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Tenascin and fibronectin expression in odontogenic cysts

Marcílio Dias Chaves de Oliveira[1], João Luiz de Miranda[2], Rivadávio Fernandes Batista de Amorim[1], Lélia Batista de Souza[1], Roseana de Almeida Freitas[1]

[1]*Department of Oral Pathology, School of Dentistry, Federal University of Rio Grande do Norte, Natal-RN, Brazil;* [2]*Department of Oral Pathology, School of Dentistry, Federal University of Diamantina, Diamantina-MG, Brazil*

BACKGROUND: Odontogenic cysts (OCs) present distinct evolution and clinical behavior. This study was performed in order to investigate if some components of the extracellular matrix (ECM) may drive these differences.
METHODS: Thirty OCs were selected: 10 radicular cysts (RCs), 10 dentigerous cysts (DCs), 10 non-syndrome odontogenic keratocysts (OKCs) and they were immunohistochemically analyzed to verify the expression pattern of tenascin and fibronectin.
RESULTS: Tenascin immunostaining was mainly intense as a thick band deep to the epithelial-mesenchymal interface in both RCs and OKCs. The intense tenascin immunoexpression observed in the RCs was usually associated with inflammation. An intense fibronectin reactivity was observed in the basement membrane region and at the cystic wall of OKCs.
CONCLUSIONS: Our results demonstrate differences between the expression of ECM proteins in the OCs studied. The higher tenascin and fibronectin expression in the capsule of OKCs suggests marked instability in the cystic structure and may contribute to its aggressive behavior.
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Keywords: dentigerous cyst; extracellular matrix; fibronectin; keratocyst; odontogenic cyst; radicular cyst; tenascin

Introduction

The extracellular matrix (ECM) plays an important role in the maintenance of a correct microenvironment for basic cell functions such as cell adhesion, proliferation and differentiation. Changes in this microenvironment may affect the epithelial-mesenchymal interactions, which are necessary for the correct control mechanism of tissues and organs in development. These interactions represent a form of local control device and can regulate the development of many lesions. Moreover, the ECM components may act as mediator molecules in several tissue interactions (1, 2).

During tooth formation, the matrix components play a crucial function in the histodifferentiation and morphodifferentiation processes. After complete tooth development, epithelial remains may continue inactive for an undetermined period. Nonetheless, under the influence of unknown stimuli, it can initiate the related odontogenic tumors and cysts (3–6).

As morphogenesis and cell differentiation in the developing tooth are controlled by series of reciprocal interactions between the epithelial and mesenchymal tissues, it's been pointed out that the development of odontogenic tumors and cysts arising from tissues remains of odontogenesis is also dependent on these interactions (1, 4, 7). The role of some ECM components such as fibronectin, tenascin, syndecan, collagen type I and IV, laminin, and heparan sulfate during odontogenesis have been studied by several reports (3, 6, 8–14). In contrast, the distribution of these proteins in odontogenic cysts (OCs) has not been widely investigated and there are only a few studies published currently (15, 16).

For a long period, it had been considered that the OCs expansion was related only to osmotic pressure exerted by the cyst contents (17). Nevertheless, some experiments have demonstrated not only the proliferative potential importance of the cystic lining epithelium but also the properties cystic wall in the growth of OCs, especially of the odontogenic keratocyst (OKC) (18–23).

Based on the fact that fibronectin is considered to be an important cell attachment protein during morphogenesis and cell differentiation, and also that tenascin has been suggested to be involved in epithelial-mesenchymal interactions during organic and neoplastic development (1, 24), the purpose of the present study was to investigate these two extracellular proteins in OCs by an immunohistochemical assessment.

