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Syndecan-1 (CD138) and Ki-67 Expression in Odontogenic Cystic Lesions

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The aim of this study was to assess the immunohistochemical expression of syndecan-1 (CD138) and Ki-67 in radicular cysts (RC), dentigerous cysts (DC) and keratocystic odontogenic tumors (KOT). Thirty-five RC, 22 DC and 17 KOT were used in the study and immunohistochemical reactions using anti-syndecan-1 and anti-Ki-67 antibodies were performed by the streptavidin-biotin-peroxidase method. Fisher's exact test and Spearman's correlation coefficient were used for statistical analysis of data. Among the studied lesions, no differences in the syndecan-1 expression were observed, but the suprabasal expression of Ki-67 was significantly higher in KOT ($p<0.0001$), when compared with RC and DC. In RC, there was positive correlation between the expression ($p=0.02$) and intensity ($p=0.0001$) of syndecan-1 and between the intensity of syndecan-1 and Ki-67 expression ($p=0.01$). In the KOT, Ki-67 expression in the suprabasal layer correlated positively with the expression ($p=0.01$) and intensity ($p=0.01$) of syndecan-1. The expression of syndecan-1 does not seem to be a determinant factor of the distinct histopathological features and biological behavior of the studied lesions. Nevertheless, positive correlation between syndecan-1 and a cell proliferation marker was observed in RC and KOT.

Key Words: keratocystic odontogenic tumor, Ki-67, odontogenic cysts, syndecan-1.

INTRODUCTION

Odontogenic cysts are common lesions of the jaws, mainly radicular cysts (RC), dentigerous cysts (DC) and odontogenic keratocysts, reclassified by the World Health Organization as benign cystic neoplasias, and renamed as keratocystic odontogenic tumors (KOT) (1).

Although the odontogenic cystic lesions are derived from remnants of odontogenic epithelium, they present distinct histopathology and biological behavior. Several studies have evaluated the expression of cell proliferation markers in the epithelial lining of odontogenic cystic lesions, such as PCNA and Ki-67. The proliferative capacity of cells, represented by mitosis, can be identified by the Ki-67 antigen, which is expressed in all active phases of the cell cycle, except in G0 (2). The epithelial cells of the cystic lining in KOT show higher Ki-67 expression when compared with other odontogenic cysts and also present some genetic alterations, which may contribute to a greater invasive capacity and higher recurrence rates observed in KOT (2,3). Moreover, the action of prostaglandins, interleukins, epithelial (EGF) and transforming growth factors (TGF), matrix metalloproteinases, tumoral necrosis factor-alpha (TNF-α), appear to play an important role in the pathogenesis and growth of RC (4,5).

Syndecan-1, also denominated CD138, is a transmembrane heparan sulfate proteoglycan expressed in endothelial cells, stromal fibroblasts and inflammatory cells (6-9). However, its maximum expression is observed in normal epithelium, particularly in stratified squamous epithelium of the skin (10). Syndecan-1 is essential for the maintenance of epithelial morphology and control of cytoskeletal organization. In addition, it

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is important for the expression of adhesion molecules, cellular growth dependent on anchorage (11,12) as well as for epithelial-mesenchymal interactions (8,13). This proteoglycan is also expressed in odontogenic epithelium and mesenchyme during tooth development (14).

The most dramatic changes in the expression of syndecan-1 occur during embryogenesis, repair and carcinogenesis, reflecting changes in the behavior, shape, growth, migration and cytoskeletal organization of the cells (9,11,12). It has been demonstrated that the absence of syndecan-1 expression in solid ameloblastomas is correlated with a more aggressive biological behavior (15). Recently, Mesquita et al. (16) observed that both odontogenic epithelium and granular cells were positive for syndecan-1 in a case of central granular cell odontogenic tumor, suggesting reciprocal interactions between odontogenic epithelium and granular cells and that syndecan-1 may be involved in the development of this tumor.

