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Immunohistochemical expression of PCNA, p53, bax and bcl-2 in oral lichen planus and epithelial dysplasia

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Abstract: The potential for malignant transformation of oral lichen planus is still controversial. The expression of proteins related to cell proliferation and apoptosis in oral lichen planus and epithelial dysplasia was analyzed to evaluate the true potential for malignant transformation of this disease. Twenty-four cases of each lesion were subjected to the streptoavidin-biotin technique for identifying the immunohistochemical expression of PCNA, p53, bax, and bcl-2 proteins. Of the 24 cases of oral lichen planus, 14 (58.33%) were positive for PCNA, 10 (41.67%) for p53, 4 (16.67%) for bcl-2 and 12 (50%) for bax, whereas of the 24 cases of epithelial dysplasia, 20 (83.33%) were positive for PCNA, 10 (41.67%) for p53, 6 (25%) for bcl-2, and 20 (83.33%) for bax. Chi-squared test showed no statistically significant differences between the expression of p53 and bcl-2 in oral lichen planus and epithelial dysplasia, regardless of the grade ($P > 0.05$). However, the expression of PCNA and bax was significantly increased in epithelial dysplasia ($P < 0.05$). The results of this study showed that alterations in expression of these proteins are observed in oral lichen planus and epithelial dysplasia, suggesting the potential for malignant transformation in both lesions. (J Oral Sci 51, 117-121, 2009)

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Introduction

Oral lichen planus is a chronic inflammatory disease of unknown cause, and its potential for malignant transformation is a subject of much controversy (1). Since the first case was reported in 1910, several studies have suggested that patients with oral lichen planus are at an increased risk of developing cancer (1-7).

However, many authors believe that there is insufficient data to prove an association between oral lichen planus and cancer. For these authors, most cases of malignant transformation are the result of errors in the initial diagnosis of the disease (1,8-13).

The true potential for malignant transformation of oral lichen planus can be evaluated by analyzing the expression of proteins related to cell proliferation and apoptosis, as alterations in the expression of these proteins are essential for carcinogenesis (14-19).

Therefore, the aim of this study was to evaluate the immunohistochemical expression of PCNA, p53, bax and bcl-2 proteins in oral lichen planus and epithelial dysplasia in order to explain the controversy regarding the potential for malignant transformation of oral lichen planus and emphasize the importance of long-term follow-up of patients with this disease.

Materials and Methods

The samples used in this study consisted of 24 cases of oral lichen planus and 24 cases of epithelial dysplasia (4 mild, 12 moderate, 8 severe) obtained from the records of

the Department of Bioscience and Oral Diagnosis of the São José dos Campos Dental School, São Paulo State University, Brazil. Five 3-µm-thick histological sections were prepared from paraffin-embedded blocks. One section was stained with hematoxylin and eosin to verify the histological diagnosis according to Eisenberg's criteria (11) for oral lichen planus and World Health Organization's criteria (20) for epithelial dysplasia (Figs. 1 and 2). Cases of oral lichen planus with doubt of epithelial dysplasia were excluded. The others sections were stained according to streptoavidin-biotin technique (Fig. 3).

Immunohistochemical reactions against proliferating cell nuclear antigen (PCNA) (PC10 clone; dilution, 1:300), p53 protein (DO-7 clone; dilution, 1:200), bax protein (dilution, 1:200) and bcl-2 (124 clone; dilution, 1:50) (all from DakoCytomation, Glostrup, Denmark) were performed for the 3-µm histological sections. Sections were dewaxed in xylene, rehydrated in graded alcohol and rinsed in water, and then immersed in two changes of 6% H_2O_2 in absolute methanol (5 min for each change)

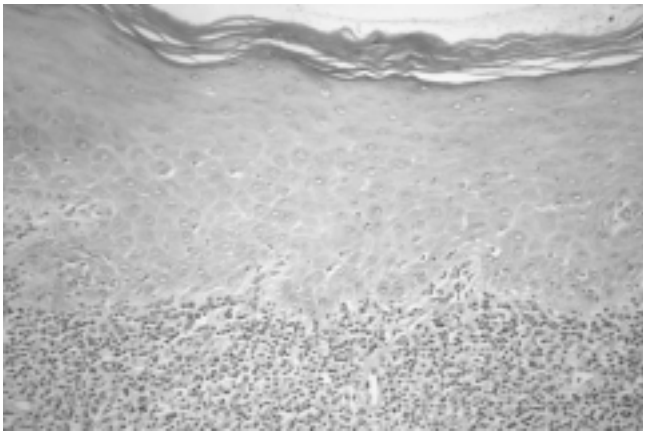

Fig. 1 Oral lichen planus (H–E staining ×200).

