

Research Article



## Molecular Targeting of Her-2/neu Protein Is Not Recommended as an Adjuvant Therapy in Oral Squamous Cell Carcinoma and Oral Lichen Planus

Maryam Kouhsoltani<sup>1</sup>, Amirala Aghbali<sup>1</sup>, Behrooz Shokoohi<sup>2\*</sup>, Ronak Ahmadzadeh<sup>3</sup>

<sup>1</sup> Dental and Periodontal Research Center and Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran.

<sup>2</sup> Hematology Oncology Research Center and Department of Pathology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

<sup>3</sup> Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

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### Abstract

**Purpose:** Target therapy against molecular markers of EGFR family has been recently used in the treatment protocol of several diseases. However, the role of Her-2/neu in OSCC is a matter of controversy and more studies are required to clarify the role of Her-2/neu in OSCC. We aimed to investigate the role of Her-2/neu in the process of carcinogenesis in oral epithelium as ELP is a premalignant and OSCC is a malignant lesion, but RLP shows no evidence of premalignancy and malignancy. To our knowledge, this is the first study comparing Her-2/neu expression in erosive lichen planus (ELP), reticular lichen planus (RLP), and oral squamous cell carcinoma (OSCC).

**Methods:** 60 samples, including 20 cases of RLP, 20 cases of ELP, and 20 cases of OSCC were evaluated immunohistochemically in this study.

**Results:** Our findings showed that there was not a significant increase in Her-2/neu expression from RLP to ELP and from ELP to OSCC. Therefore, Her-2/neu had no role in differentiating between RLP, ELP and OSCC and this protein does not contribute to the process of carcinogenesis in the oral epithelium.

**Conclusion:** The lack of Her-2/neu overexpression indicates that molecular targeting of Her-2/neu protein is not recommended as an adjuvant therapy in OSCC and OLP.

### Introduction

Lichen planus is a chronic mucocutaneous disease characterized by relapses and remissions.<sup>1,2</sup> It is an immunologically mediated disorder of unknown cause that affects stratified squamous epithelium.<sup>1,3</sup> Oral lichen planus (OLP) occurs in 1-2% of the adult population.<sup>4</sup> The importance of this disorder is due to its frequency, similarity to other mucosal disorders, and occasional painful nature.<sup>2</sup> Above all, numerous studies have suggested a 0.4–5.6% rate of malignant transformation for OLP i.e. ELP type.<sup>1,4</sup> The World Health Organization (WHO) has classified ELP as a precancerous condition, described as “a generalized state associated with a significant increased risk of cancer”.<sup>5</sup>

Clinically, there are two forms of OLP. The reticular lichen planus (RLP) is more common, characterized by interlacing white lines (Wickham's striae) that sometimes produce annular pattern. This form of OLP usually cause no symptom.<sup>2,3</sup> The erosive lichen planus (ELP) appears as atrophic areas with central ulceration. RLP, although less common, is more important because of the common symptomatic nature. Furthermore, this type of OLP is a premalignant lesion.<sup>2,3</sup> Histopathologically, OLP is

characterized by hydropic degeneration of the basal cell layer and band-like infiltration of predominantly T lymphocytes beneath the epithelium. The histopathology of OLP reveals saw-tooth-shaped elongation of the rete ridges.<sup>3,6</sup>

Since epidermal growth factor receptor (EGFR) is critical for the proliferation of keratinocytes, it is possible that EGFR overexpression is involved in the process of carcinogenesis in oral epithelium.<sup>5</sup> The proto-oncogene EGFR, also known as ERBB, is a well-documented tyrosine kinase growth factor receptor.<sup>5</sup> Two well-recognized members of EGFR family are ERBB1 and ERBB2 (Her-2/neu).<sup>7</sup> ERBB1 and ERBB2 has extensive homology in genomic sequences.<sup>8</sup> ERBB2 gene, placed on the short arm of the chromosome 17,<sup>9</sup> encodes this transmembrane protein of 185 kDa.<sup>8</sup> Activation of EGFR family members is essential for growth, differentiation and survival of epithelial tissue. Moreover, these receptors are associated with various features of malignancy, e.g. resistance to apoptosis, chemo-resistance, cell cycle dysregulation, and uncontrolled development and differentiation.<sup>4,10</sup> EGFR

\*Corresponding author: Behrooz Shokoohi, Fax: +98 41 33346977, Email: shokouhib@tbzmed.ac.ir

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is involved in the process of carcinogenesis in several malignancies, e.g. carcinomas of the larynx and the lung.<sup>5</sup>

Target therapy against molecular markers of EGFR family has recently been used in the treatment protocol of several diseases, e.g. breast cancer.<sup>10,11</sup> However, the role of Her-2/neu in OSCC is a matter of controversy and more studies are required to clarify the role of Her-2/neu in OSCC.<sup>9,10,12,13</sup> Considering the well-known role of Her-2/neu in various cancers of many sites of the body, we aimed to investigate the role of Her-2/neu in the process of carcinogenesis in oral epithelium as ELP is a premalignant and OSCC is a malignant lesion, but RLP shows no evidence of premalignancy and malignancy. To our knowledge, this is the first study comparing Her-2/neu expression in ELP, RLP and OSCC. Better understanding of the molecular mechanisms underlying these lesions (OLP and OSCC) may provide further insight into the clinical implication of Her-2/neu protein for more effective molecular treatment strategies.

