17 were higher in RANKL predominant than in OPG predominant cases.	Notch 2 expression affected CAL in AP cases/In RANKL-activated settings, the down-regulation of Notch 1 contributes to bone loss.
Mijailovic et al. (2020) [86]	130 individuals: 40 with aggressive periodontitis (AP group), 40 with chronic periodontitis (CP group), and 50 periodontally healthy controls. Reverse transcriptase - real-time polymerase chain reaction (RT-qPCR).	Significant increase of Notch 2, TNF-α, IL-17 and RANKL and a significant decrease of Notch 1 and Jagged 1 in AP compared to controls. Notch 2 and RANKL were also overexpressed in CP group compared to controls.	Notch 2 overexpression in periodontitis. The down-regulation of Notch 1 and Jagged 1 might lead to increased bone resorption levels in aggressive periodontitis.
Milinkovic et al. (2021) [108]	Peri-implantitis and peri-implant mucositis samples/Reverse transcriptase real-time polymerase chain reaction (RT-qPCR).	Significant decrease of Notch 1, and higher relative expression level of Hey 1, were found in peri-implantitis compared to healthy implant samples. In peri-implantitis versus peri-implant mucositis samples, significantly higher relative expression level was found for Hey 1, TNF-α, IL-17, IL-1β, IL-6, and RANKL.	The combined effect of Notch 1 down-regulation and elevated expression of some key inflammation modulators might result in osteoclast activity increase and subsequent osteolysis in peri-implantitis.

within the same cell (i.e., cis-interactions) [20,21]. However, the molecular basis of cis-interactions and their effects on Notch and the physiological relevance are not well understood, although they appear to be involved in a subset of Notch-dependent development events [22,23].

3.1. Notch receptors

Notch receptor family in humans consists of four large single-pass type I transmembrane proteins, that display both redundant and unique functions. The extracellular domain contains 29–36 tandem epidermal growth factor (EGF)-like repeats, a portion of which mediate the receptor-ligand interactions. Many EGF repeats bind to calcium ions, which play an important role in determining the structure and affinity of Notch in ligand binding [24] and can affect signaling efficiency [25]. The EGF repeats are followed by a unique negative regulatory region (NRR), important for the prevention of premature activation of the receptor, comprised of three cysteine rich LIN repeats and a region that links to the transmembrane and intracellular domain. The intracellular portion consists of a RAM domain, six ankyrin (Ank) repeats and a C-terminal PEST domain. It also contains nuclear localization signals.

