The immunoexpression of EGFR and Her2/neu in oral squamous carcinoma

L. P. DRAGOMIR1, C. L. MĂRGĂRITESCU2, ALMA FLORESCU2, A. D. OLIȘID3, MANUELA DRAGOMIR4, M. R. POPEȘCU1

1) Department of Dental Prosthetic
2) Department of Pathology
University of Medicine and Pharmacy of Craiova
3) Department of Biology, University of Craiova
4) Department of Public Health, University of Medicine and Pharmacy of Craiova

Abstract
In this study, we have analyzed the EGFR and Her2/neu expression in oral squamous carcinoma and adjacent dysplastic areas. The lesions were diagnosed especially in the sixth decade of life, in male patients, localized on the lips, especially as well and poorly differentiated carcinomas (34%). The EGFR immunostain has been intense in over 50% of the tumors cells in well-differentiated carcinomas, expression diminished in the moderately and poorly differentiated carcinomas. Her2/neu marker recorded a score of 3+ in moderately and poorly differentiated carcinomas, the reaction turning out positive in 25% of the cases.

Keywords: oral squamous carcinomas, EGFR, Her2/neu, immunohistochemistry.

Introduction
Clinical staging using TNM classification was, for a good period of time, used as a standard instrument for the treatment planning and the prognosis estimation. However, this method used to establish a prognosis does not provide enough predictive information for a proper course of treatment, which would be for the benefit of the patient [1].

Therefore, over the years, there have been many studies meant to identify different prognosis markers, which could provide a more reliable prediction.

The OSCC pathogenesis involves a multitude of molecular paths and, therefore, the molecular changes underlying the tumor progression represents the subject of many studies.

An interesting aspect of the OSCC is the biological behavior, which is different in patients that present clinico-pathological characteristics, some of the tumors presenting a better prognosis than other does. The identification of molecular changes responsible for this process can contribute to a better understanding of the tumor behavior.

In the past few years, there were identified some series of biological markers which can provide prognosis information, useful in managing squamous carcinoma of the head and neck [2]. Thereby, the mutation of the p53 tumor suppressor gene, cyclin D1 proto-oncogene end EGFR superexpression were all associated with an unfavorable prognosis [3–5].

This study took into consideration the EGFR and Her2/neu expression, the purpose being to identify their role in oral carcinogenesis, following, in the same time, the possible connections between them or between them and the analyzed clinic-morphological parameters and choosing those with statistical significance.

Materials and Methods
The study contained 44 surgical excisions diagnosed with OSCC, in the Pathological Anatomy Laboratory of Emergency County Hospital of Craiova, between 2008 and 2011.

The tissue fragments were fixed in 10% formalin and processed by histopathological technique by paraffin embedding and Hematoxylin–Eosin stain.

The epidemiological and histological data were analyzed and the lesions were classified according to the latest WHO criteria [6].

Subsequently, there were performed serial sections, which were used for the immunohistochemical analysis, for which there were used rabbit antihuman polyclonal antibodies (Table 1).

The working systems for the immunohistochemical reactions have been represented by CSA II, Biotin-Free, Catalyzed Amplification System (code K197, Dako) for
EGFR and LSAB+ System–HRP (Dako) for Her2/neu, their visualization being obtained with DAB (3,3’-diaminobenzidine, Dako).

Table 1 – Antibodies used for the immunohistochemical analysis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone / Source</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Polyclonal / Sigma</td>
<td>1:1000</td>
<td>Citrate, pH 6</td>
</tr>
<tr>
<td>Her2/neu</td>
<td>Polyclonal / Dako</td>
<td>1:300</td>
<td>Citrate, pH 6</td>
</tr>
</tbody>
</table>

For EGFR reactions quantification there was used a semiquantitative estimation system, with three degrees: <10%, 10–50%, and >50% marked cells. The intensity of the reactions was also evaluated [7].

Her2/neu reactions were quantified based on a system with four degrees, according to the number of marked cells and reaction intensity [8]:

- 0 – absence of the reaction or membrane reaction, in less than 10% of the cells;
- 1+ – weak or incomplete reaction, in more than 10% of the cells;
- 2+ – weak or moderate and complete reaction, in more than 10% of the cells;
- 3+ – intense and complete reaction, in more than 10% of the cells.

The validation of the reaction was achieved by using negative external controls, with primary antibody omission.

The statistical analysis used the chi-square test, using the SPSS 10 soft.

