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Oral Pathology: Gene Expression in Odontogenic Cysts

Naida Hadziabdic and Amina Kurtovic-Kozaric

Abstract

Odontogenic cysts are a group of common pathological lesions of the jaw. Typically, they can be found randomly on X-rays as round benign lesions. However, some of them can behave aggressively with a tendency toward malignancy. Among odontogenic cysts with benign pathology, up to 60% of all jaw cysts are radicular cysts, which originate from root canal infection. Pathogenesis involves the interaction between osteoblasts, osteocytes, and osteoclasts as well as the expression of RANK-RANKL/OPG signaling system. Furthermore, collagenases (e.g., MMPs) are expressed in epithelial lining of the cyst. Among odontogenic cysts with potentially aggressive behavior, odontogenic keratocysts (OKCs) have a high rate of recurrence and very debatable treatment options; they can be associated with Gorlin syndrome. Keratocysts have developmental origin and show variability in their gene expression profiles. Their etiology is closely related to genetic factors, especially mutations in different members of Shh signaling pathway, including *PTCH* gene.

Keywords: odontogenic cysts, radicular cyst, keratocyst, RANK-RANKL/OPG, PTCH gene

1. Introduction

Odontogenic cysts are pathological cavities located in the jaw bones, filled with fluid surrounded by epithelial lining and fibrous connective tissue. Two most common odontogenic cysts with epithelial lining are inflammatory and developmental cysts (**Figure 1**). Among inflammatory cysts, the most characteristic one is the radicular cyst, which can be found in 60% of all odontogenic cysts. Radicular cyst originates from root canal infection and is of benign nature [1]. Cysts that originate from tissues involved in tooth development are called developmental cysts. Among them, the most interesting one is odontogenic keratocyst because of its unique and unexplored characteristics [2].

This chapter will deal with gene expression profiles of radicular cysts as the most common member of odontogenic cysts and keratocysts as the most debatable member of epithelial developmental cysts in order to uncover possible mechanism of pathogenesis of these two types of jaw cysts.
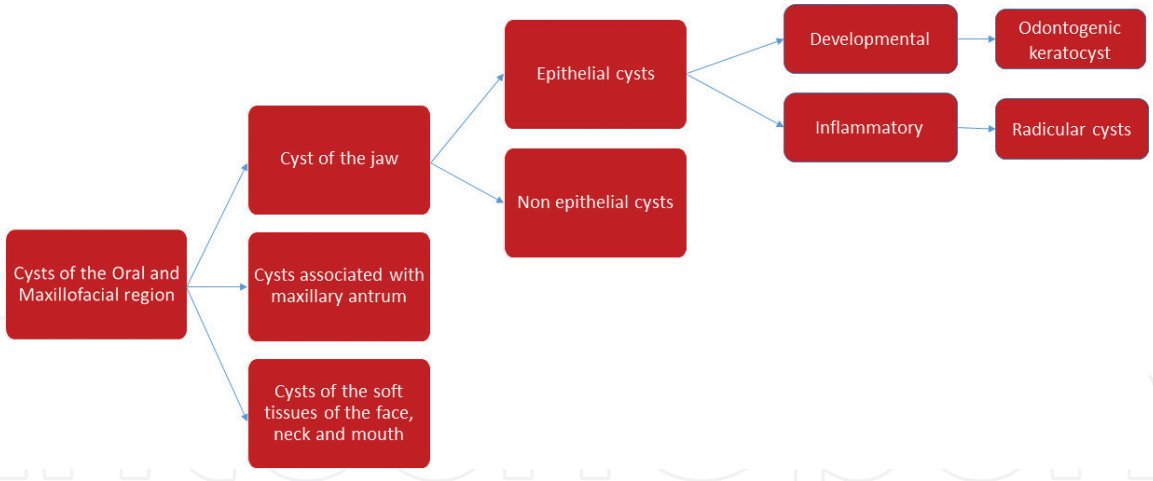
Figure 1.
Modified classification of cysts found in oral and maxillofacial regions [1].

2. Radicular cysts

2.1 Radicular and residual cysts

Radicular cysts are inflammatory cyst of jaw bones developing as a result of proliferation of the epithelium on the site of inflammation. They are the types of inflammatory lesions, which develop from epithelial residues of Hertwig lining in the process of periapical periodontitis after pulp necrosis. Cysts, which develop like this, are predominantly located around root apex and/or on the lateral side of the root, and in that case, they are called lateral radicular cysts. It often happens that radicular cyst remains in jaw bones after removal of the offending tooth, and in that case, it is called as residual cyst [1].

Radicular and residual cysts are by far the most common cystic lesions in the jaws, comprising 30% of all odontogenic cysts over a 30-year period according to results published by Jones et al. [3]. According to the age distribution of radicular cysts, most occur in patients who are in the third decade of life [3, 4]. There are many cases of patients who are between 40 and 60 years old, and after that, the number of cases decreases. Only few cases are seen in children even though dental caries commonly occurs in children.

Numerous studies have shown that radicular cysts occur more often in men than in women. The lower frequency in women may be because they take more care about mouth hygiene, especially the maxillary anterior incisors, where occurrence of cysts is very common. However, trauma to maxillary anterior teeth usually occurs to men [3].

Generally, radicular cysts show no symptoms and are discovered only after taking periapical radiographs of teeth with non-vital pulps. However, radicular cyst is the most common cause of swelling of the jaws, which patients often complain for. In the beginning, the enlargement is bony hard but as the cyst grows, the covering bone becomes very thin, and the swelling exhibits "egg shell crackling" or "springiness." The lesion becomes fluctuant only when the cyst has completely eroded the bone. In the mandible there is labial or buccal enlargement, rarely lingual, whereas in the maxilla, it is usually buccal or palatal [1].

