Histopathological findings and immunohistochemical expression of the stem cell markers CD44, ALDH1, Bmi-1, and Nanog in oral solitary fibrous tumors

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Objectives. The aim of this study was to evaluate the histomorphologic presentation and the expression of stem cell–related markers in a series of oral solitary fibrous tumors (SFTs).

Study Design. Histopathological variables and the expression of the standard stem cell markers CD34 and CD99, used for SFT diagnosis, as well as STAT6 were evaluated in 13 oral SFTs. The expression of the cancer stem cell markers CD44, ALDH1, Bmi-1, and Nanog and the tumor suppressor gene $p16^{Ink4a}$ were also investigated.

Results. The majority of oral SFTs were circumscribed and characterized by a proliferation of spindle cells arranged in a hyalinized stroma. Only 2 oral SFTs showed >4 mitoses/10 high-power fields. Hypercellularity as well as nuclear and cellular pleomorphism were classified as low and moderate in most of the oral SFTs. All oral SFTs were positive for CD34, STAT6, CD44, ALDH1, Bmi-1, and $p16^{Ink4a}$. CD99 and Nanog expression was observed in 11 and 10 oral SFT cases, respectively.

Conclusion. We suggest that STAT6 and ALDH1 have relevant diagnostic value. The expression of CD44, ALDH1, Bmi-1, and Nanog, which is observed in cancer stem cells, may confer advantages to oral SFT cells. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:444–451)

Solitary fibrous tumor (SFT) is a rare mesenchymal neoplasm of uncertain pathogenesis.[1] Although most SFTs are located in the thoracic cavity (>50%), cases in other anatomic sites such as the peritoneum, mediastinum, spinal cord, and head and neck have been described.[2,3] Head and neck SFTs account for 6% of all SFTs, with the oral cavity being the most frequently affected site.[3,4] However, only 150 oral SFTs cases have been reported so far.[5]

Clinically, an oral SFT appears as a submucosal mass of normal color, located in the buccal mucosa and tongue; it is slow-growing and asymptomatic and has a predilection for middle-aged women.[4,5] Microscopically, SFTs are composed of spindle-shaped to ovoid cells with scarce and ill-defined cytoplasm, arranged in a disorderly collagenous and hyalinized stroma, with occasional storiform or fascicular areas.[6] Although the vast majority of SFTs are benign, malignant tumors have been described.[2,6] SFTs show low risk for developing metastasis and low recurrence rates are observed in the head and neck area.[2]

Because of its nonspecific clinical presentation and histopathological variations in cell and stroma characteristics, the diagnosis of SFT can be challenging because it may share similarities with many other mesenchymal tumors including neurofibroma, myofibroma, and leiomyoma.[7] Most SFTs are positive for CD34 and, additionally, CD99 and Bcl-2 have been used as supportive markers for diagnosis.[2,8] However, CD34 and Bcl-2 expression is also common in tumors that mimic SFT traits such as well-differentiated spindle cell lipoma and synovial sarcomas.[9-11] Recent studies have identified the fusion of *NAB2-STAT6* as a driver mutation in SFTs and nuclear STAT6 expression is currently used as the gold standard marker for its diagnosis.[8,12,13]

Some studies have identified *ALDH1* gene overexpression in SFTs compared to non-SFTs, such as angiosarcomas, liposarcomas, leiomyosarcomas, schwannomas, undifferentiated pleomorphic sarcomas, meningiomas, and synovial sarcomas.[14,15] Cytoplasmic ALDH1 expression is present in 70% to 80% of SFTs and, when associated with STAT6 and CD34 expression, improves diagnostic sensitivity.[14,15] This gene acts as a cytosolic detoxification enzyme, participating in the oxidation of intracellular aldehydes and in the regulation of the stem

Statement of Clinical Relevance

Solitary fibrous tumors (SFTs) are uncommon mesenchymal neoplasms that can affect the oral cavity. STAT6 and ALDH-1 are reliable markers for SFT diagnosis, and high expression of CD44, Nanog, and Bmi-1 may confer growth advantages to SFT cells.

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cell phenotype.[16] In oral SFTs, the expression of ALDH1 has not been previously reported.

