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HEAD AND NECK

Immunohistochemical analysis of MMP-13 and EMMPRIN in epithelial odontogenic lesions

Pedro Paulo de Andrade Santos[1] · Cassiano Francisco Weege Nonaka[2] · Carlos Augusto Galvão Barboza[1] · Leão Pereira Pinto[3] · Lélia Batista de Souza[3]

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Abstract

Purpose To investigate the contribution of MMP-13 in tumor aggressiveness, by acting on the reorganization of the extracellular matrix, regulating the biological activity of cytokines in odontogenic epithelial lesions, as well as to evaluate the role of EMMPRIN as an inducer of MMP-13.

Methods Twenty solid ameloblastomas (SAs), 10 unicystic ameloblastomas (UAs), 20 odontogenic keratocysts (OKCs), and 20 adenomatoid odontogenic tumors (OATs) were selected. The expression of MMP-13 and EMMPRIN was evaluated in epithelial/connective tissue by determining the score of immunoreactive cells.

Results Higher concentration of MMP-13 was observed in epithelium of SAs and OKCs ($p=0.316$), while in connective, MMP-13 was more expressed in OKCs and UAs ($p=0.213$). OKCs exhibited the highest immunoreactivity score for EMMPRIN in the epithelium ($p=0.091$). In connective tissue, a larger number of immunoreactive cells were observed in OKCs and UACs ($p=0.357$). There was a moderate correlation ($r=0.343/p=0.004$) between MMP-13/EMMPRIN in epithelium and strong correlation ($r=0.474/p<0.001$) in connective tissue.

Conclusion We suggest that the OKCs, SAs and UAs presented greater immunoexpression for MMP-13 and EMMPRIN, since they were lesions of more aggressive behavior, with smaller expressions in the AOTs that are admittedly indolent. However, we did not find a statistically significant difference between the expression of MMP-13 and EMMPRIN in lesions studied. The positive correlation found between MMP-13 and EMMPRIN in the epithelial and connective tissue of odontogenic lesions analyzed, seems to be related to the role of EMMPRIN as an inducer of MMP-13 expression.

Keywords Ameloblastoma · Adenomatoid odontogenic tumor · Odontogenic keratocyst · MMP-13 · EMMPRIN · Immunohistochemistry

Introduction

Odontogenic tumors and cysts are a diverse group of lesions derived from tooth-forming tissues that develop during abnormal odontogenesis [1]. Most odontogenic tumors are benign and easily treated by enucleation and curettage. However, there are more aggressive benign odontogenic lesions as solid ameloblastomas (SAs), unicystic ameloblastomas (UAs) and odontogenic keratocysts (OKCs) that, like malignant lesions as metastasizing ameloblastoma, ameloblastic carcinoma and clear cell odontogenic carcinoma, require less conservative treatment. Histogenetically, odontogenic tumors are classified into epithelial, mesenchymal and mixed tumors [2, 3]. The formation of either inflammatory or developing odontogenic cysts depends on an epithelial source, a stimulus of epithelial proliferation, the capacity of bone resorption, and growth. The processes involved in the development of inflammatory cysts are well established, while the mechanisms underlying the formation of developing cysts are unclear. Several theories associated with proliferation have been suggested, including processes of aberrant development and genetic anomalies [4].

✉ Lélia Batista de Souza
leliabsouza@gmail.com

[1] Department of Morphology, Federal University of Rio Grande do Norte, Natal, RN, Brazil

[2] Department of Dentistry, State University of Paraíba, Campina Grande, PB, Brazil

[3] Department of Oral Pathology, Federal University of Rio Grande do Norte, Av. Senador Salgado Filho, 1787, Lagoa Nova, Natal, RN CEP 59056-000, Brazil

Matrix metalloproteinase (MMP)-13, also known as collagenase 3, can cleave a variety of substrates, including fibrillar collagens and other extracellular matrix components, and is stimulated by transforming growth factor β3 (TGF-β3) [5]. The gene expression of MMP-13 is induced in different cell types found in connective tissue that can be either resident or transient, such as chondrocytes, endothelial cells, osteoblasts and fibroblasts. This induction occurs through inflammatory mediators, including cytokines, prostaglandins and bacterial lipopolysaccharides [6]. MMP-13 promotes the bioavailability of growth factors that interfere with cell survival and differentiation [7].