Materials and methods

Tissue specimens

Thirty cystic odontogenic lesions were selected from the files of the Department of Oral Pathology of the Federal

Correspondence: Roseana de Almeida Freitas, Programa de Pós-Graduação em Patologia Oral, Departamento de Odontologia – Universidade Federal do Rio Grande do, Norte (UFRN), Av. Senador Salgado Filho, 1787, Cep. 59056-000, Lagoa Nova, Natal-RN, Brasil. Tel./Fax: +55 84 215 4138. E-mail: roseana@patologiaoral.com.br. Website: http://www.patologiaoral.com.br
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University of Rio Grande do Norte and they were distributed as follows: 10 radicular cysts (RCs), 10 dentigerous cysts (DCs) and 10 non-syndrome OKCs. All the diagnoses were made using criteria in the 1992 WHO classification (25).

Immunohistochemical methods

Paraffin tissue sections 3 μm thick were deparaffinized and immersed in methanol. An antigen retrieval for tenascin and fibronectin was employed in which the sections were taken through 0.4% pepsin (37°C) for 30 min and 1.0% pepsin (37°C) for 60 min, respectively. After treatment with normal serum, the specimens were incubated in a moist chamber with the monoclonal mouse anti-human primary antibody for tenascin (TN2 clone, code M0636, IgG_1 kappa isotype, Dako, Glostrup, Denmark), diluted 1:100, at 4°C overnight (18 h), and polyclonal rabbit anti-human primary antibody for fibronectin (code A-245; Dako), diluted 1:500, at room temperature for 120 min. Following incubation with the primary antibodies, the sections were washed twice in phosphate buffered saline (PBS), incubated with an appropriate biotinylated secondary antibody and treated with streptoavidin-biotin complex (Dako) for 30 min at room temperature. The sections were visualized with diaminobenzidine (D5637; Sigma Chemical, St. Louis, MO, USA), finally counterstained with Mayer's hematoxylin and coverslipped. Blood vessels in the cystic wall were used as intern positive control for tenascin and fibronectin. An irrelevant, isotype-matched antibody (IgG1, X931; Dako) was used as a negative control instead of monoclonal antibody. As negative controls for polyclonal antibody, tissues sections were treated with bovine serum albumin (BSA) in PBS instead of the primary antibody.

Immunohistochemical analysis

Under light microscope, the staining pattern of tenascin and fibronectin were evaluated considering location and intensity of immunostatining. The protein expression was graded according to its degree of intensity such as weak (+), moderate (++) or intense (+++).

Results

Tenascin was immunoexpressed as a variable fibrillar, fibroreticular and reticular band in the epithelial-connective interface of RCs. The scores of staining intensity reached are listed in the Table 1. In RCs, the intense tenascin reactivity could be detected associated with inflammatory areas and eight specimens exhibited mild intraepithelial staining (Fig. 1a). In the DCs, there was a thin positive tenascin band along the epithelial-connective interface showing a reticular pattern of staining and the majority displayed only weak expression (Fig. 2a; Table 1). In addition, five DCs showed mild intraepithelial staining. Finally, tenascin staining in OKCs showed a thick reticular and/or fibrillar positive band deep to the epithelium and an intense immunoreactivity in half of the cases (Fig. 3a; Table 1). Accordingly, it could be visualized intraepithelial staining in seven OKCs.

Table 1 Tenascin and fibronectin immunoexpression scores reached in RCs, DCs and OKCs

	Tenascin			*Fibronectin*		
Case	*RCs*	*DCs*	*OKCs*	*RCs*	*DCs*	*OKCs*
1	+	+	+++	+	+	++
2	++	+	+	+	+	++
3	+	+	+	+	+	++
4	+++	++	++	+	+	++
5	+++	+	+++	+	+	++
6	+	+	+++	+	+	++
7	+++	–	+	+	+	++
8	+++	+	+++	+	+	++
9	+	+	+	++	+	++
10	+++	+	+++	+	+	++

+, weak; ++, moderate; +++, intense; –, non-reactive.

