The immunohistochemical profile of oral inflammatory myofibroblastic tumors

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Objective. The aim of this study was to demonstrate the immunohistochemical profile of oral inflammatory myofibroblastic tumors (IMTs) along with morphologic analysis.

Study design. Three cases diagnosed as oral IMTs were selected to compile an immunohistochemical panel constituted by calponin, caldesmon, Bcl-2, desmin, fibronectin, CD68, Ki-67, S100, anaplastic lymphoma kinase (ALK), α–smooth muscle actin, cytokeratins AE1/AE3, muscle-specific actin, CD34, and vimentin. An oral squamous cell carcinoma with a focal area of desmoplastic stroma was used as control for the stained myofibroblastic cells.

Results. All oral IMTs were positive for calponin, revealing a strong and diffuse expression in the spindle-shaped cells. The lesions were also positive for vimentin (3/3), fibronectin (3/3), α–smooth muscle actin (3/3), and muscle-specific actin (1/3) and negative for h-caldesmon, Bcl-2, desmin, CD68, Ki-67, S100, ALK, cytokeratins AE1/AE3, and CD34.

Conclusions. Within the results encountered, the present panel should be of great assistance in the diagnosis of oral IMTs. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:749-756)

The term inflammatory myofibroblastic tumor (IMT) has been used to describe a wide and heterogeneous group of spindle-cell proliferations, ranging from reactive lesions to benign neoplasms and lesions that behave more aggressively or even in a malignant way.1 Such lesions present different histologic patterns, but are mainly composed of a myofibroblastic cellular population accompanied by a varying number of inflammatory cells, chiefly lymphocytes and plasma cells.2

The diagnosis of IMT is performed under light microscope and through immunohistochemistry by a panel of specific antibodies, such as vimentin, α–smooth muscle actin (SMA), muscle-specific actin (MSA), desmin, cytokeratins, CD68, and fibronectin (FN).2-4

Owing to the uncertain pathogenesis of IMT and its several histologic features, a large number of appellations, such as plasma cell granuloma, plasma cell pseudotumor, inflammatory myofibrohistiocytic proliferation, omental mesenteric myxoid hamartoma, inflammatory pseudotumor, and inflammatory fibrosarcoma, have been used to refer to this entity.2,5

IMTs are commonly found in children and young adults.3,6 The most frequent anatomic locations are the abdominopelvic region, lung, and retroperitoneum,3 but virtually any site may be involved, including the somatic soft tissues, bone, larynx, uterus, and central nervous system.7-11 Apart from the lung, which is the most prevalent site for IMT,3 head and neck IMTs account for 14%-18% of all lesions.3,12

Brooks et al. published a literature review confirming that IMTs of the oral cavity are rarely encountered13 and, excluding the patients presented herein, only 21 cases appear in the English-language literature.5,9,13-28

The rapid growth rate, as well as the nonspecific clinical appearance of oral IMTs may resemble that of malignant disorders. In that way, a comprehensive histopathologic assessment should provide precise diagnosis.20 Therefore, the present study aimed to describe the immunophenotype of IMTs of the oral cavity, which can contribute to the diagnosis of these lesions.

MATERIAL AND METHODS

Using the latest World Health Organization (WHO) classification of fibroblastic/myofibroblastic tumors,2 3 cases of oral IMTs were retrieved from the consultation files of the Department of Oral Pathology, University of São Paulo.

One case of oral squamous cell carcinoma with an intense desmoplastic area, composed mainly by myofibroblasts,29,30 was selected and functioned as a positive control of the immunohistochemical reactions.

For conventional light microscopy, tissue was fixed in 10% buffered formalin, embedded in paraffin wax,
sectioned at 5 μm, and stained with hematoxylin and eosin. Sections of 3 μm were obtained from the paraffin-embedded material, mounted on slides, treated with 3-aminopropyltriethoxy-silane (Sigma Chemical Co., St. Louis, MO, USA), deparaffinized, and hydrated. Endogenous peroxidase was quenched by incubation in 3% hydrogen peroxide in methanol (1:1) for 30 minutes at room temperature. Then sections were treated for antigen retrieval according to the antibody to be used (Table I).

Immunohistochemistry was performed on the Dako Autostainer (Dako Corp., Carpinteria, CA, USA). Briefly, primary antibodies (Dako) were incubated for 60 minutes. Sources and dilutions for each antibody are shown in Table I. Incubation with streptavidin-biotin complex (Kit LSAB Peroxidase K0690; Dako) followed by 3 minutes’ incubation with diaminobenzidine (Dako Liquid DAB plus, K3468; Dako) and subsequent counterstaining with Mayer hematoxylin. Negative control samples were treated as above, but using a solution of 1% bovine serum albumin (BSA) in Tris-HCl, pH 7.4 instead of the primary antibody.