As odontogenic cystic lesions are essentially epithelial pathologies, and considering the clinical-pathological differences existing between them, syndecan-1 could eventually play a role in the growth and biological behavior of these lesions. Nevertheless, no study has evaluated the expression of this protein in odontogenic cystic lesions. Therefore, the aim of this study was to evaluate the immunohistochemical expression of syndecan-1 in RC, DC and KOT. Moreover, in order to assess the proliferative activity and possible correlations with syndecan-1, Ki-67 expression was also evaluated.

MATERIAL AND METHODS

Between 1996 and 2006, the cases of RC that showed a well formed cystic cavity with stratified and squamous epithelial lining, cases of DC with adequate radiographic features to perform a correct diagnosis and all cases of KOT from the Oral Pathology Laboratory of the University of Ribeirão Preto, Ribeirão Preto, SP, Brazil, were selected for this study. According to the case selection criteria, 74 cases of odontogenic cystic lesions were studied, being 35 RC, 22 DC and 17 KOT. Among the KOT, there were no cases associated with the nevoid basal cell carcinoma syndrome. Clinical and epidemiological data, such as age, gender and site of the lesion, were collected from the patient charts filed in the Oral Pathology Laboratory. This study was approved by the Research Ethics Committee of the University of Ribeirão Preto, Brazil.

All cases were histologically reviewed by an oral pathologist (author D.E.C.P.) to confirm the diagnosis. The inflammatory reaction present in the cystic capsules was classified as mild (foci of inflammatory cells) or intense (diffuse inflammatory reaction).

Immunohistochemical reactions against syndecan-1 protein (clone MI15, dilution 1:200) and Ki-67 antigen (clone MIB-1, dilution 1:200) (Dako, Glostrup, Denmark) were performed in 3-µm-thick histological sections. Antigen retrieval was performed in a pressure cooker for 4 min using a 10 mM citrate buffer (pH 6.0), incubation with the primary antibodies for 18 h at 4°C, and secondary antibodies conjugated to a streptavidin-biotin-peroxidase system (strept ABComplex/HRP duet, mouse/rabbit; Dako), followed by application of diaminobenzidine as the chromogen. Slides were counterstained with Harris hematoxylin, mounted, and analyzed by two of the authors (M.R.N. and D.E.C.P.). Syndecan-1 expression was considered positive when immunoexpression was observed in the cell membrane and/or cytoplasm of the epithelial cells. Positive (tonsil for syndecan-1 and breast carcinoma for Ki-67) 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 cystic lesion, using the following criteria: negative: <5% of cells positive; low expression: 5%-50% of cells positive; and high expression: >50% of cells positive. For syndecan-1, the intensity of positive epithelial cells was also analyzed (+, weak intensity; ++, strong intensity), while for Ki-67, the basal and suprabasal layers of the epithelial cystic lining were analyzed separately.

For statistical analysis, the immunoexpression of the studied proteins was compared among the lesions; in each lesion the correlations between the studied antibodies were established separately. Correlations were evaluated by the Fisher's exact test and Spearman's correlation coefficients at a significance level of 5%.

RESULTS

Radicular Cyst

Of the 35 cases of RC, 21 (60%) occurred in men. The patients' mean age was 42.2 years (ranging from 19 to 78 years) and the maxilla was more affected (57.1%) than the mandible (42.9%). Microscopically,

non-keratinized stratified squamous epithelial tissue of variable thicknesses was observed. Twenty-three cases (65.7%) presented a fibrous capsule with intense lymphocytic inflammatory reaction and mild inflammation was observed in 12 cases (34.3%).

Syndecan-1 was expressed in 30 cases (85.7%); in 21 cases (70%) there was high expression of syndecan-1 and in 19 (90.5%) cases, strong intensity was also observed (Fig. 1). In regions with intense inflammatory reaction, syndecan-1 expression was absent or diminished (Fig. 2). The inflammatory cells of 23 lesions (65.7%) were positive for syndecan-1 (Fig. 2). Twenty-one cases (60%) were positive for Ki-67, 16 (76.2%) presenting low positivity and 5 (23.8%) showing high positivity. Ki-67 expression was restricted to the basal layer cells of the epithelial lining, except for one case that presented positive suprabasal cells.