Among other symptoms, pain and infection are very common in radicular cysts. Unless there is an infection, patients with radicular cyst usually feel no pain. But, it seems like there is no correlation between symptoms and infection. In the study done by Vier and Figueiredo, 21 out of 24 cysts were described as cavities which are filled with pus [5]. Authors did not correlate this finding and clinical symptoms, but it is unlikely that there is a relationship because most cysts are symptomless. In some cases patients complain of pain even though there is no evidence of infection and no

evidence of acute inflammation is present histologically after the cyst is removed [5]. On the other hand, some patients have histologically inflamed and clinically infected cysts, but they feel no pain [6]. A number of authors believe that there are cyst-prone individuals who are susceptible to developing radicular cysts because it often happens that more than one cyst is seen in one patient [7]. This can be supported and explained by the fact that occurrence of cysts is rare in relation to the large numbers of carious teeth which have dead pulps. Possibly, an immune mechanism inhibits formation of the cyst in most individuals, and patients who are prone to cyst development have a defect in suppression mechanism and immunological surveillance [8]. There is a possibility that some individuals are genetically susceptible to radicular cyst development.

Residual radicular cysts are cysts which remain after the non-vital offending tooth is removed. Studies have shown that they represent approximately 10% of all odontogenic cysts [9, 10]. In the study, Nair et al. considered that the type of cyst was important because of its persistence after treatment [11]. His findings confirmed the work of Simon [12] who stated that there were two types of radicular cyst: true radicular cyst, which contains a closed cavity lined by the epithelium, and the periapical pocket cyst in which the epithelium is attached to the margins of the apical foramen, so that the cyst lumen stays open to the root canal which is affected. Pocket cyst heals after treatment and tooth extraction, but true cyst, which is completely enclosed, is self-sustaining and persists even if there is no cause present [12].

2.2 Pathogenesis of radicular cysts

The pathogenesis of radicular cysts can be separated into three phases: the phase of initiation, the phase of cyst formation, and the phase of enlargement. Many studies have been done to investigate the mechanisms which are involved into these three phases.

2.2.1 The phase of initiation

Scientists agree that the epithelial cell rests of Malassez found in the periodontal ligament in periapical granulomas which are connected to necrotic and inflamed pulps are, in fact, the source of cyst linings [13]. The epithelial cell rests begin to multiply by inflammation which results from bacteria and debris discharged from the dead pulp. Bacterial endotoxins released from the necrotic pulp may be the key factor which initiates the inflammation and immune response and cause epithelial proliferation [1]. Meghji et al. investigated cyst fluids and grew cyst explants from radicular cysts and other cyst types and revealed that there are higher levels of endotoxins in radicular than in the other cysts [13].

Immunological studies are crucial in further understanding of granulomas and cysts, and they indicate that humoral and cell-mediated processes are involved in the pathogenesis of these lesions. Immunological studies have also demonstrated that inflammatory cytokines have an important role in the proliferation of epithelial cell rests. Stern et al. demonstrated that T lymphocyte infiltrates are involved in the development of periapical granulomas [1]. After a large number of studies, it became clear that endotoxins and inflammatory cytokines are highly involved in stimulation of the epithelial proliferation and they function as chemotactic and pro-inflammatory molecules as well [14, 15].

2.2.2 Phase of cyst formation

Pathogenesis of a radicular cyst is the next phase of cyst development, and it involves the process of cavity being lined by odontogenic epithelium. Two theories have been proposed, and both of them are reasonable and may function
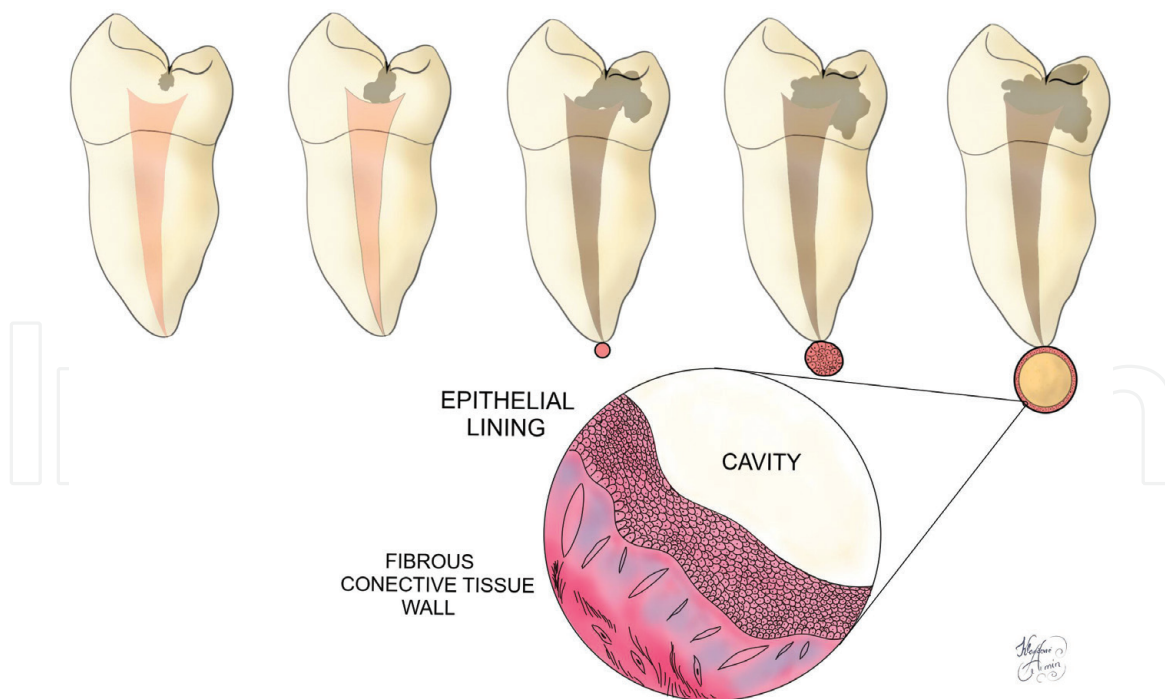
Figure 2.
Schematic representation of the development of radicular cyst. Starting from the left, superficial caries forms first, followed by medium and deep caries. Untreated deep caries leads to total pulp inflammation. Subsequently, necrosis of the pulp appears, which becomes infected. The resulting infection of the root canal initiates the epithelial remnants of Hertwig sheath (Malassez epithelial rests) to proliferate. Once the cells proliferate, the epithelial nest is formed. When the epithelial nest reaches the size of 1 cm, the center becomes necrotic leading to the formation of future cystic cavity, which becomes lined with the epithelium. For unknown reasons, this epithelium starts secreting fluid, which is called cystic fluid. These steps lead to the formation of radicular cyst, a round cavity filled with fluid and lined with the epithelium and fibrous connective tissue. This description of the radicular cyst development is the prevailing theory.

independently. The primarily accepted theory proposes that epithelial cells multiply and enclose the surface of connective tissue of an abscess cavity or cavity, which resulted from the breakdown of connective tissue by activity of proteolytic enzymes [16]. The secondary one, which is supported more, states that radicular cyst forms inside of the multiplying epithelial mass in periapical granuloma by cell death in the center (**Figure 2**) [16].