RESULTS

Clinical findings

The oral sites affected by IMTs were: case 1, tongue (dorsal side); case 2, alveolar mucoa in the canine-premolar region (Fig. 1); and case 3, buccal mucosa. The duration of these lesions was 4, 4, and 5 months, respectively. The age of the patients ranged from 31 to 60 years old, and 2 of them were women.

Gross findings

Macroscopically, the IMT lesions were fibrotic and circumscribed, described as nodular masses, with the color varying from white to light brown. Size ranged from 1.5 to 5.0 cm in diameter.

Histologic findings

Microscopically, all studied IMTs were in the submucosa and covered by keratinized and stratified squamous epithelium (Fig. 2, A). All of the tumors were composed by spindled myofibroblastic cells accompanied by an inflammatory infiltrate mainly constituted by lymphocytes and a few plasma cells (Fig. 2, B, and Fig. 3, E and I).

Various histologic patterns could be noticed, as described by Coffin and Fletcher.2 When analyzing the morphology of the lesions, some features could also be mentioned, such as the myofibroblast polymorphism characterized by elongated spindle (Fig. 3, A) and short spindle or even stellate cells (Fig. 2, C). The growth pattern included a fascicular arrangement, and each lesion demonstrated ≥1 matrix types, which were myxoid, fibrotic, or hyalinized (Fig. 2, D). Finally, several types of vascular arrangements could be noticed within the stroma. No remarkable nuclear atypia was found.

Immunohistochemical study

The immunohistochemical results are summarized in Table II. This analysis revealed that the spindle-shaped cells were strongly and diffusely positive for

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Pretreatment*</th>
<th>Dilution</th>
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<td>Citrate</td>
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</tr>
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<td>ALK1</td>
<td>Citrate</td>
<td>1:50</td>
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<td>EDTA</td>
<td>1:100</td>
</tr>
<tr>
<td>Desmin</td>
<td>D33</td>
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<td>—</td>
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<td>Vimentin</td>
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*| ALK, anaplastic lymphoma kinase; SMA, α-smooth muscle actin; MSA, muscle-specific actin.

*Citrate: 0.01 mol/L, pH 6.0, 95°C, 30 minutes; EDTA: 0.01 mol/L, pH 9.0, 95°C, 30 minutes; Pepsin: 1%, pH 1.8, 37°C, 60 minutes.
calponin (3/3; Fig. 3, D, H, and L, and Fig. 4, A) vimentin (3/3; Fig. 3, C, G, and K, and Fig. 4, B) and MSA (1/3). The expression pattern of calponin showed a tendency to be at the periphery of the stained cells’ cytoplasm (Fig. 3, L).

The expression of SMA was accentuated and diffuse in the cytoplasm of spindle-shaped cells (3/3; Figs. 3, B, F, and J, and 4, C). All the blood vessels showed positivity to this antibody and functioned as a positive control.

As for positivity in the extracellular matrix, FN immunohistochemistry revealed positivity staining in all studied cases (3/3; Fig. 4, D). The immune-staining was pericellular for the spindle-shaped cells with extracellular extension. The extracellular matrix was mainly stained on the highly organized collagen bundles. Finally, vimentin protein was diffusively expressed by the tumor cells (3/3; Fig. 3, C, G, and K, and Fig. 4, B) and localized at the periphery of the myofibroblasts’ cytoplasm (Fig. 4, B).

Table II. Three inflammatory myofibroblastic tumors (1, 2, and 3) and desmoplastic area of a squamous cell carcinoma (D)

<table>
<thead>
<tr>
<th>Case</th>
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<th>Desmin</th>
<th>FN</th>
<th>CD68</th>
<th>Ki-67</th>
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CD, h-caldesmon; FN, fibronectin; other abbreviations as in Table I.

Fig. 2. A, Morphology of the inflammatory myofibroblastic tumor revealing a circumscribed tumor covered by a keratinized and stratified squamous epithelium (hematoxylin-eosin [HE], ×100); B, Spindle myofibroblastic cells within the tumor stroma along with a chronic inflammatory infiltrate and hemorrhagic areas (HE, ×100); C, Tumor cells characterized by elongated spindle and short spindle cells with basophilic nuclei (HE, ×400); D, Hypocellular area showing hyalinized collagen bundles (HE, ×100).
The expression of h-caldesmon, Bcl-2, desmin, CD68, Ki-67, S100, cytokeratins AE1/AE3 (CK), anaplastic lymphoma kinase (ALK), and CD34 was absent in all of the tumor cells. The oral squamous cell carcinoma revealed, in its desmoplasic area, positivity for calponin (Fig. 4, A inset), vimentin (Fig. 4, B inset), SMA (Fig. 4, C inset), fibronectin (Fig. 4, D inset), and MSA and negativity for ALK, h-caldesmon, Bcl-2, desmin, CD68, Ki-67, S100, AE1/AE3, and CD34.

