Original Research
Oral Pathology

Differential protein expression of osteoclastogenic factors in odontogenic cysts and tumors

Vildeman Rodrigues de ALMEIDA JUNIOR[a]
Eder Gerardo Santos LEITE[a]
Marcus Vinicius ALMEIDA[a]
Jurema Freire Lisboa de CASTRO[b]
Roseana de Almeida FREITAS[c]
Flávia Caló Aquino XAVIER[a]
Andreia Leal FIGUEIREDO[a]
Jean Nunes SANTOS[a]
Águida Cristina Gomes HENRIQUES[a]

[a]Universidade Federal da Bahia – UFBA, Graduate Program in Dentistry and Health, Salvador, BA, Brazil.

[b]Universidade Federal do Pernambuco – UFPE, Department of Dentistry, Graduate Program in Oral Pathology, Recife, PE, Brazil.

[c]Universidade Federal do Rio Grande do Norte – UFRN, Department of Dentistry, Graduate Program in Oral Pathology, Natal, RN, Brazil.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Águida Cristina Gomes Henriques
E-mail: aguidacgh@gmail.com

https://doi.org/10.1590/1807-3107bor-2022.vol36.0072

Submitted: March 13, 2021
Accepted for publication: September 9, 2021
Last revision: October 29, 2021

Abstract: The osteolytic activity of odontogenic cysts and tumors is directly associated with their growth and aggressiveness. The influence of proteins expressed by epithelial and mesenchymal cells on this biological event differs between indolent cystic lesions, aggressive cystic lesions, and odontogenic tumors. The objective of this study was to compare the immunohistochemical expression of factors that stimulate (receptor activator of nuclear factor kappa-B ligand – RANKL, cathepsin K – CatK and matrix metallopeptidase 8 – MMP-8) and inhibit (osteoprotegerin – OPG) osteoclastogenesis between dentigerous cyst (DC), glandular odontogenic cyst (GOC), odontogenic keratocyst (OKC), and ameloblastoma (AB). Paraffin-embedded sections of nine DCs, nine GOCs, 20 OKCs, 21 ABs, and four dental follicles (DFs) were subjected to immunohistochemistry. Immunoreactivity was analyzed semiquantitatively and quantitatively in epithelium and connective tissue, respectively. The proteins were immunoexpressed in epithelial and mesenchymal cells of all lesions studied. The expression of RANKL and CatK was higher in OKC, AB, and GOC ($p<0.005$). Higher expression of OPG was found in DF and DC compared to the other markers ($p<0.005$). MMP-8 expression was high in GOC and OKC. This study demonstrated the differential expression of factors that inhibit and stimulate bone resorption during the development of DC, GOC, OKC, and AB. Higher expression of RANKL and CatK was observed in more aggressive lesions. OPG appears to be one of the molecules responsible for the slower growth of DC.

Keywords: Bone Resorption; Bone Cysts; Ameloblastoma; Biomarkers; Immunohistochemistry.

Introduction

The development, progression, and variable biological behavior of odontogenic lesions are related to their osteolytic activity.[1] The altered expression of proteins that stimulate or inhibit osteoclastogenesis, together with the activity of extracellular matrix metalloproteinases (MMPs), may influence the behavior of these lesions.[2,3,4] Bone resorption is the main biological event responsible for the progression and aggressiveness of odontogenic lesions, which depends on the formation and activation of osteoclasts. Several protein factors have been shown to participate in

the regulation of bone metabolism.[5,6,7] These factors activate or inhibit osteoblasts and osteoclasts.

The identification of the triad consisting of receptor activator of nuclear factor kappa B (RANK), its ligand (RANKL), and osteoprotegerin (OPG) has significantly contributed to the understanding of the biology of bone remodeling. Other molecules also participate in the degradation of the organic and inorganic matrix of bone tissue, favoring osteoclastogenesis.[8,9] RANK and RANKL have been linked to higher osteolytic activity and have been suggested to stimulate bone resorption.[3] On the other hand, OPG exerts the opposite effect and is known as an inhibitor of osteoclastogenesis. Changes in the RANK/RANKL/OPG ratio have been associated with the development of odontogenic lesions.[10] Cathepsin K (CatK) and MMP-8 are important proteases that participate in the degradation of bone matrix protein components, especially collagen I. The role of CatK and MMP-8 in the development of odontogenic lesions has been poorly studied and the activity of these enzymes associated with the RANK/RANKL/OPG triad can potentiate bone resorption and contribute to the growth of odontogenic lesions.[2,3,10,11]

Inasmuch as the factors that stimulate and inhibit osteoclastogenesis are likely responsible for differences in the growth of odontogenic cysts and tumors, the present study compared the protein expression of RANKL, CatK, MMP-8, and OPG between indolent odontogenic cystic lesions (dentigerous cyst – DC), aggressive odontogenic cystic lesions (glandular odontogenic cyst – GOC, odontogenic keratocyst – OKC), and odontogenic tumors (ameloblastoma – AB) in order to determine whether the expression of osteoclastogenic factors in OKC and GOC is similar to that seen in AB or DC.