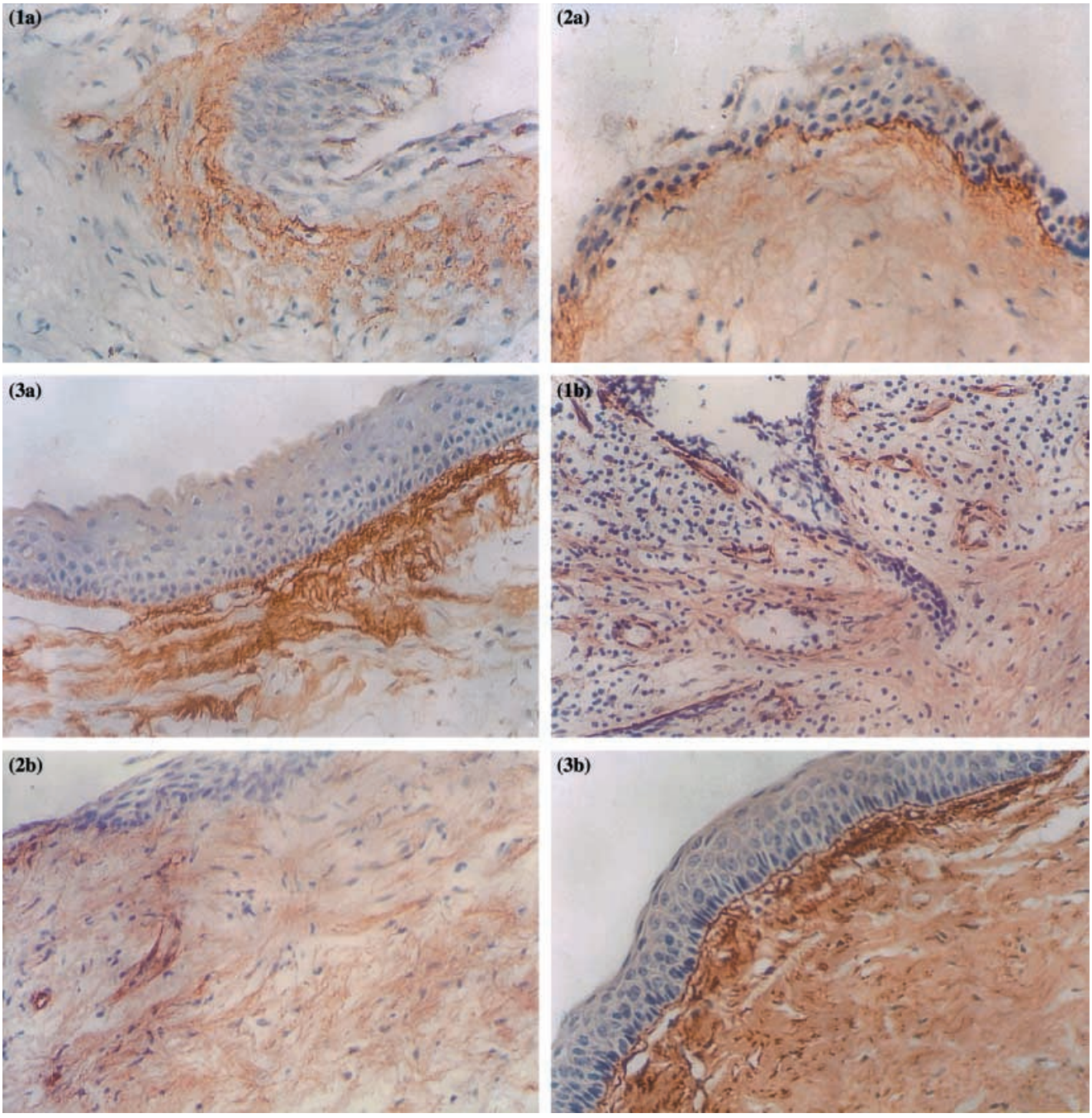
In RCs, fibronectin immunostaining was observed in all specimens showing predominantly a fibroreticular pattern (Table 1; Fig. 1b). At the basement membrane, this glycoprotein was visualized as a thin discontinuous positive line in all specimens. In the same way, DCs demonstrated a similar fibronectin staining pattern compared with RCs (Fig. 2b; Table 1). On the contrary, the OKCs demonstrated a fibrillar compacted arrangement and reticular pattern of fibronectin expression with a moderate intensity (Table 1; Fig. 3b). Fibronectin was visualized as either a continuous (six cases) or discontinuous (four cases) line at basement membrane in OKCs (Table 1).