DISCUSSION

Oral IMTs are very rare lesions that may occur predominantly in the submandibular region, the parotid duct, the retromolar area, the alveolar mucosa of the molar region, tongue, and maxilla. In contrast to soft tissue and visceral IMTs, that occur mostly in children and young adults, head and neck IMTs are most common in adults (median age of 59 years) and men. Tumor size usually varies from 0.5 to 5 cm, which was compatible with the present cases. The literature presents a follow-up that ranges from 1 month to 10 years for oral IMTs, and, including the present cases, no clinical recurrences are documented. Despite the lack of tumor reappearance or malignant transformation, a prolonged follow-up is mentioned to be necessary after surgical resection. Regarding the most frequent treatments of oral IMTs, surgical excision, radical curettage, and tumor enucleation can be mentioned.

IMTs may display >1 of the 3 histologic patterns described by WHO within the same tumor, as exemplified by our cases. Cytologic atypia with nuclear pleomorphism and increased mitotic activity are uncommon features, and may be associated with malignant transformation. In the cases presented here, no remarkable nuclear atypia was found.

Several benign and malignant spindle-cell proliferations may contribute to the differential diagnosis of IMT. Among them are proliferative fasciitis, nodular fasciitis and its homologous lesion referred to as a posttraumatic spindle-cell nodule, inflammatory...
myxohyaline tumor, infantile fibromatosis, myofi-
bromatosis, Rosai-Dorfman disease, fibrous histiocy-
toma, solitary fibrous tumor, follicular dendritic cell

tumor, low-grade myofibroblastic sarcoma, fibrosar-
coma, leiomyosarcoma, rhabdomyosarcoma, and
spindle-cell carcinoma.13

Immunohistochemistry has been a useful tool to dis-
tinguish IMTs from other soft tissue neoplasms.
Among the most applied antibodies, SMA, vimentin,
and FN have shown common positivity for the majority
of the oral and extraoral IMT studied cases.4,13,32-34
Additionally, 1 study has shown that low-grade myo-
fibroblastic sarcomas also express SMA and FN. Other
antibodies, such as ALK and cytokeratin can be used to
differentiate these lesions from IMTs.32

Other neoplastic lesions, such as the solitary fibrous
tumor shows high CD34 and CD99 positivity rate,
while the tumor cells of IMTs do not express these
markers. Alternatively, the diagnosis of spindle cell
carcinoma can be excluded based on AE1/AE3 immu-
nopositivity compared with the IMT cells, which do not
express cytokeratins.37 The cells that mainly charac-
terize Rosai-Dorfman disease show immunoreactivity
for S100 protein, differently from the IMT cells.38,39

The follicular dendritic cell tumor shows reactivity to
S100, and along with vimentin, CD21, CD23, CD35
(Ber-MAC-DRD, and Ki-M4 positivity helps in dis-
tinguishing it from IMTs.40-42 In this manner, several
antibodies should provide great assistance for the diag-
nostic process. Fibronectin, for example, has been al-
ready established as a standard marker for the extraoral
IMTs, and its importance on the diagnosis of oral
lesions was recently suggested.4 The present study re-
vealed FN positivity for all cases, this expression being

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Fig. 4. Selected positive staining for cases 1, 2, and 3 with insets of desmoplastic connective tissue of a squamous cell
carcinoma for comparison. A, Calponin expression showing a diffuse staining pattern within the tumor (×100, case 1); inset
showing the focal desmoplastic area of the carcinoma with the myofibroblasts expressing calponin (×100). B, Diffuse
positivity of the myofibroblasts for vimentin in the IMT (×100, case 3); inset showing vimentin positivity in the
desmoplastic area of the carcinoma (×100). C, Pattern of expression of the α-smooth muscle actin in the tumor cells (blood
vessels acting as positive control) (×100, case 3); inset showing α-smooth muscle actin positivity in the focal desmoplastic
area of the carcinoma (×100). D, Myofibroblasts reveal pericellular staining for fibronectin (×100, Case 2); inset—fibronectin
diffuse expression in the desmoplastic area of the carcinoma with extension to the extracellular matrix (×100).
related mostly to the highly organized collagen bundles of the lesion stroma.

