Original

# Significances of Protein Her-2-Neu Expression in Oral Carcinogenesis

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## Abstract

**Objective**: The aim of the present study was to evaluate the protein expression Her-2-Neu involved in the neoplasic progression of dysplasia until squamous cell carcinoma. **Material and method:** Ninety-four biopsy specimens from paraffin embedded archives of the Histopathology Laboratory from Positive University were analyzed. The Her-2-Neu immunohistochemical staining was performed in all specimens. The cases were classified according to the histological malignancy grading system proposed by the World Health Organization. For each case a score was established using the percentage counts of population density in all of the fields. **Results:** The results showed that the number of cells marked Her-2-Neu increased as dysplasia upgraded (from mild, moderate to severe) and squamous cells carcinoma and Her-2-Neu was detected especially in moderate and severe dysplasia, where epithelium was disorganized in specimens of squamous cell carcinoma. **Conclusion**: The results showed that the number of cells stained Her-2-Neu increased with dysplasia progression (of mild, moderate or severe) and squamous cell carcinoma, and it was detected mainly where the epithelium was disorganized and/or infiltrative.

**Keywords:** HER-2 protein. Carcinoma, Squamous Cell. Disease Progression.

## Introduction

Oral carcinogenesis is a multistep process in which genetic events lead to the disruption of the normal regulatory pathways that control basic cellular functions including cell division, differentiation, and cell death.[1-2] Several studies have identified some genetic alterations in oral carcinomas and in premalignant lesions of the oral cavity.[3]

Oncogenes confer malignant properties onto a cell, and generally results in gain of function, altering growth and promoting regulatory genes that govern the cells' signal transduction pathways.[4-5] In addiction, the overproduction or increased function of the oncogene drives excitatory proteins to cell proliferation.[6-9]

The Her-2-Neu oncogene encodes a glycoprotein with extracellular, transmembrane and intracellular domains.[7-8] This protein shares extensive sequence homology with epidermal growth factor receptor and has been associated with advanced disease, metastasis and poor clinical prognosis.[10-11]

Research studies have correlated the immunohistochemical expression of Her-2-Neu with a more aggressive behavior in breast cancer leading to a shorter overall survival,[12-14] however the role of Her-2-Neu in oral carcinogenesis is not well defined.[15]

The aim of the present study was to evaluate the protein expression Her-2-Neu involved in the progression of oral dysplasia to squamous cell carcinoma and analyze its value as a prognostic marker.

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# Material and Methods

## Histopathological Analysis

Specimens from 94 cases among which were dysplasia and squamous cell carcinoma, diagnosed in the Department of Oral Pathology, at the School of Dentistry, Positive University, were evaluated. The slides stained by hematoxylin and eosin were classified according to the histological malignancy grading system proposed by Anneroth et al. 16-17. Two independent observers performed histological grading. Five morphologic parameters were evaluated, such as the degree of keratinization, nuclear polymorphism, number of mitoses, pattern of invasion, and lympho-plasmocytic infiltration. Scores from 1 to 4 were assigned to each parameter. The mean of total malignancy score value was achieved by the sum of the scores attributed to each parameter, divided by the number of parameters used. The mean of total malignancy score values were divided into two groups of low (within the mean score range 1.0 - 2.5 points) and high (within the mean score range 2.6 - 4.0 points).

Oral epithelial dysplasia was subdivided into three prognostically significant categories, according to the WHO18. It was considered mild dysplasia (MiD) all specimens which demonstrated proliferation of atypical or immature basal cells above the parabasal region, but not extending beyond the lower third of the epithelium. Moderate dysplasia (MoD) demonstrated a similar proliferation into the middle one-third of the epithelial cross-section. The term severe dysplasia (SD) was designated for abnormal proliferation from the basal layer into the upper third of the epithelium. The dysplasia grading of the oral epithelium also took into consideration the degree of cellular atypia, as described before19.