In different types of cancer, ALDH1 expression has been used to identify cancer stem cells (CSCs), which are responsible for tumor growth, metastasis, and treatment failure.[17] In addition, CSCs demonstrate high expression levels of genes related to stem cell maintenance and differentiation, including CD44, Bmi-1, Nanog, and Oct4.[18]

Because CD34, CD99, and ALDH1 are characteristic markers of stem cells, we raise the question whether other stem cell markers are expressed in oral SFTs. Thus, the purpose of this study was to evaluate the histomorphologic characteristics of oral SFTs, as well as to investigate the expression of the stem cell−related markers CD44, ALDH1, Bmi-1, and Nanog. We have also investigated the expression of $p16^{Ink4a}$, a tumor suppressor protein that is transcriptionally repressed by Bmi-1.[19]

MATERIALS AND METHODS

SFT samples

Thirteen cases of oral SFTs, previously diagnosed according to World Health Organization classification,[1] were retrieved from the archives of the Oral Pathology Department at the School of Dentistry, University of São Paulo. Clinical information included sex, age, and tumor location. No information regarding patients' medical history or any follow-up was available. The study was approved by the Brazilian National Ethics Committee (Number 1.824.891) and was conducted in accordance with the Declaration of Helsinki.

Tumor histomorphology

Following the evaluation of histopathologic variables, scores were used to semiquantitatively investigate the number of mitotic figures, cellularity, nuclear pleomorphism, and the presence of necrosis.[2] The mitotic index was calculated per 10 high-power fields (× 400), using the highest count of the 3 to 5 areas scored.[2] The most cellular area of the tumor was scored for cellularity on a 3-point scale (1 = low, tumor predominately composed of sclerotic collagen bands with scattered, compressed spindle cells; 2 = moderate, many areas of increased cellularity with cells adjacent to one another; 3 = high, hypercellularity, with areas of nuclear overlap). Pleomorphism was scored on a 3-point scale (1 = low, cells monomorphic, with uniform nuclear features; 2 = moderate, increased nuclear pleomorphism, more prominent nucleoli, and rare multinucleated cells; 3 = intense, hyperchromatic nuclei present with foci of marked pleomorphism and bizarre cells). Necroses/hemorrhages were scored as minimal (<10%) or positive (≥10%), considering the total tumor area. The presence or absence of circumscription was also evaluated.

The tumor stroma was classified as myxoid, hyalinized, or both, and other features, such as the presence of giant cells, adipocytes, mast cells, or lymphocytes, were also described.[20,21]

To confirm the presence of amyloid deposits, Congo red staining was performed.

Immunohistochemistry

Three-micrometer sections obtained from formalin-fixed and paraffin-embedded tissues were submitted to immunohistochemistry analysis for detection of CD34, CD99, STAT6, CD44, ALDH1, Bmi-1, Nanog, and $p16^{Ink4a}$. Briefly, sections were deparaffinized and subjected to antigen retrieval with 100 mM citrate buffer target retrieval solution, pH 6.0 at 95°C, in a water bath for 30 min. Endogenous peroxidase activity was quenched by incubation with 3% hydrogen peroxide and methanol for 20 min. Sections were treated with protein block (Dako, X0909, Carpenteria, CA) for 10 min and incubated overnight at 4°C with the following monoclonal antibodies: anti-CD34 (QBEnd-10, 1:50; Dako), anti-CD99 (12 E7, 1:50, Dako), anti-STAT6 (YE361, 1:100, Abcam, Cambridge, UK), CD44 (156-3 C11, 1:300, Cell Signaling Technology, Danvers, MA), anti-ALDH1 (1:300, Dako), anti-Bmi-1 (D20 b7, 1:300, Cell Signaling Technology), anti-Nanog (CL5810, 1:100, Sigma-Aldrich, St. Louis, MO), and $p16^{Ink4a}$ (Ab 108349, 1:1000, Abcam). Next, sections were washed with PBS and incubated using the EnVision Dual Link System horseradish peroxidase method (Dako) for 30 min. Reactions were revealed by incubating the sections with 3,3′-diaminobenzidine tetrahydrochloride (Dako, K346) and counterstaining with Mayer's hematoxylin.

Ten randomly selected fields of each sample, at a magnification of × 400, were chosen by 2 independent investigators. The percentage of positive cells was recorded for each field, and the mean obtained for each tumor was used to classify the tumors as negative stain = 0, low positivity = 1 (1%-10% of cells stained), moderate positivity = 2 (11%-50% of cells stained), and high positivity = 3 (>50% of cells stained).[22]

Statistical analysis

Differences in the scores between 2 stem cell markers were obtained using the Mann-Whitney *U* test and correlation analysis was performed using Spearman's rank correlation in GraphPad Prism 6.0 (Graph-Pad Software, Inc., San Diego, CA). The level of significance was set at $P \leq .05$.

RESULTS

Clinical and histopathological evaluation

In this study, there were 8 male (61.5%) and 5 female (38.5%) patients with a mean age of 48.54 years (range, 25-73 years). In most cases the buccal mucosa

was affected (77%), followed by the tongue (15%) and floor of the mouth (8%).

The diagnosis of the oral SFTs was based on morphology (Figure 1 and Figures 2A-2C) and confirmed by immunohistochemistry using the established markers CD34, CD99, and STAT6 (Figures 2D-2F).

