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ORIGINAL ARTICLE

Signal transducer and activator of transcription expression 3 (STAT3) in potentially malignant oral lesions

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Abstract:

Objective: The present study evaluated the expression and activation of the STAT-3 in potentially malignant disorders and its association with dysplasia grade. **Methods:** Twenty-four fragments from two distinct areas, namely the buccal mucosa and the tongue, with 10 cases of hyperkeratosis without dysplasia and 14 cases of hyperkeratosis with mild, moderate or severe dysplasia, were analyzed. Immunohistochemistry for STAT-3 and P-STAT-3 was performed using the biotin-streptavidin-peroxidase method. **Results:** In both hyperkeratosis with and without dysplasia STAT-3 demonstrated cytoplasmic expression in epithelial layers. Nuclear expression in hyperkeratosis with mild or moderate dysplasia was seen. In hyperkeratosis with severe dysplasia the lower epithelial layers there were areas exhibiting loss of labeling. P-STAT-3 showed nuclear expression in all epithelial layers, except for the superficial layer, in hyperkeratosis without dysplasia. In hyperkeratosis with mild and moderate dysplasia, nuclear expression was seen in all epithelial layers, except for the superficial layer, with a few cells showing no expression. **Conclusion:** Regarding hyperkeratosis with severe dysplasia, there was loss of expression in a higher number of cells. In both hyperkeratosis with and without dysplasia, STAT-3 activation was found to be abnormal and no significant difference for STAT-3 expression at different dysplasia grades was observed.

Keywords: STAT3 Protein; Leukoplakia; Immunohistochemistry

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1. INTRODUCTION

Potentially malignant oral disorders are lesions with morphological alterations that put them at a high risk for malignant transformation when compared with normal tissue. Oral leukoplakia is considered the commonest of these lesions[1]. Clinically, presents as a mark or white plaque that cannot be defined clinical or histologically as another disease. Histologically, it can vary from a simple hyperkeratosis and acanthosis, to severe epithelial dysplasia. Cumulative epithelial dysplasia is the most important predictive factor for malignant transformation; however, oral lesions that do not histologically demonstrate epithelial dysplasia may also suffer malignant transformation, whilst dysplastic lesions are also reported to regress[2]. In addition, the fact that epithelial dysplasia grading is essentially subjective has resulted in fruitless attempts to identify a reliable prognostic marker.

Signal Transducer and Activator of Transcription (STAT) proteins remain latent in the cytoplasm, until activated by growth factors or cytokines to participate in gene control[3,4]. They are involved in normal cellular processes, such as embryonic development, regulation of cellular differentiation, growth, apoptosis and angiogenesis. However, many studies indicate that atypical activation/phosphorylation of STAT could be involved in the initiation and progression of human cancers[5]. Effectively activated STAT proteins have been detected in a large number of human cancer cell lines and primary tumors[6], including in lymphoma, myeloma, glioma, and breast, prostatic, ovarian, head and neck carcinomas and oral squamous cell carcinoma[7]. Cury et al. (2007)[8] showed that STAT-3 expression in actinic cheilitis is dependent upon dysplasia grade, and that the activation of STAT-3 in this disease is abnormal.

Considering the difficulties in establishing leukoplakia prognosis, a study of the proteins involved in the process of proliferation could shed light on the signaling pathways that precede malignant transformation. Therefore, the present study evaluated the expression and activation of the STAT-3 in oral leukoplakia, as well as its association with epithelial dysplasia grade.

2. MATERIAL AND METHODS

The present study was approved by the Sao Leopoldo Mandic, Center for Dental Research Ethics Committee (06/323).

Twenty-four tissue fragments from the archive of Center of Dentistry Research (São Leopoldo Mandic, Campinas), fixed in formalin and embedded in paraffin, from two different anatomical areas namely the buccal mucosa and the tongue, diagnosed clinically as leukoplakia, were obtained. Dysplasia was graded according to the Banóczy and Csiba criteria (1976), which are based upon the epithelial alterations established by the World Health Organization (WHO), 2005, which include basal stratification, bulbous drop-shaped rete pegs, increased mitotic figures, loss of basal cell polarity, nuclear/cytoplasmic variation, nuclear pleomorphism, nuclear hyperchromatism, increased nucleoli, keratinization of individual or groups of cells in the stratum spinosum and loss of intercellular adherence. Dysplasia was considered mild when two epithelial alterations were present, moderate when two to four epithelial alterations occurred and severe when more than five epithelial alterations were present. Of the 14 cases of hyperkeratosis with dysplasia, seven were shown to be mild, four moderate and three severe. All cases were reviewed by three pathologists.