Methodology

The sample consisted of 63 paraffin-embedded tissue specimens, including nine DCs, nine GOCs (seven multilocular and two unilocular cysts), 20 non-syndromic OKCs (seven multilocular and seven unilocular cysts, and six cysts with their classification not informed), and 21 ABs diagnosed according to the criteria proposed by WHO.[12] Four dental follicles (DFs) served as controls. Only specimens with a mild inflammatory infiltrate or without an infiltrate were included. For AB, only the conventional clinical-radiographic type was selected. The study was approved by the local Research Ethics Committee (process no. 1.091.779).

Immunohistochemistry

For immunohistochemistry, 3-µm thick sections mounted on glass slides previously prepared with organosilane adhesive (Sigma, Munich, Germany) were deparaffinized and rehydrated. Endogenous peroxidase was blocked by incubating the sections with 3% hydrogen peroxide in phosphate-buffered saline (PBS) for 45 min. For antigen retrieval, the sections were incubated with citrate buffer, pH 6.0, for 20 min in a water bath at 95°C and cooled for 20 min at room temperature. The sections were treated with 4% milk and incubated with Protein Block Serum-Free (Dako, Copenhagen, Denmark) for 10 min to block nonspecific reactions. Next, the sections were incubated with primary antibodies diluted in antibody diluents with background reducing components (Dako) for 18 h (overnight) at 4°C (RANKL: N19, Santa Cruz, 1:150; OPG: N22, Santa Cruz, 1:150; CatK: 2F1, Abcam, 1:750; MMP-8: MM0023-7, Santa Cruz, 1:50). The LSAB+System-HRP (Dako) was used for the detection of RANKL, OPG, and CatK, and the ADVANCE™ HRP system (Dako) was employed for the detection of MMP-8. The reactions were developed with 0.03% diaminobenzidine (Dako) as chromogen and the slides were counterstained with Mayer's hematoxylin for 2 min. Central giant cell lesions were used as positive control for RANKL, OPG, and CatK, and radicular cysts (RC) as positive control for MMP-8. Sections incubated with serum diluent replacing the primary antibodies served as negative control.

The presence or absence of immunoreactivity in epithelium and connective tissue was analyzed, as well as the type of positive mesenchymal cell (fibroblast or endothelial cell). Immunoexpression in the epithelium was analyzed semiquantitatively throughout the entire slide. Two observers scored immunoexpression at 100x final magnification as

follows.[4,13,14]: 0 or no staining (< 10% positive cells), 1 (11-25% positive cells), 2 (26-50% positive cells), 3 (51-75% positive cells), and 4 (> 75% positive cells). In connective tissue, the analysis was quantitative. The number of positive cells was counted in five representative histological fields corresponding to the hotspot areas for each marker at 400x final magnification, and the mean number was calculated for each case.[4,13,14] Areas containing inflammatory cells were avoided in the selection of the five histological fields. The image of each field was captured with a Nikon E200 photomicroscope. Immunostained cells were counted using the Cell Counter tool of the Image J for Windows software (version 3.0). In addition to cell counting, this tool marks positive and negative cells in different colors. The median number of positive cells was calculated for each lesion according to the protein analyzed.

Statistical analysis

Epithelial staining and mesenchymal staining were compared by the X^2 and the Kruskal-Wallis tests, respectively. The level of significance was set at 5% ($p < 0.05$).

Results

In general, positive staining was observed in the cytoplasm of epithelial and connective tissue cells. The cell types that expressed the proteins studied in connective tissue were fibroblasts and endothelial cells.

Comparison of epithelial expression of the proteins studied among lesions (Table 1) revealed a significant difference for RANKL ($p < 0.001$). The highest immunostaining for this protein was found in OKC (75% of cases classified as score 4) and AB (61.9% of cases classified as score 4). The expression of CatK also differed significantly ($p < 0.001$), with the highest expression in OKC (50% of cases classified as score 3 and 40% of cases classified as score 4), followed by GOC and AB. The highest expression of MMP-8 was found in GOC (77.8% of cases classified as score 4), followed by OKC ($p < 0.001$). Dental follicles exhibited the highest expression of OPG (100% of cases classified as score 4), followed by DC (88.9% as score 4) ($p = 0.002$).

Comparison of immunohistochemical expression of the different proteins in connective tissue among lesions (Table 2) revealed higher expression of RANKL and CatK in OKC (medians: 85.0 and 53.5, respectively) and AB (medians: 80.0 and 29.0) ($p < 0.001$). MMP-8 was expressed at higher levels in OKC (median: 78.5), DC (median: 63.0), and GOC (median: 46.0) ($p < 0.001$). The highest expression of OPG was detected in OKC (median: 52.0), DF (median: 48.0), and DC (median: 27.0) ($p < 0.001$).

Figures 1 and 2 illustrate RANKL, CatK, MMP-8, and OPG immunostaining in the epithelial and mesenchymal components of DC, GOC, OKC, AB, and DF.