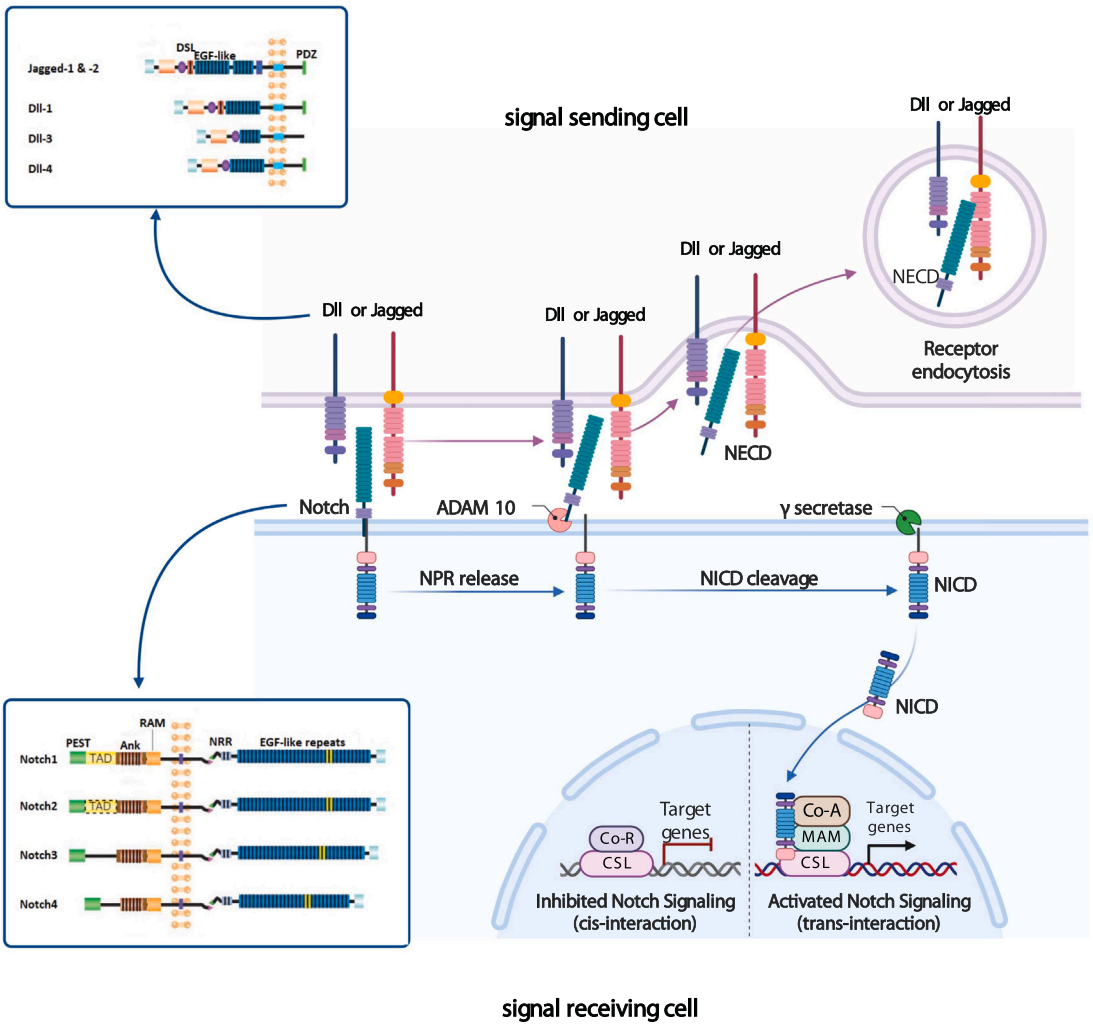

Fig. 1. A simplified illustration of the canonical Notch signaling. (Adapted from "Notch Signaling Pathway", by BioRender.com (2022). (b) Retrieved from https://app.biorender.com/biorender-templates).A Jagged or Dll ligand from the signal sending cell binds to the Notch receptor leading to activation. Ligand ubiquitination initiates the ligand-receptor complex endocytosis, causing a second proteolytic cleavage by ADAM metalloprotease, that removes the extracellular region (NECD). The membrane tethered receptor fragment is cleaved by the γ-secretase complex, releasing the Notch intracellular domain (NICD), which then translocates into the nucleus and forms a transcription activation complex, together with CSL and Master mind-like (MAM), on target genes promoters, such as like Hes1 or Hes5. Binding of the receptor and ligand present on the same cell surface can lead to a so-called cis-inhibition, with yet not well-known molecular basis of the cis-inhibitory complex, however including similar regions of receptor and ligand to those involved in trans-activation.

3.2. DSL ligands

The DSL ligands family of proteins are designated as either Delta-like or Serrate-like (also known as Jagged) based on the structural homology to the two Drosophila ligands, Delta and Serrate [13,21]. Like the Notch receptors, the DSL ligands are single pass cell surface proteins containing multiple EGF-like repeats; however, these ligands contain a signature motif dubbed DSL, which together with N-terminal (NT) sequences constitutes the ligand-binding domain. In mammals, three Delta-like (Dll) genes have been identified (Dll-1, −3, and −4). On the other hand, only two Serrate-like subtypes (Serrate1/Jagged-1 and Serrate2/Jagged-2) have been isolated from humans, with almost twice the number of EGF repeats as Delta-like ligands [26]. The intracellular regions of DSL ligands lack obvious sequence homology except that most, but not all, contain multiple lysine residues required for ligand signaling activity and a C-terminal PDZ (PSD-95/Dlg/ZO-1)-ligand motif responsible for the interactions with the cytoskeleton [27].