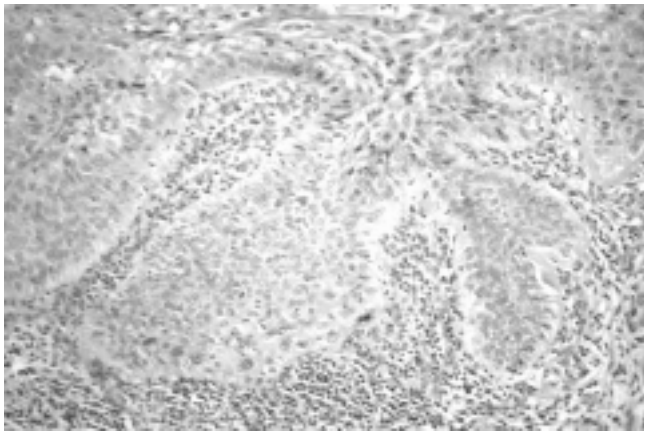

Fig. 2 Moderate epithelial dysplasia (H–E staining ×200).

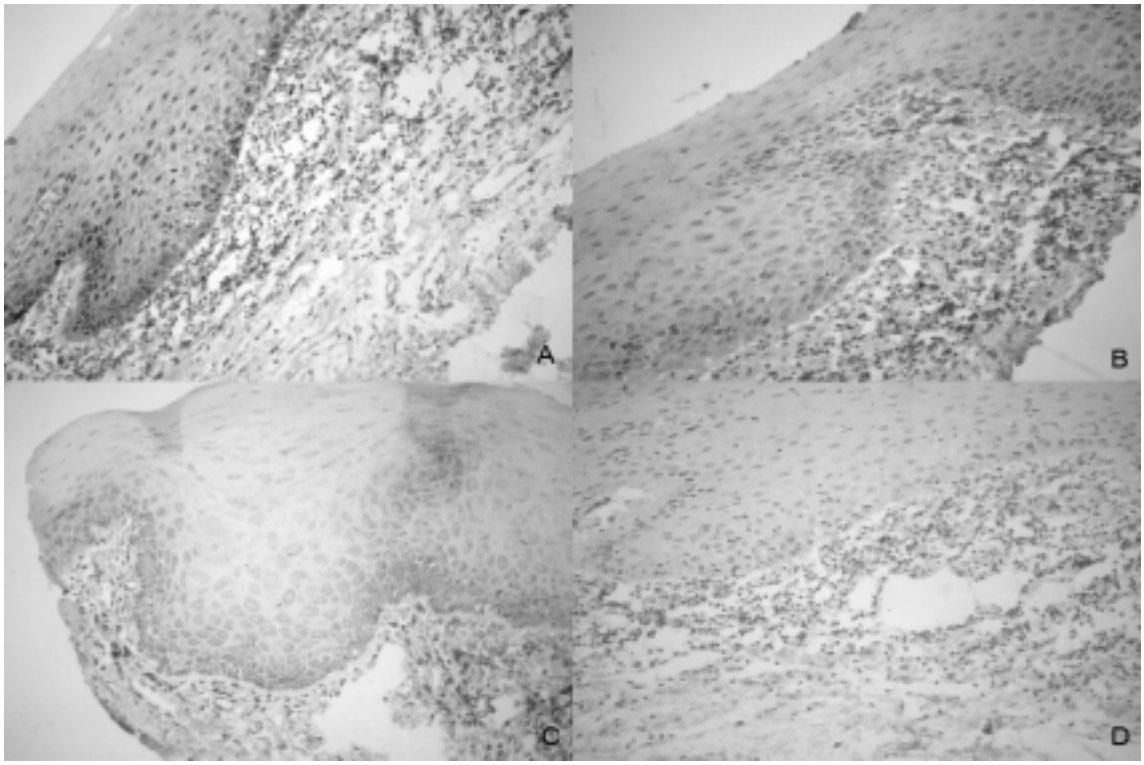

Fig. 3 Oral lichen planus (A: PCNA, ×200; B: p53, ×200; C: bax, ×200; D: bcl-2, ×200).