EMMPRIN is a highly glycosylated type I transmembrane protein of the immunoglobulin superfamily that shows wide tissue distribution and is expressed by different cells in normal, inflammatory or neoplastic tissues [8]. This glycoprotein mediates heterotypic cell–cell interactions such as the interplays between tumor cells and fibroblasts and between tumor cells and endothelial cells, as well as homotypic interactions such as the relationships between the same population of tumor cells [9]. In neoplastic processes, EMMPRIN expressed by tumor cells acts as an inducer of MMPs, stimulating peritumoral fibroblasts to produce high levels of these proteinases which, in turn, degrade the extracellular matrix and facilitate tumor invasion [10]. EMMPRIN does not only activate MMP genes, but can also mediate the secretion or activation of preexisting MMP proteins. The exact mechanism whereby EMMPRIN induces the expression of MMP genes in still not clear. However, studies have demonstrated the interaction of EMMPRIN with some molecules such as integrins α3β1 and α6β1, lactate transporters (MCT1 and MCT4), cyclophilin A and B, and caveolin-1 [11].

In view of the multi-functional role of MMPs in both physiological and pathological mechanisms [12] and of their strong correlation with EMMPRIN in extracellular matrix degradation and tumor invasion [10, 13], the objective of the present study was to evaluate the possible relationship between MMP-13 and EMMPRIN in selected epithelial odontogenic lesions of recognized aggressiveness that are the OKCs, SAs and UAs, besides odontogenic lesions of indolent behavior that are the AOTs by analyzing the immunohistochemical expression of these proteins to understand how this correlation may interfere with the biological behavior of the lesions studied.

Materials and methods

Seventy specimens, including 20 solid ameloblastomas (SAs), 10 unicystic ameloblastomas (UAs), 20 odontogenic keratocysts (OKCs), and 20 adenomatoid odontogenic tumors (AOTs), obtained from the Oral Pathology Department of the Federal University of Rio Grande do Norte (UFRN), were selected for this study according to the diagnosis. The pathological diagnosis of UA was confirmed based on the clinical, radiographic, macroscopic, and microscopic criteria described by Philipsen and Reichart [14]. The study was approved by the Research Ethics Committee of UFRN, Natal, Brazil (Protocol no. 354/2010).

Immunohistochemistry

Sections (3 µm) were cut from paraffin-embedded tissue blocks. The tissue sections were deparaffinized and immersed in 3% hydrogen peroxide to block endogenous peroxidase activity. The sections were then washed in phosphate-buffered saline (PBS). Antigen retrieval, antibody dilution, and clone type for MMP-13 (Santa Cruz Biotechnology; Dallas, Texas, USA) and EMMPRIN (Invitrogen; Camarillo, California, USA) are shown in Table 1. After treatment with normal serum, the sections were incubated with the primary antibodies in a moist chamber. The sections were washed twice in PBS and treated at room temperature with a polymer-based complex (Advance™ HRP; Dako) for anti-MMP-13 and anti-EMMPRIN antibodies. Peroxidase activity was developed by immersing the tissue sections in diaminobenzidine (Liquid DAB+ Substrate; Dako), which resulted in a brown reaction product. Finally, the sections were counterstained with Mayer's hematoxylin and coverslipped.

According to the manufacturer's specifications (Santa Cruz), validation of MMP-13 (sc-80200), routinely undergoes Western Blot (WB) analysis on membrane-enriched extracts of HeLa cells, MCF-7 cells, zebrafish tissue, MDA-MB-453 s cells. Using WB technique with lysates/

Table 1 Catalog number, specificity, clone, company, type, species, dilution, antigen retrieval and incubation of the primary antibodies

Catalog no.	Specificity	Clone/PAD	Manufacturer	Type	Species	Dilution	Antigen retrieval	Incubation (min)
Sc-80200	MMP-13	72B-01	Santa Cruz Biotechnology	Monoclonal	Mouse	1:250	Citrate, pH 6.0 Microwave, 10 min	60
34-5600	EMMPRIN	ZMD.182	Invitrogen	Polyclonal	Rabbit	1:400	Citrate, pH 6.0 Pascal, 3 min	60

proteins at 10 µl per lane, the predicted band size is 48 kDa and observed band size of 48 kDa. As expected, a band corresponding to MMP-13 was detected in all four of the lysates by Western Blot using anti-MMP-13 antibody sc-80200.

