



HEALTH SCIENCES

Oral and uterine leiomyomas exhibit high immunoexpression of Cripto-1 compared to normal myometrium

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Abstract: Leiomyomas are the most common benign tumors in women. Many of them are associated with significant morbidity. The present study aimed to analyze histomorphological and histochemical characteristics and immunoexpression of Cripto-1 in oral leiomyomas (OL), uterine leiomyomas (UL), and normal myometrium (NM). Sample was composed of ten OL, 11 UL and 11 NM. Histomorphological characteristics were analyzed at 100 and 400x magnifications with HE staining. The immunoexpression of Cripto-1 was analyzed in five high-power fields. Statistical analysis considered a significant difference when $p < 0.05$. Six OL disclosed moderate/intense inflammatory infiltrate, while ten UL exhibited mild infiltrate ($p = 0.024$). When analyzing all leiomyomas together, 20 exhibited hyalinization, whereas no NM exhibited this alteration ($p < 0.001$). There was no statistical difference in the distribution of mast cells among the lesions. The median Cripto-1 was higher in UL (9.0), followed by OL and NM (4.0). Associations of the Cripto-1 expression between leiomyomas (separately and together) and NM were statistically significant ($p < 0.001$). These results indicate that OL and UL exhibit similar histomorphological and histochemical characteristics, as well as differences to NM. The higher immunoexpression of Cripto-1 in leiomyomas compared to NM suggests that this protein may influence cell proliferation and tissue architecture of oral and uterine leiomyomas.

Key words: Leiomyoma, Cripto-1, Immunohistochemistry, Neoplasia.

INTRODUCTION

Leiomyomas are benign smooth muscle-derived mesenchymal neoplasms. They are often found in the gastrointestinal tract and genitourinary tract, but may occur in various body locations where smooth muscle cells subsist (Gianluca et al. 2011, Islam et al. 2016).

Uterine leiomyomas (UL) are the most common benign tumors in women of reproductive age. They occur in about 77% of these women, but are clinically detectable in about 25% (Falcone & Parker 2013, Islam et al. 2013b, 2016). Generally, UL are asymptomatic, but

can significantly affect the woman's quality of life (Falcone & Parker 2013, Islam et al. 2013a). UL are responsible for most indications for hysterectomies, and cause significant morbidity (Islam et al. 2013b). Approximately 45% of U.S. women underwent hysterectomy (Stewart 2015). The morbidity related to UL may include: urethral obstruction, ureteral obstruction/hydronephrosis, lower urinary tract symptoms, vesicouterine fistula, renal failure, hematuria, sexual dysfunction, neurogenic bladder, and numerous non-urological complications (Dagur et al. 2016). Knowledge about the etiology and pathophysiology of uterine leiomyomas

is limited; consequently, the alternatives of medical treatments are restricted (Wegienka 2012, Flake et al. 2013, Islam et al. 2013a).

On the other hand, oral leiomyomas (OL) are rare, with a frequency varying from 0.016% to 0.065% among all leiomyomas (Nonaka et al. 2010), and probably have perivascular origin (El-Naggar et al. 2017). Clinically, an OL consists of a small, slow growing, and asymptomatic nodule, and is mostly located on the tongue, lips, palate and oral mucosa (Gaitan Cepeda et al. 2008).

The literature reports several variants of UL, including: cellular, epithelioid, palisaded, symplastic, lymphocytic rich, lipoleiomyoma, and less commonly, vascular variants (Kurman et al. 2014, Naz et al. 2019, WHO 2020). With respect to the OL variants, they can be solid, vascular or epithelioid (Neville et al. 2016). Some OL and UL variants share similar histological aspects. Cellular uterine leiomyomas and solid oral leiomyomas are composed of intersecting fascicles of spindle cells presenting pale staining, and blunt end. Angioleiomyomas exhibit numerous blood vessels with thickened walls. Intervening smooth muscle bundles are found between the vessels (Kurman et al. 2014, Neville et al. 2016, WHO 2020).