Although membrane attachment is thought to be important for activation of Notch, soluble forms of Delta have been identified in embryos and cultured cells, produced through cell surface proteolytic shedding by metalloproteases, and it is possible that their activity may be regulated through interactions with the extracellular matrix as found for soluble growth factors.

3.3. Nuclear target genes

The primary target genes of Notch signaling are the members of the Hes family of basic helix-loop-helix (bHLH) type trancriptional repressors, acting through negative regulation of tissue-specific transcription factors expression [28,29]. Another bHLH family has been isolated and named as Hey/Hesr/HRT/CHF/gridlock/HERP (or simpler Hey), with characteristic protein domains demonstrating close relation of these proteins to the Hes family. Hey expression is detected in tissues expressing and not expressing Hes proteins. In addition to their function as homodimers, they have been found to function as Hes-Hey heterodimers as well, in co-expressing tissues [30]. Although structurally and functionally similar, Hes and Hey function via different transcriptional repression mechanisms [30].

3.4. Canonical Notch signaling pathway

As mentioned, the canonical Notch signaling involves cell-to-cell interaction via Notch receptor and DSL ligand, unfolding of the negative regulatory region (NRR) within the Notch receptor, thereby allowing the proteolytic cleavage and the release of the NICD, which then translocates from the membrane to nucleus where it binds a conserved transcription factor (CSL; CBF1/RBPJ, Su(H), Lag-1) to upregulate Notch target genes [20].

3.5. Non-canonical Notch signaling pathway

Contrary to canonical Notch signaling, the non-canonical pathway is CSL-independent and can be ligand-dependent or independent. The most well-studied effect of non-canonical Notch function is the regulation of Wnt/β-catenin signaling, i.e., Notch binds and titrate levels of the obligate Wnt-signaling component - active β-catenin [31]. In progenitor cells, Notch levels are inversely correlated with active β-catenin. Notch regulation, however, does not appear to affect total β-catenin protein or transcript levels, but rather targets active β-catenin, hence active β-catenin activity may serve as a useful readout for non-canonical Notch signals [32].

4. Alveolar bone homeostasis

The alveolar bone is the terminal part of the maxilla and mandible bone that forms and supports the socket of the teeth. This unique entity is intimately connected with teeth since the alveolar bone forms and evolves with the development and the eruption of the teeth. Conversely, its height gradually decreases in case of trauma or injury, periodontal disease, or tooth loss.

The architecture of the alveolar bone consists of two compact bone walls: (i) the outer layer formed by haversian (i.e., cortical) bone and (ii) the inner socket wall. The latter consists in a 0.1–0.4 mm thick layer of bone that supports the attachment of periodontal ligament via Sharpey's fibers, and surrounds cancellous trabeculae [33,34]. This unequal architecture provides both rigidity and low weight to the alveolar bone, allowing it to endure mechanical loads transmitted by teeth during mastication. Alveolar bone also serves as a source of hematopoietic and mesenchymal stem cells, and acts as a reservoir for calcium phosphate, hydroxyl and carbonate, citrate, magnesium, sodium, potassium, or fluoride [35,36].

Alveolar bone is a connective tissue with cells, fibers, and ground substance containing an inorganic part made of hydroxyapatite crystals, several ions, collagens (mainly Type I (95 %), Type V and XII) and non-collagenous proteins (osteocalcin, bone sialoprotein, osteonectin, osteopontin) [2,36].