According to Invitrogen (Thermo Fisher) for validation of the EMMPRIN (CD-147) primary antibody is routinely performed Western blot analysis on membrane-enriched extracts of SH-SY5Y (lane 1), U-87 MG (lane 2) and HeLa (lane 3).

Immunohistochemical analysis

The immunoexpression of MMP-13 and EMMPRIN was evaluated in epithelial and connective tissue cells of all epithelial odontogenic lesions. Tissue sections were examined by light microscopy to identify ten fields (five in epithelial tissue and five in connective tissue) that contained the largest number of immunostained cells. The number of positive and negative cells was determined in each field at 400× magnification permitting the calculation of the percentage of MMP-13- and EMMPRIN-positive cells in each case. Using this percentage, the following scores were assigned to each sample, adapted from that proposed by Nonaka et al. [15]: 0 (≤ 10% immunostained cells), 1 (11–25% immunostained cells), 2 (26–50% immunostained cells), 3 (51–75% immunostained cells), and 4 (> 75% immunostained cells).

Statistical analysis

The results were submitted to statistical analysis using the Statistical Package for the Social Sciences 17.0 (SPSS, Inc. Chicago, IL, USA). The nonparametric Kruskal–Wallis test was applied to compare the percentage of MMP-13 and EMMPRIN immunopositive cells between SAs, OKCs, UAs, and AOTs. Spearman's correlation test was used to determine possible correlations between the percentage of MMP-13 and EMMPRIN immunopositive cells. A level of significance of 5% ($p < 0.05$) was adopted for all tests.

Results

Analysis of the immunoexpression of MMP-13 in the epithelial component showed expression in all SAs, OKCs, UAs, and AOTs (Fig. 1a–d). Epithelial expression was higher in SAs (Table 2), but the nonparametric Kruskal–Wallis test (Table 3) revealed no statistically significant differences between groups ($p = 0.316$). In connective tissue, MMP-13 was expressed in all groups (Fig. 1a–d). Higher expression of MMP-13 was seen in OKCs and UAs (Table 2), but the difference was not significant ($p = 0.213$) (Table 4).