There is a considerable difference between the frequency of leiomyomas in the oral cavity and in the uterus, and both the etiological factors and pathophysiology of these lesions remain obscure (Ciarmela et al. 2011a, Islam et al. 2013a). Several studies in literature have sought to establish molecular markers that may be associated with the development of leiomyomas and to clarify their variable behaviors (Gaitan Cepeda et al. 2008, Ciarmela et al. 2011b). Among these markers, there is Cripto-1.

Cripto-1 belongs to the family of EGF-CFC proteins (epidermal growth factor - Cripto/FRL1/Cryptic). These proteins are involved in the activation of several different signaling

pathways during embryonic development and cell transformation (Yoon et al. 2011). This marker has been related to several neoplastic processes, such as cell proliferation, migration, invasion and tumor angiogenesis (Ravisankar et al. 2011). Besides its' known association with endometrial and myometrial tumors (Strizzi et al. 2007, Ciarmela et al. 2011a), several studies have also reported the association of the expression of Cripto-1 in neoplasms of different origins such as: tumors of the breast, gastrointestinal tract, lung, ovary, testis, bladder, prostate, oral squamous cell carcinoma, and salivary glands (Silva et al. 2018). Additionally, this protein is thought to be poorly expressed or absent in the normal counterparts of these tissues (Strizzi et al. 2007, Ciarmela et al. 2011a, Yoon et al. 2011).

Considering that knowledge about the etiopathogenesis of oral leiomyomas is not yet entirely understood, and important morbidity is associated with uterine leiomyomas, new biological and molecular markers are needed to aid in the understanding of the development and behavior of these lesions. Thus, the objective of this study was to comparatively analyze the clinical-pathological characteristics of oral leiomyomas, uterine leiomyomas and normal myometrium.

MATERIALS AND METHODS

Sample

This was a cross-sectional study that was approved by the Research Ethics Committee of the Onofre Lopes University Hospital from Federal University of Rio Grande do Norte (protocol number 762.331), and conducted according to the resolution number 466/12 of the National Health Council.

This research was performed at Department of Pathology from the Federal University of Rio Grande do Norte, Natal, Brazil. The period of

sample selection, collection of the data, and analysis of the results was between 2015 – 2016.

The sample consisted of ten cases of oral leiomyomas, 11 uterine leiomyomas and 11 cases of normal myometrium. Considering the rarity of oral leiomyomas, we selected a similar number of uterine leiomyomas and normal myometrium in order to standardize the sample. Cases were selected intentionally, according to the presence of sufficient tissue in the paraffin blocks and adequate storage conditions of the specimen in paraffin. From the uterine leiomyomas, the vascular and typical variants were selected because they presented histopathological characteristics similar to most of the oral leiomyomas (Weiss et al. 2007). Samples of normal myometrium were taken from areas away from the lesion areas previously removed by hysterectomy from the same patients.

Histomorphological and histochemical analysis

Histomorphological and histochemical analysis were performed using an optical microscope (DM500, Leica Microsystems, Heerbrugg, Switzerland). Slides stained with hematoxylin and eosin (HE) were obtained, and the presence and/or intensity of: inflammatory infiltrate, hyalinization, hemorrhage, edema, hydropic degeneration, calcification, adipocytes, giant cells and necrosis were analyzed – morphological findings that are commonly observed in these lesions, according to literature (Nucci & Oliva 2009). With regard to inflammatory infiltrate, it was classified as mild, moderate, or intense. For the subsequent statistical analysis, the cases that were classified as moderate and intense were joined together.

The amount and distribution of collagen and smooth muscle were analyzed over the entire length of the specimen slides by using Masson's Trichrome technique (Suvarna et al.

2013). A classification of the amount of collagen was standardized according to the cross system (+, predominance of smooth muscle in the lamina; ++, balance in the proportion of collagen and muscle; +++, prevalence of collagen). For the statistical analyses, the cases in which there was a predominance of collagen and those in which there was a balance in the proportion of collagen and muscle tissue were joined together. Regarding localization, the collagen was categorized as focal or diffuse on the slide.