Discussion

Odontogenic lesions arise from the activation of epithelial (cysts and tumors) and/or ectomesenchymal (tumors) remnants of odontogenesis that persist in the bone. These remnants can be stimulated by proteins that activate biological events that are essential for the growth and progression of the lesion. One important event is osteoclastogenesis, which is directly linked to the onset of intraosseous lesions. The molecular events involved in bone resorption and growth of odontogenic lesions are not fully understood, but studies have shown some proteins seem to influence the osteolytic activity and expansion of these lesions.[1,4,11,15] The present study suggests differential expression of RANKL, CatK, MMP-8, and OPG during the development of DC, GOC, OKC, and AB.

The expression of osteoclastogenic factors was evaluated in the epithelial and mesenchymal components in order to determine whether the two cell types influence the osteolytic activity of the lesions. Previous studies have demonstrated positive expression of RANKL, CatK, MMP-8, and OPG by epithelial and mesenchymal cells.[15-19] Areas in the lesions with positive expression of these molecules most likely represent active bone remodeling sites, whereas downregulation of these factors would indicate quiescent areas. [15] In the present study, the expression of osteolytic factors was similar in epithelial and mesenchymal cells, suggesting these cells play an

Table 1. Comparison of epithelial expression of the proteins studied in dentigerous cyst, glandular odontogenic cyst, odontogenic keratocyst, ameloblastoma, and dental follicle.

Protein	Score					p-value
	0	1	2	3	4	
	(< 10%)	(11% to 25%)	(26% to 50%)	(51% to 75%)	(> 75%)	
	n (%)	n (%)	n (%)	n (%)	n (%)	
Rankl						
DC	0 (0)	1 (11.1)	3 (33.3)	3 (33.3)	2 (22.2)	
GOC	0 (0)	1 (11.1)	3 (33.3)	3 (33.3)	2 (22.2)	< 0.001
OKC	0 (0)	0 (0)	1 (5)	4 (20)	15 (75)	
AB	0 (0)	0 (0)	0 (0)	8 (38.1)	13 (61.9)	
DF	3 (75)	1 (25)	0 (0)	0 (0)	0 (0)	
CatK						
DC	2 (22.2)	3 (33.3)	2 (22.2)	1 (11.1)	1 (11.1)	
GOC	1 (11.1)	1 (11.1)	0 (0)	4 (44.4)	3 (33.3)	< 0.001
OKC	0 (0)	0 (0)	2 (10)	10 (50)	8 (40)	
AB	6 (28.6)	1 (4.8)	8 (38.1)	2 (9.5)	4 (19)	
DF	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
MMP8						
DC	0 (0)	1 (11.1)	1 (11.1)	5 (55.6)	2 (22.2)	
GOC	0 (0)	0 (0)	0 (0)	2 (22.2)	7 (77.8)	< 0.001
OKC	0 (0)	0 (0)	3 (15)	10 (50)	7 (35)	
AB	7 (33,3)	5 (23.8)	5 (23.8)	3 (14.3)	1 (4.8)	
DF	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	
OPG						
DC	0 (0)	0 (0)	1 (11.1)	0 (0)	8 (88.9)	
GOC	1 (11.1)	2 (22.2)	4 (44.4)	2 (22.2)	0 (0)	0.002
OKC	0 (0)	0 (0)	2 (10)	5 (25)	13 (65)	
AB	0 (0)	8 (38.1)	8 (38.1)	5 (25)	0 (0)	
DF	0 (0)	0 (0)	0 (0)	0 (0)	4 (100)	

DC: Dentigerous cyst; GOC: Glandular odontogenic cyst; OKC: Odontogenic keratocyst; AB Ameloblastoma; DF: Dental follicle.

equally important role in bone resorption, which is essential for the onset, development, and progression of the intraosseous lesions studied. Moraes et al.[4] evaluated the participation of RANK, RANKL, and OPG in the expansion of DC and RC. Similar to the present study, immunohistochemical expression was evaluated in the epithelium and connective tissue. The results suggested the ratio between RANKL and OPG in capsular cyst was associated with different stages of lesion progression. On the other hand, the concentration of OPG was increased in the epithelium compared to RANKL. The function of OPG would be to create a barrier against cystic expansion. The differential distribution of osteoclastogenesis stimulating and inhibitory factors in the epithelial and mesenchymal components is believed to favor higher or lower bone resorption and consequently mediate the progression of the lesion.

Analysis of the immunoexpression of osteoclastogenic factors in DC demonstrated high expression of OPG. However, significant expression of MMP-8 was also observed in this cyst. Marked expression of OPG in DC is expected because of the indolent nature of the lesion. OPG inhibits the proliferation and differentiation of osteoclasts and competes with RANKL by preventing its binding to RANK, thus inhibiting osteolytic activity.[7,15] Suojanen et al.[20] found higher expression of OPG in DC when compared to OKC and AB, in agreement with our results.

Table 2. Comparison of immunohistochemical expression of the studied proteins in connective tissue in dentigerous cyst, glandular odontogenic cyst, odontogenic keratocyst, ameloblastoma, and dental follicle.