Five fragments of normal lower labial mucosa, present in submucosal lesions without epithelial alterations, were used as the control group. The paraffin blocks where cut in to 3 µm sections for immunohistochemistry, following the Streptavidin-Biotin method using the STAT-3 (#9132 Cell Signaling Technology Inc) and P-STAT-3 (Tyr 705) (#9131 Cell Signaling Technology Inc.) polyclonal antibodies. The primary antibody reaction was omitted as a negative control. Quantitative analysis of the STAT-3 and P-STAT-3 labeled samples was performed by three pathologists, using photographs from five random fields at 400X magnification, to a minimum of 500 cells for each case.

Positive and negative cells were presented in terms of percentage of labeled nuclei and cytoplasm, taking into account dysplasia grade and epithelial layer (basal, parabasal, squamous and superficial or keratinized). Subsequently, the average percentage between each group (hyperkeratosis without dysplasia, mild, moderate and severe dysplasia) was described.

3. RESULTS

Histologically, the 24 samples comprised: 10 cases of hyperkeratosis without dysplasia, with eight from the buccal mucosa and two from the tongue, six being male and four female, with an average age of 49.7 years

(range 22-72 years) and; 14 cases of hyperkeratosis with dysplasia, seven from the buccal mucosa and seven from the tongue, nine being male and five female, with an average age of 60.2 years (range 42-77 years)

3.1 STAT-3

In the normal labial mucosa, STAT-3 was expressed in the cytoplasm of the basal, parabasal and lower squamous layers (Figure 1A). Strong nuclear STAT-3 positivity was demonstrated in all epithelial layers, with a decreasing intensity from the basal to the superficial layers, with no labeling in the keratinized layer (Figure 1B).

STAT-3 was positive in the cytoplasm of 89.97% of the epithelial cells with decreasing intensity from the basal and parabasal to the superficial layers in the 10 cases of hyperkeratosis without dysplasia (Figure 2B). The superficial keratinized layers, in addition to a few cells of the upper squamous layer, were negative. A few positive nuclei in the basal layer (17.86%) were also present (Figure 2B).

Regarding the seven cases of hyperkeratosis with mild dysplasia (Figure 2D), 90.38% of the cells were positive in the cytoplasm (Figure 2E). In the four cases of moderate dysplasia (Figure 3A), 87.60% of the cells were also positive in the cytoplasm (Figure 3B). STAT-3 showed cytoplasmic expression in 96.05% of the epithelial cells with severe dysplasia (Figure 3E). In all cases, labeling was seen in the cells of all layers, except for the superficial keratinized layer, generally with a decreasing intensity from the basal and parabasal towards the superficial cell layers. In the cases of mild and moderate dysplasia, the most superficial layers were negative, whereas in hyperkeratosis with severe dysplasia, some basal and parabasal layer cells (two cases), or in the squamous layer (one case) were negative. In moderate and severe dysplasia, there were also positive nuclei in some cells (3.32% and 26.40%, respectively), mainly found in the basal layer for moderate dysplasia and the basal and squamous layers for severe dysplasia in areas where there was also no cytoplasmic immunolabeling in the lower layers.

3.2 P-STAT-3

P-STAT-3 immunolabeling was observed in 72.18% of the nuclei in the hyperkeratosis without dysplasia group, 86.37% of mild dysplasia, 87.17% of moderate dysplasia, and 88.68% of severe dysplasia. For all cases, the labeling intensity was heterogeneous, varying from intense to mild, and was distributed throughout the epithelium, with the exception of the superficial layer. A few isolated negative cells were observed in the basal and parabasal layers. In mild, moderate and severe dysplasia, negative cells were observed both in the basal and parabasal layers, and the squamous layer. In mild and moderate dysplasia, negative cells were sparsely present, whilst they formed groups in severe dysplasia. Interestingly, two cases of severe dysplasia also showed cytoplasmic positivity in 18.97% of cells in the basal and squamous layers (Figures 2C, 2F, 3C and 3F).