With the use of the Toluidine Blue technique (Suvarna et al. 2013), the presence and quantity of mast cells were assessed in the different tissue specimens. Thus, ten high-power fields (400x) exhibiting the highest densities of the cells were selected. All cells with purple staining and histologically compatible with mast cells were counted. Thus, the median of the ten fields was obtained, which represented the value of each case, as adapted from Santos et al. (2011).

Immunohistochemical analysis

Tissue sections of 3µm were obtained and submitted to the immunohistochemical technique through a biotin-free polymer system. The deparaffinization and antigenic recovery were performed through the Trilogy solution (Cell Marque, Rocklin CA, USA). The specimens were incubated for 1h at 4°C with the anti-Cripto-1 antibody (clone H-10, Santa Cruz Biotechnology, Inc., Dallas, Texas, USA), diluted at 1:500. The amplification reaction was done through EnVision+HRP (#K8021; Dako, Glostrup, Denmark) and visualized by diaminobenzidine. Positive control was performed using mammary adenocarcinoma tissue according to manufacturer's instructions. The negative control was obtained by replacing the antibody with normal rabbit serum.

Images of the slides were obtained through an optical microscope coupled to a digital

camera (ICC50, Leica Microsystems, Heerbrugg, Switzerland). Images of five random fields for each case were analyzed (400x magnification). The intensity and area of the immunostaining were analyzed in absolute numbers through ImageJ software (1.6.0_24 version; NIH, Bethesda, MD, USA), with the FIJI package (Schindelin et al. 2012), as described by Sysel et al. (2013). For intensity, scores were set from "1 to 3 (1, weak; 2, moderated; 3, intense), as well as for the percentage of the immunopositive area (1, <33%; 2, 33-67%; 3, >67%). The immunoreactivity score for each case was obtained by multiplying the intensity scores by those of the area (value from 1 to 9), according to Ertoy et al. (2000).

Statistical Analysis

The data obtained were analyzed using SPSS software (Statistical Package for Social Sciences, version 22, Chicago, IL, USA). The results were submitted to the Pearson chi-square, Mann-Whitney and Kruskal-Wallis statistical tests, considering the level of significance when $p < 0.05$.

RESULTS

The average age of patients with UL/NM was 54.7 years of age (ranging from 36 to 88 years), and for the patients with OL, it was 25.9 years of age (ranging from 7 to 44 years). Four patients (36.4%) were brown-skinned and three (27.2%) were white. For four patients (36.4%), there was no information about the skin color in the medical records. Of the patients with OL, three (30%) were male and six (60%) were female. One of the medical records did not have information about the patient's sex. Most were brown-skinned ($n=10/47.6\%$). Lesions were located in the alveolar ridge (4 / 40%), tongue (2 / 20%),

palate (2 / 20%), mouth floor (1 / 10%), and bottom of buccal sulcus (1 / 10%).

Histomorphological and histochemical analysis

The inflammatory infiltrate was moderate or severe in six (60%) of the OL, and was classified as mild in ten (90.9%) of the UL; this result was statistically significant ($p=0.016$) (Figure 1a, b; Table I). Hyalinization was present in all OL and in ten (90.9%) UL. When analyzing all leiomyomas (oral and uterine), 20 (95.2%) revealed areas of hyalinization, whereas no case of NM exhibited such alteration ($p < 0.001$) (Figure 1c, d). Intra-tissue hemorrhages were detected in eight (80%) OL and in one (9.1%) NM. No UL revealed presence of hemorrhages. There was edema in eight (80%) OL, one (9.1%) UL and two (18.2%) NM. Hydropic degeneration was observed in five (50%) OL, six (54.4%) UL and three (27.3%) NM. When analyzing oral and uterine leiomyomas together, 11 (78.6%) revealed hydropic degeneration, whereas three NM (21.4%) exhibited this alteration ($p=0.266$). Calcification was present in hyalinized areas of two (20%) OL. One (9.1%) NM showed calcification of perivascular areas. Adipocytes were identified in only one slide (10%) of OL. Also, there were giant cells in just one (10%) OL. There was no necrosis in any of the slides.