The histologic findings of the SFTs included in this study are provided in Table I. SFTs were circumscribed in 11/13 (85%) cases (Figure 1A), and 2 samples (15%) demonstrated muscular (Figure 1B) and salivary gland (Figure 1C) involvement. Most oral STFs were characterized by a proliferation of uniform, basophilic spindle cells with scant cytoplasm and predominantly dense ovoid to cigar-shaped nuclei with inconspicuous nucleoli (Figure 1D), but vesicular nuclei were also seen (Figure 1E).

The overall architectural pattern was diffuse and characterized by sheets of neoplastic cells. All cases demonstrated numerous ectatic, thin-walled vessels, often in a staghorn configuration, and perivascular hyalinization, sometimes extending beyond the perivascular areas (Figure 1F). One of the oral SFTs showed periductal hyalinization, probably due to a stromal response to neoplastic proliferation (Figure 1G). The stroma was hyalinized in all the oral SFTs and 2/13 cases also showed myxoid areas (15%; Figure 1H). Amyloid deposits were observed in 1/13 cases as evidenced by the Congo red staining (7.5%; Figure 1I).

Only 2 oral SFTs showed >4 mitoses/10 high-power fields (15%). Regarding hypercellularity, 6/13 SFTs were scored as 1 (45%) and were predominately composed of sclerotic collagen bands with scattered, compressed spindle cells (Figure 2A), 5/13 were scored as 2 (37.5%), with many areas of increased cellularity (Figure 2B), and 2/13 showed hypercellularity (15%; score 3), with areas of nuclear overlap (Figure 2C). In