### Materials and Methods

Paraffin-embedded tissue samples were obtained from the laboratory archives. Sixty samples, including 20 cases of RLP, 20 cases of ELP, and 20 cases of OSCC were evaluated in this study. Criteria for the inclusion of the patient was availability of sufficient clinical documents, the histologically confirmed diagnosis, and the presence of adequate microscopic fields for evaluation. The patient was excluded if he had a history of a systemic disease, or local or systemic allergic reactions e.g. drug or metal allergies.

Immunohistochemical assessment was performed on 4 micrometer thick biopsy material from formalin-fixed, paraffin-embedded specimens. Staining was performed through standard immunohistochemical methods according to the manufacturer's instructions (DAKO, Glostrup, Denmark). Tissue sections were deparaffinized in xylene and rehydrated in a graded series of alcohol. The endogenous peroxidase activity was blocked in 3% hydrogen peroxide, and the antigens were retrieved in citrate buffer solution (0.01 M, PH 6.0) for 20 min. The slides were then incubated with the 1:800 diluted primary anti-Her-2/neu antibody for 20 minutes at room temperature. The secondary antibody was applied to the slides at room temperature. 3, 3'-diaminobenzidine was then used as a chromogen and the slides were counterstained with Harris hematoxylin. The samples of breast cancer, previously positive for Her-2/neu, were positive controls.

All the slides were scored according to the percentage of the stained cells and the degree of membrane staining. This means that the slides were scored 0 if no membrane staining was observed or when incomplete membrane staining was observed in less than 10% of the lesional cells. The slides were scored 1 when incomplete membrane staining was observed in more than 10% of the lesional cells. When complete membrane staining was seen in less than 10% of the lesional cells score 2

was considered for that case. Score 3 was given for the complete membrane staining of more than 30% of the lesional cells.

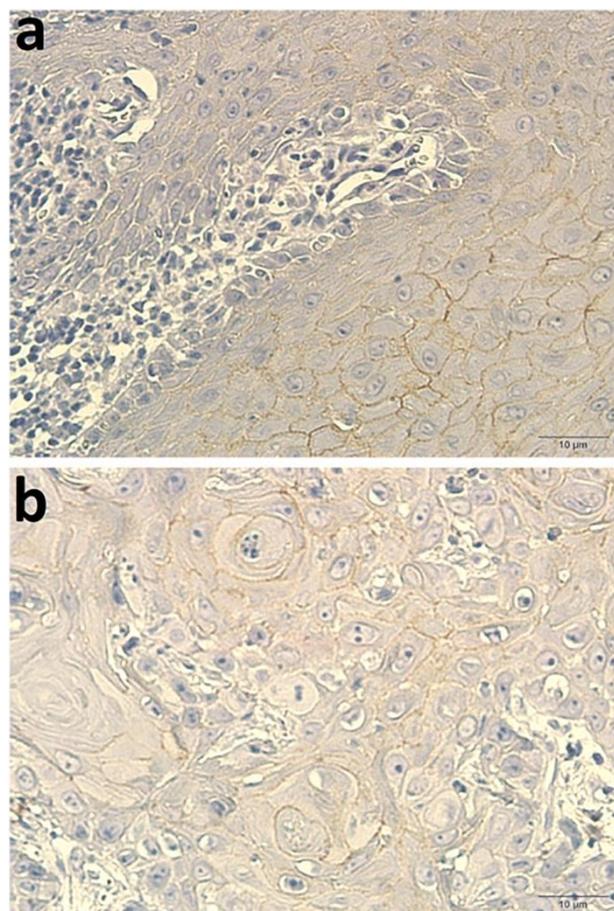
### Statistical analysis

The statistical analysis of the collected data was performed using Statistical Package for Social Sciences (SPSS) 20.0 (SPSS, Chicago, IL). To compare Her-2/neu expression amongst the studied groups, Kruskal–Wallis test was used and a p-value of <0.05 was considered as statistically significant.

### Results

The basic histopathologic patterns for both ELP and RLP were similar and showed classical histopathologic features described in the literature. All the lesions consisted of a band-like infiltration of predominantly lymphocytes subjacent to the epithelium and hydropic degeneration of the basal cell layer. OSCC consisted of a dysplastic epithelium with invasion of the dysplastic cells to the underlying connective tissue.

Her-2/neu Expression, assessed by immunohistochemistry, is illustrated in Figure 1.