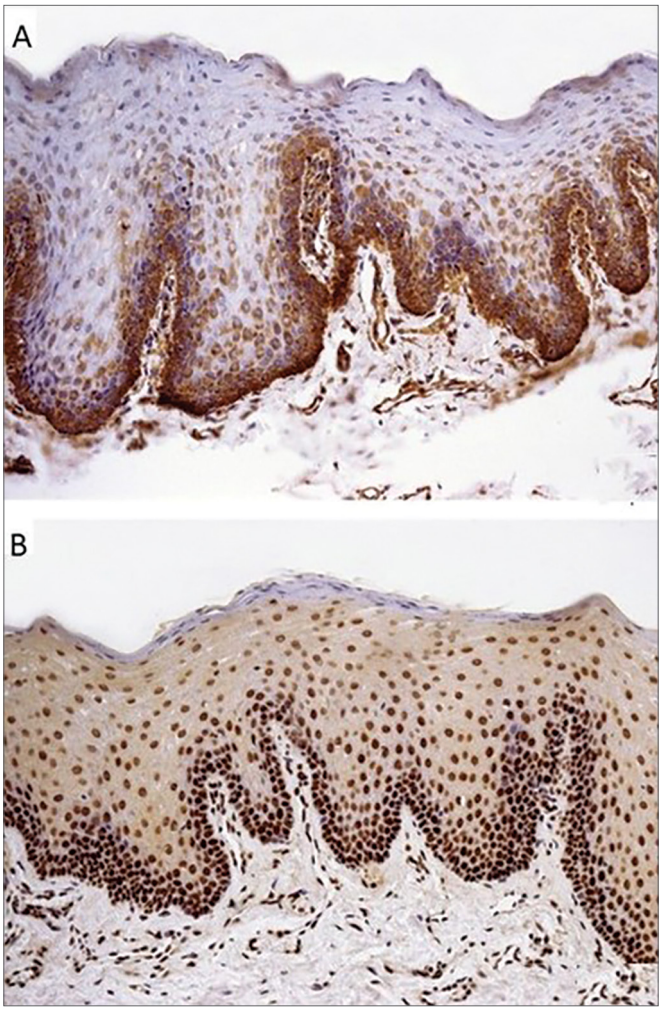

Figura 1. Normal labial mucosa. A - Immunohistochemistry for STAT-3 (200x). Cytoplasmic STAT-3 positivity in the basal, parabasal and lower squamous cell layer. The superficial layers are negative. B - Immunohistochemistry for P-STAT-3 (200x). Nuclear expression in all epithelial cell layers, except the parakeratinized layer.

4. DISCUSSION

In normal cells, STAT-3 activation is tightly controlled to prevent deregulated gene transcription.

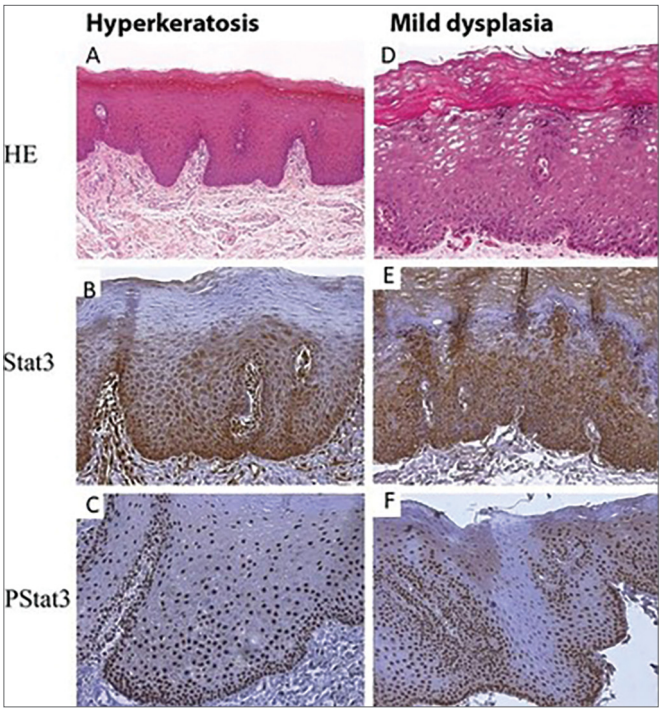

Figura 2. - A-C & D-F - Hyperkeratosis without and with mild dysplasia, respectively. A&D - HE 100x and 200x. B&E - Immunohistochemistry for STAT-3 (200X). C&F - Immunohistochemistry for P-STAT-3 (200X).