*2.2.3 Growth and enlargement of the radicular cyst*

The cyst enlargement is the final stage in radicular cyst pathogenesis. Toller's studies showed that osmosis contributes to the increase in the size of cysts [17]. Ward et al. proved this by simulating the growth of odontogenic cyst by mathematical modeling [18]. This modeling not only confirmed the results of Kubota et al. but also demonstrated that as the cyst became larger, cell proliferation played bigger part than osmotic pressure [19]. Harris and Toller suggested that cyst enlargement depends on epithelial proliferation which continues if inflammatory stimulus is present [20]. **Figure 3** shows large radicular cyst in the upper jaw.

2.3 Gene expression in radicular cysts

As described previously, among odontogenic cysts with benign pathology, up to 60% of all jaw cysts are radicular cysts, which originate from root canal infection caused by various microorganisms. The consequence of the radicular cyst development is the concomitant resorption of the surrounding bone tissues and periradicular periodontal ligament (PDL; [21]). Studies that have analyzed gene expression in radicular
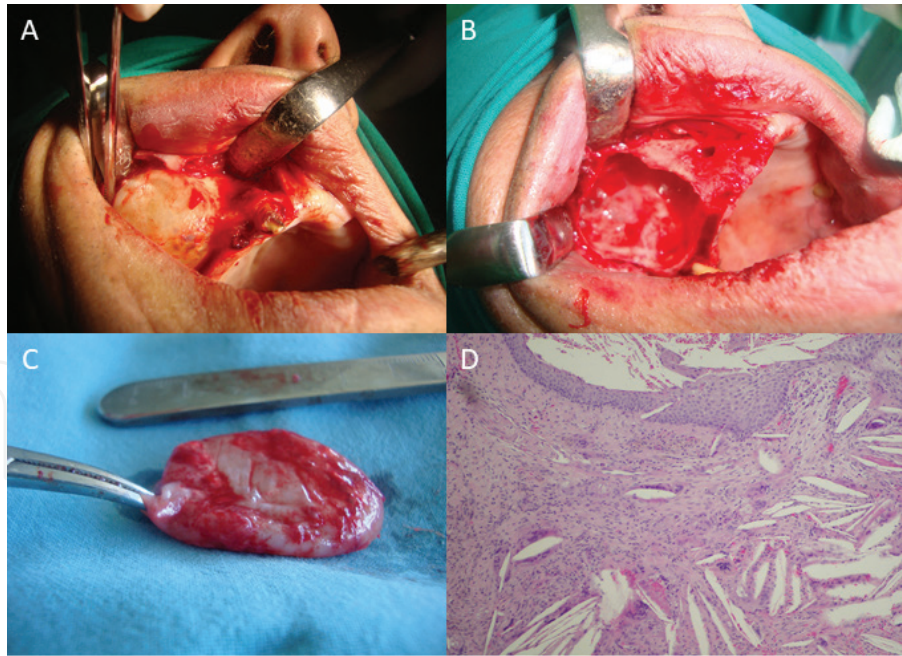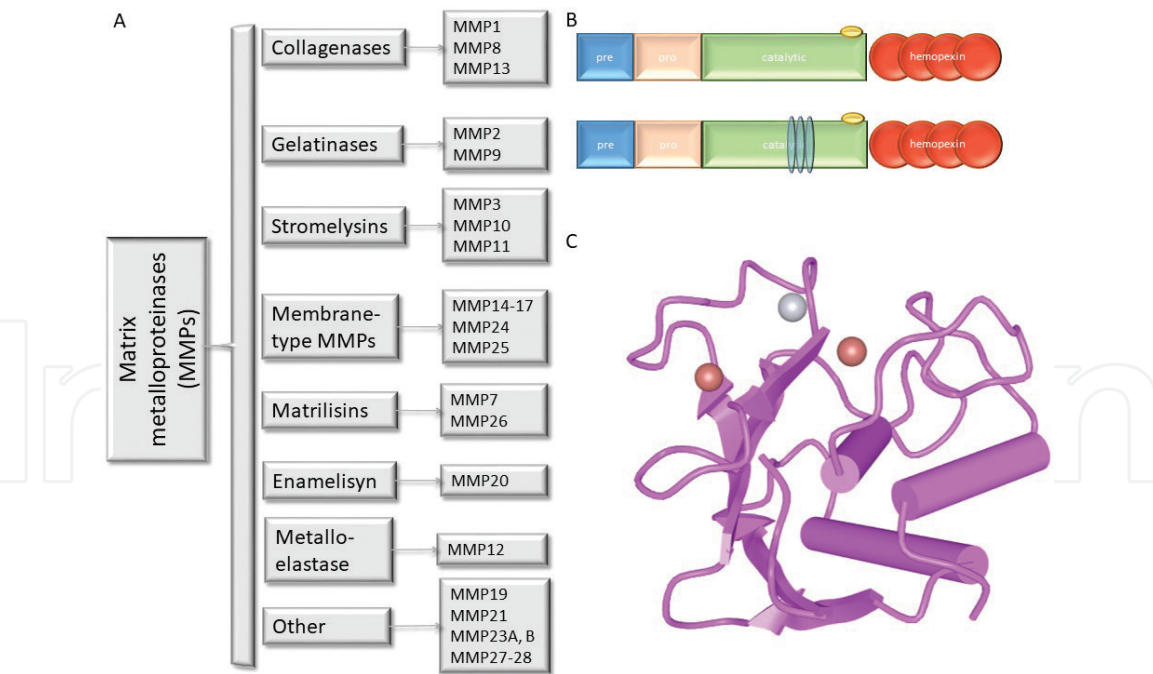
Figure 3.
Large radicular cyst located in the upper jaw on the right side. The appearance of the radicular cyst after mucoperiosteal flap was raised (A). Bone cavity after the cyst enucleation (B). The macroscopic view of the enucleated cyst (C). The histological morphology of radicular cyst with typical cholesterol crystals in the form of clefts, stained with H&E, 40× magnification (D).