Immunohistochemical staining was performed using the streptavidin-biotin method. The 3 μm sections from the paraffin-embedded materials were dewaxed with xylene, hydrated through graded alcohols and fully rehydrated in water. For Her-2-Neu, the sections were placed in EDTA (pH = 8.0, 1 mM) in a steamer for 20 min. at 95°C. The sections were placed in 0.6% hydrogen peroxide in methanol for 10 min to block endogenous peroxidase activity.

The sections were incubated with the primary antibodies, and diluted in a solution of TRIS (pH = 7.4) and 1% of bovine albumin (BSA, Biotest S/A, São Paulo, Brazil). Monoclonal antibody against Her-2-Neu (1:100, clone DCS-6, Dako Corporation, Golstrup, Denmark) was applied over the sections for 18 h at 4°C. This was followed by the use of the Streptavidin-Biotin Kit (Dako Corporation, KO-690). Sections were then incubated in diamine benzidine solution (0.3%) for 3 min., and then counter-stained with Mayer's hematoxylin.

Sections of breast cancer that were strongly positive for Her-2-Neu were used as positive control. The same sections were used as negative control, replacing the primary antibody with buffer solution.

## Quantitative Analysis

The sections stained by immunohistochemistry were evaluated using a light microscope, with 400× magnification. The images were transferred to a computer and the positive membrane for Her-2-Neu, which were individualized by the software ImageTool ®, were counted. Fields were chosen randomly and the positive cells were counted in 1000 cells.

For analysis, the immunohistochemical expression was considered over the absolute percentage of Her-2-Neu positive cells, which was obtained for each section analyzed.

The expression of Her-2-Neu were graded according to the scores 0 = less than 1% of positive cells, 1 = 1 - 10% of positive cells, 2 = 11 - 20% of positive cells, 3 = 21 - 30% of positive cells, 4 = 31 - 40% of positive cells, and 5 = more than 40% of positive cells.

## Statistical Analysis

Data obtained from the expression of Her-2-Neu were submitted to analysis using Moda, in order to verify a possible difference in the immunoexpression of Her-2-neu between the five groups studied.

# Results

Our results showed 24/94 specimens Her-2-Neu negative, or 0 graded. The scores of histological grading and the scores of positive cells attributed to each pathological condition are shown in table 1.

Among dysplasia specimens, immunohistochemical expression of Her-2-Neu and the number of marked cells were much varied. For Mild Dysplasia, only 8/22 specimens were positive and all marked cells were stained in the basal layer, while in other specimens the stain was heterogeneous, especially where epithelium was disorganized.

In our study, differences were found among

**Table 1** – Histopathological diagnosis and Her-2-Neu score

| Diagnostic | Score Her-2-Neu positive | | | | | | |
|---|---|---|---|---|---|---|---|
| | Score 0 | Score 1 | Score 2 | Score 3 | Score 4 | Score 5 | ModA |
| Mild Dysplasia (MiD) | 14/22 | 8/22 | 0/22 | 0/22 | 0/22 | 0/22 | 0 |
| Moderate Dysplasia (MoD) | 3/14 | 6/14 | 4/14 | 1/14 | 0/14 | 0/14 | 1 |
| Severe Dysplasia (SV) | 2/26 | 0/26 | 6/26 | 14/26 | 4/26 | 0/26 | 3 |
| Low grade oral squamous cell carcinoma | 3/20 | 1/20 | 1/20 | 6/20 | 9/20 | 0/20 | 4 |
| High grade squamous cell carcinoma | 2/12 | 1/12 | 1/12 | 1/12 | 7/12 | 0/12 | 4 |

groups. In addition, the immunostain frequency increased in accord with degree of dysplasia (Figs. 1A and 1B).

Among oral squamous cell carcinoma group, the oncoprotein Her-2-Neu expressed similar frequency, mainly in the peripheral layers of invasion islands and in endophytic projections inside low graded malignancy neoplasia. Neoplasms with high grade of malignancy expressed Her-2-Neu in both peripheral and central area of the invasion islands (Figs. 1C and 1D). However, there were not important differences among SD, low graded squamous cell carcinoma and high graded carcinoma.