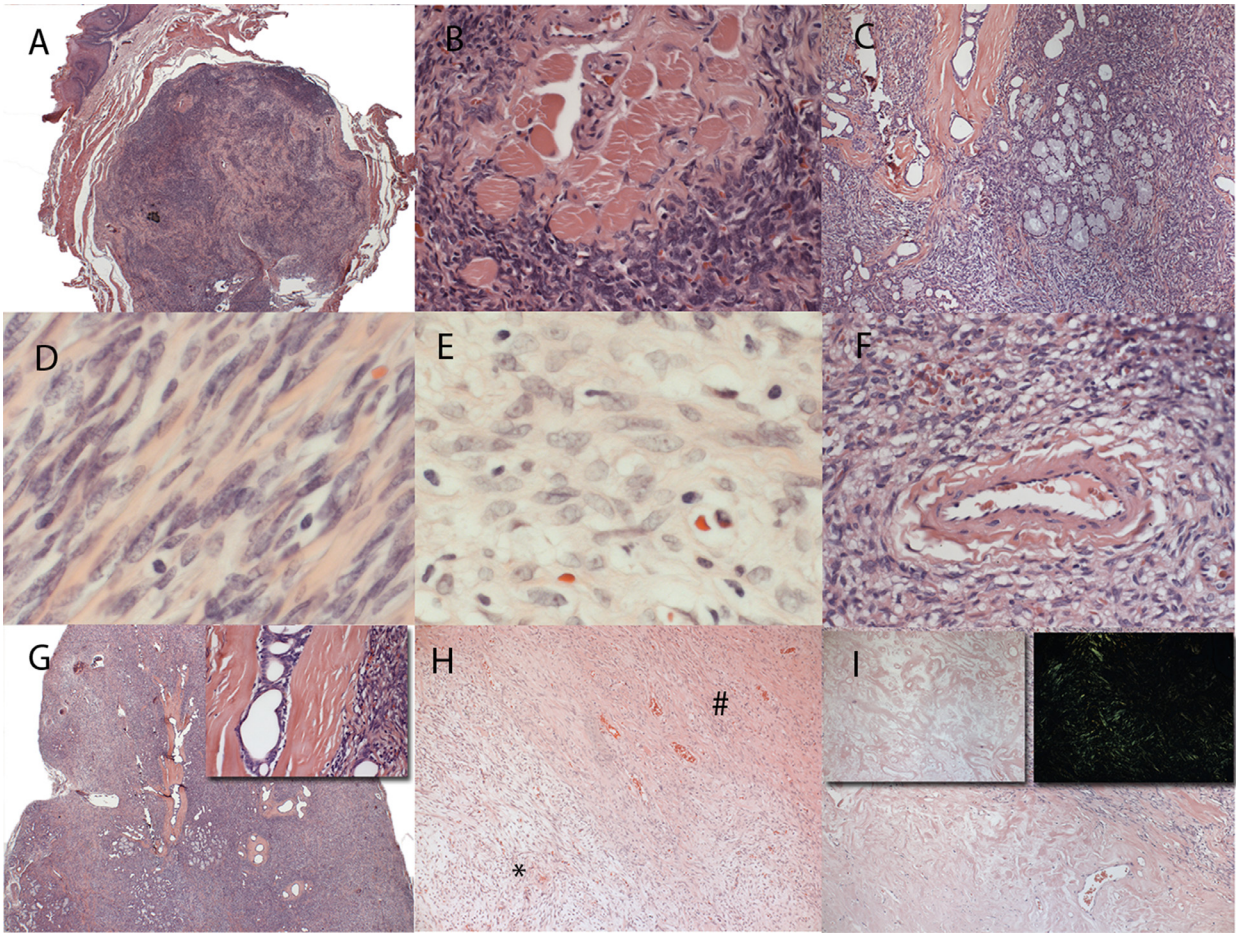

Figure 1. Histopathological findings observed in oral solitary fibrous tumor samples. **(A)** Well-circumscribed tumor (25 ×). **(B)** Tumor invasion into muscle tissue (100 ×). **(C)** Tumor invasion into salivary gland (100 ×). **(D)** Spindle cells with scant cytoplasm and predominant dense ovoid to cigar-shaped nuclei (400 ×). **(E)** Cells demonstrating vesicular nuclei (400 ×). **(F)** Typical perivascular hyalinization in oral solitary fibrous tumors (400 ×). **(G)** Periductal hyalinization (25 ×, upper box 400 ×). **(H)** Tumor stroma (*areas of myxoid features; #areas of dense connective tissue; 100 ×); **(I)** One case showed amyloid deposits, positive for Congo red staining (100 ×).

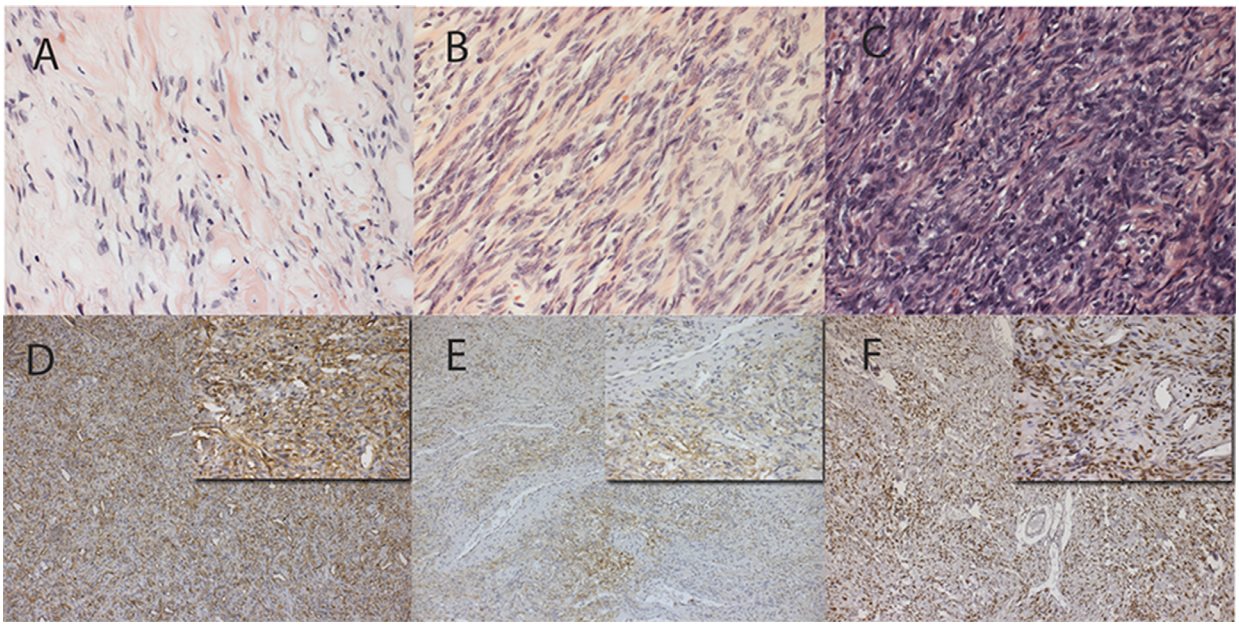

Figure 2. Cellularity of the tumor on a 3-point scale. **(A)** Oral solitary fibrous tumors (SFTs) were composed predominately of sclerotic collagen bands with scattered, compressed spindle cells (score 1). **(B)** Oral SFT showing many areas of increased cellularity with cells adjacent to one another (score 2). **(C)** Oral SFT with hypercellular areas characterized by nuclear overlap (score 3). **(D)** Positive staining for CD34 in all cases (100 ×, upper box 400 ×). **(E)** Positive staining for CD99 in 11/13 cases (100 ×, upper box 400 ×). **(F)** Positive staining for STAT6 in all cases (100 ×, upper box 400 ×).