Discussion

After complete tooth formation, the epithelial residues of the odontogenesis remain inactive for an indeterminate period. When these epithelial rests are stimulated, they proliferate and might initiate the odontogenic cystic lining. These epithelial cell properties are regulated by ECM (24, 26). Uitto et al. (27) demonstrated that the periodontal ligament epithelial cells attach and spread on different kinds of ECM proteins.

It is well recognized that the endotoxins from necrotic tooth pulp stimulate the epithelial rests of Malassez giving origin to RC (28, 29). According to Nishimura et al. (30), the epithelial rests of Malassez perform migrate, proliferate and differentiate in the formation of the epithelial lining of the RC and that these properties of the epithelial cells are regulated by the ECM. However, the stimulus and the mechanisms for the development and progression of DC and OKC are still discussed (31–34).

Several studies have been performed regarding the epithelial and mesenchymal features in various OCs in order to elucidate the distinct biological behaviors and also for diagnostic reasons. Scharffetter et al. (35) observed that fibroblasts of the OKC cystic capsule as well as the epithelial cells lining have a higher proliferative potential compared with DCs and RCs. Indeed, Stenman et al. (36) and Hume et al. (18) demonstrated that the OKCs epithelial cells are more capable of proliferating than epithelial cells of DCs and RCs



Figures 1–3 Immunohistochemical detection of tenascin (1) and fibronectin (2) in odontogenic cysts. (1a) Fibroreticular pattern of tenascin beneath the epithelium in RC and presence of intraepithelial staining (SABC-200×). (2a) Tenascin distribution in DC showing a fibrillar pattern in juxtaepithelial position (SABC-200×). (3a) Intense tenascin staining in OKC as a fibrillar band in the epithelia-connective interface (SABC-200×). (1b) Fibronectin distribution in RC showing a reticular band immediately beneath the epithelium and a fibrillar arrangement in the boundaries of the field (SABC-200×). (2b) Fibrillar pattern of fibronectin throughout the cystic capsule in DC (SABC-200×). (3b) Intense immunostatining of fibronectin at the basement membrane and a fibrillar arrangement in the cystic capsule of OKC (SABC-200×).

in vitro. By analyzing 31 OCs, Li et al. (37) reported an intense proliferating cell nuclear antigen (PCNA) immunoreactivity in OKCs, especially in the suprabasilar cell epithelial layer, and concluded that these findings may be related to the more aggressive nature of this lesion. Other comparative studies have also demonstrated that the OKCs present a higher proliferative and aggressive potential compared with DCs and RCs by using cellular biological markers such as PCNA (38), Ki-67 (34), p53 (39–42) and argyrophilic nucleolar organizer regions (AgNOR) (18, 43).

The aggressive behavior of OKCs is related to factors inherent in the epithelium, cystic capsule and to its proneness of recurrence after treatment (44–46). The elevated recurrent potential is associated to the difficulty in its complete removal due the thinly and friable nature

of the cyst wall (47). As stated by Browne (48), the principal cause for OKCs recurrence is the disruption of the cystic epithelium, which is probably associated with deficient formation of the basal membrane of the lining cyst. Giving support to this hypothesis, the experiment of Oliveira et al. (49) showed weak expression of laminin and collagen type IV in OKCs compared with DCs and RCs.

