Histochemical and immunohistochemical differences between solitary oral fibroma and fibrous papule of the face*

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Abstract: Background: The morphological similarities between fibrous papules of the face and multiple sporadic oral fibromas were mentioned long ago and a relationship between them has been reported in the literature.

Objective: The aim of this study was to evaluate the participation of mast cells, elastin and collagen in a series of oral fibromas and fibrous papules of the face in order to better understand the possible role of these factors in fibrosis and the formation of these lesions.

Methods: Thirty cases of oral fibroma involving the buccal mucosa and 30 cases of fibrous papules of the face were selected. Tissue samples were submitted to picrosirius red staining and immunohistochemistry using anti-elastin and anti-tryptase antibodies.

Results: The percentage of tryptase-positive mast cells and expression of elastin were higher in cases of fibrous papules of the face (p < 0.05). In contrast, a higher intensity of collagen deposition was observed in oral fibromas. The results showed mast cell accumulation and higher elastin synthesis in fibrous papules of the face, and mast cell accumulation with higher collagen fiber synthesis in oral fibromas.

Conclusion: These findings support the hypothesis that mast cells influence the development and growth of these lesions through different mechanisms.

Keywords: Collagen; Elastin; Immunohistochemistry; Mast cells

INTRODUCTION

Fibrous papules (FP) of the face are small, benign swellings on the skin characterized by an increase in blood vessels in the dermis and underlying fibrous stromal tissue. Some authors regard FP as regressed melanocytic nevi, whereas others have suggested these lesions are fibromas with a melanocytic component, or a type of angiofibroma.4-6 Oral fibroma (OF) is the most common benign tumor of the oral cavity and it is typically characterized by dense, fibrous tissue with numerous fibroblasts and an overlying epithelium that is usually thinned. The term “irritation fibroma” is also used as a synonym for solitary OF, in contrast to OF associated with a phakomatosis, which is usually multiple.5 The morphological similarities between FP of the face and multiple OF were mentioned long ago, especially when OF appeared in a context of phakomatoses.4 A recent study has demonstrated that solitary OF shares many morphological features with FP of the face, including a fibrous and collagenized stroma, dilated blood vessels, concentric perivascular fibrosis and multinucleated cells.6 This seems to support the hypothesis that sporadic OF is a member of the group of angiofibromatous lesions, which includes FP of the face and multiple fibromas, seen in some phakomatoses.4

Much attention has been paid to the participation of mast cells (MC) in the events of extracellular matrix synthesis and remodeling. Mast cells are bone marrow-derived cells, widely found in human tissues.7-9 The role of MC in connective tissue is still a matter of speculation and it has been suggested that these cells participate in cell regulation and in controlling the accumulation of connective tissue components. Previous reports indicate that MC are poten-
tially fibrogenic since they secrete potent mediators of fibrosis.\textsuperscript{5,10} The identification of MC subpopulations requires the detection of enzymes secreted by these cells, mainly tryptase and chymase.\textsuperscript{11,12} Tryptase is found in all human MC, but has not been detected in any other cell type. Therefore, the detection of tryptase in human biological fluids and tissues is interpreted as an indicator of mast cell activation.\textsuperscript{13}

The secretion of endopeptidases by MC modulates inflammation, matrix destruction, tissue remodeling, and angiogenesis.\textsuperscript{14,17} Studies have shown that tryptase upregulates fibroblast proliferation, indicating that, once activated, MC can affect the mitogen-induced proliferation of fibroblasts and stimulate fibrosis.\textsuperscript{18,19} Direct and/or indirect interactions between MC and fibroblasts promote connective tissue reorganization. The process of tissue remodeling involves the synthesis of all extracellular matrix components by fibroblasts, including collagen and elastin fibers. Elastin and microfibrils comprise the elastic fiber system. Studies have demonstrated the participation of elastin in the development of fibrous lesions in the oral mucosa.\textsuperscript{20} In this respect, fibroblasts that are stimulated by chemical mediators released by MC are expected to produce higher amounts of the fibrillar component of extracellular matrix, thus contributing to tissue fibrogenesis.

The aim of this study was to evaluate the participation of mast cells, elastin and collagen in a series of sporadic OF and FP of the face using immunohistochemical and histochemical methods, in order to better understand the possible role of these factors in fibrosis and the formation of these lesions.

METHODS

The sample consisted of 60 paraffin-embedded tissue specimens, including 30 cases of solitary OF of the buccal mucosa and 30 FP of the face, obtained from the Pathological Anatomy Service of the Department of Oral Pathology and from a private surgical pathology laboratory, respectively. None of the patients had hematomatosis.

Histochemistry

To evaluate the intensity of collagen deposition, 5-µm-thick sections were stained with picrosirius red and analyzed under an Olympus CX31 light microscope. The following scores were attributed: (weak) ≤ 50% stained fibers, and (strong) > 50% stained fibers.