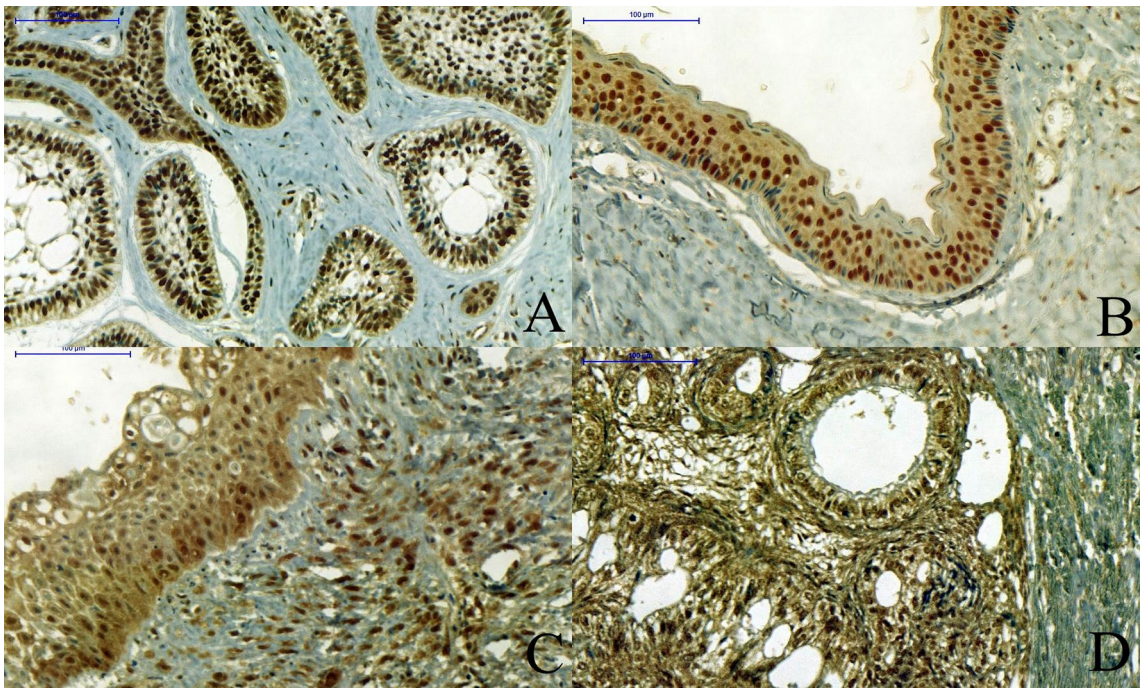

Fig. 1 Immunohistochemical reactivity for MMP-13 in epithelial and connective tissues: **a** solid ameloblastoma; **b** odontogenic keratocyst; **c** unicystic ameloblastoma; **d** adenomatoid odontogenic tumor (ADVANCE, Pannoramic Viewer 100 µm)

Table 2 Absolute and percentage values of MMP-13 and EMMPRIN immunoexpression, according to the scores used for analysis in the epithelial and connective component of solid ameloblastomas (SAs), odontogenic keratocysts (OKCs), unicystic ameloblastomas (UAs), and adenomatoid odontogenic tumors (AOTs)

Groups	Epithelial tissue					Connective tissue				
	Scores					Scores				
	0	1	2	3	4	0	1	2	3	4
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
MMP-13										
SA	2 (10)	2 (10)	0 (0)	6 (30)	10 (50)	11 (55)	0 (0)	3 (15)	6 (30)	0 (0)
OKC	0 (0)	2 (10)	0 (0)	12 (60)	6 (30)	2 (10)	4 (20)	9 (45)	4 (20)	1 (5)
UA	0 (0)	0 (0)	5 (50)	3 (30)	2 (20)	3 (30)	1 (10)	2 (20)	2 (20)	2 (20)
AOT	0 (0)	0 (0)	2 (10)	12 (60)	6 (30)	2 (10)	12 (60)	3 (15)	3 (15)	0 (0)
EMMPRIN										
SA	0 (0)	0 (0)	1 (5)	11 (55)	8 (40)	1 (5)	10 (50)	6 (30)	3 (15)	0 (0)
OKC	0 (0)	0 (0)	2 (10)	7 (35)	11 (55)	3 (15)	7 (35)	7 (35)	2 (10)	1 (5)
UA	0 (0)	0 (0)	1 (10)	8 (80)	1 (10)	0 (0)	5 (50)	2 (20)	1 (10)	2 (20)
AOT	0 (0)	0 (0)	2 (10)	14 (70)	4 (20)	4 (20)	10 (50)	4 (20)	1 (5)	1 (5)

Table 3 Parameters used for the calculation of the Kruskal–Wallis (KW) test for the evaluation of the scores of the immunoexpression of MMP-13 and EMMPRIN in the epithelial tissue of solid ameloblastomas, odontogenic keratocysts, unicystic ameloblastomas, and adenomatoid odontogenic tumors

Group	*n*	Median	Q_{25}–Q_{75}	Mean of ranks	KW	*p*
MMP-13						
Solid ameloblastoma	20	3.50	3.00–4.00	38.85	134.00	0.316
Odontogenic keratocyst	20	3.00	3.00–4.00	36.00		
Unicystic ameloblastoma	10	2.50	2.00–3.25	25.70		
Adenomatoid odontogenic tumor	20	3.00	3.00–4.00	36.55		
EMMPRIN						
Solid ameloblastoma	20	3.00	3.00–4.00	38.15	6.470	0.091
Odontogenic keratocyst	20	4.00	3.00–4.00	41.80		
Unicystic ameloblastoma	10	3.00	3.00–3.00	27.40		
Adenomatoid odontogenic tumor	20	3.00	3.00–3.00	30.60		