Dentigerous Cyst

In the 22 cases of DC, the patients presented a mean age of 29 years (ranging from 7 to 68 years). Thirteen cases (59.1%) occurred in men. The maxilla was affected in 17 cases (77.3%). Microscopically, the epithelium was arranged in 2 to 5 cellular layers, frequently formed by cubic epithelial cells. Eleven cases (50%) presented a mild and 2 (9.1%) an intense chronic inflammation in the fibrous capsule.

Syndecan-1 was positive in 21 cases (95.5%), with high positivity in 14 cases (66.7%), and 13 of these (92.8%) also presenting intense marking (Fig. 3). The inflammatory cells present in the fibrous capsule of 13 DC were also positive for syndecan-1. Twelve cases (54.5%) were positive for Ki-67 and all presented low expression; of these 10 (83.3%) showed positivity restricted to the basal layer, and in 2 cases (16.7%) there was expression in both basal and suprabasal cells.

Keratocystic Odontogenic Tumor

Most cases occurred in men (58.8%), with a mean age of 45.8 years (ranging from 13 to 78 years) and the mandible (70.6%) was the most affected site. Microscopically, epithelial lining composed of 6 to 8 cellular layers with corrugated superficial parakeratin and basal layer composed of cells disposed in palisade were observed. All the KOT presented a parakeratin surface. Inflammatory infiltrate in the fibrous capsule was observed in 7 cases (41.2%). In regions that presented a more intense inflammatory reaction, the epithelium frequently presented thickening, with rete processes, loss of keratinized surface and consequently of its conventional architecture.

Syndecan-1 was positive in all cases, 16 (94.1%) with high expression and strong intensity (Fig. 4) and 1 (5.9%) with low expression and weak intensity. In regions in which there was an alteration in epithelial morphology due to an inflammatory infiltrate, the

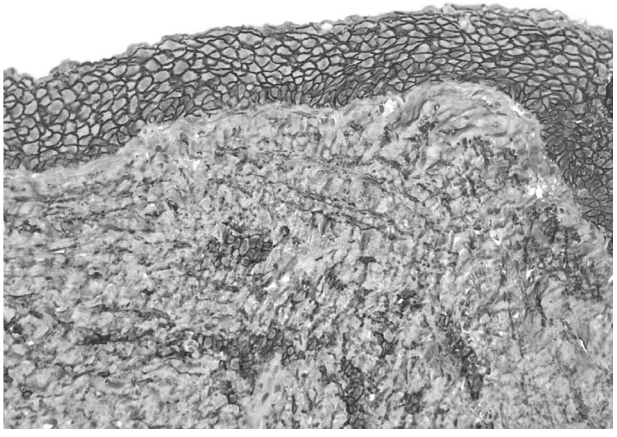

Figure 1. Strong intensity and high expression of syndecan-1 in the epithelial lining of a radicular cyst. In the fibrous capsule, there are scarce lymphocytes positive for syndecan-1 (original magnification, ×200).

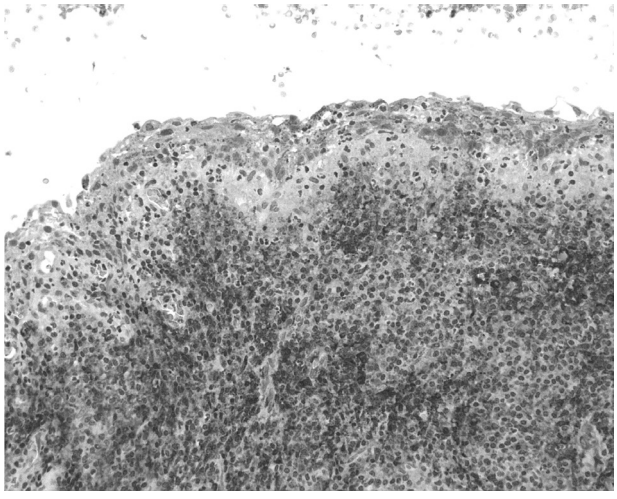

Figure 2. Decrease in syndecan-1 expression in regions with inflammatory infiltrate in radicular cyst. Note that inflammatory cells present high expression for syndecan-1 (original magnification, ×200).

expression of syndecan-1 was absent or low (Fig. 5); however, if the epithelial morphology was preserved, expression was not altered, even in the presence of inflammation. Similar to RC and DC, syndecan-1 expression was observed in some inflammatory cells present in 7 cases of KOT.