Table I. Clinical data and histologic findings of the oral SFTs included in this study*

Case	*Gender*	*Age*	*Localization*	*Circumscribed*	*Mitoses/10 high-power fields*	*Pleomorphism*	*Myxoid*	*Hyalinized*	*GC/Fat/MC/L*	*Necrosis*
1	F	37	Buccal mucosa	+	–	–	–	+	MC/L	–
2	M	N/I	Tongue	+	+	–	–	+	L	–
3	F	56	Buccal mucosa	+	–	–	–	+	MC/L	–
4	F	25	Buccal mucosa	+	–	–	+	+	L	–
5	M	42	Buccal mucosa	Invading muscle	–	–	–	+	L	–
6	F	N/I	Buccal mucosa	+	–	+	–	+	MC/L	–
7	F	57	Buccal mucosa	+	–	–	–	+	L	–
8	M	30	Tongue	+	–	+	+	+	MC/L	–
9	M	33	Floor of the mouth	Invading salivary gland	–	–	–	+	MC/L	–
10	M	52	Buccal mucosa	+	–	+	–	+	MC/L	–
11	M	66	Buccal mucosa	+	–	+	–	+	MC/L	–
12	M	63	Buccal mucosa	+	–	–	–	+	L	–
13	M	73	Buccal mucosa	+	+	+	–	+	MC/L	–

SFT, solitary fibrous tumor; *GC*, giant cell; *MC*, mast cell; *L*, lymphocytes; *F*, female; *M*, male; *N/I, not informed*.
*+ = yes; – = no.

addition, low and moderate cellular and nuclear pleomorphisms were observed in 8/13 (61.5%) and 5/13 (38.5%) of the oral SFTs, respectively.

Mast cells were seen in 8/13 of the oral SFTs (60%), and all of the samples showed a discrete and predominant lymphocytic inflammatory infiltrate. Necrosis was not observed in the oral SFTs evaluated here and only 1/13 demonstrated minimal areas of hemorrhage (<10% of the histologic section; 7.5%).

Stem cell markers expression in SFTs

CD34 and STAT-6 were expressed in all oral SFTs (13/13) and CD99 was expressed in 11/13 of oral SFT cases (Figures 2D-2F).

Table II and Figure 3A summarize the semiquantitative evaluation of the CD44, ALDH1, Bmi-1, Nanog, and $p16^{Ink4a}$ expression in oral SFTs.

CD44 showed diffuse membranous expression (Figure 3B), with a high positivity observed in 12/13 cases (92.5%) and moderate positivity in 1/13 cases (7.5%).

Table II. Expression of CD44, ALDH-1, Bmi-1, Nanog, and $p16^{Ink4a}$ in oral SFTs*

Case	*CD44 score*	*ALDH-1 score*	*Bmi-1 score*	*Nanog score*	*$p16^{Ink4a}$ score*
1	3	3	3	3	3
2	3	3	3	3	3
3	3	2	3	2	2
4	3	2	1	0	2
5	3	1	2	3	2
6	3	3	1	2	1
7	3	3	3	3	2
8	3	3	3	2	3
9	2	3	2	0	2
10	3	2	3	3	2
11	3	3	3	0	2
12	3	3	3	3	2
13	3	3	3	3	1

SFT, solitary fibrous tumor.
*Percentage of tumor areas stained and scored as negative (score 0), low positivity (score 1; 1%-10% of stained cells), intermediate positivity (score 2; 11%-50% of stained cells), and high positivity (score 3; >50% stained cells).

ALDH1 was expressed in the cytoplasm and cell membrane of tumor cells (Figure 3C). ALDH1 high positivity was seen in 9/13 cases (67.5%), moderate positivity in 2/13 cases (15%), and only 1 case exhibited less than 10% ALDH1 positive cells.

Bmi-1 was expressed only in the nucleus of tumor cells (Figure 3E) and 9/13 SFTs showed high positivity for Bmi-1 (67.5%). Moderate and low positivity for Bmi-1 was observed in 2/13 SFTs (15%).

Nanog positivity was mainly detected in the nucleus (Figure 3D) of tumor cells and 7/13 showed high positivity (52.5%). In addition, moderate positivity for Nanog was observed in 3/13 SFTs (22.5%) and 3/13 samples were negative for Nanog (22.5%). Mast cells showed a cytoplasmic positivity to Nanog, which was interpreted as a possible nonspecific reaction.

The expression of $p16^{Ink4a}$ was restricted to the nucleus of tumor cells (Figure 3F), showing a high positivity in 3/13 cases (22.5%), moderate positivity in 8/13 cases (60%), and low positivity in 2/13 cases (15%).

