Correlation of β-catenin expression and metastasis in tongue squamous cell carcinoma

Correlação da expressão da β-catenina e presença de metástases em carcinoma de células escamosas de língua

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ABSTRACT

Purpose: It has been reported that the oral squamous cell carcinoma (OSCC) in tongue shows a more infiltrative profile, aggressive clinical course and poor prognosis, which may be related to a higher metastatic potential. The aim of the present study was to assess the expression of β-catenin in OSCC of the tongue and its correlation with tumor metastasis.

Methods: Twenty four cases were selected and divided in two groups: metastatic group (n=12) and non-metastatic group (n=12). A semi-quantitative analysis of the β-catenin expression was performed in the invasive tumor front and cases were graded as follows: negative (score 0), positive (score +), and strongly positive (score ++).

Results: It was detected that 33%, 50% and 17% of the cases in metastatic group were scored 0, + and ++, respectively, and the non-metastatic group showed that 42% were scored “0”, 33% scored + and 25% scored ++. Statistical analysis showed no difference between the studied groups.

Conclusions: Based on these results, it can be concluded that the immunoexpression of β-catenin does not represent a valuable tool to predict metastatic potential of OSCC in tongue.

Key words: Carcinoma, Squamous Cell. Tongue. Neoplasm Metastasis. beta Catenin. Cell Adhesion.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents over 90% of malignant tumors of the oral mucosa. There are strong clinical evidences that OSCC has a worse prognosis when present in the tongue. Moreover, it usually shows a more infiltrative invasion and, therefore, predispose to the formation of metastasis.

Tumor invasion and metastasis formation involve a series of steps including proteolytic degradation of basement membrane and extracellular matrix, changes in cell adhesion and tumor cells movement on tissues. In particular, it has been suggested that the reduction of cell adhesion mediated by β-catenin/E-cadherin complexes is associated with the development and progression of squamous cell carcinomas of the head and neck.

β-catenin is a multifunctional protein crucial both in regard to cell-cell adhesion and also in transduction of cellular signals. A junctional portion of β-catenin links E-cadherin to alph-catenin and consequently to the structure of the actin.
microfilaments of the cytoskeleton; playing this way a very representative role with respect to cell adhesion. Corroborating this importance, several studies have shown that deregulation of cadherin-catenin complex, in addition to reduced expression of β-catenin, is present in several types of malignancies, especially those of a more aggressive nature and a higher metastatic potential⁶.⁷

On the other hand, non-junctional portion of β-catenin is rapidly phosphorylated and degraded in the cytoplasm. However, the activation of the Wnt induces the stabilization of cytoplasmic β-catenin, facilitating its translocation to the nuclei. In the nuclei, the protein binds to members of the family of transcription factors T-cell factor/ lymphoid enhancer factor - TCF/LEF. Such deposits can contribute to the development of malignancies⁶.⁷

Thus, this study was to evaluate the immunohistochemical expression of β-catenin in squamous cell carcinoma of tongue with and without metastasis, in order to establish a correlation between the expression of this protein and the tumor metastatic potential.

**Methods**

Sample selection

To perform this study, 24 cases of OSCC were selected from Pathology-Anatomy Service, Oral Pathology Department, Federal University of Rio Grande do Norte. The cases were divided into two groups: with metastasis (n=12) and without metastasis (n=12). It was used, as a randomization criterion for inclusion in each group, the presence or absence of regional or distant metastasis at the time of diagnosis or before the initial therapeutic protocol.

**Immunohistochemistry**

Histological sections of 3μm in thickness were obtained from tissue samples fixed in 10% formalin and embedded in paraffin. The specimens were processed by the immunohistochemical technique by the streptavidin-biotin method, using anti-β-catenin polyclonal antibody at a dilution of 1:500 (Ab-1 clone, LabVision/ Neomarkers). The sections were subjected to antigen retrieval through steamer treatment, for 25 minutes, in citric acid (pH 6.0). After incubation with primary, secondary antibodies and streptavidin-biotin complex (DAKO, A/S, Glostrup, Denmark), revelation of the sections using a diaminobenzidine chromogen solution and a counter-staining with Mayer’s hematoxylin were conducted.

Based on the histological grading proposed by Bryne et al., two observers identified the front of tumor invasion (light microscopy - Olympus CX-31) and at a 1000x magnification and analyzed the presence or absence of immunostaining in the cell membrane, cytoplasm, nucleus or a combination of these. Then, it was performed a semi-quantitative analysis of the β-catenin protein expression according to the technique described by Lee et al., adapted for this study. It was considered as immunostained the cell with color ranging from dark yellow to brown, regardless of staining intensity. It was assigned therefore the following scores: “0”, when less than 10% of the cells were immunostained; “+”, when between 10 to 50% of the cells were immunostained; and “++”, when more than 50% of the cells were immunostained.

**Statistical analysis**

The results were statistically analyzed by the software STATISTICA 6.0 (StarSoft Inc.), using the nonparametric U Mann-Whitney test for exploratory descriptive analysis. It was considered statistically significant a p value less than 0.05.