The images were obtained using the Nikon Eclipse E600 microscope and Lucia 5 soft.

Results

The study of the 44 CSO selected pieces indicated predominance of neoplasias in patients aged between 59 and 79 years, in over 75% of the male cases (29 cases).

Regarding their topography, we observed that most of the cases presented carcinomas around the lips (23 cases), followed by lingual carcinomas (18 cases) and palatal carcinomas (three cases).

We did not record any statistical associations between the analyzed epidemiological factors and the two markers’ expression.

In terms of the histopathological behavior, the tumors corresponded with well-differentiated forms in 15 cases, moderately differentiated forms in 14 cases and poorly differentiated forms in 15 cases. In nine cases, there were identified adjacent dysplastic areas.

The immunoreactions analysis for EGFR indicated positive results in 32 cases, representing 72.7% of the analyzed cases (Table 2).

The cases with negative results corresponded with poorly differentiated OSCC.

The immunomarking for EGFR was cytoplasmic and membranous, with variable intensity and distribution, frequently heterogeneous. There has been no correlation between the immunostain OSC and the differentiation levels, but we observed that all well and moderately OSC cases have been positive with moderate or high intensity, while poorly differentiated OSCC corresponded to a moderate or low intensity immunostain.

Table 2 – The distribution of the immunomarkers EGFR and Her2/neu

<table>
<thead>
<tr>
<th>Dysplasia</th>
<th>OSCC</th>
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<tbody>
<tr>
<td></td>
<td>EGFR</td>
</tr>
<tr>
<td>Score</td>
<td>Negative</td>
</tr>
<tr>
<td>0%</td>
<td>10–50%</td>
</tr>
<tr>
<td>%</td>
<td>6.8</td>
</tr>
<tr>
<td>Score</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>13.6</td>
</tr>
</tbody>
</table>

In 15 cases the tumors have been positive for less than 10% of the tumor cells, with moderate intensity (Figure 1a), which included well differentiated OSCC in five cases, moderately differentiated in six cases, and poorly differentiated, in four cases. For other 11 cases, the tumors presented immunostain for 10–50% of the tumor cells, with high intensity and included moderately differentiated OSCC in eight cases and well-differentiated in three cases (Figure 1b).

For seven cases, the immunostain has been of high intensity in over 50% of the tumor cells and included only well-differentiated OSCC.

The review of the correlation between the tumor differentiation and EGFR expression, observed during the chi-square test, indicated a highly significant association, \( \chi^2(4, N=44)=41.51, p=0.00 \).

The immunomarking analysis for the EGFR in adjacent dysplastic areas indicated positive results in six (66.6%) of the nine studied cases but with no correlation with their stage (Figure 1c).

The analysis for Her2 expression indicated positive results in a small number of cases, respectively in 11 cases (25% of analyzed OSCC) (Table 2). The marking was a membranous one, complete or incomplete, with different proportions of intensity.

We observed that the 11 cases corresponded in three cases with the score of 1+, other three cases with 2+ and five cases with 3+. Regarding the three cases with the score of 1+ the tumors corresponded with well differentiated OSC. In two cases with the score of 2+, the tumors were moderately differentiated (Figure 1d).

In one case of 2+ tumor and five cases with score of 3+ there were poorly differentiated SCO. In these cases, the expression was continuous in the membrane in over 30% of the cells (Figure 1e).

Among the nine cases of oral mucosa dysplasia, the immunomarking for Her2/neu has been positive in three cases with moderate and severe dysplasia. The pattern of the immunomarking did not correspond with the level of dysplasia, the score being 2+ (Figure 1f).

The analysis of the relation between the level of tumor differentiation and Her2/neu expression, observed during the chi-square test, indicated a highly significant association, \( \chi^2(6, N=44)=18.34, p=0.005 \).
The receptor for the epidermal growth factor (EGFR) is an oncogene (a tyrosin-kinase receptor from the erbB family) identified in more than one affections as malign, including breast cancer, prostate cancer, pulmonary cancer, bladder cancer and head and neck cancers [9]. EGFR appears to be, part of it at least, the dominating control factor of the malign phenotype in head and neck squamous carcinomas, through the adjustment of the molecules involved in the invasive processes angiogenic and lymphangiogenic ones [10].

Discussion

In OSC, the EGFR expression was associated with a lower global surviving rate, lymph node metastases and tumor invasion [7, 9].