cysts have mostly focused on genes that are involved in processes such as bone metabolism, inflammation, and tumorigenesis. Regarding bone metabolism, a gene known as receptor activator of nuclear factor-B ligand (RANKL) has been extensively studied because of its role in bone resorption around the tooth apex. This gene is part of a pathway that activates osteoclasts and is inhibited by a protein called osteoprotegerin (OPG). The role of RANK-RANKL-OPG signaling pathway in radicular cyst pathogenesis is further described in Section 2.3.2. Regarding genes involved in inflammatory processes, studies have analyzed expression of genes that code for chemokines and chemokine receptors that are involved in T helper type 1 (Th1) and Th2 responses that are characterized by the generation of interleukin-2 (IL-2), IL-12, and interferon-c (IFN-c) and by IL-4, IL-5, IL-6, IL-10, and IL-13, respectively [22, 23]. Regarding genes involved in tumorigenesis, *TP53* has been well analyzed in radicular cysts, where it shows low expression. Besides, *TP53*, *PCNA*, *FHIT*, and *Ki67* genes were analyzed and also showed insignificant changes compared to controls [24–26].

However, the most extensively studied genes in the pathogenesis of radicular cysts belong to the family of matrix metalloproteinases (MMPs). Their role in the cyst formation and development is described in Section 2.3.1.

2.3.1 Matrix metalloproteinases (MMPs)

The family of genes that are most commonly associated with the development of these lesions are matrix metalloproteinases (MMPs), which are metal-dependent endopeptidases that represent the major class of enzymes responsible for extracellular matrix degradation. It has been shown that MMPs have a crucial role in collagen degradation during periodontal tissue destruction [27–29]. Schematic representation of different classes of MMPs and an example of molecular structure is shown in **Figure 4**. Most commonly differentially expressed MMPs in oral diseases are presented in **Table 1**. For example, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, and MMP-13 play a role in the pathogenesis of periapical inflammatory lesions [27–31]. MMP-1 is a crucial enzyme in the initiation of osteoclastic bone

Figure 4.
Schematic classification, domain, and crystal structure of MMPs. Functional classification of MMPs is shown in A. MMPs are classified according to their function into collagenases, gelatinases, stromelysins, membrane-type MMPs, matrilysin, enamelin, metalloelastases, and others. Domain structure of collagenases (MMP-1, MMP-8, MMP-13) and gelatinases (MMP-2, MMP-9) is shown in B. The domain structure consists of signal peptide sequence (in blue color, labeled "pro"); prodomain that inhibits the catalytic domain, making it inaccessible to substrates (in pink color, labeled "pre"); catalytic domain that contains zinc atom (in green color, labeled "catalytic"); and hinge domain which links the catalytic and hemopexin domain (in red color, labeled "hemopexin"). Gelatinases also contain fibronectin-like domain repeats which aid in substrate binding (in blue color). Crystal structure of MMP-1 is shown in C.

resorption [32], because it degrades the collagen of the unmineralized layer found on the surface of the bone. After collagen degradation, the collagen fragments are produced, which function in osteoclast activation [33]. Studies in rats have shown that MMP-1 expression is elevated in the active phase of periapical lesion development [34]. MMP-1 also causes expansion of radicular cysts in humans [35].

MMP-2 and MMP-9 function in the degradation of the extracellular matrix (ECM), particularly during the active stages of lesion development [30], and their expression is increased in those lesions compared to control healthy tissue (gingiva, PDL, or oral mucosa).

MMPs play an important role in normal cellular processes such as tissue growth, bone resorption, and remodeling (wound healing and angiogenesis) [36]. MMPs are secreted in their proenzyme state and require extracellular activation. They are regulated by endogenously secreted inhibitors, called TIMPs (the tissue inhibitor of metalloproteinases). MMPs cleave native, nondenatured collagens with long uninterrupted triple helices and can function as collagenases (**Table 1**) [37]. Thus, the molecular basis of MMP function involves proteolytic cleavage of different substrates and subsequent activation of transforming growth factor-beta (TGF-beta), insulin-like growth factors (IGF), vascular endothelial growth factors (VEGF), and RANKL pathway.

Normal tissues generally show low expression of MMPs. However, during pathological states that require destruction of extracellular matrix, expression of MMPs can be drastically increased [29]. In normal and healthy tissues, components of extracellular matrix are in constant balance between degradation and protein synthesis. It has been shown that elevated MMP levels correlate with nonhealing [38, 39].