Sixteen cases (94.1%) were positive for Ki-67, among which 15 (93.7%) presented low expression. The suprabasal layer showed positivity in all the Ki-67 cases, while basal layer cells were positive in 8 cases (50%).

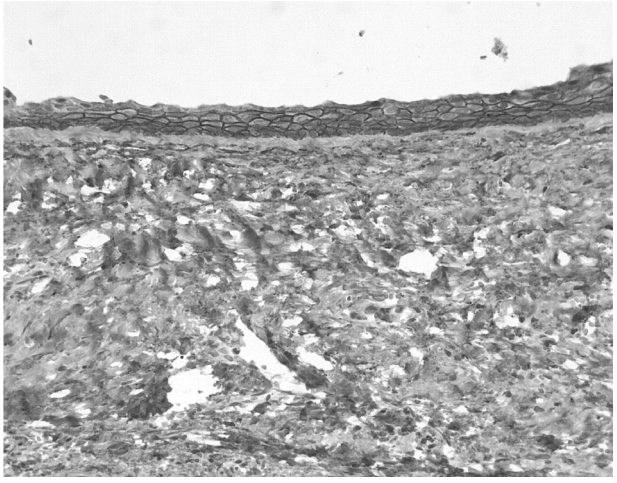

Figure 3. Epithelial cells from a dentigerous cyst presenting strong intensity and high syndecan-1 expression (original magnification, ×200).

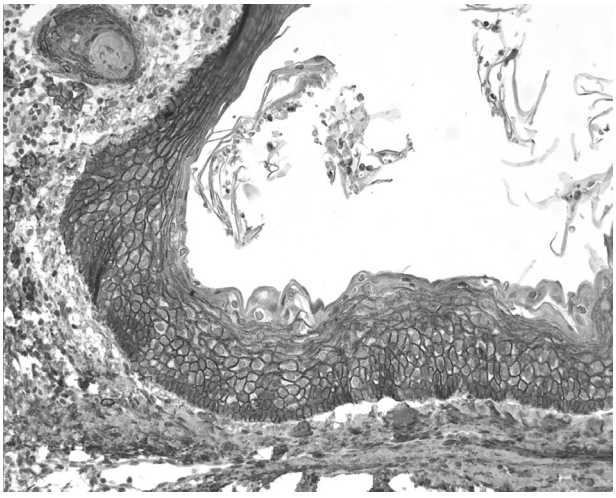

Figure 4. Strong intensity and high syndecan-1 expression in the epithelial lining of a keratocystic odontogenic tumor (original magnification, ×200).

Statistical Analysis

Although differences were observed among the lesions regarding the number of positive cases for basal and suprabasal Ki-67 and syndecan-1 expression and intensity, these differences were not statistically significant. However, KOT presented significantly higher positivity for Ki-67 in the suprabasal layer ($p<0.001$) than the other studied lesions (Table 1).

In the RC cases, positive correlation was observed between Ki-67 expression and intensity of syndecan-1 ($p=0.01$) and between the expression and intensity of syndecan-1 ($p<0.001$). In the KOT cases, positive correlation was observed between the expression and intensity of syndecan-1 ($p<0.001$). Moreover, Ki-67 expression in the suprabasal layer correlated positively with the expression ($p=0.01$) and intensity ($p=0.01$) of syndecan-1. Similar to other lesions, positive correlation was observed between the expression and intensity of syndecan-1 ($p=0.03$) in the DC cases.