and rinsed in water to inhibit endogenous peroxidase activity. For antigen retrieval, sections were immersed in 10 mM sodium citrate buffer (pH 6.0) and boiled for 15 min in a microwave oven (700 W). After washing with Tris buffer (pH 7.4), the slides were incubated at 4°C for 18 h with monoclonal antibodies. After incubation, immunodetection was performed with the LSAB Visualization System (DakoCytomation) using 3,3'-diaminobenzidine chromogen as substrate, according to the manufacturer's instructions. Slides were counterstained with Mayer's hematoxylin, dehydrated, and mounted in Permount@ (Fisher Scientific, Fair Lawn, NJ, USA).

Paraffin-embedded oral squamous cell carcinoma biopsied cases (for PCNA and p53) and tonsils (for bax and bcl-2) served as positive controls. As negative control, the primary antibodies were replaced with antibody diluent solution.

PCNA, p53, bax and bcl-2 expression were classified according to the number of positively stained cells per 1,000 counted cells. The percentage of positive cells was scored according to the method of Nakagawa et al. (21) as follows: 3+ = strong staining (more than 50% stained); 2+ = moderate staining (between 25 and 50% stained); 1+ = weak staining (between 5 and 25% stained); 0 = negative (less than 5% stained).

Data were analyzed by the Chi-squared test. Values of $P \leq 0.05$ were considered statistically significant.

This study was approved by the São Paulo State University Local Ethics Committee (protocol # 081/2006-PHCEP).

Results

Of the 24 cases of oral lichen planus, 14 (58.33%) were positive for PCNA, 10 (41.67%) for p53, 4 (16.67%) for bcl-2 and 12 (50%) for bax, whereas of the 24 cases of epithelial dysplasia, 20 (83.33%) were positive for PCNA, 10 (41.67%) for p53, 6 (25%) for bcl-2, and 20 (83.33%) for bax. Results are shown in Figs. 4 and 5.

The Chi-squared test showed no statistically significant difference between the expression of p53 and bcl-2 in oral lichen planus and epithelial dysplasia, regardless of the grade ($P = 1$ and $P = 0.349$, respectively). However, the expression of PCNA and bax was significantly increased in epithelial dysplasia ($P = 0.031$ and $P = 0.046$, respectively). Significant difference was observed between the expression of PCNA, p53 and bcl-2 in mild, moderate and severe epithelial dysplasia ($P = 0.001$, $P = 0.033$ and $P = 0.003$, respectively), but there was no significant difference regarding the expression of bax ($P = 0.070$).

Discussion

Alterations in the expression of proteins related to cell proliferation and apoptosis are a strong indicator of the malignant transformation potential of a certain lesion. The obtained results suggested that oral lichen planus presents a possibility of evolution to cancer similar to epithelial dysplasia. Therefore, cases of malignant transformation of oral lichen planus are not just a consequence of error in their initial diagnosis, but natural evolution of this disease.

The observed increase in the cell proliferation index in epithelial dysplasia favours the accumulation of genetic alterations and consequently cancer development, suggesting that the epithelium in epithelial dysplasia is more susceptible to carcinogenic transformation than oral lichen planus. In addition, according to Da Silva Fonseca and Do Carmo, the epithelium in oral lichen planus is even more susceptible than normal epithelium (22).