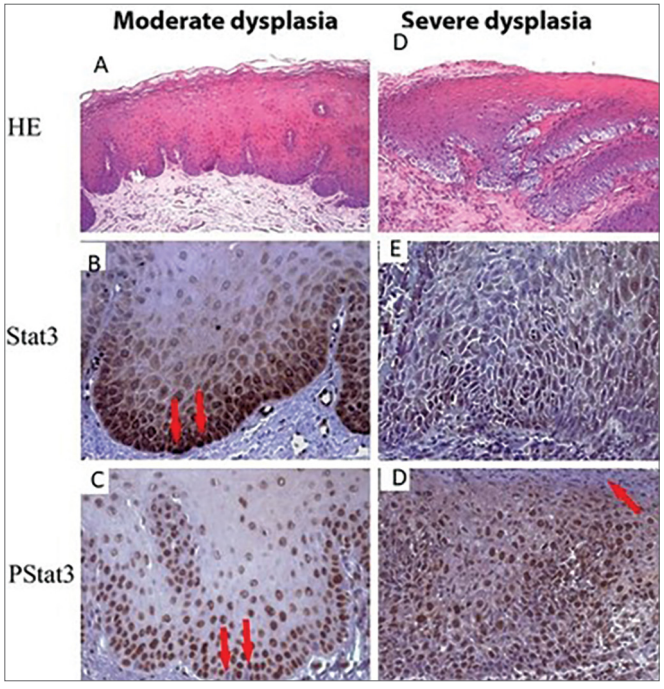

Figura 3. A-C & D-F - Hyperkeratosis with moderate and severe dysplasia, respectively. A&D - HE 100x and 200x. B & E - Immunohistochemistry for STAT-3 (200X). In B, rare nuclear expression is noted in the basal layer (arrow). In E, loss of cytoplasmic expression, as well as nuclear positivity in some cells of the basal and squamous layer is observed. C & F - Immunohistochemistry for P-STAT-3 (400X). In C, a few negative cells in the basal layer are noted (arrows). In F, cytoplasmic expression in some cells of the basal and squamous layers, as well as absence of expression in the keratin layer (arrow) can be observed.

However, in tumorigenesis activated STAT-3 plays an important role via the upregulation of genes involved in anti-apoptosis, proliferation and angiogenesis.

The literature is controversial in terms of the expression of STAT-3 in normal epithelium. As with the present study, Quadros et al.[9] and Cury et al.[8] demonstrated that STAT-3 and P-STAT-3 are expressed by normal keratinocytes, however, Nagpal et al.[10] did not detect their expression, attributing this absence to the fact that STAT3 binding-dependent activation is a transient process, which may take minutes to hours[11]. In this study, as found by Cury et al.[8], immunolabeling was observed mainly in the lower epithelial layers (basal, parabasal and deep squamous), which corroborates its role in cell cycle progression.

In hyperkeratosis, both with and without dysplasia, STAT-3 and P-STAT-3 immunolabeling was observed throughout the epithelium, with the exception of the superficial layers, especially the keratinized layer. In addition, some nuclei demonstrated positivity for STAT-3 and negativity for P-STAT-3, as well as the heterogeneous intensity of nuclear labelling by P-STAT-3 throughout the epithelium and cytoplasmic labelling in severe dysplasia. However, there was no significant quantitative difference in expression of either antibody between hyperkeratosis with or without dysplasia, or between the different dysplasia grades. Although a small difference could be noted, such as the loss of cytoplasmic STAT-3 expression and nuclear labeling in some cells in the lower squamous cell layer in severe dysplasia, and loss of P-STAT-3 nuclear positivity for some cells in the squamous layer for all dysplasias, with cellular grouping and cytoplasmic positivity in some cells in severe dysplasia.

It was expected that immunolabeling of STAT-3 would be observed not only in the cytoplasm, but also in the nuclei of the positive cells when labeled with P-STAT-3 since, according to the manufacturer, the STAT-3 antibody detects the total level of endogenous STAT-3 protein, and the P-STAT-3 antibody detects the endogenous level of STAT-3, only when phosphorylated with Tyrosine 705. The results from this study, in accordance with Araújo et al.[12] suggests that the STAT-3 antibody detects the total level of endogenous STAT-3 protein, except for Tyrosine 705 phosphorylated STAT-3.