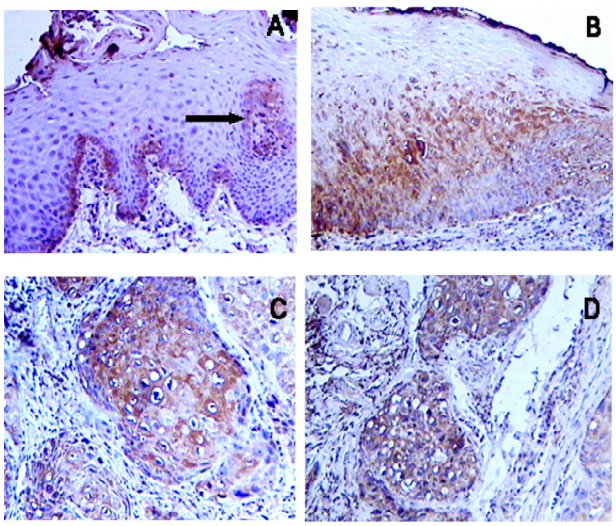


**Figure 1** (A-D) - Immunohistochemical expression of Her-2-Neu in dysplasia and oral squamous cell carcinoma (A) Her-2-Neu expression in basal and disorganized layers of moderate dysplasia - original magnification 50×. (B) Positive staining for Her-2-Neu in severe dysplasia - original magnification 50×. (C and D) Diffuse Her-2-Neu expression in the invasion island of the oral squamous cells carcinoma - original magnification 100× and 50×.

## Discussion

With the advent of the refined modern techniques, innumerable methodologies and new findings in the genetic semantics have been carried through to establish and to favor a higher effectiveness in the diagnosis of several types of cancer.

Regarding the exhausting research in which the main purpose seems to be the characterization of biological markers, especially genetic, for better interpretation from the development and progression of carcinogenesis to the invasive neoplasic transformation, the specific target has been the cellular biological population, relating the kinetic of the pathways that take the signaling of the growth factors and pathways of the differentiation and cellular growth of a certain neoplasm 20.

The biological elements of the cancer as well as the protein mechanisms lead to the alteration of several regulatory pathways that may function as inhibitory or excitatory effects on the tissue environment, and the differences in the cascades that take to the development of beginning and growth of the process of mitosis. Commercially, the target of this signaling can be identified through the use of monoclonal antibodies that disclose the specific protein sequence from amplified genetic codification or mutant. The use of these specific anti-protein antibodies by immunohistochemical techniques results in an excellent alternative, considering it as an accessible methodology of low cost and easy manipulation (allowing for the use of paraffin embedded specimens from laboratory archives), when compared to elaborated techniques of molecular biology1,20-22.

Currently, the scientific attention is turned to the immunoexpression of oncogenes considered potential prognostic and initiating factors. That seems to be the case of the oncoprotein Her-2-Neu, the genetic product of a homonym protein gene, with its function intimately connected to the cellular growth, progression of mitosis and cell differentiation. Her-2-Neu has also been used in the anatomic-pathological routine for the diagnosis and prognostic of the breast cancer23-25.

The Her-2-Neu presents an initial point in

the process of carcinogenesis. The overexpression or amplification of this oncogene is considered a maleficent reply to hormone-therapy and is suggest that the Her-2-Neu could be a poor prognostic factor, 26-27 although predictive to the adjuvant chemotherapy reply. On the other hand, van ofVijver 28 Harris et al. 29 Wright et al. 30 emphatically consider the Her-2-Neu as an excellent prognostic factor.

Although not too profound, this study tried in its purpose to establish the immune reactivity of the Her-2-Neu in cellular dysplasia, with diverse dysplastic degrees in primary oral squamous cell carcinoma.

It was found in this study a low percentile of positive areas with discrete cellular dysplasia (36.3%), with approximately 63% (14 among 22) of all specimens without any immune-marking.The cellular content that showed reactivity to the antibody in specimens of mild cellular dysplasia was restricted to the basal layer of the epithelium.