If the properties of epithelial remains can be regulated by ECM (30), it is possible that the cystic growth is dictated not only by the proliferative potential of cystic lining but also by the characteristics and components of the ECM.

Tenascin and fibronectin are adhesion glycoproteins of the ECM that have been associated with several interactions in cell-matrix during normal embryonic development, tissue repair, inflammatory and neoplastic processes (50, 51). These proteins interact with several other macromolecules at the cell surface, basal membrane and interstitial ECM (52).

Bellinghieri et al. (53) demonstrated a striking role of ECM proteins in the development of renal cysts in patients with renal polycystic disease. However, the role of matrix proteins in the development of OCs have not yet been demonstrated. To date, data on the ECM immunoexpression in OCS are scarce, with no previous attempts having been reported to compare the presence and distribution of these proteins between them.

In the present study, tenascin immunoreactivity was more evident in the OKCs capsule and basement membrane than in the DCs and RCs. In addition, the OKCs basement membrane displayed a more continuous tenascin positivity than DCs and RCs did. The epithelial–mesenchymal interactions are intense during embryonic development period (50–52). As the OKC arises from more primitive odontogenic epithelia, the epithelial–mesenchymal interactions during its development must be very intense. This may be one of the reasons of a stronger tenascin expression in this cyst.

The results of this study demonstrated a diffuse distribution of fibronectin at the fibrous capsule of all studied cysts in a fibrillar or reticular pattern and was more intense in the OKCs. The immunostatining of this protein was also observed in the basement membrane of the investigated cysts, but in the OKCs this reaction was more intense and showed a markedly continuous pattern. During odontogenesis, Thesleff et al. (3) demonstrated the role of fibronectin in the polarization process of the odontoblasts. In addition, Medeiros (54) studied the role of ECM proteins in all histological types of ameloblastoma and detected the presence of fibronectin in the epithelial-stroma interface in all types except in the desmoplastic ameloblastoma. Inasmuch as the desmoplastic type does not shown a striking polarization in the outer layer of epithelial tissues and that the other types commonly do exhibit this feature, the author stated that the fibronectin could be related to the polarization of the cells located at that site. Based on this data, it can be hypothesized that the characteristic pattern of the OKCs lining epithelial basal cells might be related to the strong presence of fibronectin in the basement membrane.

Apart from the previous considerations, another aspect concerning the evaluation of tenascin and fibronectin should be pointed out: taken together, the pattern of expression of these proteins in the OKCs suggests strong instability in the cystic structure. This statement is consistent with data from previous studies such as those performed by Scharffetter et al. (35) and Oliveira (2002). In the same way, Hirshberg et al. (21) demonstrated that the collagen fibers of the OKC capsule are structurally disorganized which indicates instability in the cystic architecture. Thus, it is reasonable to propose that the enhanced fibronectin and tenascin expression in OKCs may influence the epithelial–mesenchymal signalizing relations and propitiate a more favorable microenvironment for epithelial proliferation and migration.

One interesting finding observer in the present experiment was the weak immunoexpression of fibronectin in the cystic wall of SOKC. According to Labat-Robert, during tumor growth, invasion, metastasis, angiogenesis, ECM proteolysis is a crucial step. Tumor cells secrete high levels of proteolytic enzymes degrading ECMs.

An unexpected and surprising finding was the mild intraepithelial tenascin expression in the lining of studied cysts. This finding is similar to that of Lukinmaa et al. (16) who demonstrated intraepithelial tenascin immunostaining in odontogenic calcifying cyst. In view of this, it is worth investigating whether tenascin has an influence on the epithelial cavitation process of OCs.

In summary, the present study demonstrates substantial differences between tenascin and fibronectin expression in the studied OCs. Particularly, the more evident expression of tenascin in OKCs capsule suggests marked instability in this cystic structure and may contribute to the more aggressive behavior of OKCs compared to RCs and DCs.

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