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FEATURES OF EGFR EXPRESSION IN THE HEAD AND NECK SQUAMOUS CELL CARCINOMA

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ABSTRACT. Head and neck comprise a wide spectrum of neoplasms with different tumor biologies, prognosis and response to therapies. The most common histology of head and neck tumor is squamous cell carcinoma. Disappointigly, until now, survival has not markedly improved because the patients still frequently develop locoregional recurrences, distant metastasis and different second tumours. The purpose of the present study was the assessment of the EGFR expression in the squamous cell carcinoma with different localization in the area of the head and neck. We included in the study a number of 17 biopsy fragments from patients diagnosed with squamous cell carcinoma: from the larynx area (5), from the pharynx area (2), the hard palate (1), tongue (2), submandibular (1), lip (2), gingival sulcus (1), nasal pyramid (1), maxilla (1), zygomatic (1). The immunoreaction for EGFR was evaluated according with the following score: 0 (0% positive cells), 1 (<10% positive cells), 2 (10-30%), 3 (>30%). The value 3 of score was noticed in half of the cases included in the study. This was present in each type, excepted the squamous cell carcinomas from the submandibulary, maxillary and zygomatic areas. Heterogeneous distribution, more intense in the cells from the periphery of the tumoral areas were noticed in the case of the squamous cell carcinoma from the areas of the larynx, lip, nasal pyramid. A new classification of HNSCC based on molecular features of each tumor subtype could improve the diagnosis of these diseases and also can cause a better stratification of the patients who will be followed then by a more targeted therapeutic schemes.

Keywords: EGFR, head and neck squamous cell carcinoma

INTRODUCTION

The receptor of the epidermal growth factor (EGFR, erbB1, HER1) is a surface cellular protein of 170 kDa involved in the adjustment of the cellular differentiation and growth. EGFR is a member of the erbB family of the tyrosine kinases receptor, four of them being closely related to the receptors of the cellular membrane: EGFR (HER1 or erbB1), erbB2 (HER2), erbB3 (HER3), erbB4 (HER 4). They all are involved in the proliferation and invasion of the tumoral cells, including those from the head and neck squamous cell carcinoma, as a response to the stimulation with growth factors. (Baselga, 2009)

The head and neck squamous cell carcinomas represent the sixth neoplasia as prevalence at a global scale and is responsible for over 90% of the cases of cancer that emerge at this level. The statistical data have indicated the emergence of 40.000 new cases per year and of 12.000 cases of death per year in the United States of America (Zhang et.al., 2007). Moreover, approximatelly 600.000 new cases are being reported annualy at a global scale (et al. 2008, Parkin et.al. 2005). The risk factors for the development of the squamous cell carcinoma in the area of the head and neck are smoking, alcohol consumption, HPV – 16 (et al., 2001, Furniss et al., 2007).

The current classification is based on morphology and anatomical distribution, which leads to a homogenous treatment for different illnesses. Even if the oncogenesis of the head and neck squamous cell carcinoma (HNSCC) was investigated (Estilo et al., 2003, Kinoshita et al., 2012) the exact mechanism of the tumor development, the progression of the illness and the aggresivity are not currently very well characterized.

EGFR is involved in the HNSCC pathogenesis, being supraexpressed in over 90% of the cases of head and neck neoplasias if compared to the level of expression in the area of the regular mucosa (Kalyankrishna et al., 2006, et al., 1997). The inhibition of EGFR by means of different methods, determines the blocking of the tumoral growth in the preclinical patterns of study of HNSCC (Erjala et al., 2006). Despite the EGFR supraexpression in the head and neck squamous cell carcinoma, only one subset of patients responded favourably to the therapy with inhibitors of EGFR, among which Cetuximab (Nozawa et al.,2006).

Starting from such a premise, the purpose of the present study was the assessment of the EGFR expression in the squamous cell carcinoma with different localization in the area of the head and neck.

MATERIALS AND METHODS

We included in the study a number of 17 biopsy fragments from patients diagnosed with squamous cell carcinoma: from the larynx area (5), from the pharynx area (2), the hard palate (1), tongue (2), submandibular (1), lip (2), gingival sulcus (1), nasal pyramid (1), maxilla (1), zygomatic (1). The biopsy fragments have been fixed in buffer formalin 10% for 48 hours and paraffin embedded. Five μm thick sections were performed from each case. The dewaxing and rehydration of the sections was followed by heat-induced epitope retrieval in citrate buffer pH6 for 30 minutes (with PT link module, Dako Cytomation, Denmark). The immunohistochemical technique continued with the blocking of the endogeneous peroxidases, using hydrogen peroxide 3%. Incubation with the EGFR primary antibody (clone EGFR 25, RTU, Novocastra, Newcastle upon Tyne, UK) had a duration of 30 minutes. NovoLink Max Polymer Detection System, was applied for 30 minutes, as visualisation system. The chromogene used was 3,3 – diaminobenzidine, and for counterstainig Lille's modified hematoxylin was used. The entire immunohistochemical technique was performed with DakoAutostainer Plus (DakoCytomation), according to the work protocol. After dehydration in absolute alcohol, the sections were clarefied in benzene and fixed, by using Canada balm.