In pathological state such as apical periodontitis, MMP expression and secretion are increased, suggesting the direct role of MMPs in tissue remodeling and

Name of enzyme	Class	Substrate	Expression in normal tissue and oral disease
MMP-1 (collagenase-1, interstitial collagenase)	Collagenase	Collagen I, II, III, VII, VIII, and X and gelatin	Bone resorption in periapical lesions and human periapical lesions degrades nonmineralized extracellular matrix and stimulates osteoclastogenesis by collagen degradation on bone surface, expression in cystic wall and cystic fluid, pulpitis, squamocellular carcinoma, and normal tissues (stomach and gallbladder)
MMP-8 (collagenase-2, neutrophil collagenase)	Collagenase	Collagen I, II, III, V, VII, VIII, and X and gelatin	Chronic pulp inflammation, human periapical lesions, pulpitis, caries, and normal tissues (bone marrow and spleen)
MMP-13 (collagenase-3)	Collagenase	Collagen I, II, III, IV, V, VII, IX, and X	Chronic pulp inflammation, human periapical lesions, pulpitis, and normal tissue (vagina, lung)
MMP-9 (gelatinase B)	Gelatinase	Collagen III, IV, V, VII, X, and XI	Periodontitis, caries, oral squamocellular carcinoma metastasis, invasiveness and shorter survival, and human periapical lesions
MMP-2 (gelatinase A)	Gelatinase	Collagen I, II, III, IV, V, VII, X, and XI	Periodontitis, caries, oral squamocellular carcinoma metastasis, invasiveness and shorter survival, collagen type IV degradation, ameloblastoma, human periapical lesions, and normal tissue (gallbladder, uterine, endometrium, cervix)
MMP-3		Collagen III, IV, V, VII, IX, X, and XI	Oral squamocellular carcinoma, human periapical lesions, and normal tissue (endometrium, salivary gland)

Table 1.
List of MMPs most commonly associated with and expressed in oral disease and healthy tissues.

destruction during lesion development [40–42]. Regulation of their expression is primarily controlled at the transcriptional level even though regulatory mechanisms have still not been fully elucidated [27, 43, 44]. Some of the reasons include the influence of promoter polymorphisms, epigenetic mechanisms, and posttranscriptional processes [43, 45].

Promoter polymorphisms have been detected in MMP promoter regions, suggesting that the changes in MMP expression can predispose individuals to develop periapical inflammatory lesions. Similarly, these MMP promoter polymorphisms can lead to progression of disease [42, 46]. For example, Menezes-Silva et al. investigated genetic predisposition to periapical disease by testing 16 SNP polymorphisms in *MMP2*, *MMP3*, *MMP9*, *MMP13*, *MMP14*, and *TIMP2* genes [42]. They found that polymorphisms in *MMP2* and *MMP3* genes are associated with the development of periapical lesions, suggesting that these markers could assist in prevention and healing process [42].

Besides MMP promoter polymorphisms, another regulatory mechanism for MMP expression has been found at the epigenetic level, where methylation of MMP promoters can lead to gene inactivation and subsequent decrease in transcription [47]. Campos et al. have studied the methylation state of *MMP-2* and *MMP-9* in periapical inflammatory lesions [48]. Their results show that *MMP-2* gene was partially methylated in periapical granuloma, radicular cysts, and normal oral mucosa,

and subsequent association between methylation status and gene expression was not possible [48]. Regarding *MMP-9*, the study found that this gene was more unmethylated in periapical granulomas and radicular cysts than in healthy mucosa, which implies that *MMP-9* mRNA expression is increased and may be epigenetically controlled [48]. Effects of DNA methylation on MMP genes can contribute to individual susceptibility to the development of periapical granuloma and radicular cysts as periapical inflammatory lesions. Moreover, it may also play a role in the patient's response to therapy [48].

Besides MMP studies, other genes such as *FOXP3* have shown interesting results in periapical granulomas and radicular cysts. It has been shown that the *FOXP3* gene promoter methylation was inversely correlated with *FOXP3* transcript levels, suggesting that *FOXP3* may be crucial in determining periapical lesion development [49].

In conclusion, here we presented studies that suggest that genetic predisposition to frequent development of periapical inflammatory lesions could be caused by the presence of polymorphisms in MMP gene promoters or by epigenetic mechanisms such as differential methylation status of MMP genes.

2.3.2 RANKL expression

RANKL-RANK-OPG system discovery has changed our understanding of bone biology (**Figure 5**). This is a signaling system that is crucial for skeletal homeostasis because it maintains the balance between bone resorption by osteoclasts and bone formation by osteoblasts. RANKL is a ligand for its receptor RANK which can be found on osteoclast progenitor cells. After binding, RANK-RANKL system activates NF-κB pathway and upregulation of NFATc1 protein, which is a master regulator of cytokines important for osteoclastogenesis. The disruption of RANKL-RANK system leads to the inhibition of bone resorption. RANKL belongs to the TNF superfamily of proteins. The name RANKL stands for receptor activator of nuclear factor-κB ligand, also known as osteoprotegerin ligand (OPGL), osteoclast differentiation factor (ODF), TNF-related activation-induced cytokine (TRANCE), and TNF ligand superfamily member 11 (TNFSF11) [50, 51]. It is a homotrimeric protein that is membrane bound on osteoblasts and activated T cells. It can also be
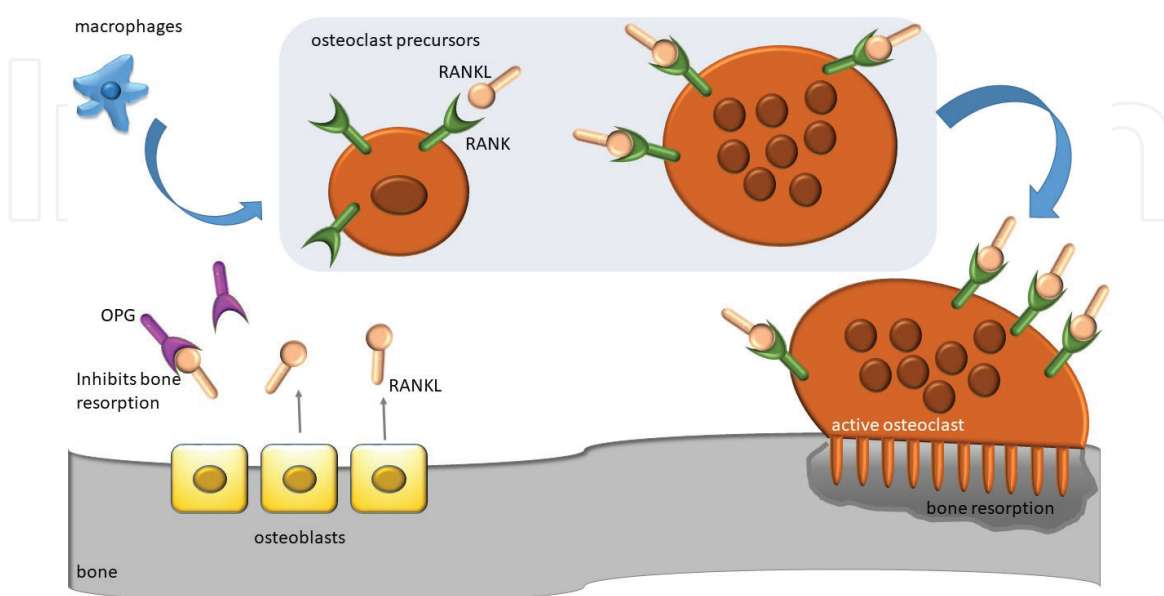
Figure 5.
RANKL-RANK-OPG system as regulators of bone resorption. Bone metabolism is a dynamic process that balances bone formation and bone resorption. RANKL (ligand) is secreted by osteoblasts and binds to the RANK receptor on osteoclast precursors and activated osteoclasts, which in turn result in bone resorption. The inhibition of this process is mediated by OPG.