Protein	Median	Q_{25}.Q_{75}	p-value
RankI			
DC	40.0	20.5–58.5	< 0.001
GOC	25.0	1.5–48.5	
OKC	85.0	40.25–92.0	
AB	80.0	74.5–87.5	
DF	0	0.0–3.0	
CatK			
DC	4.0	0.5–6.0	< 0.001
GOC	8.0	74.5–87.5	
OKC	53.5	33.25–69.0	
AB	29.0	0.0–70.0	
DF	0	0,0–0,0	
MMP8			
DC	63.0	27.5–92.0	< 0.001
GOC	46.0	31.0–64.0	
OKC	78.5	66.75–84.25	
AB	17.0	10.0–33.0	
DF	16.0	10.75–43.0	
OPG			
DC	27.0	17.0–59.5	< 0.001
GOC	3.0	0.0–13.0	
OKC	52.0	33.5–57.75	
AB	13.0	1.5–20.0	
DF	48.0	39.75–58.5	

DC: Dentigerous cyst; GOC: Glandular odontogenic cyst; OKC: Odontogenic keratocyst; AB: Ameloblastoma; DF: Dental follicle.

Studies investigating the expression of MMP-8 in odontogenic lesions are scarce in the literature and there is only one study on DC.[20] Our findings are consistent with the results of Suojanen et al.,[20] who also found high immunoexpression of MMP-8 in DC. MMP-8 is one of the most important endopeptidases that mainly degrades collagen I.[20,21] By acting together with other factors that stimulate osteolytic activity, this protease would contribute to the growth and progression of intraosseous lesions. We believe the expression of MMP-8 in DC evaluated in the present study was important for the onset of the lesion. However, the predominance of OPG is responsible for the indolent behavior of DC. With respect to CatK, low expression of this protein was detected in DC. CatK participates in the degradation of bone matrix protein components, especially collagen I, the most abundant collagen in bone. However, the low expression of CatK in DC suggests a minor role of this protein in the development of this lesion. There are no studies evaluating the immunoexpression of CatK in DC.

In GOC, MMP-8, RANKL, and CatK were expressed at higher levels than OPG. This finding may partly explain the aggressive nature of this cyst given that it exhibits higher expression of molecules that favor bone resorption.

The highest immunoexpression of RANKL was observed in OKC. This result is consistent with the literature in that OKC is considered a more aggressive lesion compared to other odontogenic cysts[22,23] and is associated with higher bone resorption.[24,25] Matos et al.,[1] Tekkesin, Mutlu and Oglac,[11] and Siar et al.[15] also found high immunoexpression of RANKL in OKC. Furthermore, our results showed significant expression of OPG in the capsule of OKC. This result raises the possibility that, although considered an aggressive lesion, OKC normally possesses slower growth than a tumor lesion such as AB. Similar results have been reported by Tekkesin, Mutlu and Oglac,[11] who evaluated the levels of RANKL/OPG in OKC and found higher expression of OPG in 62.4% of the cases studied. By contrast, in our sample, OPG had a lower expression than RANKL, CatK, and MMP-8 in the capsule of OKC. The different ratios of stimulating and inhibiting factors are believed to favor higher or lower bone resorption.

Significant staining for CatK was observed in OKC, suggesting this protein is important for the development of this lesion. The immunohistochemical expression of MMP-8 in OKC was more significant in the capsule. There are no studies on the expression of CatK or MMP-8 in OKC. Some authors, however, have demonstrated a relationship of CatK with higher bone resorption in the progression of rheumatoid arthritis and periodontitis,[26] and of MMP-8 with reduced bone repair and an increased risk of developing malignant tumors.[2,21]

High levels of RANKL were detected in the parenchyma and stroma of the AB cases studied, a finding compatible with an aggressive lesion. Low expression of OPG was also observed in AB, which is consistent with the fact that this protein is an inhibitor

