Presence of Myofibroblasts and Matrix Metalloproteinase 2 in Radicular Cysts, Dentigerous Cysts, and Keratocystic Odontogenic Tumors: A Comparative Immunohistochemical Study

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Abstract

Introduction: The aim of this study was to analyze the presence of myofibroblasts and matrix metalloproteinase 2 (MMP-2) in radicular cysts (RCs), dentigerous cysts (DCs), and keratocystic odontogenic tumors (KOTs). **Methods:** For the study, 29 RCs, 19 DCs, and 15 KOTs were selected. Immunohistochemical reactions were performed by using anti–MMP-2 and anti–α-smooth muscle actin (SMA) antibodies. For the analysis, 10 high-power fields were observed in each case to determine the percentage of positive cells, which was classified as negative, weak, or strong. **Results:** The presence of myofibroblasts (α-SMA–positive cells) was most common in KOTs (46.67%), followed by DCs (36.84%) and RCs (31.04%); however, it was not statistically significant (P = .8). The stromal MMP-2 expression was positive in all lesions but 1 case of KOT. Most cases of RC and DC presented strong MMP-2 expression in the stroma, whereas half of the KOTs showed similar classification. The MMP-2 expression was commonly found in the epithelial lining of the lesions; it was strong in almost all KOTs. No correlation between epithelial and stromal MMP-2 and α-SMA expressions was observed. **Conclusions:** Myofibroblasts and MMP-2 are frequent in RCs, DCs, and KOTs and eventually can contribute to bone resorption, favoring the progression and growth of these lesions. *(J Endod 2012;38:1363–1367)*

Key Words

Dentigerous cyst, keratocystic odontogenic tumor, matrix metalloproteinase-2, myofibroblasts, odontogenic keratocyst, radicular cyst

Myofibroblasts (MFs) are specialized cells that contain both cytoplasmic β-and γ-actins present in fibroblasts as well as the isoform α of smooth muscle actin (α-SMA), typically expressed in the smooth muscle cells (1). They were initially found in the granulation tissue of open wounds and afterwards identified in normal tissues that require mechanical force, in hypertrophic scars, fibromatosis, and fibro-contractile lesions, as well as in the reactional stroma of epithelial tumors (2). Several molecules could be involved in the differentiation of MFs, but the most important is transforming growth factor beta 1 (TGF-β1) (1–4).

In the reactive or desmoplastic stroma from neoplasias, MFs may be associated with both the defensive mechanism of isolating the lesion and active participation in its progression, either by modulating angiogenesis, facilitating the invasion of tumor cells, or by promoting metastases. In addition to the capacity to produce collagen, MFs are also responsible for the synthesis of enzymes such as matrix metalloproteinase 2 (MMP-2), which are capable of degrading the extracellular matrix and favoring the invasive growth of the lesion (3, 5).

Few studies have evaluated the expression of MFs in odontogenic lesions (6, 7). In odontogenic cysts, MFs are present in different degrees of expression in practically all cases of radicular and dentigerous cysts (6, 7). Nevertheless, in odontogenic keratocysts, reclassified by the World Health Organization as benign cystic neoplasia and renamed as keratocystic odontogenic tumor (8), the presence of MFs was related to lesions with the more aggressive phenotype (6, 7). Regarding an enzyme synthesized by MFs, the identification of MMP-2 in stroma from ameloblastomas indicates that it may digest bone matrix and contribute to tumor invasion (9, 10). Thus, the presence of stromal MFs and MMP-2 could be related to the biological behavior of the odontogenic cystic lesions.

Because the odontogenic cystic lesions show variable biological behavior in terms of invasiveness, growth, and recurrence rate and the presence of stromal MF and MMP-2 is predicted to be a critical step in growth and progression of bone lesions (7, 9), the aim of this study was to analyze the presence of MFs by immunohistochemical evaluation of α-SMA and the immunohistochemical expression of MMP-2 in radicular cysts, dentigerous cysts, and keratocystic odontogenic tumors.

Materials and Methods

Twenty-nine radicular cysts, 19 dentigerous cysts, and 15 keratocystic odontogenic tumors from the files of the Oral Pathology Laboratory, University of Ribeirao

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Preto, Ribeirao Preto, Sao Paulo, Brazil were used in this study. Radicular cysts that presented a well-formed cystic cavity with stratified and squamous epithelial lining were selected. In the keratocystic odontogenic tumors, there was no case associated with nevoid basal cell carcinoma syndrome. This study was approved by the local Research Ethics Committee.

All cases were histologically reviewed to confirm the diagnosis. The inflammatory reaction eventually present in the cystic fibrous capsules was classified as slight (foci of inflammatory cells) or intense (diffuse inflammatory reaction).

For immunohistochemical reactions, 3-μm histologic sections were obtained from the paraffin-embedded tissue blocks and mounted on silane-coated glass slides. Antigen retrieval was performed in a pressure cooker for 4 minutes in 10 mmol L^{-1} citrate buffer (pH 6.0), followed by a washing step with phosphate-buffered saline. The incubations with the anti–MMP-2 (polyclonal, dilution 1:200, NeoMarkers; Lab Vision Corporation, Fremont, CA) and anti–α-SMA (clone 1A4, dilution 1:200; Dako, Glostrup, Denmark) primary antibodies were carried out for 18 hours at 4°C. The tissue sections were incubated with Post Primary Block (Novolink Max Polymer; Novocastra, Newcastle, UK) for 30 minutes at 37°C, followed by application of diaminobenzidine (Dako) as the chromogen. Slides were counterstained with Harris hematoxylin, mounted, and analyzed by 2 oral pathologists who were previously calibrated. The expression of α-SMA was evaluated in the stroma (fibrous capsule) and MMP-2 in the stroma and epithelial lining of the studied lesions. Positive (blood vessels in normal oral mucosa for α-SMA and breast carcinoma for MMP-2) and negative (omission of primary antibodies) controls were included in all reactions.