There was a predominance of smooth muscle on the collagen in four (40%) OL, four (36.4%) UL and 11 (100%) NM. A balance or predominance of collagen in relation to the muscle was found in six (60%) OL and seven (63.6%) UL ($p=0.867$). In the joint analysis of leiomyomas (oral and uterine) versus normal myometrium, there was balance (of collagen and muscle) or predominance of collagen in 13 of the 21 leiomyomas (61.9%), while muscle tissue predominated in 100% of the 11 normal myometrium ($p=0.001$) (Figure 1e-g; Table I). The distribution of collagen was diffuse in nine (90%)

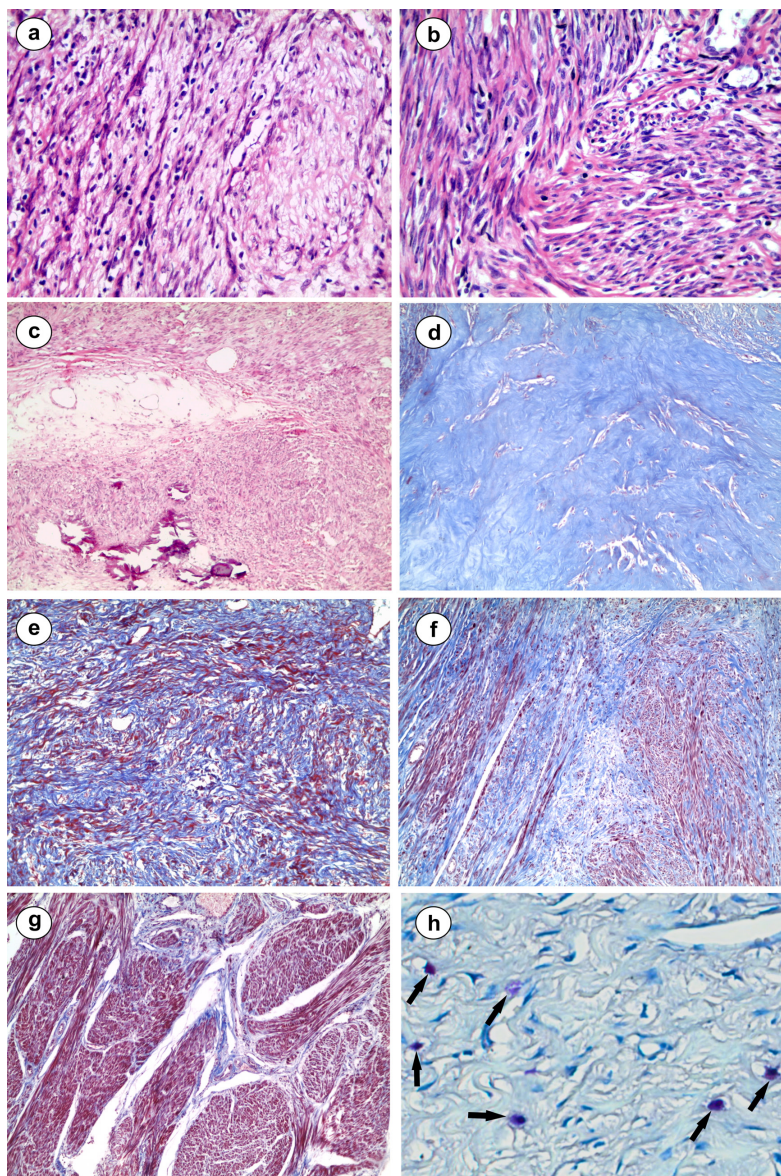


Figure 1. Histomorphological and histochemical characteristics of the lesions. (a) Intense inflammatory infiltrate in oral leiomyoma (OL) (HE, 400x). (b) Mild inflammatory infiltrate in uterine leiomyoma (UL) (HE, 400x). (c) Area of hyalinization and calcification in OL (HE, 100x). (d) Extensive hyalinization in UL (Masson's Trichrome, 100x). (e), (f) Prevalence of collagen versus muscle tissue in OL and UL, respectively (Masson's Trichrome, 100x). (g) Predominance of muscle tissue versus collagen in normal myometrium (Masson's Trichrome, 100x). (h) Distribution of mast cells in OL (arrows, Toluidine blue, 400x).