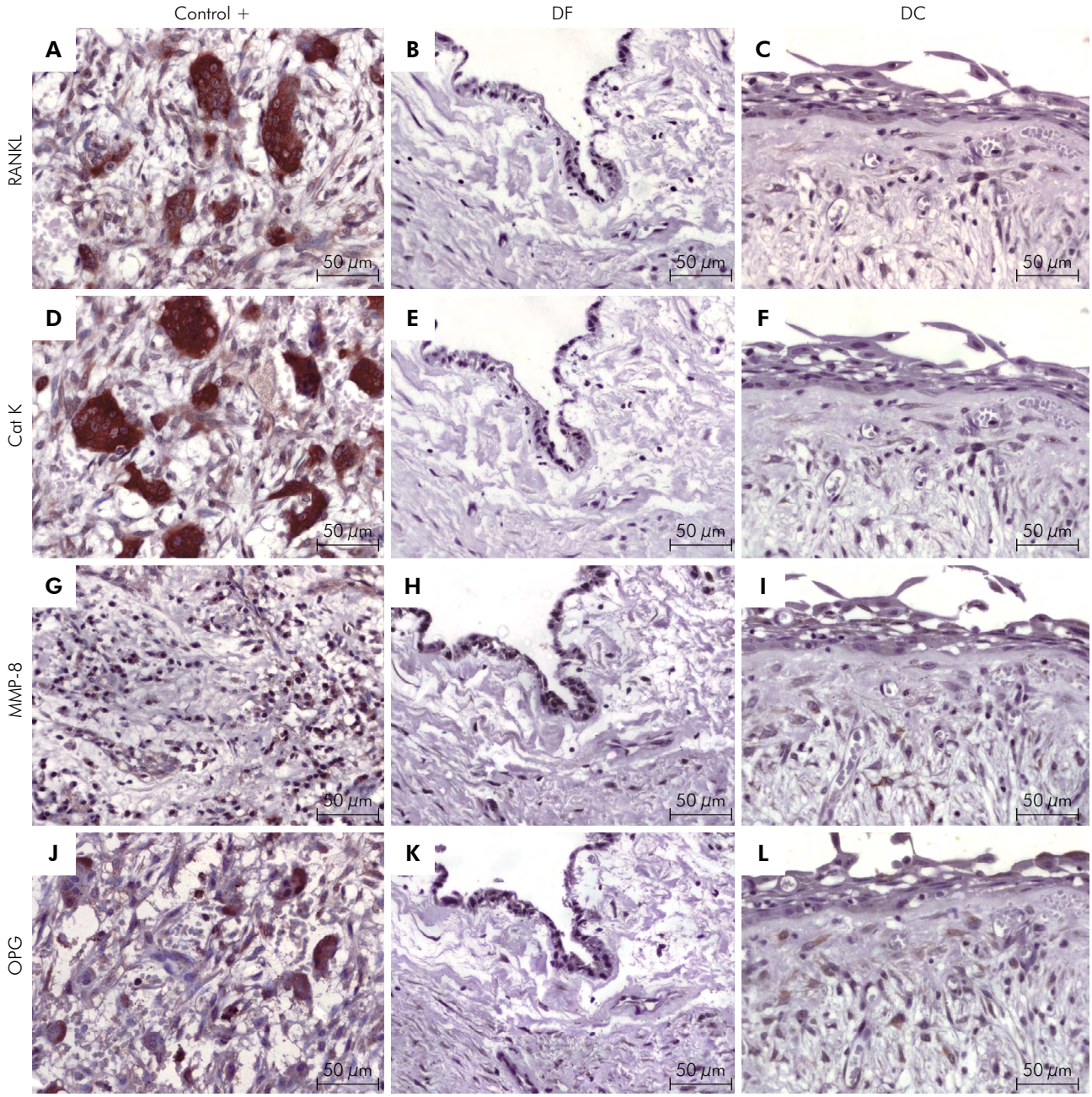

Figure 1. Immunoexpression of RANKL, CatK, MMP8 and OPG in dental follicle (DF), dentigerous cyst (DC), and positive control (A, D and J: central giant cell lesion, G: radicular cyst). Note the absence of expression of RANKL (B) and CatK (E) in DF and weak expression in the cystic epithelium and capsule of DC (C and F). Expression of OPG is more evident in the cytoplasm and nucleus of epithelial and mesenchymal cells of DF (K) and DC (L). MMP8 present in the cytoplasm and nucleus of mesenchymal cells of DF (I).

of osteoclastogenesis. These results corroborate several studies in the literature.[1,11,27,28] Kumamoto et al.[28] found higher expression of RANKL compared with OPG in the parenchyma of AB. Tekkesin, Mutlu and Oglac[11] detected higher expression of RANKL in the stroma of AB when compared to OPG.

In the present study, significant CatK staining was observed in the parenchyma and in the stroma of AB. Cathepsin K is a biological marker of osteoclastogenesis whose expression, together with RANKL, may explain the bone resorption and expansion potential of this lesion. Kim et al.[29] observed important expression of