The percentage of positive cells in 10 high-power fields was used to classify each lesion. The presence of cells positive for α-SMA and MMP-2 was evaluated semiquantitatively and classified as negative (<5% of positive cells), weak (5%–50% of positive cells), or strong (>50% of positive cells), according to Fregnani et al (10). The evaluators were blinded with respect to the types of cystic lesions during the analysis, and any disagreements were discussed until a consensus was reached.

For statistical analysis, the lesions were compared with regard to the expression of the studied antibodies (Fisher exact test), and whether there was correlation between α-SMA and MMP-2 in each cystic lesion (Spearman correlation coefficients) was verified, adopting a significance of 5%.

Results

Radicular Cyst

Of the 29 cases of radicular cyst, 21 (72.41%) presented a fibrous capsule with diffuse inflammatory reaction, and there was focal inflammation in 8 (27.59%). Twenty cases (68.96%) did not express α-SMA, 8 (27.59%) presented weak positivity for α-SMA, and 1 case (3.45%) presented strong immunoexpression (Fig. 1). In the fibrous capsule, MMP-2 had weak expression in 8 cases (27.59%) and strong positivity in 21 cases (72.41%) (Fig. 2). In the same way, most cases presented epithelial lining positive for MMP-2; 8 (27.59%) showed weak immunoexpression, and 18 (62.07%) had strong positivity (Fig. 2). However, the epithelial cells were negative in 3 cases (10.34%).

Dentigerous Cyst

Inflammatory reaction was observed in 11 cases (57.89%) of dentigerous cyst, of which 9 showed focal inflammation and 2 diffuse inflammatory reaction.

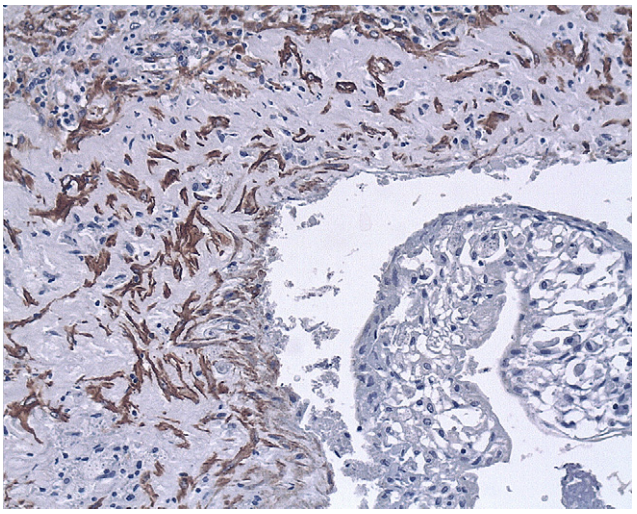

Figure 1. Strong positivity for α-SMA (MFs) in the fibrous capsule of radicular cyst (original magnification, ×200).

The expression of α-SMA was negative in 12 cases (63.16%), 6 (31.58%) presented weak positivity for α-SMA, and 1 case (5.26%) had strong immunoexpression. In the stroma, all cases were positive for MMP-2, of which 17 (89.47%) presented weak positivity and 2 cases (10.53%) strong positivity. Of the 19 cases of DC, 3 (15.79%) showed weak immunoexpression for MMP-2 in the epithelial cells, and 13 (68.42%) showed strong positivity in the epithelial lining (Fig. 3). In 3 cases (15.79%), the epithelial lining was negative for MMP-2.

Keratocystic Odontogenic Tumor

Of the 15 cases of keratocystic odontogenic tumor, 8 (53.33%) did not express α-SMA, 6 (40%) showed weak positivity, and 1 (6.67%) showed strong immunoexpression for α-SMA. MMP-2 was weakly expressed in the fibrous capsule of 7 cases (46.66%) and strongly positive in 7 (46.66%) (Fig. 4), and 1 case (6.67%) was negative. All cases presented epithelial lining positive for MMP-2; 1 case (6.67%) had weak expression, and 14 (93.33%) had strong positivity (Fig. 4). Moreover, 5 cases (33.3%) presented focal inflammation in the fibrous capsule.

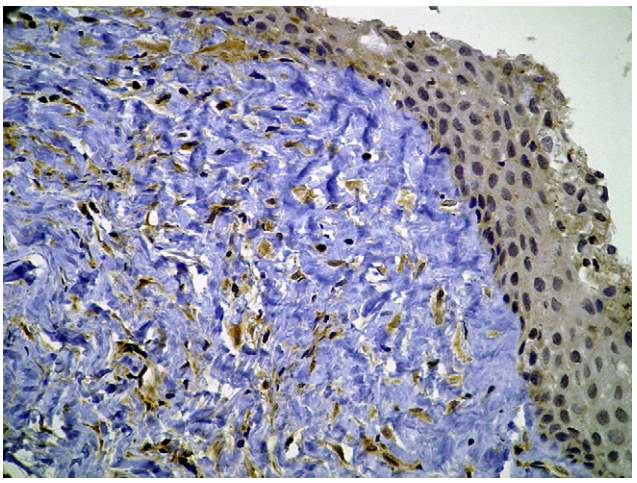

Figure 2. Strong positivity for MMP-2 in epithelial and mesenchymal cells in radicular cyst (original magnification, ×200).