The basal cellular content has important functions, once this cellular layer contains the called stem cells, responsible for the epithelial cell differentiation and basal proliferation, indispensable functions to the tissue maintenance, such as the replacement of cells that were lost by apoptosis or by exfoliation, keeping the stratification and tissue balance.

Specifically, one of the characteristics of the proto-oncogene is to keep the tissue homeostasis, by sending biochemical signaling of initiation in cellular cycle. By immunohystochemistry, it is possible to detect the epitope, once it inherits the loci of lowest mutational point. In the specimens of this study, the monitoring of the oncogene suggested a behavior of homeostatic factor, not as one of mutation.

In agreement with Whyte et al. 31 this study related the increase of the basal activity, transforming the respective cellular content by the transduction pathway of stimulated signals, chronically, resulted of the importance in the regulation of the cellular growth. In addition, through a genomic amplification methodology, Khan et al. 15 report that Her-2-Neu is only involved in invasive carcinomas. Contradictorily, in potentially malignant lesions Pavelic et al. 32-33 detect the participation in intra-epithelial alterations in situ, suggesting that Her-2-Neu could be an initiating factor of the neoplastic process.

Wilkman et al. 34 analyze the expression of the oncoprotein Her-2-Neu in benign and malignant lesions. All of these authors studied lesions demonstrate some expression of the Her-2-Neu, but the distribution and intensity of the expression has strong variance related to the degree of differentiation. As the degree of indifferentiation rise in the sample, the index of the expression of the oncoprotein Her-2-Neu also rises. This way, it is suggested that in oral carcinoma, the carcinogenesis pathway can be interacted with the Her-2-Neu.

Those findings are analogous to ours. The evidence becomes clear at the moment in which the immunoexpression of the protein were proportional to the increase of the histological grading of tissue and cellular dysplasia.

However, we considered the evident participation of the Her-2-Neu as an effective prognostic factor from the diagnosed specimens with moderate and intense cell dysplasia. It was correlated that the areas with reactivity in the trans-membrane cellular portion coincided, in loci, with the areas where the morphologic aspect disclosed alterations of loss of the stratification, especially where the loss of cell-cell adhesion occurred.

In interesting research, Khan et al. 15 study by immunohystochemistry and by fluorescence in situ hibridization (FISH), 77 specimens of oral pharynx carcinoma.The authors present a result of 25% immunoexpressed cases. However, their study does not consider the quantitative analysis of immunoexpression within each neoplastic specimen, but strongly report that all the immuno-positive specimens concomitantly show genetic alteration detected by FISH and immunohystochemistry.

Our results showed that all carcinomas had immunoexpression for the Her-2-Neu. The differences in the percentage can be related with the initiating factors of the neoplasia, associated with ambient factors, 35 or perhaps with some other factor that occurs in the South region of Brazil.We considered important the percentage of immuno-reactive cells when comparing the moderate differentiated carcinomas, the mild differentiated, and even the carcinomas in situ, considered as intense/severe dysplasia.Although the score disclose differences between intense/severe dysplasia and moderate differentiated carcinomas, we interpreted that the Her-2-Neu, as said previously, was related mainly with areas of intense/severe dysplasia.We do not observed immuno-reactivity in any of the locci that showed keratinization, an interesting criterion for cellular differentiation. However, it was evident the immuno-reactivity in the adjacent connective tissue cell content, mainly in the invasion islets, that as smaller they were the greater the immunoexpression, which means a higher invasive degree, possible infiltration and metastasis.

The intense/severe dysplasia classified specimens

showed different score to the moderate differentiated carcinoma. This fact can be related mainly to intra-epithelial acantolisys. However, not only the dysplasia itself could be developing the invasive carcinoma in a carcinogenesis process, but also it could be an adjacent area to the carcinoma, without any infiltration, due to the incisional biopsy. From our results, we suggest that the Her-2-Neu can be considered as a prognostic factor of oral cancer, although continuing research need to be carried, in order to establish a wider span that may better reflect the prognostic of the oral squamous cell carcinoma.

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