Therefore, alveolar bone homeostasis is essential since it participates in the regulation of serum calcium levels and its homeostasis is under the control of parathyroid hormone and calcitonin [37]. Moreover, alveolar bone metabolism is influenced by endocrine signaling (sex hormones and circulating inflammatory factors) [38,39]. Alveolar bone tissue undergoes perpetual renewal in response to the concentration of circulating calcium, as well as mechanical, nutritional, and hormonal influences. Like other bone tissues, alveolar bone remodeling is carried out thanks to several types of cells: osteoclasts, involved in the resorption of the bone matrix, and osteoblasts in charge of the production of this matrix. Osteoblasts secrete mainly type I collagen, small amount of type V collagen, proteoglycans, and several non-collagenous proteins. Osteocytes, which are differentiated osteoblasts trapped in the bone matrix, are currently considered to be the conductors of the remodeling process. Under physiological conditions, a balance exists between osteoclastic bone resorption, and osteoblastic bone formation activity [36,40]. Alveolar bone turnover remodeling rate has been reported to be faster in the mandible and maxilla than other skeletal sites such as in the femur [41,42]. Moreover, this turnover is significantly higher at the alveolar crest compared to the mandibular canal [42].

Owing to the association between the tooth and periodontium, the alveolar bone is exposed to several different stress or stimuli, such as pathogen invasion from the oral environment or mechanical stress from orthodontic treatments, resulting in the alveolar bone remodeling. Any change in the homeostasis of alveolar bone leads to pathogenic processes, where the crosstalk between bone and immune cells is a major component for the regulation of bone turnover and is called osteoimmunology [1,3,43]. Resident cells of alveolar bone, such as fibroblasts, keratinocytes, or immune cells produce antimicrobial agents, reactive oxygen species and release proinflammatory cytokines. This promotes the migration of multiple inflammatory cells (e.g., neutrophils, macrophages, and T or B cells) to the site of inflammation and their gradual infiltration deeper into the periodontal connective tissue, including alveolar bone [44].

Osteoblasts arise from pluripotent stem cells, whose differentiation is regulated by runt domain–containing transcription factor (RUNX), osterix (OSX), and activating transcription factor 4 (ATF4) [45–47]. A complex network of signaling pathways also interfere with osteoblast differentiation and function. Indeed Wingless-integrated (Wnt), bone morphogenetic protein (BMP), fibroblast growth factor (FGF), and insulin-like growth factor (IGF) signaling support osteoblast differentiation and function. In contrast, Notch signaling might inhibit osteoblast differentiation and be considered as a key factor for the coupling of osteogenesis and angiogenesis in skeletal repair [46,47].

Osteoblasts may undergo apoptosis, become quiescent bone lining cells, or develop into osteocytes entombed within the mineralized bone matrix. Osteocytes account for more than 90 % of total bone cells and play a crucial role in sensing mechanical loading forces on bone. Moreover, recent evidence suggests that osteocytes are involved in regulating bone anabolism and catabolism in the progression of periodontitis [48]. Osteocytes may also secrete factors that critically regulate osteoblast activity including Dickkopf-related protein 1 (DKK1), acting as a Wnt inhibitors, and sclerostin [48–50].

Osteoclasts are giant multinucleated bone resorbing cells derived from monocyte-macrophage precursor. The osteoclastogenesis is mediated by the signaling factors macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kappa B ligand (RANKL) [51,52]. The secretion of proinflammatory cytokines TNF-α and IL-β, through activation of B and T lymphocytes results in osteoclast differentiation and activation that will ultimately lead to bone resorption by osteoclast activating factors, including prostaglandins (PGs), endotoxin bacteria, and complement activator products (e.g., IL-1β, TNF-α, IL-6, and IL-11) [53].

Alveolar bone homeostasis is therefore affected by the oral microbiota and biofilms, and a balanced host-immune response. Chronic inflammation may shift the “coupled” osteoclast-osteoblast actions, which ultimately results in alveolar bone destruction that is dependent of several mechanisms related to RANKL, Notch, and Wnt signaling, as well as the nucleotide oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome [54].