secreted by T cells. RANKL is proteolytically cleaved by MMP-3 or MMP-7. Besides T cells and osteoclasts, RANKL expression can be seen in lymph nodes, thymus, mammary glands, spleen, and bone marrow. In tumor cells, RANKL is associated with migration and bone metastasis. Thus, RANKL is a key regulator of bone metabolism, specifically a regulator of osteoclastogenesis and osteoclastoactivation in a normal and pathological states [50, 51]. RANK is a receptor for RANKL and also a member of TNF superfamily. It is a homotrimeric transmembrane protein. Its expression is generally less than RANKL, and high expression is seen in mammary glands and cancer cells. OPG is an inhibitor of RANK-RANKL system. It stands for osteoprotegerin, also known as osteoclastogenesis inhibitory factor (OCIF) or tumor necrosis factor receptor superfamily member 11B, is a cytokine receptor of the TNF receptor superfamily encoded by the *TNFRSF11B* gene. It is a 380 amino acid glycoprotein that is found in soluble form as either monomer or dimer. The OPG dimer is crucial for RANK-RANKL inhibition because OPG dimer increases the affinity of OPG for RANKL.

RANKL affects the development of the periapical inflammatory lesions by activating osteoclasts, thus inducing pathological bone resorption [52]. RANKL protein expression was first shown in radicular cysts [53], which colocalized with osteoclasts. Subsequent studies analyzed transcript levels in inflammatory granulomas, which were increased compared to healthy PDL tissue [52]. RANKL expression was found in infiltrating leucocytes, specifically monocytes and dendritic cells, which were shown to be the main cells that secrete this protein. Another study compared RANKL and OPG levels in apical granulomas and radicular cysts [54], finding that both OPG and RANKL expressions were higher in granulomas than in cysts, but their ratio was comparable in these two types of periapical inflammatory lesions. Fukada and colleagues found that RANKL transcript levels were significantly higher in granuloma than in radicular cysts [55]. At the protein level, no difference was observed in RANKL and OPG levels in a study conducted by Fan and colleagues [56]. In a study on endodontically involved disease, RANKL expression was higher in lesions with more intense inflammation, but the ratio RANKL/OPG in relation to inflammation was not increased [57].

3. Odontogenic keratocysts

Odontogenic keratocysts (OKCs) represent a rare form of odontogenic cysts which originate from dental lamina remnants or eventually from the basal layer of upper and lower jaw oral epithelium before the odontogenesis ended. Since it was first described in 1876, this form of cysts grabs scientific attention mostly because of its developmental variabilities, histological appearance, and genetic basis [2, 58]. In the past few years, the World Health Organization (WHO) made an attempt to create more appropriate classification of these cysts. Recently, they were considered as keratocystic odontogenic tumors for their aggressive behavior, high mitotic rate, and association with genetic and chromosomal abnormalities. The newest WHO classification reclassifies them again as odontogenic keratocysts because *PTCH1* gene mutations were detected, similarly to other developmental cysts such as dentigerous cyst [59–61]. Despite many classifications, pathologists and surgeons face difficulties in the establishment of proper diagnosis. This is because keratocysts cannot be clinically and radiographically distinguished from other odontogenic cysts. Moreover, it is still debatable what the optimal therapeutic approach is in the treatment of keratocysts in order to prevent recidive, which is a characteristic of this disease [2].

Odontogenic keratocyst is histologically characterized with stratified squamous epithelium, which is five to eight layers thick with palisaded hyperchromatic basal cell layer and "corrugated" parakeratotic epithelial cells on luminal surface [2, 62, 63].
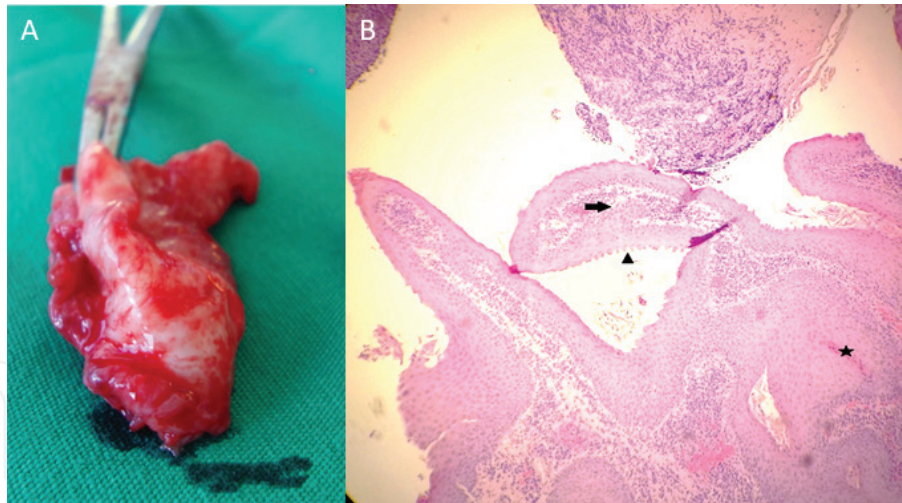
Figure 6.
Macroscopic view (A) and histological morphology (B) of odontogenic keratocyst. Inflamed fibrous wall lined with nonspecific stratified squamous epithelium (arrow) with focally corrugated surface (arrow head). The presence of satellite cysts and solid islands in the cystic wall is marked with a star.