**Figure 1.** Her-2/neu expression in: a) reticular lichen planus, b) oral squamous cell carcinoma (IHC stain, x400).

### Reticular lichen planus

14 cases (70%) of RLP showed negative Her-2/neu expressions, 4 cases (20%) were 1+, and 2 (10%) were 2+.

**Erosive lichen planus**

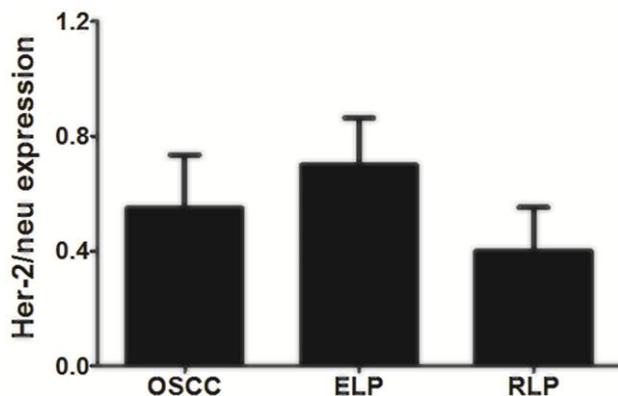
Her-2/neu expression was negative in 9 samples (45%), 8 cases (40%) were 1+, and 3 (15%) were 2+.

**Squamous cell carcinoma**

Of the 20 cases of RLP, 12 (60%) were negative, 6 (30%) were 1+, 1 (5%) was 2+, and 1 (5%) was 3+.

**Statistical analysis**

Kruskal–Wallis test did not reveal a statistically significant difference between the studied groups ( $p=0.32$ ). Her-2/neu expression in RLP, ELP and OSCC is illustrated in Figure 2.



**Figure 2.** Histogram of Her-2/neu expression in a) RLP: reticular lichen planus, b) ELP: erosive lichen planus, c) OSCC: oral squamous cell carcinoma

**Discussion**

In the present study, we showed that there were no shifts in HER2/neu expressions from RLP to ELP and from ELP to OSCC. Previous studies have reported associations between Her-2/neu overexpression and advanced disease in several premalignant and malignant lesions e.g. ovarian, breast and salivary carcinomas.<sup>14-16</sup> However, less is known about the nonglandular diseases and the role of Her-2/neu in OSCC is a matter of controversy<sup>9,10,12,13</sup> and more studies are required to clarify the role of Her-2/neu in OSCC. Her-2/neu has been reported to be overexpressed in a very small number to all of the OSCC samples in various studies.<sup>9,10,12,13</sup>

Sardari et al. found that Her-2/neu expression was not significantly elevated in OSCC and this protein could not be used as a salivary marker for detection of OSCC.<sup>10</sup> Likewise, Hanken et al. declared that Her-2/neu overexpression was rarely detectable in OSCC, but they suggested those few patients with HER-2/neu overexpression could benefit from anti-Her-2 therapy.<sup>13</sup> Conversely, in a study by Chen et al., ELISA test showed that Her-2/neu mean levels were significantly reduced after treatment of OSCC and Her-2/neu overexpression correlated with lymph node metastasis in these patients.<sup>12</sup> Similarly, Khan et al. observed Her-2/neu overexpression in OSCC.<sup>9</sup>

To our knowledge, HER-2/neu (ERBB2) in OLP has been evaluated in one study previously.<sup>5</sup> Kumagai et al.

assessed 10 cases of OLP using RT-PCR. They showed enhanced EGF-like ligands on the keratinocytes and concluded that this could be associated with the carcinogenesis and pathogenesis of OLP.<sup>5</sup> Signaling in OLP may lead to the activation of the proto-oncogenes and the transcription factors, thus the disordered growth of epithelial cells, and ultimately causing malignancy. In another study by Ebrahimi et al., no overexpression of ERBB1 was seen in 20 cases of OLP.<sup>4</sup>

In the present study, we immunohistochemically assessed HER-2/neu expression in 40 cases of OLP, including 20 samples of RLP and 20 samples of ELP (classified by WHO as a premalignant lesion). These two groups were compared with each other and also with OSCC. However, it is important to say that immunohistochemistry is a more predictable method compared with genomic evaluation. It is because immunohistochemistry evaluates the expression of a specific protein e.g. HER-2/neu., but the assessed gene in genomic evaluation may not be expressed.

**Conclusion**

Our findings showed that Her-2/neu protein had no role in differentiating between RLP, ELP and OSCC and this protein does not contribute to the process of carcinogenesis in the oral epithelium. This lack of overexpression indicates that molecular targeting of Her-2/neu protein is not recommended as a chemical prevention and adjuvant therapy. Further studies based on the investigation of the signaling pathways will provide invaluable help to clarify the pathogenesis of OLP and aid in diagnosis and treatment.

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**Ethical Issues**

Not applicable.

**Conflict of Interest**

Authors declare no conflict of interest in this study.

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