5. The Notch signaling pathway in pathogenesis of alveolar bone resorption in apical periodontitis

Apical periodontitis (AP) represents a chronic inflammatory reaction within tooth-supporting tissues of teeth with an infected root canal system. It is most often the result of an irreversible infection by different microorganisms within the root canal system that leads to pulp tissue necrosis and subsequent progression of inflammatory reaction in the periapical region of the affected teeth [55]. As a result

of the harmful effect of different microorganisms and their virulence factors, the host's immune system is activated leading to the recruitment of various cell types and the production of cell-specific mediators. Finally, it results in the break-down of tooth supporting tissues, alveolar bone resorption and the formation of periapical lesions [56]. Having in mind the burden of AP in world adult population [57] and its significant potential to impair general health [58–60], it is imperative to continuously investigate its pathogenesis in order to prevent the development and progression of this disease in the future. In this regard, several investigations evaluated the role of the Notch signaling pathway in the pathogenesis of AP.

At the beginning of 21st century, Mitsiadis and co-workers investigated the involvement of the Notch signaling pathway in the dental pulp cells (DPCs) biology [61–63]. The authors have shown that different Notch signaling molecules are expressed in developing and injured rodent teeth, as well as in developed lesion, suggesting their involvement in developmental and regenerative processes. These findings have been confirmed by Løvschall et al. [64], who also reported that Notch signaling is activated in response to injury and associated with the differentiation of DPCs into perivascular cells and odontoblasts. Altogether, these primary experimental investigations in animal models corroborate the involvement of the Notch signaling pathway, as an important element, in pulpal physiological and pathogenic conditions.

In 2011. Meliou et al. [65] investigated the involvement of Notch signaling molecules in cell proliferation of the lining epithelium of periapical cysts. The authors evaluated immunohistochemical expression of receptors Notch1 and Notch2, ligand Delta1, and transcription factors Hes1 and Hes5 in 55 formalin-fixed and paraffin-embedded, well-defined periapical cysts with minimum inflammation. It was revealed that immune staining reaction of all Notch signaling components was observed in the cytoplasm and/or the cytoplasmic membrane, and occasionally in the nucleus, of the majority of periapical cysts epithelial cells (e.g., strong staining of Notch2 in 52.83 % of the cases). Based on the observed results, the authors concluded that the Notch pathway is activated in the lining epithelium of periapical cysts, suggesting its potential involvement in lining epithelium cells differentiation, proliferation, and apoptosis. These findings were confirmed by Gonçalves et al. [66], who also revealed the involvement of the Notch signaling components in the development, maintenance, and integrity of cystic odontogenic epithelial lining, favoring lesion persistence.

In order to better understand the contribution of this important signaling cascade in alveolar bone resorption, Nikolic et al. [67] analyzed the expression of Notch signaling molecules (Notch2, Jagged1, and Hey1) and proinflammatory cytokines (TNF-α, IL-1β, and IL–6) in human apical periodontitis lesions with different RANKL/OPG ratios. The authors observed that the gene expression of Notch2, Jagged1, and Hey1 was significantly higher in periapical lesions with RANKL predominance compared to periapical lesions with OPG predominance, suggesting the activation of the Notch signaling pathway [67]. These results were in line with an in vitro study performed by Fukushima et al. [11], which revealed the positive association between Notch2 and RANKL-induced osteoclastogenesis, and showed that activated Notch2 and Jagged1 in bone marrow–derived macrophages resulted in the induction of osteoclast differentiation in a RANKL dose-dependent manner. In addition, Nikolic et al. [67] found significant positive correlation between gene expression levels of RANKL and Notch2, RANKL and Jagged1, Jagged1 and Notch2, Jagged1 and TNF-α, Jagged 1 and Hey 1, and TNF-α and IL-1β. Also, a significant negative correlation between Notch2 and OPG, and Jagged1 and OPG was reported. 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 (i.e., Notch signaling components, bone resorption regulators and proinflammatory cytokines) corroborate their interrelationship and potential involvement in alveolar bone resorption in AP.