Parakeratinization of the surface layer is one of the histological features that predicts recurrence together with the higher level of cell proliferative activity in the epithelium, binding in the basal layer of the epithelium, supraepithelial split of the epithelial lining, presence of daughter cells or also called satellite cells (**Figure 6**) [64].

Considering clinical and histological features together with unclear etiology and therapeutic strategy odontogenic keratocyst still remains debatable. Controversies in nature of these lesions are reflection of limited knowledge about their origin. Recent molecular and genetic discoveries try to elucidate these pathological entities and thus approach closer to better therapy modalities.

3.1 Molecular basis of odontogenic keratocysts

Odontogenic keratocysts are locally aggressive and have a tendency to recur over time. Keratocysts can happen sporadically or in conjunction with Gorlin-Goltz syndrome (Gorlin syndrome, OMIM 109400) [62, 64]. Gorlin syndrome is an autosomally inherited disease with a germline mutation in *PTCH* gene, located on chromosome 9q22. The incidence is 1:60,000 newborns. Patients with this syndrome present with a spectrum of developmental abnormalities which affect skin, nervous system, eyes, endocrine system, and bones. Skin abnormalities include basal cell carcinoma, benign dermoid cysts and tumors, palmar and plantar keratosis, and dermal calcinosis. Dental and bone abnormalities include multiple keratocysts, mild mandibular prognathism, kyphoscoliosis, and other vertebral defects like bifurcation of ribs, spina bifida, and others. Eye abnormalities include hypertelorism, wide nasal bridge, congenital blindness and strabismus. Neurological abnormalities include mental retardation, calcification of dura mater, and others. For the diagnosis of Gorlin syndrome, two major or one major and two minor criteria are needed [62, 64, 65].

The major criteria are as follows: multiple basal cell carcinoma or one tumor diagnosed before 20 years of age, histologically proven odontogenic keratocyst, three or more palmar or plantar pits, bilamellar calcification of the falx cerebri, fused or markedly splayed ribs, and first-degree relatives with Gorlin syndrome. The minor criteria include macrocephaly; congenital cleft lip or palate, frontal bossing, coarse face, or hypertelorism; other skeletal abnormalities; radiological deformities like bridging of the sella turcica, vertebral abnormalities, and ovarian fibroma; and medulloblastoma.
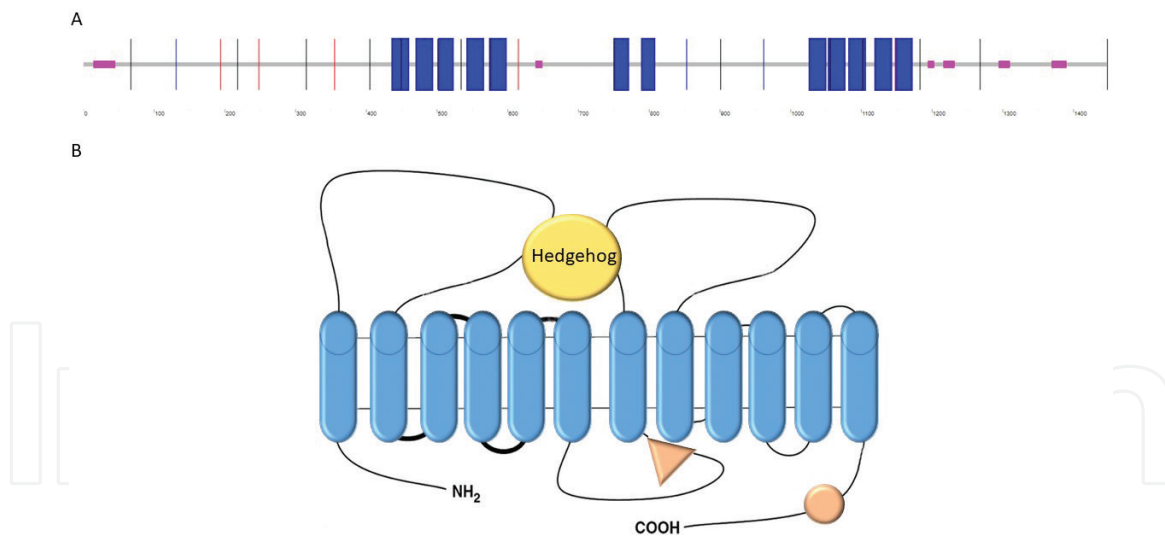
Figure 7.
Schematic representation of PTCH molecular structure. (A) Domain structure of PTCH protein with 12 transmembrane domains (blue) and low complexity regions (pink). It contains 23 coding exons spanning 74 kb and encoding a 1447 amino acid receptor glycoprotein. (B) PTCH is a membrane-bound receptor for hedgehog protein (yellow). PTCH1 gene produces patched protein, which is a homolog of Drosophila segment polarity gene. This receptor is a part of the hedgehog signaling cascade, which include downstream proteins such as smoothened (Smo) and disheveled (Dsh).

The appearance of keratocysts in Gorlin syndrome patients can be seen in up to 92% of all patients. Even though it has been shown that the syndromic form of keratocysts contains much higher numbers of proliferating epithelial cells and satellite cysts within the cystic wall, pathological examination cannot differentiate between syndromic and sporadic forms of this disease. Similarly, it has been shown that the syndromic form has higher rate of recurrence than the sporadic form. However, the syndromic form has shown more aggressive behavior than the sporadic form. Thus, radiographic appearance of multiple keratocyst formations should raise suspicion of a possible Gorlin syndrome [63].