Table 4 Parameters used for the calculation of the Kruskal–Wallis (KW) test for the evaluation of the scores of the immunoexpression of MMP-13 and EMMPRIN in the connective tissue of solid ameloblastomas, odontogenic keratocysts, unicystic ameloblastomas, and adenomatoid odontogenic tumors

Group	*n*	Median	Q_{25}–Q_{75}	Mean of ranks	KW	*p*
MMP-13						
Solid ameloblastoma	20	0.00	0.00–3.00	29.83	4.488	0.213
Odontogenic keratocyst	20	2.00	1.00–2.75	41.60		
Unicystic ameloblastoma	10	2.00	0.00–3.25	40.15		
Adenomatoid odontogenic tumor	20	1.00	1.00–2.00	32.75		
EMMPRIN						
Solid ameloblastoma	20	1.00	1.00–2.00	36.93	3.237	0.357
Odontogenic keratocyst	20	1.50	1.00–2.00	36.48		
Unicystic ameloblastoma	10	1.50	1.00–3.25	42.25		
Adenomatoid odontogenic tumor	20	1.00	1.00–2.00	29.73		

EMMPRIN was expressed in the epithelial component of all SAs, OKCs, UAs, and AOTs (Fig. 2a–d). Epithelial expression of EMMPRIN was higher in OKCs when compared to SAs, UAs, and AOTs (Table 2), but the difference was not significant ($p = 0.091$) (Table 3). In connective tissue, EMMPRIN was expressed in all groups (Fig. 2a–d) and higher expression was seen in UAs and OKCs (Table 2). The nonparametric Kruskal–Wallis test (Table 4) revealed no statistically significant differences between the groups ($p = 0.357$).

Spearman's correlation test showed statistical significant ($p = 0.004$) and moderate correlation ($r = 0.343$) between the expression of MMP-13 and EMMPRIN in the epithelial component. In connective tissue, there was a statistic