The expression of all cancer stem cell markers, CD44, ALDH1, Bmi-1, and Nanog, was observed in 76.9% of

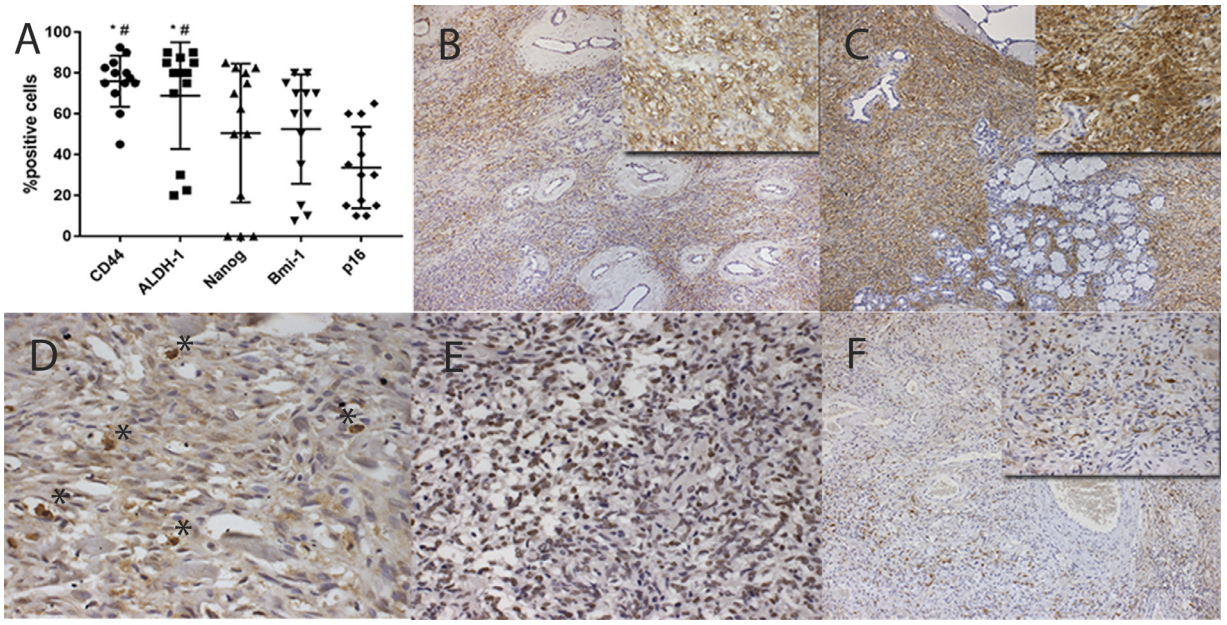

Figure 3. Evaluation of the CD44, ALDH-1, Bmi-1, Nanog, and $p16^{Ink4a}$ protein expression in solitary fibrous tumors. **(A)** Percentage (mean ± SD) of CD44, ALDH-1, Bmi-1, Nanog, and $p16^{Ink4a}$ positive cells (*$P < .05$ compared to Bmi-1 expression; #$P < .001$ compared to $p16^{Ink4a}$ expression). **(B)** Positive staining for CD44 (100 ×, upper box 400 ×). **(C)** Positive staining for ALDH-1. **(D)** Positive staining for Nanog (*mast cells, 100 ×, upper box 400 ×). **(E)** Positive staining for Bmi-1 (100 ×, upper box 400 ×). **(F)** Positive staining for $p16^{Ink4a}$ (100 ×, upper box 400 ×).

Table III. Correlation analyses between CD44, ALDH1-1, Bmi-1, Nanog, and $p16^{Ink4a}$ expression in the oral SFTs (Spearman's rank test)

Protein expression	*CD44*	*ALDH-1*	*Bmi-1*	*Nanog*
CD44	—	$r = 0.2$, $P = .52$	$r = -0.06$, $P = .83$	$r = -0.06$, $P = .81$
ALDH-1	$r = 0.2$, $P = .52$	—	$r = 0.5$, $P = .08$	$r = 0.12$, $P = .7$
Bmi-1	$r = -0.06$, $P = .83$	$r = 0.5$, $P = .08$	—	$r = 0.47$, $P = .11$
Nanog	$r = -0.06$, $P = .81$	$r = 0.12$, $P = .7$	$r = 0.47$, $P = .11$	—
$p16^{Ink4a}$	$r = -0.35$, $P = .23$	$r = -0.09$, $P = .23$	$r = 0.47$, $P = .09$	$r = 0.05$, $P = .84$

SFT, solitary fibrous tumor.

oral SFTs, and the concomitant expression of CD44, ALDH1, and Bmi-1 was observed in 23.1% of cases.