OL, nine (81.8%) UL and in all (100%) NM. Only one (10%) OL and two (18.2%) UL disclosed focal distribution of collagen, which was concentrated at specific extensive areas of hyalinization.

Mast cell distribution values revealed a higher median for OL and UL (2.0) than for NM (1.5), although not statistically significant ($p > 0.05$ in both) (Table II). There was no significant association between the number of mast cells and other histomorphological parameters ($p > 0.05$).

Immunohistochemical analysis

Cripto-1 median scores were higher for the UL (9.0), followed by the OL (4.0) and finally by NM (2.0) (Figure 2a-c). The associations between UL x NM and OL x NM revealed statistically significant differences ($p < 0.001$). When all leiomyomas were analyzed along with NM, a significant difference ($p < 0.001$) was also observed. The association between UL x OL showed no statistically significant difference ($p = 0.378$) (Table III).

The median Cripto-1 was higher in the specimens with presence of hyalinization (6.0) than in those with absence (2.0). This result was statistically significant ($p < 0.001$). Other associations of Cripto-1 immunoeexpression with histological parameters were not statistically significant ($p > 0.05$).

DISCUSSION

In the present study, most oral leiomyomas exhibited moderate or severe inflammatory infiltrate, whereas most of the uterine leiomyomas presented mild infiltrate. The presence of inflammatory infiltrate in oral

leiomyomas has also been reported in previous studies (Gaitan Cepeda et al. 2008, Liu et al. 2014). According to the literature, this process of inflammation may occur secondarily in response to mechanical factors (Liu et al. 2014). Regarding uterine leiomyomas, studies suggest that chronic inflammatory processes could be associated, in part, with the pathogenesis of these neoplasms (Wegienka 2012, Protic et al. 2016), which may lead to an increase in the amount of estrogen produced (Wegienka 2012). Protic et al. (2016) observed a greater amount of inflammatory cells around and near UL compared to around NM, indicating an influence of the chronic inflammation in these lesions. Based on these studies, our results suggest that

Table I. Associations between the different lesions and inflammatory infiltrate and distribution of muscle tissue and collagen.

Lesions/tissue	Inflammatory infiltrate – n(%)		p value*
	Mild	Moderate/Intense	
UL x	10 (90.9)	1 (9.1)	0.016
OL	4 (40)	6 (60)	
UL x	10 (90.9)	1 (9.1)	0.061
NM	6 (54.5)	5 (45.5)	
OL x	4 (40)	6 (60)	0.515
NM	6 (54.4)	5 (45.5)	
UL + OL x	14 (66.7)	7 (33.3)	0.508
NM	6 (54.5)	5 (45.5)	
	Muscle x Collagen – n(%)		
	MUS > COL	MUS = COL / MUS < COL	
UL x	4 (36.4)	7 (63.6)	0.867
OL	4 (40)	6 (60)	
UL x	4 (36.4)	7 (63.6)	0.002
NM	11 (100)	0 (0)	
OL x	4 (40)	6 (60)	0.003
NM	11 (100)	0 (0)	
UL + OL x	8 (38.1)	13 (61.9)	0.001
NM	11 (100)	0 (0)	

*Pearson’s Chi-square test.

Abbreviations: UL – uterine leiomyoma, OL – oral leiomyoma, NM – normal myometrium, MUS – muscle, COL – collagen.

the inflammatory process may influence the development of OL, and that traumatic factors in the oral cavity may explain the greater amount of inflammatory cells in OL compared to in UL.

All oral leiomyomas presented hyalinization, and in half of the cases it was moderate or intense. The presence of hyalinization in oral leiomyomas is controversial, since some authors affirm that this finding is common in leiomyomas of any localization (Montague et al. 2014), while others state that oral cavity leiomyomas do not have hyalinized collagen (Chang & Kessler 2008). Our results point to the similarity between oral and uterine leiomyomas in relation to the production of the collagen matrix, since – as in oral cases – almost all of the uterine cases exhibited hyalinization. Another feature found in certain oral tumors was foci of dystrophic calcification; although rare, this process has already been described in oral leiomyomas and originates from progressive degeneration in the hyalinized stroma (Nonaka et al. 2010, Gianluca et al. 2011).