Another study investigated the involvement of the Notch signaling pathway in pathogenesis of AP [68]. It was related to the identification of Epstein-Barr virus (EBV) as putative causative agent of AP [69], and its involvement in alveolar bone resorption via excessive production of reactive oxygen species and imbalance between bone resorption regulators [70,71]. In this regard, Jakovljevic et al. [68] evaluated whether EBV positive AP lesions exhibited increased expression of Notch signaling pathway molecules (Notch2 and Jagged1), bone remodeling markers (RANKL and OPG) and cytokines (TNF-α, IL-1β and IL-6) compared to EBV negative lesions. The authors revealed significantly higher mRNA levels of Notch2, Jagged1, RANKL and IL-1β in EBV positive compared to EBV negative lesions. In addition, significant positive correlation was found between Notch2 and Jagged1, Jagged1 and RANKL, and IL-b and TNF-α in EBV positive periapical lesions [68]. These results corroborate previous hypothesis, by which EBV nuclear antigen EBNA-2 and Notch receptors are able to activate different genes in the same way, by interacting with cellular repressor protein RBPJj located in the nucleus. In this regard, it has been suggested that activated Notch serves as a potential functional cellular equivalent to EBNA-2, which is used for B cells immortalization, and which enables lifelong latent EBV infection [72,73].

These findings suggest that alveolar bone resorption in AP is mediated by a multifaceted relationship between Notch signaling, bone resorption molecules, and proinflammatory cytokines. Further studies are needed to determine whether inhibition of these pathways might be beneficial and safe as a novel approach in AP treatment.

6. Notch signaling pathway in the pathogenesis of alveolar bone resorption in periodontitis

Periodontitis is a progressive inflammatory disease which destroys periodontal tooth supporting tissues, causing loss of attachment (measured clinically as clinical attachment level, CAL) between the gingival tissues and the tooth, and leading to loss of the supporting bone (visible radiographically). The bone loss occurring in periodontal disease can, if left untreated, lead to tooth loss [74].

The prevalence of periodontal disease is extremely high, to the point that it is considered the most common chronic non-communicable disease in human beings [75]. The prevalence of severe periodontitis is estimated to be 11.2 % [76], while milder forms of disease are more common [77]. Furthermore, periodontitis may impact the general health negatively and has been linked (with varying degrees of evidence) to cardiovascular disease, type-2 diabetes, obesity, rheumatoid arthritis, respiratory infections and certain cancers, to name but a few [78,79].

Due to its extremely high prevalence, negative effects on quality of life and significant costs in dental care, the importance of understanding the underlying mechanisms that regulate the pathophysiology of periodontitis is critical.

The pathogenesis of periodontal disease engages a complex immune/inflammatory cascade that is initiated by the bacteria of the oral biofilm. The susceptibility to periodontitis appears to be determined by the host response; specifically, the magnitude of the inflammatory response and the differential activation of immune pathways [80].

Evidence suggests that human periodontal ligament (hPDL) cells may be involved in osteoclast formation via the RANK/RANKL/OPG pathway, because these cells stimulate osteoclastogenesis in vitro (despite low levels of RANKL expression) [78]. Furthermore, when

osteoprotegerin (OPG) expression increases, osteoclast formation is inhibited [81].

The Notch pathway appears to play a role in osteoclastogenesis and bone resorption related to periodontal tissue destruction. In hPDL cells, Jagged1 attenuates OPG expression, while both RANKL mRNA and protein expression increase slightly. Further, Jagged1 was found to significantly enhance osteoclast formation [82]. Taken together, this evidence appears to suggest that the Notch signaling mechanism may play a part in osteolytic processes taking place in periodontal disease.

The role of Notch signaling in alveolar bone resorptive mechanisms still remains to be clarified in detail, although scarce evidence in the literature has confirmed its role in periodontitis related bone loss.

In subjects with aggressive periodontitis [83], a significant increase in Notch2, TNF-α, IL-17 and RANKL was observed, while Notch1 and Jagged1 levels were decreased compared to controls. Notch2 and RANKL were also overexpressed in chronic periodontitis patients, although Notch1 expression levels did not differ compared to controls. The loss of alveolar bone observed in periodontitis cases is associated with the overexpression of Notch2 receptor, because Notch2 has been found to play a major role in pro-resorptive processes [11]. For example, Notch2 is involved in the suppression of osteoblastogenesis [84], while inducing the expression of RANK on osteoblasts which leads to bone resorption via activation of the RANK-RANKL cascade [6,85,86]. Increased levels of pro-inflammatory cytokines such as TNF-α are associated with periodontitis. The molecular crosstalk between TNF-α and RANKL contributes to increased alveolar bone resorption by increasing the expression of RANK on osteoblast precursors and RANKL expression in osteoblasts, as well as increasing production of osteoclasts directly in the bone marrow [87–89].