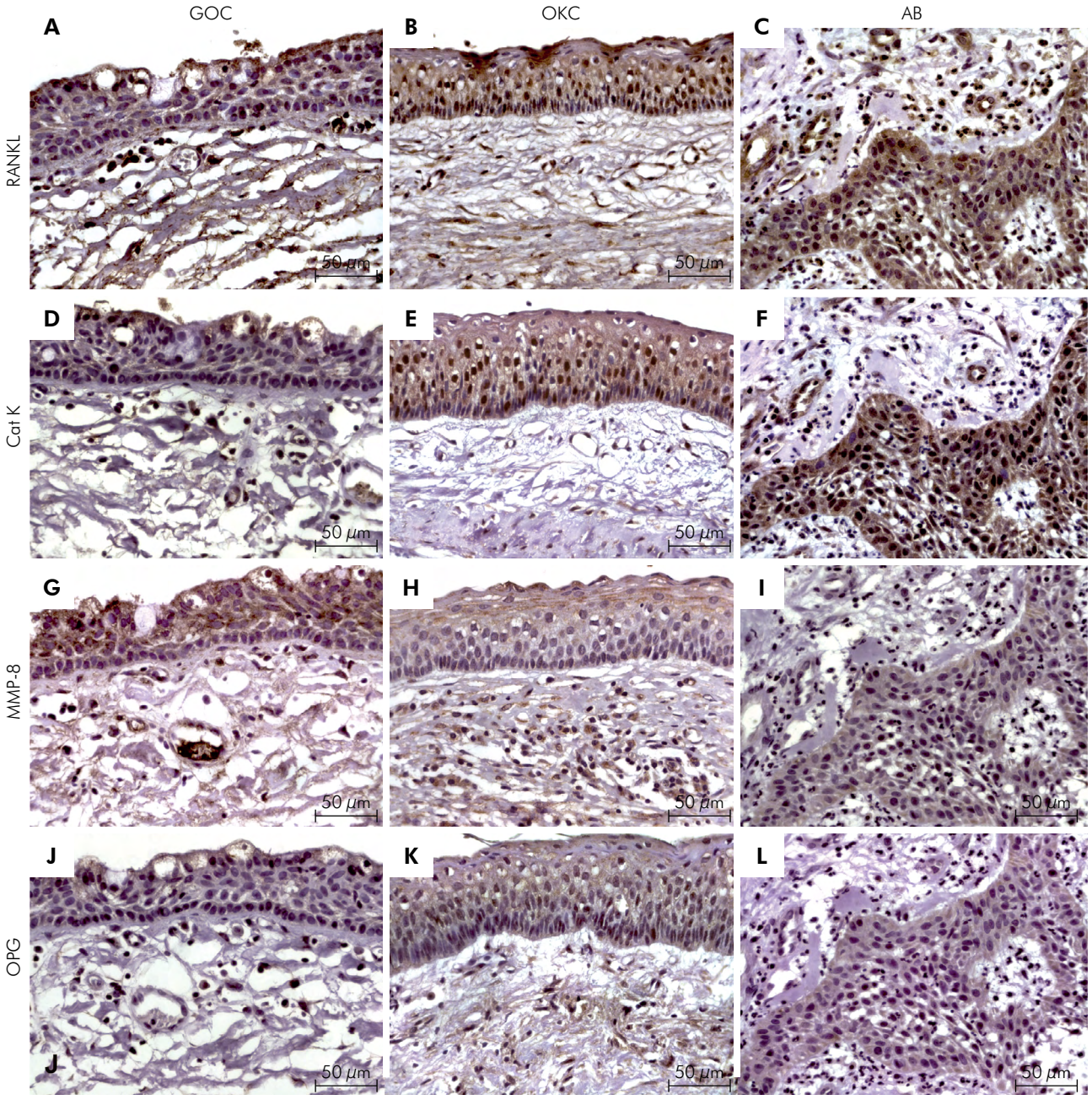

Figure 2. Immunoexpression of RANKL, CatK, MMP8 and OPG in glandular odontogenic cyst (GOC), odontogenic keratocyst (OKC), and ameloblastoma (AB). Note the high expression of RANKL (C) and CatK (F) in the parenchyma and stroma of AB. High expression levels are seen in the cystic epithelium and capsule of OKC (B and E) and lower expression levels in GOC (A and D). Expression of MMP8 is evident in the cytoplasm and nucleus of epithelial and mesenchymal cells of GOC (G, OKC (H) and AB (I). OPG is less evident in GOC (J), OKC (K) and AB (L).

CatK in peripheral AB. There are no previous studies evaluating the expression of CatK in intraosseous AB. The immunohistochemical expression of MMP-8 in AB was not significant. The studies published so far did not investigate the participation of MMP-8 in the pathogenesis of AB. However, other MMPs have been explored and an important role in the development, bone resorption, and growth of this tumor has been demonstrated.[3,30]

Comparison of the immunohistochemical expression of the proteins among the lesions studied

revealed an apparently greater participation of RANKL and CatK in the pathogenesis of OKC, AB, and GOC than in that of DC. This result might be important to explain the greater bone resorption, growth, and aggressiveness of AB, OKC, and GOC.[3,7,11] Tekkesin, Mutlu and Oglac[11] evaluated the immunohistochemical expression of RANK, RANKL, and OPG in OKC, AB, and RC. In their study, the expression of RANKL was high and similar in AB and OKC, but low in RC. RANK expression was higher in OKC, whereas OPG expression was low, without differences between lesions. Brito et al.[31] evaluated the expression of the RANK/RANKL/OPG triad in unicystic AB, OKC, and DC. Higher expression of RANKL was observed in unicystic AB, followed by OKC. The latter exhibited higher expression of OPG when compared to the other lesions studied. Higher expression of RANK and lower expression of RANKL were found in DC. The present study also compared the immunohistochemical expression of RANKL and OPG in DC, AB, and OKC. Higher expression of RANKL was observed in OKC, followed by AB. Taken together, these results suggest considerable osteolytic activity in AB and OKC, as indicated by the high expression of factors that stimulate osteoclastogenesis.

Higher immunoexpression of OPG was observed in DC and DF. This finding might explain the lower osteolytic activity associated with these processes. Likewise, Tekkesin, Mutlu and Oglac[11] found high immunopositivity for OPG in 70% of DF cases when compared to AB, OKC, and DC. According to Moraes et al.[4] and Siar et al.[15], higher expression of OPG is expected in more indolent lesions because of the inhibitory activity of this protein on osteoclastogenesis.

This study provides a better understanding of the osteoclastogenic activity involved in the development of aggressive and indolent cysts compared with conventional AB, which is considered the most aggressive lesion with the greatest osteolytic activity in the sample, especially when OKC is considered, given that the discussion about its possible neoplastic nature persists. In this study, the profile of OKC related to the signaling pathways in osteoclastogenesis was similar to that of conventional AB. Another important contribution of this study was the better understanding of the bone resorption process that occurs in GOC, a rare lesion that has not been described in previous studies. Furthermore, the role of CatK and MMP-8 in the development of odontogenic lesions has been poorly studied and the activity of these enzymes associated with the RANK/RANKL/OPG triad can potentiate bone resorption and contribute to the growth of odontogenic lesions. These findings may thus shed new light on the different roles of these molecules in the induction of bone remodeling activities in DC, GOC, OKC, and AB, and may also indicate targets for the clinical treatment of these lesions.