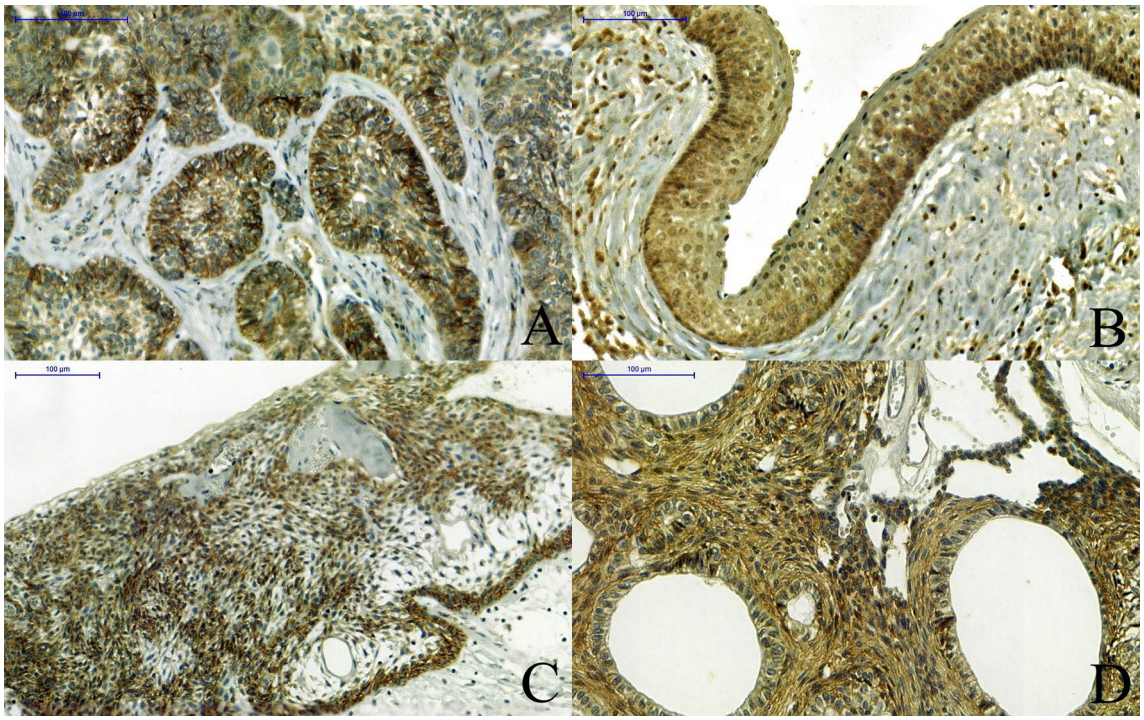

Fig. 2 Immunohistochemical reactivity for EMMPRIN in epithelial and connective tissues: **a** solid ameloblastoma; **b** odontogenic keratocyst; **c** unicystic ameloblastoma; **d** adenomatoid odontogenic tumor (ADVANCE, Pannoramic Viewer 100 µm)

Table 5 Spearman correlation (*r*) and statistical significance (*p*) between the MMP-13 and EMMPRIN in the epithelial and connective tissue of solid ameloblastomas, odontogenic keratocysts, unicystic ameloblastomas, and adenomatoid odontogenic tumors

Epithelial tissue		Connective tissue	
MMP-13		MMP-13	
r	0.343	*r*	0.474
p	0.004	*p*	< 0.001
	EMMPRIN		EMMPRIN
	r		*R*
	p		*P*

significant ($p < 0.001$) and strong correlation ($r = 0.474$) between MMP-13 and EMMPRIN in this component (Table 5).

Discussion

Ameloblastoma is a locally invasive benign tumor that arises from the dental lamina and accounts for about 1% of all neoplasms affecting the oral cavity. Clinically, ameloblastoma appears as a painless growth that causes expansion of the jaw bones. Ameloblastoma can be classified as solid or multicystic, unicystic and peripheral [3, 16]. Ameloblastoma of the solid (conventional) type is characterized by its aggressive behavior, with a tendency towards recurrence when not treated correctly. On the other hand, UA is known for a less aggressive behavior when compared to the solid type and is treated more conservatively, except for cases of mural growth [3, 17].

Odontogenic keratocyst is the third most common cystic lesion of the jaw bones, accounting for 10–20% of all odontogenic cysts, which arises from remnants of the dental lamina. One characteristic of these cysts is postoperative recurrence when the lesion is not removed adequately. However, recurrence rates are currently declining with complete removal of the lesion, resulting in a rate of less than 2%. Another cause of recurrence reported is the presence of satellite cysts in the connective tissue wall of these lesions [18]. In some cases, OKC is part of nevoid basal cell carcinoma syndrome [15, 19]. AOTs are encapsulated lesions of slow growth and indolent behavior when compared to the other odontogenic lesions studied here, although they belong to the same group of epithelial lesions [20].

MMPs comprise a family of zinc- and calcium-dependent proteolytic enzymes that can degrade extracellular matrix components into macromolecules such as collagen, gelatin, fibronectin, tenascin and laminin [7], and participate in both physiological and pathological events [21]. More than 20 different MMPs are known, which are classified according to their domain organization: collagenases (MMP-1, 8, 13, and 18), gelatinases (MMP-2 and

9), stromelysins (MMP-3 and 10), matrilysins (MMP-7, 11, and 26), membrane-type MMPs (MMP-14, 15, 16, 17, 24, and 25), and other MMPs (MMP-12, 19, 20, 21, 23, 27, and 28) [22].

MMP-13 is a metalloproteinase with a broad substrate spectrum whose expression is highly regulated. This proteinase is related to a large number of pathological processes, including tumors, cardiovascular diseases, osteoarthritis, rheumatoid arthritis, and periodontal diseases [23]. Several studies have shown a relationship between the expression of MMP-13 and tumor aggressiveness, more specifically demonstrated in malignant tumors [24, 25]. Within this context, the role of mi-RNAs in the epigenetic regulation of MMP-13 should be highlighted; for example, the antitumor activity of miR-125b, miR-143 and miR-375, with the observation of a correlation between the activation of MMP-13 and low expression of these mi-RNAs [26]. The results of the present study revealed the presence of MMP-13 in all odontogenic lesions studied, but no relationship with the biological behavior of these lesions was found.