STAT-3 promotes an increase in cellular proliferation and inhibition of apoptosis[13]. Therefore, its presence in basal epithelial cells of the normal mucosa and in the epithelium of hyperkeratosis with or without dysplasia, in addition to the absence of labelling in the

superficial (keratinized) epithelial layer was observed in this study. Thus explaining the findings of active proliferation in hyperkeratosis with dysplasia, whilst in the more advanced stages of cellular differentiation in hyperkeratosis without dysplasia, a lack of proliferative activity was noted.

The results showed that STAT-3 labeling seemed to be deregulated in hyperkeratosis and that the cells remained persistently phosphorylated. Only one study was found in the literature for the STAT-3 protein in potentially malignant lesions of the epithelial lining. In this study, Cury et al.[8], compared normal epithelium with the epithelium of actinic cheilitis, confirming, as in the present study, that deregulation of STAT3 in these lesions is directly associated to the histological dysplasia grade. The authors suggested that the activation and deregulation of STAT-3 in this lesion could be acting to inhibit apoptosis following DNA damage by ultraviolet radiation, in addition to stimulating cellular proliferation, which could result in a malignant neoplasm.

Shah et al.[14] analyzed 135 oral squamous cell carcinomas for cytoplasmic or nuclear STAT-3 labeling with immunohistochemistry. They found that nuclear STAT-3 was an independent predictor for poor outcome in early-stage disease, concluding that STAT-3 activation is an early event in head and neck carcinogenesis. In the present study, STAT-3 nuclear immunolabeling was observed in cases of hyperkeratosis with a more significant dysplasia grade (moderate and severe), corroborating the findings of Shah et al.[14], also suggested that nuclear STAT-3 expression may represent an important initial aspect in carcinogenesis.

Pectasides et al.[15] performed the first study to quantitatively assess nuclear STAT-3 in head and neck cancer, in association with patient prognosis. They used AQUA, a method that allows quantitative measurement of protein expression within subcellular compartments. Since activation of STAT-3 signaling involves translocation to the nucleus, they hypothesized that assessment of nuclear STAT-3 expression could act as an activation marker of a STAT3-mediated signaling pathway. Their findings showed that a higher nuclear STAT-3 level is predictive of a favorable clinical outcome. Furthermore, nuclear STAT-3 was the only identified prognostic factor when other commonly used prognostic markers and pathological parameters were considered.

Many studies place STAT-3 at the core of development, progression, and maintenance of many human tumors. STAT-3 has been confirmed as an anticancer target in several contexts. It modulates the transcription of a variety of genes involved in the regulation of critical functions, including cell differentiation, proliferation, apoptosis, angiogenesis, metastasis, and immune responses. For many cancers, elevated levels of activated STAT-3 have been associated with a poor prognosis[16].

It is known that in a large proportion of head and neck carcinomas, usually of squamous cell origin, activation of STAT-3 is due to the aberrant signaling of the EGF receptor, whilst in cases where such aberration is not present, the cause of STAT-3 activation is unknown[7].

As stated by some of the aforementioned studies, it can be observed that STAT-3 is deregulated and activated, and according to Bowman et al.[17] within the members of the STAT family, STAT-3 is most frequently associated with abnormal cellular growth and neoplasia. This factor may play a crucial role in the process of carcinogenesis of oral lesions, both in potentially malignant lesions and squamous cell carcinoma, confirming it as a potential prognostic marker, where in the future it may have a therapeutically benefit.

The understanding of the mechanism by which STAT-3 is activated in potentially malignant and malignant lesions is of upmost importance for the development of adequate therapeutic targets for such lesions. Therefore, pharmacologically safe and effective agents, which block STAT-3 activation have the potential for both the prevention and treatment of carcinoma[18].

5. CONCLUSIONS

From the results obtained, one may conclude the following:

- STAT-3 expression is deregulated when hyperkeratosis with and without dysplasia are qualitative analyzed;

- There was no difference in STAT-3 expression with regard to epithelial dysplasia grade.

ETHICAL APPROVAL

The present study was approved by the Sao Leopoldo Mandic, Center for Dental Research Ethics Committee (06/323)).

CONFLICT OF INTEREST

The authors have no conflicts of interests to declare in this study.

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