Lastly, when comparing the expression between 2 individual markers in oral SFTs, CD44 and ALDH1 were the most expressed and significant differences were found when these 2 stem cell markers were compared to Bmi-1 ($P < .05$) and $p16^{Ink4a}$ ($P < .001$), respectively (Mann-Whitney U test). Bmi-1 expression was higher than the expression of $p16^{Ink4a}$, but no significant difference was observed ($P = .06$). No correlation was observed between the expression of CD44, ALDH1, Bmi-1, Nanog, or $p16^{Ink4a}$ (Table III).

DISCUSSION

In this study, we have demonstrated for the first time that oral SFTs express the cancer stem cell markers CD44, ALDH1, Bmi-1, and Nanog, in addition to the previously known expression of CD34, CD99, and STAT6. Moreover, the low positivity for $p16^{Ink4a}$ suggests that it may be involved in the deregulated cellular proliferation found in SFTs.

In agreement with previous studies, the mean age at diagnosis observed was 48.54 years and the most affected site was the buccal mucosa, followed by the tongue.[2,3] In a recent systematic review, Morais et al.[3] observed a discrete female predilection (53.2%). In our series, oral SFTs were more prevalent in males (60%), but because of the low number of samples, this result may not be representative of the gender prevalence.

The histomorphologic criteria for SFT diagnosis include the characteristics of circumscription, hyper- or hypocellularity, and spindle-shaped or ovoid cells with scarce and ill-defined cytoplasm arranged in a collagenous to myxoid, well-vascularized stroma with perivascular hyalinization.[2,20,21] In our series of oral SFTs, we evaluated the pathologic variables including mitotic figures, cellularity, nuclear pleomorphism, and the presence of necrosis, according to Demicco et al.,[2] a large cohort study of SFTs (110 cases) with clinical follow-up. In this study, the authors demonstrated that patient age, tumor size, and mitotic index predicted both time to metastasis and disease-specific mortality, whereas necrosis predicted metastasis only.[2]

Most oral SFT cases evaluated in this study were circumscribed (11/13), and 2/13 presented a pseudoinfiltrative pattern of growth, with muscle and salivary gland involvement. However, additional characteristics of malignant transformation, such as cellular and nuclear atypia, hypercellularity, and mitotic figures, were not observed in these tumors with an infiltrative pattern. In addition, minimal pleomorphism and mitosis were observed in some cases, but these findings alone are not enough to confirm a diagnosis of malignancy.[6] Nunes et al.[5] reported that in malignant SFTs, more than 4 mitoses/10 high-power fields and areas of necrosis were present in 78.6% and 21.4% of the oral SFTs reviewed, respectively. Moreover, only 14/150 reported SFT cases were malignant and only 1 case showed metastasis, indicating that oral tumors have a more favorable prognosis then SFTs in other sites.[5] Unfortunately, data regarding the treatment and follow-up of the patients with SFTs included in this study were not available and no association with survival could be performed.

The "classic" histologic pattern described by Chan[20] was the most common histopathologic presentation in our series, in agreement with other studies.[2,6] Only 2 of our cases were primarily hypercellular, with less conspicuous hypocellular/myxoid changes. Mast cells were also seen in 61.5% of the cases. O'Regan et al.[21] also reported the presence of mast cells in 38% of their cases. It has been suggested that mast cells may release proteases in the intercellular matrix and around blood vessels, contributing to tumor sclerosis.[21,23] We noticed the presence of amyloid in 1 SFT, which was positive for Congo red stain. To the best of our knowledge, there is no reported case demonstrating amyloid deposits in SFTs, but this was an isolated finding. In addition, in 1 case of SFT, periductal hyalinization was seen nearby salivary gland, an unusual finding, because hyalinization is usually observed around blood vessels. O'Regan et al.[21] observed marked periductal hyalinization in 2 SFTs in which the remnant salivary gland tissue was found within the bulk of the tumor, suggesting that the tumor may arise within the parenchyma of minor salivary glands.

Because the microscopic features of SFTs are shared with other mesenchymal neoplasms, the final diagnosis

is based on immunophenotypical features. In this context, CD34 and STAT6 are considered the most reliable markers for SFTs, because CD99 and Bcl-2 expression may be variable.[3,5] CD34 is a transmembrane cell surface glycoprotein found in myeloid progenitor cells and in different neoplasms, including SFTs, in which its expression is almost 100%.[3,5] Moreover, the identification of the chromosomal fusion involving the *NAB2* (NGFI-A-binding protein 2) and *STAT6* genes contributed significantly for the diagnosis of SFTs because *STAT6* nuclear expression has been found to be highly sensitive and specific to SFTs.[9,12,24] In our series, all SFTs were positive for CD34 and STAT6 and 85% of the cases were positive for CD99, confirming the diagnostic value of the first 2 markers.[8,9,14,15] In addition, this result corroborates the findings of the recent systematic review by Morais et al.,[3] in which CD34, STAT-6, and CD99 were positive in 99.2%, 100%, and 76.2% of the oral SFTs, respectively.