PTCH1 gene produces Patched protein, which is a homolog of *Drosophila* segment polarity gene. It contains 23 coding exons spanning 74 kb and encoding a 1447 amino acid receptor glycoprotein. This receptor is a part of the Hedgehog signaling cascade, which includes downstream proteins such as Smoothened (Smo) and Disheveled (Dsh). Molecular structure including schematic domain representation is shown in **Figure 7**.

PTCH is a tumor suppressor and as such requires both gene copies to be mutated in tumors. Thus, patients with Gorlin syndrome already have one inherited mutation and can acquire the second mutation easier than patients with sporadic form. It has been shown that mutations in this gene often occur as LOH (loss of heterozygosity), meaning that both copies are mutated through different genetic mechanisms such as deletions, mutations, gene silencing, and others. After PTCH mutation, keratocysts can acquire additional genetic alterations which accelerate tumor development. When LOH was analyzed in sporadic form, several additional tumor suppressor genes were affected such as *TP53*. The association between these mutations and appearance of satellite microcysts in the cystic wall is particularly interesting [64].

3.2 Gene expression in odontogenic keratocysts

The hallmark of odontogenic keratocysts is mutation in *PTCH* gene, which is a receptor in Sonic hedgehog signaling (Shh) pathway. This pathway is important for proper differentiation of embryonic cells, and mutations in this pathway lead

to abnormal cellular proliferation and differentiation, which is strongly associated with the development of certain tumors [66, 67]. This section will describe genes that are involved in pathogenesis of odontogenic keratocysts through Sonic hedgehog pathway (Shh). The members of this pathway include secreted SHH ligand, which binds to its receptor *PTCH*, a 12-pass transmembrane protein, as described previously (**Figure** 7). In the absence of SHH ligand, PTCH inhibits a transmembrane protein called Smoothened (SMO).

In odontogenic keratocysts, *PTCH* mutations include LOH, deletions, point mutations, and others. The consequence of *PTCH* mutations are the constitutive activation of Shh pathway. When *PTCH* is mutated and inactivated, it is expected that SMO would be disinhibited and thus activated. This is exactly what is found in odontogenic keratocysts, where Smo overexpression has been shown by transcriptional and immunohistochemical studies [67–69]. Besides overexpression of SMO and PTCH, other downstream genes such as *GLI1*, *CCND1*, and *BCL-2* have been shown to be overexpressed in odontogenic keratocysts, indicating that these SHH pathway genes contribute to the development of these lesions.

Another downstream target of Shh signaling is a transcription factor SOX-2 [70] that is expressed in progenitor cells in epithelial tissues. SOX-2 expression is associated with elements of tooth development, especially in the region of the third molars in the lower jaw, which is the place where OKCs are usually located [71, 72].

Besides studies on Shh pathway, a list of genes that are upregulated in odontogenic keratocysts is shown in **Table 2**. Bioinformatic analysis has shown that other genes, such as *TP53* and *PCNA*, appear as the leaders and initiators of gene expression that is important for the development of odontogenic keratocysts [61, 73, 74]. Their analysis has shown that genes related to cell cycle and apoptosis are often dysregulated in these cysts, implying the recurrence of these cysts. Studies have shown that *TP53*, *PCNA*, p63, and Ki-67 expression is higher in keratocysts than in other types of odontogenic cysts. *TP53* is a tumor suppressor gene with several different functions in the cell including apoptosis, cell cycle arrest, and DNA repair.

The second gene that is found to be associated with odontogenic keratocysts is proliferating cell nuclear antigen (*PCNA*), which encodes a protein located in the nucleus and associated with DNA polymerase delta. It acts as a homotrimer and is

Gene name	Method of detection	References
PCNA	IHC	[74]
CCND1	IHC and real-time PCR	[67]
IL-6	IHC	[75]
VEGFA	IHC	[76]
BCL2	IHC, real-time PCR, Western blot	[77]
FHIT	IHC	[25]
GLI1	IHC	[78]
TP63	IHC	[76]
KRT6B	IHC	[79]
TP53	Bioinformatic analysis	[73]
SMO	IHC and transcriptional analysis	[67–69]

IHC, immunohistochemistry; PCR, polymerase chain reaction.

Table 2.
Genes that show high expression in odontogenic keratocysts, detected either at the RNA or protein level (modified from [63]).

implicated in the leading strand synthesis during DNA replication. DNA damage induces ubiquitination of this protein through RAD6-dependent DNA repair pathway. Expression of both *TP53* and *PCNA* in different odontogenic lesions was higher in the suprabasal layer of keratocysts than in radicular cyst. Overexpression of *PCNA* in the suprabasal layer implies its neoplastic nature and a tendency toward recurrence.

4. Conclusion

This chapter summarizes gene expression profiles of radicular cysts as the most common member of odontogenic cysts and keratocysts, a specific entity of epithelial developmental cysts, in order to uncover possible mechanism of pathogenesis that would help in the timely diagnosis and discovery of novel therapeutic options for these two types of jaw cysts. Pathogenesis of radicular cysts is associated with differential expression of genes involved in bone metabolism (RANK-RANKL-OPG pathway) and inflammation (chemokines and their receptors). However, the most extensively studied genes in the pathogenesis of radicular cysts belong to the family of matrix metalloproteinases (MMPs), which show increased expression.

Specific entities of odontogenic cysts are odontogenic keratocysts, which are prone to recidive. This trait of keratocysts to recur makes them similar to tumors, which can be also seen in their gene expression profiles. The hallmark of odontogenic keratocysts is mutation in *PTCH* gene, which is a receptor in Sonic hedgehog signaling (Shh) pathway. Mutations in PTCH gene lead to the constitutive activation of this pathway. Besides overexpression of PTCH, other downstream genes such as *SMO*, *GLI1*, *CCND1*, and *BCL-2* have been shown to be overexpressed in odontogenic keratocysts, indicating that these SHH pathway genes contribute to the development of these lesions.

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Conflict of interest

Authors declare no conflict of interest.

Author details

Naida Hadziabdic[1]* and Amina Kurtovic-Kozaric[2]

1 Department of Oral Surgery, Faculty of Dental Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

2 Department of Pathology, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina

*Address all correspondence to: nsulejma@yahoo.com

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