In most leiomyomas, there was balance or predominance of collagen in relation to the smooth muscle. In all the cases of normal myometrium, the predominance of muscle tissue on the collagen was verified. This result is in line with Protic et al. (2016), which reported significantly higher amounts of collagen in uterine leiomyomas compared to normal myometrium. Furthermore, according to Flake et al. (2013), in the initial stage of leiomyomas, the collagen matrix is minimal (as well as in normal myometrium) and, as the tumor reduces its mitotic activity, the extent of fibrosis increases, so that in the final phases there is a clear predominance of collagen over the muscle. The accumulation of aggressive factors, like reproductive events, mechanical trauma, oxidative stress, and others, contribute to induce an inflammatory status. Such chronic inflammatory condition induces myofibroblasts to continuously produce extra cellular matrix, resulting in an excessive response and tissue growth (Islam et al. 2018). Thus, our results suggest that the specimens in our sample were

Table II. Distribution of mast cells between the different lesions.

Lesions/tissues	n	Median	Q ₂₅ -Q ₇₅	Mean of ranks	Sum of ranks	U	p
UL x	11	2.0	1.5-2.5	9.73	107	41	0.296*
OL	10	2.0	2-4.75	12.4	124		
UL x	11	2.0	1.5-2.5	12.27	135	52	0.569*
NM	11	1.5	1-2.5	10.73	118		
OL x	10	2.0	2-4.75	12.7	127	38	0.221*
NM	11	1.5	1-2.5	9.45	104		
UL x	11	2.0	1.5-2.5	16.00			0.389**
OL x	11	1.5	1-2.5	14.18			
NM	10	2.0	2-4.75	19.60			

*Mann-Whitney U test ; **Kruskal Wallis test.

Abbreviations: UL – uterine leiomyoma, OL – oral leiomyoma, NM – normal myometrium.