IL-17, another pro-inflammatory cytokine, was also found to be increased in aggressive periodontitis subjects. Similar to TNF- α, IL-17 also increases the expression of RANK in osteoclast precursor cells and increases the expression of RANKL in osteoclast-supporting cells such as osteoblasts. Thus, this pro-inflammatory cytokine also contributes to alveolar bone resorption in aggressive periodontitis [11,84].

Interestingly, Jagged1 and Notch1 levels were found to be lower in aggressive periodontitis cases (though not in chronic periodontitis) suggesting that the loss of expression of Notch1 may be cumulated with the pro-resorptive effects of Notch2, RANKL and pro-inflammatory cytokines in determining bone loss in these subjects. Additionally, Notch1 suppresses osteoclastogenesis, thereby protecting the bone, and its inhibition reduces osteoprotegerin levels [82]. Notch1 and Jagged1 levels in chronic periodontitis subjects were similar to those of control subjects, which suggests that there is a certain extent of balance between pro-resorptive and anti-resorptive signaling in these subjects [85]. Notch receptor expression was also found to correlate with clinical parameters commonly used to measure periodontal destruction and inflammation. In aggressive periodontitis patients, Notch2 levels were directly proportional to CAL, while an inverse relationship was found between OPG and CAL though OPG remained a borderline predictor ($p = 0.506$) for CAL. The direct relationship between Notch2, (a pro-resorptive factor) and CAL is understandable and suggests that Notch2 could contribute to the observed periodontal destruction in advanced cases. On the other hand, OPG is a well-established anti-resorptive factor acting as a decoy receptor and neutralizes the effects of RANKL by binding to RANK. With lower CAL values, observed OPG levels increased. This suggests that OPG's protective effects also correlate with a milder clinical presentation.

Furthermore, when considering both subjects with chronic and aggressive periodontitis, Notch1 expression acted as a borderline predictor of pocket probing depth (PPD). Notch1 acts as a promoter of bone synthesis, and it is hypothesized that an inverse correlation may exist between PPD and Notch1 expression in aggressive periodontitis [90].

7. Notch signaling pathway in the pathogenesis of alveolar bone resorption in peri-implantitis

Biological complications occurring around osseointegrated dental implants are related to microbiologically induced inflammatory response. They manifest in two entities: peri implant mucositis (PM) and peri-implantitis (PI) [91–93]. Both conditions feature an inflammatory lesion, although PI additionally involves loss of supporting bone. It is known that PM precedes PI but, conditions characterizing the conversion from PM to PI, have not been fully clarified [94]. Biological peri-implant complications, although bearing many similarities with periodontal diseases, demonstrate certain differences. PI demonstrates a faster, more aggressive, and a non-linear pattern of bone loss [95]. Due to its high prevalence in patients rehabilitated with dental implants [96,97], it represents one of the major topics and challenges of the contemporary dentistry.

Undeniable similarities in both etiopathogenesis and clinical features of periodontitis and peri-implant diseases [98] are observed mainly in dysbiotic biofilm formation and altered immune response to the biofilm resulting in a non-reversible progressive destruction of supporting tissues [99]. Additionally, since periodontitis and peri-implant conditions have numerous risk factors in common, the history of periodontal disease presents a risk factor for peri-implant diseases [100].

Etiopathogenetic mechanisms behind PM and PI have not been fully determined yet. As a result of a polymicrobial infection, the host's immune inflammatory reaction is activated represented by altered levels of proinflammatory cytokines. Cytokine activity via different signaling pathways contributes to bone loss that might ultimately result in dental implant loss [101]. IL-1β, IL-6, IL-10, IL-17 and TNF-α levels are found to be significantly higher in patients diagnosed peri-implantitis compared to healthy periodontium [102–104].