The stem cell markers CD44, ALDH1, Bmi-1, and Nanog have recently been investigated in a wide range of benign and malignant tumors, because they confer stem cell properties to tumor cells. Additionally, they can be used, in isolation or in combination, to identify CSC subpopulations, which are responsible for tumor progression, metastasis, resistance to therapy, and tumor recurrence.[17]

CD44 is a transmembrane glycoprotein that participates in the proliferation of mesenchymal stem cells, cellular adhesion, and migration and cytokine and proapoptosis signaling.[25] This protein is highly expressed in different types of cancer and has been identified as a predictor of worse prognosis in soft tissue sarcomas.[26] In the present study, we observed a diffuse membranous positivity in all oral SFTs. Previous reports have also demonstrated high CD44 expression in SFTs in different locations, but no association with tumor behavior or prognosis was found.[9,27] We speculate that due to the multifunctional role of CD44 in normal and malignant stem cells, it may participate in tumor development, as observed in other tumor types.[25]

In this study, ALDH1 was highly expressed in 10/13 of oral SFTs and showed intermediate and low expression in 2/13 and 1/13 SFTs, respectively. ALDH1 is highly expressed in stem cells, and its high expression is linked to stem cell−like features in different malignant tumors, conferring advantages properties in relation to cellular proliferation, differentiation, invasion, and cell survival.[16,17,28] The *ALDH*1 gene has been identified as the most overexpressed gene in SFTs compared to the gene signature observed in soft tissue sarcomas.[29] Although its functional role in SFTs is unknown, it is possible that the high expression of *ALDH1* may contribute to the maintenance of the dedifferentiation and proliferation of tumor cells. Additionally, previous studies have indicated that ALDH1 is a sensitive and specific marker for differential diagnosis of SFTs, together with STAT6.[14,15]

Bmi-1 is a stemness-related gene that integrates the polycomb repressive complex 1, a key epigenetic regulator.[30] Through chromatin and histone alterations, Bmi-1 regulates the cell cycle and self-renewal of normal stem cells and CSCs via its suppressive effects on the INK4 a locus, which encodes the tumor suppressor genes $p16^{Ink4a}$ and $p14^{arf}$, leading to the deregulated expression of the downstream target genes p53 and Rb.[19,31] It is important to highlight that Bmi-1 is also a central gene in controlling other cellular processes, such as differentiation, apoptosis, senescence, epithelial to mesenchymal transition (EMT), and drug resistance, and its expression has been associated with poor prognosis in different cancers.[30,32]

In oral SFTs, we observed a high expression of Bmi-1 (9/13), which showed a tendency to be associated with a reduced expression of $p16^{Ink4a}$. However, the evaluation of both markers in a large cohort of SFTs is needed to establish the relationship between these interconnected molecules. Machado et al.[6] also found low/negative expression of $p16^{Ink4a}$ in 5/28 SFTs having at least one pathologic factor associated with aggressive behavior. Taken together, these results suggest that the elevated expression of Bmi-1 may result in the downregulation of $p16^{Ink4a}$, leading to the uncontrolled cellular proliferation seen in SFTs. However, functional studies are necessary to better address this question. Recently, Liang et al.,[33] in a series of 23 SFTs (14 in the central nervous system), reported longer, disease free survival in patients with low $p16^{Ink4a}$ expression. However, the authors emphasized the need for multi-institutional studies because the limited number of SFTs evaluated could have resulted in overestimation of the prognostic value of $p16^{Ink4a}$.[33]

In this study, we observed that the majority of the oral SFTs showed high expression of Nanog. This protein is a homeodomain-containing transcription factor with a critical role in regulating the self-renewal of embryonic stem cells and CSC, G1-S transition, epithelial-to-mesenchymal transition, and the metastatic potential of malignant cells.[34] Thus, we considered that the expression of this protein might favor SFT development, although additional studies are necessary to address this issue.

In summary, our results confirm the diagnostic value of STAT6 and ALDH1 in SFTs. The immunohistochemical findings showed that oral SFTs cells express the stem cell markers CD44, ALDH1, Bmi-1, and Nanog, which are also observed in CSCs from different tumors, conferring growth advantages. In addition, $p16^{Ink4a}$ low expression may contribute to deregulated cellular proliferation in oral SFTs. However, further functional studies using SFT cells and large cohorts of cases are necessary to gain a better understanding of the role of these stem cell markers in the development of SFTs as well as its diagnostic or prognostic relevance.

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