As stated, FN expression is more abundant in the stroma of benign and malignant soft tissue neoplasms, but can also appear as intracellular dots in lung IMT cells and in a pericellular mode in IMTs and low-grade myofibroblastic sarcomas. Accordingly, the present study showed FN staining on the cell surface with extension to the extracellular matrix.

Extraoral IMTs may also be diffusely positive for MSA. Because myofibroblasts contain peripheral myofilaments that are effectively stained by the actin-associated proteins SMA, MSA, and vimentin, it was expected to find such expression at the periphery of the myofibroblast cytoplasm. In fact, IMTs are collectively positive for SMA (92%) and all the oral tumors studied herein showed expression in the cytoplasm of the spindle-shaped cells.

Calponin is expressed by myofibroblasts and is a constituent of smooth muscle thin filaments; therefore, it is considered to modulate smooth-muscle contraction. IMTs of the urinary bladder have shown positivity for calponin in the majority of cases, with a range of 86%-100%. The expression of calponin in a recent case of cardiac IMT also helped in diagnosing the lesion. In addition, IMTs arising from lungs, respiratory tract, abdomen, pelvis, retroperitoneum, trunk, extremities, and head and neck are positive for calponin, confirming the effectiveness of this antibody as an adjuvant in the diagnostic process.

Our results show that calponin can play an important role when diagnosing lesions arising from the oral mucosa. In the present work, the myofibroblasts of all of the studied tumors showed calponin expression with enhanced staining at the periphery of their cytoplasm, as already described in the literature. Nevertheless, calponin is a nonspecific marker that can be positive in a wide range of myofibroblastic and myoid cells, which highlights the importance of an adjuvant marker, such as h-caldesmon, to differentiate myofibroblastic lesions from smooth-muscle tumors.

Accordingly, Miettinen et al. noticed the interesting and simultaneous calponin positivity and h-caldesmon negativity in all lesions with myofibroblastic differentiation. Those authors therefore proposed the use of both antibodies in a panel for tumors with myofibroblastic differentiation. High-molecular-weight caldesmon (h-caldesmon) is an isoform of caldesmon widely distributed in smooth and nonsmooth muscle cells and is thought to regulate cellular contraction. This antibody was very useful in guiding the diagnosis of the cases presented here while differentiating the IMT lesions from smooth-muscle neoplasms, such as leiomyomas. In addition, fibromatosis, nodular fasciitis, myofibroblastic sarcoma, and rhabdomyosarcoma can be distinguished from smooth-muscle tumors by means of their h-caldesmon negativity.

Overexpression of ALK protein as well as the proliferation marker Ki-67 and the apoptosis protein inhibitor Bcl-2 has been correlated with tumorigenesis. Regarding ALK reactivity, a slightly higher degree of malignant transformation has been related to the IMTs that showed positivity for this protein. On the other hand, Ki-67 nuclear staining can be associated with aggressive tumoral behavior, multifocal origin, and rapid growth of IMTs, and Bcl-2 is related to IMT recurrence and malignant transformation.

Although none of the present cases showed immunoreactivity for Ki-67 antibody, the literature demonstrates a certain degree of nuclear staining in IMTs. Nevertheless, Ki-67 expression is generally low for these tumors (0-10%), whereas in malignant lesions the percentage is usually higher (up to 80%). Moreover, the prognostic significance of IMT tumors that present Ki-67 expression is considered to be ambivalent, because not all Ki-67–positive IMTs are related to worse clinical outcomes. Nonetheless, all tumors presented in this study were negative for Ki-67 and had good clinical prognosis with no signs of recurrence after the follow-up.

We used an oral squamous cell carcinoma with a focal area of desmoplastic stroma as control for the immunohistochemical reactions performed. Desmoplastic stromas of carcinomas are mainly composed of myofibroblasts or myofibroblastic cells and the deposition of extracellular matrix proteins. As expected, the myofibroblasts were positive for calponin, fibronectin, SMA, MSA, and vimentin. The oral IMTs seem to behave differently from the ones that occur in other anatomic sites, once they do not produce significant systemic symptoms nor are detected with routine blood investigations. The basic components though, are the myofibroblastic cells, which have to be identified within the characteristic stroma of the lesion. Thus, the present study demonstrated the immunohistochemical profile of oral IMTs and the consistent staining pattern of myofibroblast-associated markers while identifying the characteristic tumor cells. Such an immunohistochemical panel should be of great assistance in the diagnosis of oral IMTs.

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REFERENCES