Conclusion

Our results indicate higher expression of RANKL and CatK in lesions with a more aggressive behavior such as OKC, AB, and GOC. OPG was more significantly expressed in DF and DC and seems to be one of the molecules responsible for the slower growth of DC. MMP-8 appears to play an important role in the growth of GOC and OKC. Further studies on odontogenic lesions and on these markers, especially CatK and MMP-8, are necessary to improve our understanding of the process of bone resorption in these lesions.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, for their financial support. The authors declare that they have no financial interests or involvement with any commercial organization. The authors deny any conflicts of interest related to this study.

References

1. Matos FR, Moraes M, Silva EBN, Galvão HC, Freitas RA. Immunohistochemical detection of receptor activator nuclear κB ligand and osteoprotegerin in odontogenic cysts and tumors. J Oral Maxillofac Surg. 2013 Nov;71(11):1886-92. https://doi.org/10.1016/j.joms.2013.05.023

2. Ahmed Haji Omar A, Haglund C, Virolainen S, Häyry V, Atula T, Kontio R, et al. MMP-7, MMP-8, and MMP-9 in oral and cutaneous squamous cell carcinomas. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015 Apr;119(4):459-67. https://doi.org/10.1016/j.oooo.2014.12.019
3. Dutra KL, Cordeiro MM, Vieira DS, Rivero ER. Immunohistochemical expression of matrix metalloproteinases in ameloblastomas and pericoronal follicles. J Oral Pathol Med. 2016 Sep;45(8):586-90. https://doi.org/10.1111/jop.12411
4. Moraes M, Matos FR, Souza LB, Freitas RA, Costa ALL. Immunoexpression of RANK, RANKL, OPG, VEGF, and vWF in radicular and dentigerous cysts. J Oral Pathol Med. 2013 Jul;42(6):468-73. https://doi.org/10.1111/jop.12036
5. Chen B, Wu W, Sun W, Zhang Q, Yan F, Xiao Y. RANKL expression in periodontal disease: where does RANKL come from? BioMed Res Int. 2014;2014:731039. https://doi.org/10.1155/2014/731039
6. Guerrini MM, Takayanagi H. The immune system, bone and RANKL. Arch Biochem Biophys. 2014 Nov;561:118-23. https://doi.org/10.1016/j.abb.2014.06.003
7. Sun H, Li Q, Zhang Y, Bi Y, Li X, Shu Y, et al. Regulation of OPG and RANKL expressed by human dental follicle cells in osteoclastogenesis. Cell Tissue Res. 2015 Nov;362(2):399-405. https://doi.org/10.1007/s00441-015-2214-8
8. Hua Y, Robinson TJ, Cao Y, Shi GP, Ren J, Nair S. Cathepsin K knockout alleviates aging-induced cardiac dysfunction. Aging Cell. 2015 Jun;14(3):345-51. https://doi.org/10.1111/acel.12276
9. Guha M, Srinivasan S, Koenigstein A, Zaidi M, Avadhani NG. Enhanced osteoclastogenesis by mitochondrial retrograde signaling through transcriptional activation of the cathepsin K gene. Ann N Y Acad Sci. 2016 Jan;1364(1):52-61. https://doi.org/10.1111/nyas.12709
10. Gao B, Chen W, Hao L, Zhu G, Feng S, Ci H, et al. Inhibiting periapical lesions through AAV-RNAi silencing of cathepsin K. J Dent Res. 2013 Feb;92(2):180-6. https://doi.org/10.1177/0022034512468757
11. Tekkesin MS, Mutlu S, Olgac V. The role of RANK/RANKL/OPG signalling pathways in osteoclastogenesis in odontogenic keratocysts, radicular cysts, and ameloblastomas. Head Neck Pathol. 2011 Sep;5(3):248-53. https://doi.org/10.1007/s12105-011-0271-1
12. El-Naggar AK, Chan JK, Grandis JR, et al. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. 4th ed. Lyon: IARC Press; 2017.
13. Sá MC, Matos FR, Conceição TS, Leitão AC, Freitas AR. Immunoexpression of tumour necrosis factor-α, interleukin 1- α and interleukin-10 on odontogenic cysts and tumours. Int Endod J. 2017 May;50(5):437-55. https://doi.org/10.1111/iej.12640
14. Santos SC, Couto LA, Fonseca JM, Xavier FC, Figueiredo AC, Freitas VS, et al. Participation of osteoclastogenic factors in immunopathogenesis of human chronic periapical lesions. J Oral Pathol Med. 2017 Oct;46(9):846-52. https://doi.org/10.1111/jop.12618
15. Siar CH, Tsujigiwa H, Ishak I, Hussin NM, Nagatsuka H, Ng KH. RANK, RANKL, and OPG in recurrent solid/multicystic ameloblastoma: their distribution patterns and biologic significance. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015 Jan;119(1):83-91. https://doi.org/10.1016/j.oooo.2014.09.017
16. Aslan F, Küçük Ü. Kuçuk u. RANK and RANKL expression in salivary gland tumors. Ear Nose Throat J. 2020 Aug;99(7):475-80. https://doi.org/10.1177/0145561320930640
17. Guo Y, Xu C, Wu X, Zhang W, Sun Y, Shrestha A. Leptin regulates OPG and RANKL expression in gingival fibroblasts and tissues of chronic periodontitis patients. Int J Med Sci. 2021 Apr;18(11):2431-7. https://doi.org/10.7150/ijms.56151
18. Heo SC, Kim YN, Choi Y, Joo JY, Hwang JJ, Bae MK, et al. Elevated expression of cathepsin K in periodontal ligament fibroblast by inflammatory cytokines accelerates osteoclastogenesis via paracrine mechanism in periodontal disease. Int J Mol Sci. 2021 Jan;22(2):695-708. https://doi.org/10.3390/ijms22020695
19. Kelppe J, Thorén H, Haglund C, Sorsa T, Hagström J. MMP-7, -8, -9, E-cadherin, and beta-catenin expression in 34 ameloblastoma cases. Clin Exp Dent Res. 2021 Feb;7(1):63-9. https://doi.org/10.1002/cre2.331
20. Suojanen J, Lehtonen N, Färkkilä E, Hietanen J, Teronen O, Sorsa T, et al. Common matrix metalloproteinases (MMP8, -9, -25 and -26) cannot explain dentigerous cyst expansion. J Clin Diagn Res. 2014 Sep;8(9):ZC82-5.
21. Lawal AO, Adisa AO, Kolude B, Adeyemi BF. Immunohistochemical expression of MMP-2 and MMP-8 in oral squamous cell carcinoma. J Clin Exp Dent. 2015 Apr;7(2):e203-7. https://doi.org/10.4317/jced.52047
22. Pogrel MA. The keratocystic odontogenic tumour (KCOT): an odyssey. Int J Oral Maxillofac Implants. 2015 Dec;44(12):1565-8. https://doi.org/10.1016/j.ijom.2015.03.008
23. Leung YY, Lau SL, Tsoi KY, Ma HL, Ng CL. Results of the treatment of keratocystic odontogenic tumours using enucleation and treatment of the residual bony defect with Carnoy's solution. Int J Oral Maxillofac Implants. 2016 Sep;45(9):1154-8. https://doi.org/10.1016/j.ijom.2016.02.002
24. Barnes L, Eveson JW, Reichart P, et al. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC Press; 2005.
25. Stoelinga PJ. The management of aggressive cysts of the jaws. J Maxillofac Oral Surg. 2012 Mar;11(1):2-12. https://doi.org/10.1007/s12663-012-0347-9