DISCUSSION

Syndecan-1 presents greater expression in well-differentiated epithelial tissues, mainly the stratified and squamous tissues that compose the skin. On the other hand, decrease of its expression alters the cellular morphology and organization, arrangement

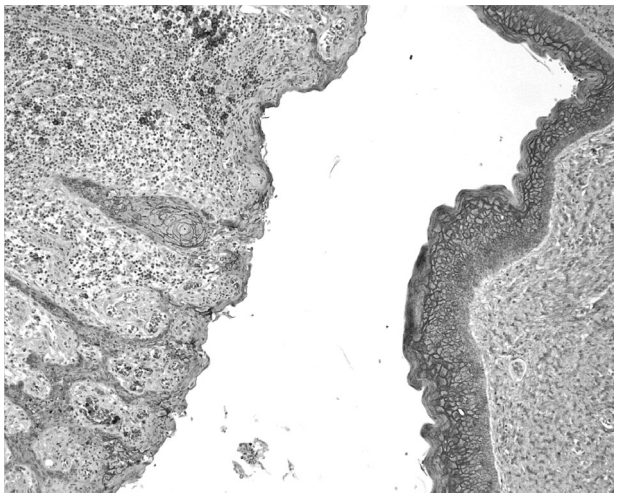

Figure 5. On the right, typical epithelial lining of keratocystic odontogenic tumor presenting strong intensity and high syndecan-1 expression; on the left, decrease in syndecan-1 expression in the epithelium with loss of keratinized surface and formation of rete processes, with adjacent inflammation (original magnification, ×200).

and expression of adhesion molecules and the growth control dependent on anchorage, which represents an indicator of malignant transformation, invasive growth and cellular atypia (11,15). Diminished syndecan-1 expression in head and neck squamous cell carcinomas has been shown to correlate with poor prognosis (9,17). Nevertheless, the greater expression of syndecan-1 correlated with worse prognosis in breast and pancreas carcinomas, and in multiple myeloma (6,18).

As syndecan-1 is typically expressed in epithelial cells, its expression can be important not only in malignant neoplasias, but also in benign tumors and lesions of the epithelial tissue, as are the majority of the odontogenic lesions. Recently, some authors reported that syndecan-1 expression in the extracellular matrix of ameloblastomas and diminished expression in tumor cells can determine a more aggressive local behavior of these lesions (7,15). This protein was also identified in the odontogenic epithelium and granular cells in a central granular cell odontogenic tumor, suggesting an important role in cell-cell adhesion and reciprocal interaction between these cell groups (16). Nevertheless, there are no studies evaluating the expression of syndecan-1 in odontogenic cystic lesions.

In the present study, syndecan-1 expression was intense in the epithelial lining of all the cystic lesions, being positive in 85.5% of RC, 95.5% of DC and in all KOT cases. However, differences among the cystic lesions were not statistically significant. Interestingly, in RC, the epithelium located in regions with intense inflammatory reaction presented diminished or absent syndecan-1 expression. It is likely that the alterations caused by the inflammatory cells in the epithelial lining contributed to decreasing the expression of the protein. On the other hand, in KOT, in areas of typical epithelial lining, high and intense expression was maintained even in the presence of inflammatory cells. However, in areas in which there was epithelial

Table 1. Comparative analysis of Ki-67 and syndecan-1 expression in radicular cysts (RC), dentigerous cysts (DC) and keratocystic odontogenic tumors (KOT).

Variables	Odontogenic cysts			p value
	RC n=35 (%)	DC n=22 (%)	KOT n=17 (%)	
Ki-67 expression (basal layer)				
Negative	15 (42.9)	10 (45.5)	9 (52.9)	
Low	15 (42.9)	12 (54.5)	7 (41.2)	0.4
High	5 (14.2)	0 (0.0)	1 (5.9)	
Ki-67 expression (suprabasal layer)				
Negative	34 (97.1)	20 (90.9)	2 (11.8)	
Low	1 (2.9)	2 (9.1)	14 (82.3)	<0.0001*
High	0 (0.0)	0 (0.0)	1 (5.9)	
Syndecan-1 expression				
Negative	5 (14.3)	1 (4.6)	0 (0.0)	
Low	9 (25.7)	7 (31.8)	1 (5.9)	0.08
High	21 (60.0)	14 (63.6)	16 (94.1)	
Syndecan-1 intensity				
Negative	5 (14.3)	1 (4.6)	0 (0.0)	
Weak	6 (17.1)	3 (13.6)	1 (5.9)	0.3
Strong	24 (68.6)	18 (81.8)	16 (94.1)	