Notch signaling pathway is involved in skeletal development and bone remodeling processes, including bone regeneration and bone resorption, depending on the conditions [6,88,105]. Osseointegration presents a unique biological process, with complex underlying cellular and molecular mechanisms. In dental implant osseointegration processes, the signalling pathways associated with the increase of skeletogenesis-related gene expression are TGF-b/BMP, Wnt and Notch [106]. It has been shown that the osteogenesis process takes place mainly at 7 and 14 days of healing on different implant surfaces. An upstream activation of Notch1 was reported at 14 days on implant surfaces, demonstrating its role in bone formation around dental implants, aiding the process of osseointegration [107].

On the other hand, the impact of Notch signaling cascade on bone loss, its correlation to certain proinflammatory cytokines' levels, as well as alterations in clinical parameters, have been recently documented in apical periodontitis and periodontal disease [67,68,71,86,90].

The study conducted by Milinkovic and co-workers [108] analyzed clinical periodontal parameters, relative expression levels (REL) of Notch1, Notch2, Jagged1, Hes1, Hey1, TNF-α, IL-17, IL-1β, IL-6, RANKL and OPG mRNA by means of reverse transcriptase-real-time polymerase chain reaction (RT-qPCR), as well as quantity of Notch1, Il-17 and IL-6 protein levels by means of ELISA tests. The above-mentioned factors were assessed for three groups of patients: peri-implantitis (PI), peri-implant mucositis (PM) and healthy implants (HI) group.

All clinical parameters were, as expected, altered in PI group. Significantly higher gene expression levels of Hey1, TNF-α, IL-17, IL-1β, IL-6 and RANKL were found in this study, coupled by higher IL-17

and IL-6 protein levels in the PI, compared to the PM group. The Notch1 gene downstream in conjunction with Hey1 gene upstream was found in PI in contrast to HI, accompanied with a trend of lower Notch1 values at the protein level too. Similar findings were reported for Notch1 gene expression in PM vs. HI.

Regarding inflammation markers, there have been some reports in the literature. Similar data were presented in a recent meta-analysis [109], with higher IL-6 and TNF-α levels in peri-implantitis than in peri-implant mucositis sites. It might be assumed that the increase of IL-6, IL-1β and TNF-α from peri-implant mucositis to peri-implantitis lesions increases the pathological bone loss owing to their synergistic involvement in the osteoclast activation [110]. Opposing the previous research [104], IL-17 expression levels in this study showed significantly elevated levels in PI then in PM. Along with TNF-α, IL-17 induces RANKL expression, therefore indirectly stimulating bone loss [87,88].

Peri-implantitis appears be accompanied by higher levels of some crucial cytokines' such as TNF-α, IL-17, IL-6 and IL-1β, along with the RANKL up-regulation, as reported in this study. Such an increased cytokine level, together with the loss of the osteoprotective function of Notch1, might be responsible for the osteoclast over-activation and bone resorption in PI. This study suggests that expected clinical differences between PM and PI are accompanied by explicit differences at molecular level. Seven of the eleven analyzed molecules (Hey1, TNF-α, IL-17, IL-1β, IL-6 and RANKL) appeared as useful markers allowing the subtle molecular distinction between PI and PM. This study seems to be the first dealing with the potential role of Notch1 in peri-implant diseases, therefore, future studies are needed to confirm the present findings.

8. Conclusions

This work highlights the importance of the Notch signaling system in alveolar bone homeostasis. The Notch signaling components, along with complex network of different biomolecules (e.g. proinflammatory cytokines, oxidative stress parameters, etc.), are involved in pathological process of bone resorption in apical periodontitis, periodontitis, and peri-implantitis. In this regard, there is a considerable interest to control the activity of this pathway in the treatment of disorders associated with its dysregulation. 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. Therefore, further investigations are necessary to determine whether inhibition of the Notch signaling pathways might be beneficial and safe as a novel approach in the treatment of these pathological conditions.

Conflict of interest

None.

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