Immunohistochemical analysis

For the immunohistochemistry, 3-µm-thick sections were cut from paraffin-embedded tissue blocks, deparaffinized, and immersed in 3% hydrogen peroxide to block endogenous peroxidase activity. The sections were then washed in phosphate-buffered saline (PBS) and submitted to antigen retrieval (Table 1). After treatment with normal serum, the sections were incubated with the primary anti-trypase and anti-elastin antibodies in a moist chamber (Table 1). Next, the sections were washed twice in PBS and incubated at room temperature with the labeled streptavidin biotin complex (LSAB+ System-HRP, Dako, Carpinteria, CA, USA) for anti-elastin and anti-trypase antibodies. Peroxidase activity was visualized 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. Sections of pyogenic granuloma and actinic cheilitis were used as positive controls for MC tryptase and elastin, respectively. As a negative control, the sections were treated as described above, except that the primary antibody was replaced with a solution of bovine serum albumin in PBS.

The slides were examined under an Olympus CX31 light microscope. Immunoreactivity of MC to the anti-trypase antibody was analyzed quantitatively in the lining epithelium and in connective tissue of the specimens. Areas of high MC density were identified at 100x magnification. The number of tryptase-positive MC was determined in up to 10 fields at 400x magnification, using a digital camera for recording. Mast cells were counted using the Image J program and the mean number of positive cells was calculated for each case. The expression of elastin was analyzed in up to 10 fields at 100x magnification, applying scores ranging from +3 to 0, adapted from Fukushima et al. and Araújo et al.\textsuperscript{21,22} A score of +3 corresponds to a diffuse increase of elastic fibers with mass pattern; +2 signifies a diffuse increase of elastic fibers; +1 reflects partial increase with focal clusters of elastic fibers; while 0 denotes the same characteristics as observed in the control group. The thickness and fragmentation of elastic fibers were also described.

Differences between groups were evaluated by the Chi-square test, Student t-test and Mann-Whitney test. All statistical calculations were performed using the Statistical Package for the Social Sciences 17.0 (SPSS, Inc., Chicago, IL, USA). A p value < 0.05 was deemed statistically significant.

RESULTS

A fibrotic and collagenized hypocellular stroma with a sparse inflammatory cell infiltrate was observed in all lesions. Multinucleated cells were scattered and non-abundant in FP. The number of dilated vessels ranged from minimal in OF to substantial in FP. Concentric fibrosis around the vessels was not a prominent feature. Hair follicles, sebaceous glands and periadnexal dermis were seen in FP, but not in OF. Vascular ectasia and perivascular fibrosis were also less striking in OF.

| Antibody | Manufacturer | Clone | Antigen retrieval | Dilution | Incubation |
|----------|--------------|-------|-------------------|----------|------------|
| Tryptase | Abcam        | AA1   | Citrate, pH 6.0, Pascal | 1:200    | 30'        |
| Elastin  | Novocastra   | BA4   | 0.1% trypsin, 30', 37°C | 1:50     | 60'        |

\textsuperscript{590}Henriques ACG, Freitas RA, Pires BC, Gurgel CA, Santos JN

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Comparison of the intensity of collagen deposition between the two groups of lesions showed strong picrosirius red staining in all OF cases, whereas weak staining was observed in most FP cases (p < 0.001, X²) (Figure 1).

Mast cells were detected in all OF and FP cases. The mean total number of tryptase-positive MC was 167.4 (SD: 51.171) in FP and 64.63 (SD: 17.022) in OF (Figure 2). The parametric t-test revealed a statistically significant difference between groups (p < 0.001). Tryptase-positive MC were detected only in connective tissue, mainly at the periphery of blood vessels (perivascular MC). The presence of MC in FP or OF was not associated with an inflammatory response since the infiltrate of inflammatory cells was sparse.

Analysis of the expression of elastin in FP showed a score of +3 in four cases, +2 in twelve, +1 in five, and 0 in nine (Figure 3). For OF, twenty-four cases were scored as zero; only six had a score of +1, while there were no scores of +2 or +3 (Figure 3). Scores +2 and +3 were more frequent in FP. The nonparametric Mann-Whitney test revealed a statistically significant difference between groups (p < 0.001). The elastic fibers exhibited variable degrees of thickness, but were thin and fragmented in most cases. Elastic fibers were also present in the submucosa, though in lower quantities.

**DISCUSSION**

The results reflected differences in the number of MC, collagen and elastin between FP and OF, indicating that these components seem to influence the development of these lesions.