In the present study, expression of MMP-13 was detected in the epithelial and in the connective tissue component, corroborating the findings of Kumamoto et al. [27] for colon carcinomas and of Nielsen et al. [28] for breast carcinomas. However, we observed no significant difference in the expression of MMP-13 between the lesions studied, either in the epithelial component ($p = 0.316$) with higher expression in SAs or in the connective tissue component ($p = 0.213$) with higher expression in OKCs and UAs. Wahlgren et al. [29] suggested the presence of MMP-13 in the epithelium of OKCs to be related to features such as the migration and proliferation of epithelial cells and detachment of the epithelial component from the connective tissue capsule. Although the study emphasized the importance of MMP-13 in the epithelial component, it should be remembered that the main source of MMPs, including MMP-13, are stromal cells as demonstrated by Nielsen et al. [28, 30] in breast carcinomas and by Leonardi et al. [31] in OKCs. These studies emphasized that the behavior of lesions is the result of epithelial–mesenchymal interactions. In our study, despite the non-statistical significance, this higher expression of MMP-13 observed in the epithelial component of SAs suggests that this MMP produced by the tumor cells of this neoplasia acts in the degradation of collagen IV and in this way confers to these lesions the invasiveness of the neoplastic epithelial nests through the basement membrane. Regarding the greater expression of MMP-13 in the connective tissue found in both OKCs and UAs, it can explain the behavior of these two distinct lesions, with OKC being an aggressive cystic lesion and UA being a cystic neoplasm it is observed a more aggressive behavior, as well as the similarity in the process of growth of these two lesions in which the connective tissue would be the main source of MMP-13, promoting the expansion of these lesions through the degradation of the surrounding tissues.

EMMPRIN is a glycoprotein that can induce the production of various metalloproteinases and has been suggested to be the main regulator of MMP production [4, 32]. This protein is frequently expressed in tumor cells, stimulating fibroblasts and endothelial cells to produce MMPs that degrade the extracellular matrix, and plays an important role in tumor progression [33]. A number of studies have associated higher expression of EMMPRIN with tumor aggressiveness, a fact demonstrated in malignant tumors and often related to a poor prognosis [13, 33, 34].

Few studies have evaluated the expression of EMMPRIN in odontogenic lesions. One of these studies that analyzed the expression of this metalloproteinase inducer in the epithelial component of OKCs, dentigerous cysts, periapical cysts and dental follicles found higher immunoreactivity in OKCs [35]. Another study comparing the immunoexpression of EMMPRIN in the epithelial and connective tissue component of calcifying odontogenic cysts demonstrated higher expression in the former [36]. This MMP inducer was also detected in ameloblastomas, malignant ameloblastic tumors, and tooth germs [37]. Another study highlighted the presence of EMMPRIN during different stages of tooth development, suggesting the involvement of this MMP inducer in mechanisms of morphogenesis [11]. In the present study, higher immunoexpression of EMMPRIN was observed in the epithelial component of OKCs compared to the other odontogenic lesions, exhibiting marked membrane or sometimes cytoplasmic expression in the basal and parabasal portions. On the other hand, weak or absent immunoreactivity was seen in the superficial layer. Similar results have been reported by Ali [35] who found higher EMMPRIN immunoreactivity in the epithelial component of OKCs compared to the other odontogenic lesions studied. Comparison of EMMPRIN expression between the epithelial and connective tissue components of the odontogenic lesions analyzed here showed higher immunoreactivity in the epithelial component of OKCs while UAs exhibited higher expression in the connective tissue component, agreeing in part with the findings of Prosdócimi et al. [36]. This higher immunoexpression, verified in the epithelial component of OKCs and SAs in our study, associated with evidence of the relationship between elevated levels of EMMPRIN and aggressiveness of several lesions in previous studies, suggests that it has a role in the more aggressive behavior evidenced in both OKCs and SAs, and further studies are needed to confirm the exact role of EMMPRIN present in the epithelium of the odontogenic lesions studied.

When we analyzed the expression of EMMPRIN in connective tissue, we found higher immunoreactivity in UAs, with staining of inflammatory and endothelial cells and occasional immunoreactivity in some fibroblasts.