26. Hao L, Zhu G, Lu Y, Wang M, Jules J, Zhou X, et al. Deficiency of cathepsin K prevents inflammation and bone erosion in rheumatoid arthritis and periodontitis and reveals its shared osteoimmune role. FEBS Lett. 2015 May;589(12):1331-9. https://doi.org/10.1016/j.febslet.2015.04.008
27. Bansal S, Desai RS, Shirsat P, Prasad P, Karjodkar F, Andrade N. The occurrence and pattern of ameloblastoma in children and adolescents: an Indian institutional study of 41 years and review of the literature. Int J Oral Maxillofac Implants. 2015 Jun;44(6):725-31. https://doi.org/10.1016/j.ijom.2015.01.002
28. Kumamoto H, Ooya K. Expression of parathyroid hormone-related protein (PTHrP), osteoclast differentiation factor (ODF)/receptor activator of nuclear factor-kappaB ligand (RANKL) and osteoclastogenesis inhibitory factor (OCIF)/osteoprotegerin (OPG) in ameloblastomas. J Oral Pathol Med. 2004 Jan;33(1):46-52. https://doi.org/10.1111/j.1600-0714.2004.00204.x
29. Kim YS, Lee SK. Different protein expressions between peripheral ameloblastoma and oral basal cell carcinoma occurred at the same mandibular molar area. Korean J Pathol. 2014 Apr;48(2):151-8. https://doi.org/10.4132/KoreanJPathol.2014.48.2.151
30. Henriques ÁC, Vasconcelos MG, Galvão HC, de Souza LB, de Almeida Freitas R. Comparative analysis of the immunohistochemical expression of collagen IV, MMP-9, and TIMP-2 in odontogenic cysts and tumors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011 Oct;112(4):468-75. https://doi.org/10.1016/j.tripleo.2011.05.033
31. Brito-Mendoza L, Bologna-Molina R, Irigoyen-Camacho ME, Martinez G, Sánchez-Romero C, Mosqueda-Taylor A. A comparison of Ki67, sSyndecan-1 (CD138), and molecular RANK, RANKL, and OPG triad expression in odontogenic keratocytes, unicystic ameloblastoma, and dentigerous cysts. Dis Markers. 2018 Jul;2018:7048531. https://doi.org/10.1155/2018/7048531