The immunoreaction for EGFR was evaluated according with the following score: 0 (0% positive cells), 1 (<10% positive cells), 2 (10-30%), 3 (>30%). The intensity of the immunohistochemical reaction was quantified with values ranging between 1 - low, 2- moderate and 3 – intense. The expression patterns included in the assessment were the cytoplasmic and the membrane one. The microscopical evaluation was performed with the Nikon Eclipse E 600 microscope, and the images were obtained by using the LUCIA G. system.

RESULTS

The internal positive control for the immunohistochemical technique was represented by the reaction in the area of the basal cells stratum of the epidermis. (figure 1). Of the five cases of squamocellulare carcinoma with origin in the larynx area, 3 (60%) had score 2 and 2 (40%) had score 3. The intensity of the immunohistochemical reaction in these cases was in conformity with the score that percentually quantifies the positive cells for EGFR (figure 3). At the level of the tumoral spectrum we identified a heterogenity of the expression, with a higher intensity of the reaction at the periphery of the tumoral surfaces (figure 4), but the homogeneous aspect as well, with a uniformity of the intensity of expression in the tumoral area (figure 2). The latter aspect was mainly noticed in the cases quantified with a maximum percentual score.

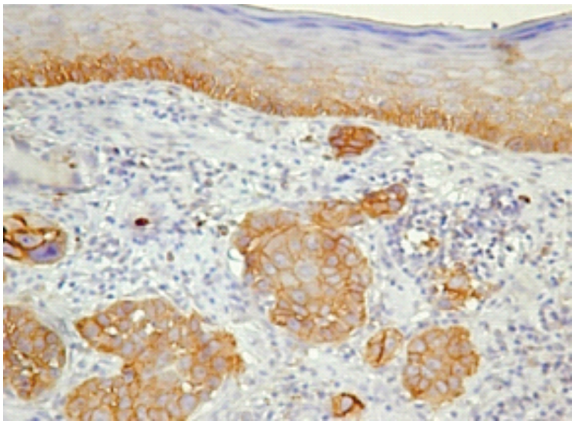

Fig. 1 EGFR expression in the basal cells layer of the epidermis, X200

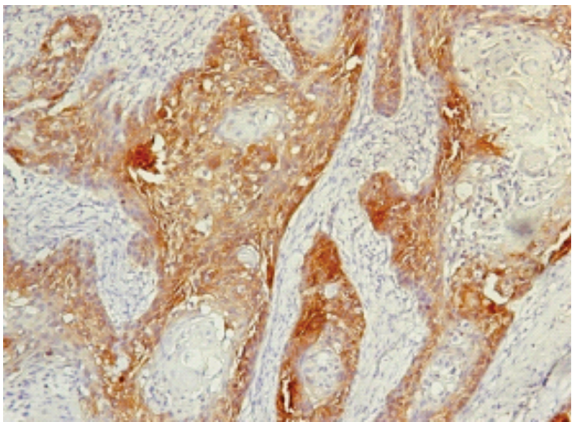

Fig. 2 EGFR, score 3, squamous cell carcinoma with origin in the larynx area, homogeneous expression, X100

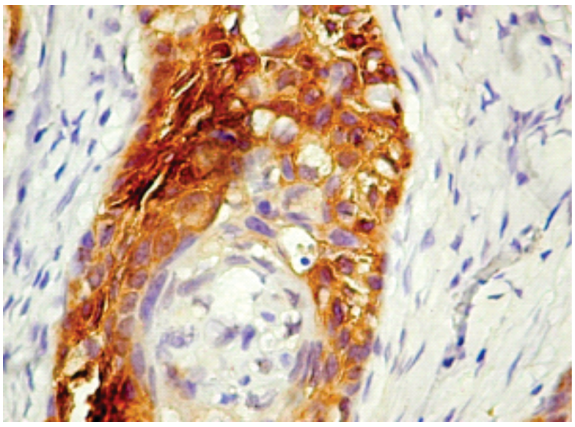

Fig. 3 EGFR expression, intense, cytoplasmic and membrane pattern, score 3, squamous cell carcinoma of the larynx, X400