This higher expression of EMMPRIN in UAs indicates the greater stimulatory effect of collagenases in relation to the other lesions studied, suggesting that EMMPRIN can participate in the tumor progression of these lesions through the induction of MMPs in stromal cells. We also found less immunoreactivity for EMMPRIN in the connective component of TOAs, which are indolent lesions. Thus, we may suggest that EMMPRIN in connective tissue may play a role in the aggressiveness of the MMP-dependent lesions.

Regarding the observation of higher EMMPRIN expression in the epithelial component of OKCs and considerable immunoreactivity in connective tissue of these lesions, we suggest a role of this metalloproteinase inducer in the biological behavior of OKCs, acting at the epithelial–mesenchymal interface. This suggestion partially disagrees with Ali [35] who only considers the presence of EMMPRIN in the epithelial component as important for the behavior of these lesions. Chen and Zhu [33], Kefeli et al. [34] and Urbaniak-Kujda et al. [38] reported a significant association between higher expression of EMMPRIN and a more aggressive biological behavior of lesions. However, we found no significant difference in EMMPRIN expression in the epithelial ($p = 0.091$) or connective tissue ($p = 0.357$) component.

Comparison of the immunoexpression of EMMPRIN and MMP-13 in the present study showed a positive correlation that was strong in connective tissue ($r = 0.474$; $p < 0.001$) and moderate in the epithelial component ($r = 0.343$; $p = 0.004$). These findings support the role of EMMPRIN as an inducer of MMP-13 expression, highlighting its importance for the formation of a microenvironment that favors tumor growth and progression [33, 39]. It should be noted that Kumamoto et al. [27] found another positive correlation of MMP-13 mRNA expression with inhibitor of growth 2 (ING2) in colon carcinomas, in which the expression of MMP-13 is stimulated by the binding of ING2 to other molecules such as HDAC1 (histone deacetylase) and mSin3A (mammalian Sin3A), forming the ING2-HDAC1-mSin3A complex. These molecules, in turn, confer an essential role to ING2 in the induction of MMP-13 through chromatin remodeling. Leeman et al. [40] also highlight that the expression of MMP-13 is also regulated by MT1-MMP, MMP-2, MMP3, MMP-9, and plasmine. We, therefore, suggest that MMP-13 may be regulated not only by EMMPRIN, but also by other transcription factors in lesions studied.

MMPs have been considered as promising targets for therapy and so far numerous synthetic and natural MMP inhibitors have been identified. However, therapeutic approaches aiming to block MMPs have not yet been successful in the treatment of cancer patients, for example [41]. In our study, we suggest that therapeutic inhibition of MMP-13 may aid in the treatment of these odontogenic lesions.

Any therapeutic strategies directed to down-regulate EMMPRIN, however, should take into account that EMMPRIN represents a multi-functional cell surface protein and that consequences might also arise for physiological processes influenced by EMMPRIN [41]. In our study, epithelial cells, endothelial cells and fibroblasts were EMMPRIN positive, suggesting that systemic anti-EMMPRIN therapy might cause undesirable effects.

In conclusion, we can suggest that the OKCs, SAs and UAs presented greater immunoexpression for MMP-13 and EMMPRIN, since they were lesions of more aggressive behavior, with smaller expressions in the AOTs that are admittedly indolent. However, we did not find a statistically significant difference between the expression of MMP-13 and EMMPRIN in lesions studied. The positive correlation found between MMP-13 and EMMPRIN in the epithelial and connective tissue of odontogenic lesions analyzed, seems to be related to the role of EMMPRIN as an inducer of MMP-13 expression.

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Compliance with ethical standards

Conflict of interest None of the authors has any conflict of interest, financial or otherwise.

Ethical approval This study was approved by the UFRN Research Ethics Committee, Natal, Brazil (Protocol no. 354/2010).

Patient consent Not required.

Statement of agreement Certify that all the authors (Pedro Paulo de Andrade Santos; Cassiano Francisco Weege Nonaka; Carlos Augusto Galvão Barboza, Leão Pereira Pinto and Lélia Batista de Souza) have viewed and agreed to the manuscript submitted.

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