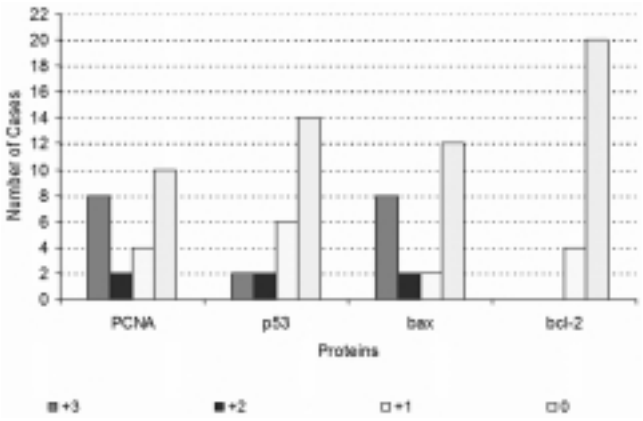

Fig. 4 Expression of PCNA, p53 and bcl-2 in oral lichen planus.

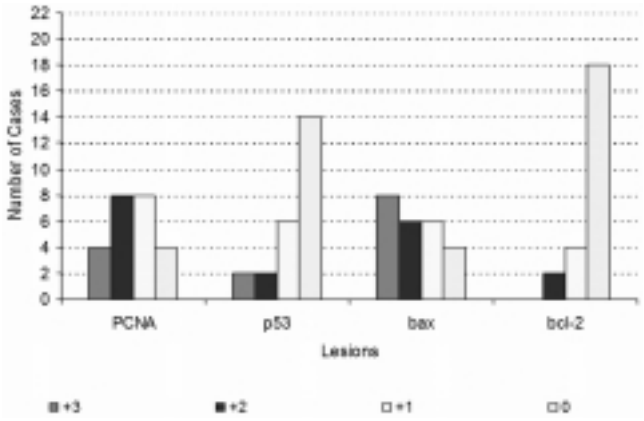

Fig. 5 Expression of PCNA, p53, bax and bcl-2 in epithelial dysplasia, regardless of grade.

On the other hand, the expression of p53 in 41.67% of cases of oral lichen planus and epithelial dysplasia in the present study is a relevant finding. p53 is a nuclear protein, whose mutation is strongly associated to several cancer types. Many studies showed that alterations in the expression of p53 are essential for carcinogenesis and can indicate an important step in transformation of normal to neoplastic epithelium (16,19,23,24). According to Stoll et al., the loss of p53 function is found in at least half of oral cancer cases (24). Therefore, the similar expression of p53 in oral lichen planus and in epithelial dysplasia can be an important indicator of malignant transformation potential of these lesions.

Another protein strongly related to cancer development is bcl-2, whose function is to inhibit apoptosis in different stages, increasing the genetically altered cell survival rate and consequently, facilitating the appearance of new mutations (19,25). In oral cancer, bcl-2 is observed from the initial stages of carcinogenesis up to the appearance of metastasis (14-17,23,26). In the present study, a significant statistical difference between the expression of bcl-2 in oral lichen planus and epithelial dysplasia was not observed ($P > 0.05$).

The expression of bax was significantly lower in oral lichen planus than in epithelial dysplasia. This fact can indicate a deregulation of the apoptosis mechanisms in oral lichen planus, preventing the death of genetically damaged cells and consequently, increasing the malignant transformation risk (27). Some studies suggested that decrease in the expression of bax is essential for the development and progression of oral cancer (14,17,19,28). Although bax and bcl-2 are strongly associated in apoptosis, no correlation between these proteins was observed in this study, which can be explained by the existence of different mechanisms of apoptosis regulation.

In general, the data obtained in the present study are in agreement with those of several other authors who evaluated the expression of PCNA, p53, bax and bcl-2 in addition to other proteins related to cell proliferation and apoptosis in oral lichen planus (21,26,29-32). For these authors, the alterations in the expression of these proteins were a strong indicator of the potential for malignant transformation of oral lichen planus, as these proteins participate actively in oral carcinogenesis.

The results of the present study showed that alterations in expression of these proteins are observed in oral lichen planus and epithelial dysplasia, suggesting the potential for malignant transformation in both lesions. Therefore, there is a need for long-term rigorous follow-up of patients with oral lichen planus, aiming at precocious identification of any alteration that can indicate a possible malignant transformation.

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