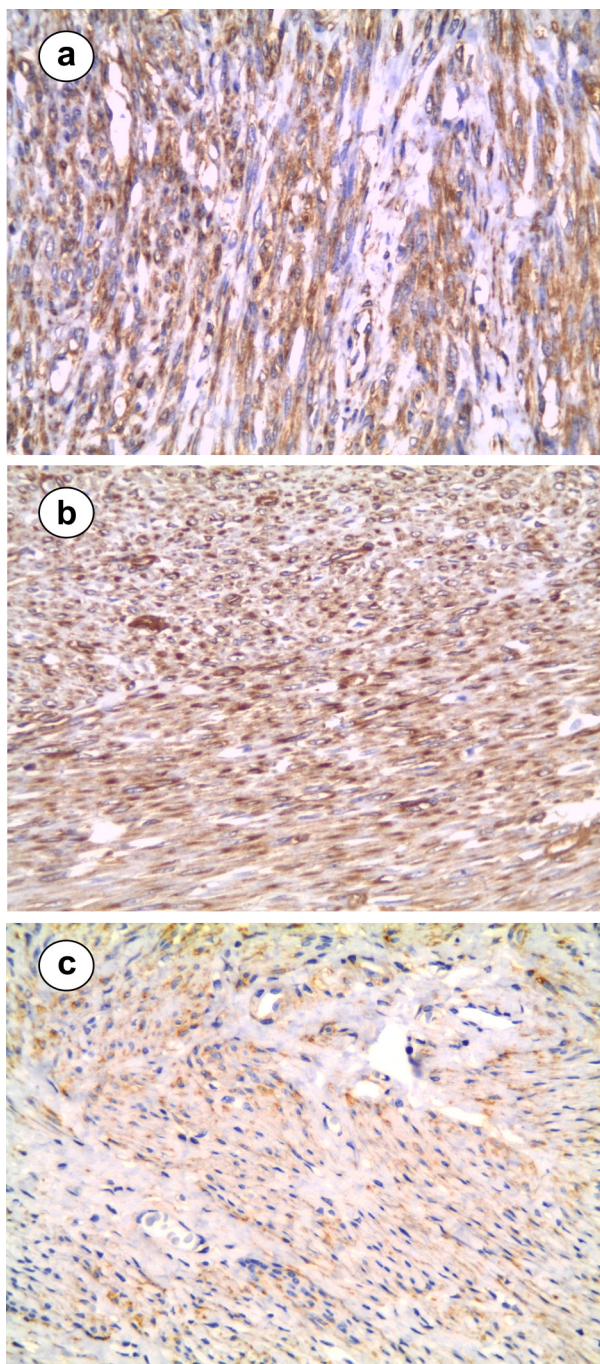


Figure 2. Immunoeexpression of Cripto-1 in the lesions. (a) Intense and diffuse immunoeexpression of Cripto-1 in oral leiomyoma (400x). (b) Intense and diffuse immunoeexpression of Cripto-1 in uterine leiomyoma (400x). (c) Mild and diffuse Cripto-1 immunoeexpression in normal myometrium (400x).

in more advanced stages of development, with less muscle proliferation and more stroma.

The localization of collagen was diffuse in most leiomyomas and in all normal myometrium. In these cases, collagen was mainly present in septa of fibrous tissue between smooth muscle fascicles, surrounding muscular tissue. This finding of interfascicular fibrosis is described in literature by Chang & Kessler (2008) and Veeresh et al. (2013), as being typical of oral leiomyomas; and by Flake et al. (2013), in uterine leiomyomas and normal myometrium. Flake et al. (2013) add that – unlike the adjacent normal myometrium – collagen is visualized by Masson’s Trichrome in uterine leiomyomas not only around, but also within, muscular bundles, that is, intrafascicular fibrosis. This finding is consistent with our results, in which fibrosis was more prominently and disseminated in the tumors, while the cases of normal myometrium presented comparatively less collagen.

Our study did not observe significant differences in the number of mast cells among the studied specimens. Other studies reported in the literature also did not observe significant differences in the amount of these cells between uterine leiomyomas and normal myometrium (Goksu Erol et al. 2011, Protic et al. 2016). Protic et al. (2016) state that the amount of mast cells in these tissues may be an occasional finding, associated to the high variability of these cells among the patient samples. However, according to Jiang et al. (2013), the involvement of mast cells in neoplasms has revealed contradictory results, so that these cells may promote or inhibit tumor growth depending on local stromal conditions. Considering these results, new studies in different populations are necessary to clarify if there is any role of mast cells in leiomyomas.

Our study observed a greater immunoeexpression of Cripto-1 in oral and uterine leiomyomas compared to normal

Table III. Distribution of Cripto-1 immunoexpression scores according to the type of lesion.

Lesions/tissues	n	Median	Q ₂₅ -Q ₇₅	Mean of ranks	Sum of ranks	U	p
UL x OL	11 10	9 4	4 – 9 4 – 9	12.05 9.85	132.5 98.5	43.5	0.378*
UL x NM	11 11	9 2	4 – 9 2 – 3	16.32 6.68	179.5 73.5	7.5	<0.001*
OL x NM	10 11	4 2	4 – 9 2 – 3	16.5 6.0	165.0 66.0	<0.0	<0.001*
UL x OL x NM	11 10 11	9 4 2	4 – 9 4 – 9 2 – 3	22.36 20.85 6.68			<0.001**

*Mann-Whitney U test ; **Kruskal Wallis test.

Abbreviations: UL – uterine leiomyoma, OL – oral leiomyoma, NM – normal myometrium.

myometrium. Several studies have reported that Cripto-1 is expressed in normal tissues, although at very low concentrations, but it exhibits high expression in neoplastic tissues (de Castro et al. 2010, Silva et al. 2018). Ciarmela et al. (2011a), in turn, identified the expression of Cripto-1 mRNA in uterine leiomyomas, but not in normal myometrium. Literature states that in normal tissues, Cripto-1 has the role of regulating embryonic development and has been known to be a marker for undifferentiated human embryonic stem cells (James et al. 2005, de Castro et al. 2010). The expression of Cripto-1 in normal adult tissue has been better described in breast tissue. In this tissue, Cripto-1 participates in the morphogenesis and specification of the cell fate of the mammary stem cells, which will originate the basal/myoepithelial and luminal cell compartments of the mammary gland (Rangel et al. 2016). To date, our study is the first to identify the immunoexpression of this protein in normal myometrium. Its real role in this specific tissue and in other normal tissue still needs to be clarified in further research.