Analyzing the immumomarker for EGFR, we can see it indicates positivity in 32 cases, representing 72.5% of the total number of analyzed cases, ruling in well-differentiated forms. Literature facts confirm as well the EGFR overexpression in over three quarters of the OSC analyzed cases, with values like 73.42% [10], 87.5% [11], 72% [12], and 75% [13]. Another recent study that investigated the EGFR expression reports positivity in 50% of the cases, more frequent in well-differentiated forms. Figure 1 – EGFR immunostain: (a) Moderately differentiated carcinoma, over 40% marked cells, high intensity, ×100; (b) Poorly differentiated carcinoma, 25% marked cells, low intensity, ×100; (c) Mild dysplasia, over 50% marked cells, high intensity, ×40. Her2/neu immunostain: (d) Moderately differentiated carcinoma, 2+ score, ×100; (e) Poorly differentiated carcinoma, 3+ score, ×100; (f) Moderate dysplasia, 2+ score, ×40.
differentiated forms [14]. Another possible explanation could be the fact that this receptor is connected to the degree of differentiation of the neoplastic keratinocytes [15]. Other studies have reported, on the contrary, an association between the EGFR overexpression and poorly differentiated CSC [16].

The EGFR expression in OSC was associated with the advanced T stage of the primary tumor, with an advanced tumor progression and high metastases incidence [17]. More studies confirm that EGFR is an independent prognosis marker at patients with OSC and oropharyngeal [7].

Following, the EGFR antigen represents an attractive target for specific therapies using monoclonal antibodies or tyrosine kinase inhibitors to those patients [7, 12]. More than that, a series of reports have shown a connection between the EGFR expression and the resistance to ionizing radiation [18, 19], the signaling paths of the erbB family receptor, being responsible for the radiosensitivity modifications. In vivo, inhibition of EGFR by a monoclonal antibody, cetuximab, increases the response to irradiation of the squamous carcinomas [20]. Recently, a randomized study showed a doubled average surviving rate of the patients with squamous carcinomas of the head and neck in an advanced stage, treated both with radiotherapy and cetuximab, comparing to just a radiotherapy treatment [21].

The review of the EGFR immunomarker positivity for all the nine cases of dysplasia analyzed from the surgical safety margin indicated its presence in three cases. Recent literature data reports that for the evaluation of the surgical safety margin, the histopathological examination is conventionally not enough, the detection of some oncogenes such as EGFR, can identify patients with a high risk of tumor recurrence that can benefit of an anti EGFR treatment [13].

The purpose of the HER2/neu in head and neck squamous carcinomas is not well defined. HER2/neu is a proto-oncogene, with a homologous EGFR sequence. Its overexpression was observed in multiple types of cancer and used in treatment measures against cancer. It has been proved that the HER2/neu overexpression increases the metastatic potential, by promoting through multiple stages the invasion and the metastatic cascade [22], suggesting that this gene might play an important role in the carcinogenesis.

The analysis of the HER2/neu expression indicates a positivity in a low number of cases, namely 11 cases, representing 25% of the analyzed OSC, ruling in poorly differentiated forms. From all the nine cases of the oral mucosa dysplasia, the immunomarker for HER2/neu was positive in three cases corresponding to severe and moderate dysplasia.

Literature studies concerning HER2/neu in OSC communicated the protein overexpression in a small number of tumors and it does not appear to have any importance in prognosis [10, 14, 22–23].

Another study indicates a very low HER2/neu immunoreactivity, with HER2/neu(+) in 10% of normal mucosa cases, 25% of dysplasia cases, comparing to CSO cases, in which HER2/neu(+) expressions are 40%, and 10% HER2/neu(++) [23]. Authors never found a significant connection between the HER2/neu expression and the clinical and pathological covariates.

A similar study reports a percentage of 17% of the positive tumors for HER2/neu [22], without statistic significant correlation between HER2/neu and the T or N stage, the degree of the tumor, the general surviving rate, and the surviving rate without a disease. However, the authors find a correlation between the HER2/neu expression and the VEGF.

Conclusions

The presence of the EGFR and HER2/neu expression in both lesional groups, dysplasias and carcinomas, indicate their intervention in the oral carcinogenesis, even from their early stages, at least for a part of the tumors.

References

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Corresponding author
Claudiu Mărgăritescu, Associate Professor, MD, PhD, Department of Pathology, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Romania; Phone/Fax +40251–599 228, e-mail: c_margaritescu2000@yahoo.com

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