Mast cells are found in almost all organs and tissues of the human body and are the main source of histamines, proteases, and other important chemical mediators. The major component present in the secretory granules of MC is tryptase, an enzyme released along with other mediators in the extracellular matrix after the activation/degranulation of MC. It has been suggested that tryptase stimulates the proliferation of fibroblasts and synthesis of collagen, and that it is involved in fibrinogenesis. In addition, tryptase plays a role in other biological activities, such as: the stimulation of the proliferation of smooth muscle cells and bronchial epithelial cells, the degradation of vasoactive intestinal peptide, and angiogenesis. In the oral mucosa, MC are involved in the induction of fibrosis and modulation of endothelial cell function in inflammatory fibrous hyperplasia and giant cell fibroma. Furthermore, there is evidence of a parallel increase in tryptase-positive MC and angiogenesis in normal oral mucosa, epithelial dysplasia and squamous cell carcinoma.

In this study, the number of tryptase-positive MC was higher in FP of the face than in solitary OF. The presence of MC in FP and OF suggests aberrant extracellular matrix remodeling during the growth of these lesions. One possible explanation for the larger number of MC in FP is the marked vascularization observed in these lesions, which correspond to a type of angiofibroma. Another plausible explanation is that FP can be triggered by exposure to ultraviolet radiation, which seems to affect directly MC, altering their potential to release mediators. Some investigators have shown

**FIGURE 1:**
Picrosirius red. Histo logic sections showing strong picrosirius red staining in OFs (A), compared with FP with less intensely staining (B) (200X)

**FIGURE 2:**
Tryptase. Note the tryptase-positive MCs in FP (A) and in OF (B) (400X). Tryptase-positive MCs (arrow) at the periphery of blood vessels (B) (400X)
that the number of MC increases significantly in skin exposed to sunlight.\textsuperscript{32,33}

In the lesions studied (FP and OF), most tryptase-positive MC were detected in connective tissue, mainly at the periphery of blood vessels. Nevertheless, there appears to be intimate cellular communication between this cell population and the vascular system. Mast cells produce and release proteolytic enzymes, tryptase and chymase, which mediate the migration of endothelial cells and the release of angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (b-FGF), transforming growth factor-beta (TGF-b), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-8, creating a microenvironment that favors neoplastic development.\textsuperscript{24,34}

The large mean number of MC in FP suggests that these cells may be responsible for the stimulation of fibroblast proliferation and fibrous stroma formation in lesions. However, high concentrations of MC in areas of fibrosis have been observed in different types of lesions. Vidal \textit{et al.} detected high concentrations of MC in many cases of pleomorphic adenoma and in the fibrous matrix of malignant, minor, salivary gland tumors.\textsuperscript{17} Ahmed \textit{et al.} suggested a direct relationship between MC and intramedullary fibrosis. Pereira \textit{et al.} raised the hypothesis of the growth and expansion of odonto-
We observed no association between the number of MC and the presence of a significant inflammatory process. These results agree with the findings of Smith et al. and Pereira et al., who also found no relationship between the presence of these cells and inflammation in other lesions.33, 40

Interestingly, in this study, the expression of collagen fibers was higher in OF than in FP, as would be expected, since the latter exhibited a larger number of MC. On the other hand, we observed a higher expression of elastin in FP than in OF. The high expression of elastin in FP may be related to the high concentration of MC, as fibroblasts also synthesize elastic fibers. It is therefore possible that MC also influence the deposition of elastin by stimulating fibroblasts through the secretion of chemical mediators. In addition, we believe that ultraviolet radiation has a significant effect on tissue remodeling and fibrosis in FP, based on the hypothesis that fibrocytes altered by ultraviolet radiation are related to induce the synthesis and excessive deposition of elastic material.22, 41, 42 Hence, fibroblasts involved in tissue remodeling in FP differ phenotypically from those involved in the development of OF, suggesting differences in matrix composition between these lesions. The stronger association between MC accumulation and elastin synthesis in FP -in contrast to OF in which MC accumulation was associated with the synthesis of collagen fibers- supports the hypothesis that MC influence the growth and expansion of OF and FP through different mechanisms.

A relationship between FP and OF has been reported in the literature. According to Fernandez-Flores, these lesions seem to be part of the same group of angiofibromatous lesions, since they share some microscopic features.6 However, the morphological findings of this study indicate that blood vessels, multinucleated cells, vascular ectasia and perivascular fibrosis are less frequent in OF, as also reported by Reed and Ackerman.4 Moreover, hair follicles, sebaceous glands, and periadnexal dermis are absent in OF. The differences in the expression of collagen, elastin and MC observed in this study between FP and OF suggest these lesions have a distinct etiopathogenesis. This does not seem to support the hypothesis that sporadic OF is a member of the group of angiofibromatous lesions, which includes FP of the face and multiple fibromas, seen in some phakomatoses.6 The results suggest that a greater involvement of MC in the formation of fibrous tissue in FP, and that ultraviolet radiation influences both the concentration of MC and the synthesis of the fibrillar component found in the fibrous matrix of these lesions.

CONCLUSION

MC tryptase activates fibroblasts to produce collagen and elastin, thus contributing to fibrosis in FP and OF. The results suggest that the MC population and the intensity of collagen and elastin expression can contribute for the morphological differences between FP and OF.
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