*Statistically significant.

hyperplasia secondary to inflammatory reaction, expression was diminished or absent. Thus, similar to other epithelial tissues, syndecan-1 seems be essential for maintaining the epithelial morphology and eventually controlling the cytoskeletal organization of the epithelial lining in odontogenic cystic lesions (11,12).

Syndecan may bind several extracellular matrix molecules and thus, can be considered as a multipotent matrix receptor (19). During odontogenesis, syndecan expression is similar to that of tenascin, a known ligand for syndecan (19). Thus, the simultaneous expression of syndecan and tenascin during tooth development suggests that they could form ligand-matrix receptor complexes, which can play an important role in the mesenchymal condensation and differentiation (19), essential phases of tooth formation. In odontogenic cystic lesions, originating from the odontogenic epithelium remnants, these complexes formed by syndecan and tenascin can be important for the maintenance of the cystic lining epithelium, since tenascin is largely expressed in these lesions (20).

There is evidence that syndecan-1 participates in various processes involved in the modulation of inflammation, mainly in interactions between leukocytes and endothelial cells (13), which explains its expression in the inflammatory cells - 65.7% of RC, 59.1% of DC and 41.1% of KOT that presented secondary inflammation. Therefore, particularly in RC, an inflammatory odontogenic cyst, the expression of syndecan-1 in inflammatory cells could indicate another pathway of action of this proteoglycan in this lesion.

Ki-67 is commonly used to evaluate the proliferative activity in neoplastic and non-neoplastic lesions, including odontogenic cysts (21,22). Ki-67 is significantly more expressed in the suprabasal layer of KOT than the other odontogenic cystic lesions (3,22), as occurred in the present study. This finding may explain the infiltrative growth and higher recurrences rates presented by KOT, when compared to other cystic lesions, including RC and DC. Moreover, positive correlation was found between the expression of Ki-67 and syndecan-1 in the RC and KOT in this study.

In summary, syndecan-1 expression does not seem to be a determinant factor of the distinct histopathological features and biological behavior of the studied cystic lesions. Nevertheless, positive correlation between syndecan-1 and a cell proliferation marker was obserevd in RC and KOT.

RESUMO

O objetivo deste estudo foi avaliar a expressão imunoistoquímica de syndecan-1 (CD138) e Ki-67 em cistos radiculares (CR), cistos dentígeros (CD) e tumores odontogênicos queratocísticos (TOQ). Trinta e cinco CR, 22 CD e 17 TOQ foram utilizados no estudo e as reações imunoistoquímicas usando os anticorpos anti-syndecan-1 e anti-Ki-67 foram realizadas pelo método estreptavidina-biotina-peroxidase. Para análise estatística, o teste exato de Fisher e o coeficiente de correlação de Spearman foram utilizados. Entre as lesões estudadas, não foram observadas diferenças na expressão de syndecan-1, mas a expressão suprabasal de Ki-67 foi significativamente maior nos TOQ ($p<0,0001$), quando comparado aos CR e CD. No CR, havia correlação positiva entre a expressão ($p=0,02$) e intensidade ($p=0,0001$) de syndecan-1 e entre a intensidade de syndecan-1 e expressão de Ki-67 ($p=0,01$). No TOQ, a expressão de Ki-67 na camada suprabasal correlacionou positivamente com a expressão ($p=0,01$) e intensidade de expressão ($p=0,01$) de syndecan-1. A expressão de syndecan-1 não parece ser um fator determinante das características histopatológicas distintas e do comportamento biológico das lesões estudadas. Entretanto, correlação positiva entre syndecan-1 e um marcador de proliferação celular foi evidenciado em CR e TOQ.

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