There was no significant difference in Cripto-1 immunoexpression between uterine and oral leiomyomas. The immunoexpression of Cripto-1 in uterine leiomyomas has previously been reported in literature (Strizzi et al. 2007, Ciarmela et al. 2011a). In these lesions, Ciarmela et al. (2011a) claim that Cripto-1 has the role of inhibiting two cyostatic proteins, activin and myostatin, thus contributing to tumor growth. These authors state that the presence of the Cripto-1 mRNA observed in leiomyomas suggests that this molecule may be an auxiliary factor for the tumorigenesis of these lesions. From these results, we can suggest that the immunopositivity of Cripto-1 in uterine leiomyomas observed in this study indicates that this protein may be involved in the development of these lesions.

In the present study, all oral leiomyomas were immunopositive for Cripto-1. Previous studies that correlated the expression of Cripto-1 in breast cancer found no correlation between this protein and the estrogen and progesterone hormone receptors (Normanno et al. 1995, Panico et al. 1996). In their study of uterine tumors from nulliparous and multiparous transgenic murine,

Strizzi et al. (2007) suggest that the oncogenic effects of Cripto-1 do not depend on ovarian hormones. Previous studies have failed to identify the presence of estrogen (ER) (Chang et al. 2014, Grimm et al. 2016) or progesterone (PR) receptors in normal oral mucosa or hyperplastic processes. The progesterone receptor was also not observed in precursor or malignant oral lesions (Grimm et al. 2016). Through these results, we can suggest that the expression and oncogenic effect of Cripto-1 on OL probably is not hormone dependent. Although there are no previous studies investigating this protein in oral leiomyomas, the results observed in our study suggest that Cripto-1 similarly influences tumorigenesis in uterine and oral leiomyomas.

The median Cripto-1 was higher in the specimens with presence of hyalinization than in those with its absence. The hyalinization commonly observed in leiomyomas results from the excessive production of extracellular matrix components (Flake et al. 2013). Yang & Mutter (2015) found that necrotic/infarcted areas of uterine leiomyomas underwent a process of replacement of dead tissue by dense hyalinized tissue. These authors also observed an increase in mitotic activity in the interface regions between repair tissue and dead tissue. Considering the result of the present research, we suggest that the observed increase in the expression of Cripto-1 in hyalinized leiomyomas may indicate that this protein contributes to cell proliferation and to the process of tissue repair with extracellular matrix production and consequent hyalinization in these lesions.

The main limitations observed during the development of the present study are related to the size of the sample. The rarity of the oral leiomyomas combined with the low amount of tissue in the paraffin blocks, contributed to the limited quantity of cases. Some paraffin blocks also failed to reveal an adequate condition of

use; therefore, they could not be included in the experiments.

The present results indicate that there are histomorphological and immunoexpression similarities between oral and uterine leiomyomas, as well as differences between these neoplasms and normal myometrium. The findings associated with Cripto-1 suggest that this protein may contribute to the development of both oral and uterine leiomyomas, emphasizing its role in cell proliferation and repair processes with hyaline matrix production. Further studies using different techniques that can confirm these results are recommended.

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How to cite

SILVA AF, DA SILVA ECC, OLIVEIRA CN, ROCHA KBF, MIGUEL MCC & PEREIRA JS. 2020. Oral and uterine leiomyomas exhibit high immunoreexpression of Cripto-1 compared to normal myometrium. *An Acad Bras Cienc* 92: e20200519. DOI 10.1590/0001-3765202020200519.

*Manuscript received on April 10, 2020;
accepted for publication on September 21, 2020*

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JSP and MCCM: Conception and design of the work. JSP, AFS, ECCS, CNO; KBFR and MCCM: Acquisition, analysis and interpretation of data; JSP, AFS and MCCM: Drafting and revising the work for intellectual content; JSP, AFS, ECCS, CNO, KBFR and MCCM: Final approval of the version to be published.