**Results**

Data related to β-catenin immunoeexpression are shown in Table 1 and Figures 1 and 2. It was observed a variable expression in relation to the topography of β-catenin, which was detected in cytoplasmic membrane, cell nucleus or both (Figures 3 to 6). However, there was not identified significant statistical differences in the topography of β-catenin immunosuppression between the groups analyzed (p=0.9539).

| TABLE 1 - Scores of β-catenin immunoeexpression in the group with metastasis and group without metastasis |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Case | Score | Location | Case | Score | Location |
| 1 | 0 | - | 1 | + | M/C |
| 2 | + | M/C* | 2 | + | M |
| 3 | + | M | 3 | 0 | - |
| 4 | + | M | 4 | + | C/N |
| 5 | + | M | 5 | 0 | - |
| 6 | 0 | - | 6 | 0 | - |
| 7 | + | M/C* | 7 | 0 | - |
| 8 | 0 | - | 8 | + | M |
| 9 | ++ | M/C* | 9 | 0 | - |
| 10 | ++ | M/C* | 10 | ++ | C |
| 11 | + | M/C | 11 | ++ | M/C |
| 12 | 0 | - | 12 | ++ | M/N/C |

0 = no staining or insignificant; + = positive; ++ = strongly positive; * = predominance of the staining; M = staining on cell membrane; N = staining in nucleus; C = staining in cytoplasm.
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FIGURE 1 - Percentual distribution of β-catenin scores in the groups with metastasis and without metastasis

FIGURE 2 - Representation of the β-catenin immunoexpression location in the group with metastasis and in the group without metastasis (M = staining on cell membrane; N = staining in nucleus; C = staining in cytoplasm; * = predominance of the staining)

FIGURE 3 - Intense expression of β-catenin in metastatic group of tongue OSCC. Cytoplasmic and membrane reaction (score ++) (streptavidin-biotin, original magnification×400)

FIGURE 4 - Strong expression β-catenin in the group without metastasis (Score ++) (streptavidin-biotin, original magnification×200)
FIGURE 5 - Immunoreactivity reduced for ß-catenin in the group with metastasis (Score +) (streptavidin-biotin, original magnification×100)

FIGURE 6 - Staining of ß-catenin in the central portion of tongue OSCC without metastasis (Score +) (streptavidin-biotin, original magnification×200)

Discussion

It has been shown a close association between the reduction of the intercellular adhesion and the loss of cell differentiation, accompanied by a greater mobility and invasion potential of neoplastic epithelial cells in several types of human malignancies, including OSCC. One of the molecules related to cell adhesion is ß-catenin, with a dichotomous effect: it is able not only to actively participate in the process of intercellular adhesion but also related to intracellular signals transduction.

In our study, a clear reduction of ß-catenin reactivity was observed. More than 80% of the lesions in the group with metastasis and 75% of lesions in the group without metastasis had a score “0” or “+”. Overall, these results are in line with other studies of OSCC, as has been observed reduced expression of ß-catenin molecule, with decreased levels ranging from 62% to 85% in OSCC. However, the statistical analysis showed no significant differences between ß-catenin expression between the groups.

One possible explanation for the lack of difference in ß-catenin immunoexpression among the groups may be the sample selection. For this study, only patients with metastasis at diagnosis of carcinoma were selected to compose the metastatic group. In other words, patients with development of secondary tumors after the diagnosis of primary tumor were not considered, as well as patients with subclinical metastasis. Therefore, it was not detected a positive relationship of this protein with the tumor metastatic potential, which corroborates the results of previous studies. However, some studies were able to establish a statistically significant relationship between the decreased ß-catenin expression with metastasis.

Different results can be also explained by the existence of variations in the methodology adopted in relation to the immunostaining analysis. Some researches used quantitative methods, while others used semi-quantitative techniques. In addition, there are studies which used a percentage distribution in relation to immunostaining, while others adopted single and absolute count of positive cells.

Another factor that limits possible comparisons of data with previous studies is we analyzed only cases of OSCC in tongue, since the anatomical location of oral squamous cell carcinoma influences the biological behavior of this tumor. It is also possible that the loss of intercellular adhesion is only one of the stages required for the occurrence of metastases. Soon, there could be a need for other phenomena, such as loss of cell adhesion to the extracellular matrix, which can be caused by the metaloproteinases expression.

According to what was found by Lopes et al., the presence of ß-catenin in nucleus and cytoplasm was a striking finding in this study, despite the lack of statistical correlation with metastatic potential. This fact suggests a possible role of ß-catenin in other tumor etiopathogenic aspects that are not exclusively related to the formation of metastasis, such as cell proliferation and angiogenesis. These processes need studies to assess these relationships in an attempt to better understand the clinical value of altered expression of ß-catenin in OSCC.

Conclusion

In view of these findings, it is appropriate to infer that the individual analysis of the ß-catenin immunoexpression does not represent a good criterion to predict the metastatic potential of tongue OSCC to the extent that there was no significant difference in its expression in the groups with metastasis and without metastasis.

References


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