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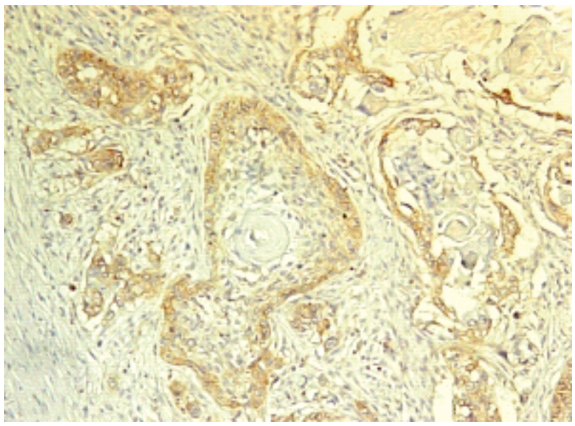


Fig. 4 EGFR, score 2, squamous cell carcinoma of the larynx, heterogeneous distribution, prevailing in the peripheral area, X100

In the case of squamous cell carcinoma with origin in the pharynx area, in one of the cases, we noted a score 0, and the other one had score 3. For the latter one, as in the case of the squamous cell carcinoma with origin in the larynx area, we noticed a homogeneous distribution of the EGFR expression in the tumoral area, cytoplasmic granular pattern with membrane intensification and maximum of intensity of the immunohistochemical reaction (figure 5). The 0 score was also noted in the only case of squamous cell carcinoma with origin in the zygomatic area.

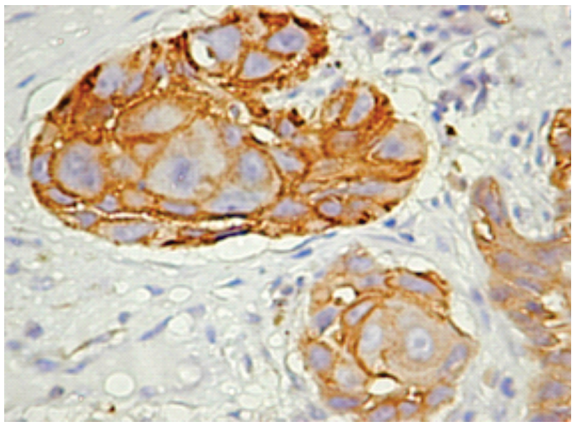


Fig. 5 immunohistochemical expression of EGFR- squamous cell carcinoma with origin in the pharynx area, score 3, homogeneous distribution, membrane, cytoplasmic granular pattern, X400

The squamous cell carcinoma with origin in the hard palate, had a score of 3, over 30% of the tumoral cells have expressed EGFR (figure 6), homogeneous distribution, membrane and cytoplasmic granular pattern, and an average intensity of the immunohistochemical reaction.

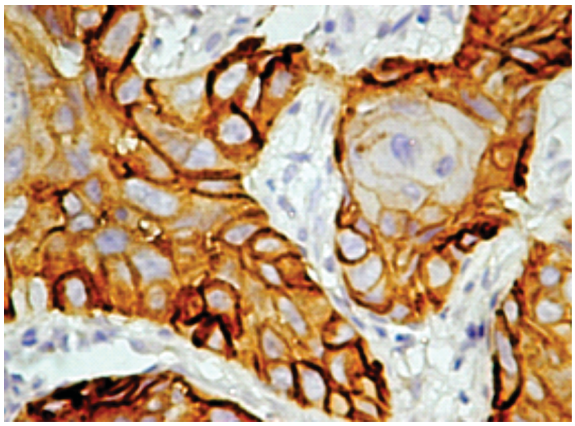


Fig. 6 EGFR, score 3, squamous cell carcinoma, hard palate, X400

The scores observed in the case of squamous cell carcinoma in the area of the tongue were: 1 (under 10% positive EGFR tumoral cells) for one of the cases and 3 (over 30% of positive EGFR tumoral cells) for the second case (figure 7). Compared to the squamous cell carcinoma from another area, the membrane pattern prevailed (figure 8).

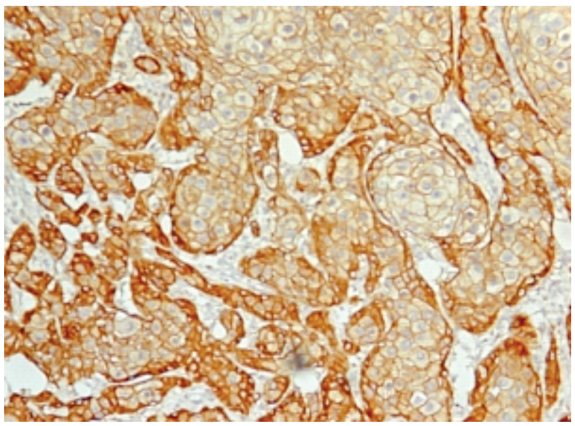


Fig. 7 EGFR, score 3, squamous cell carcinoma in the area of the tongue, X100

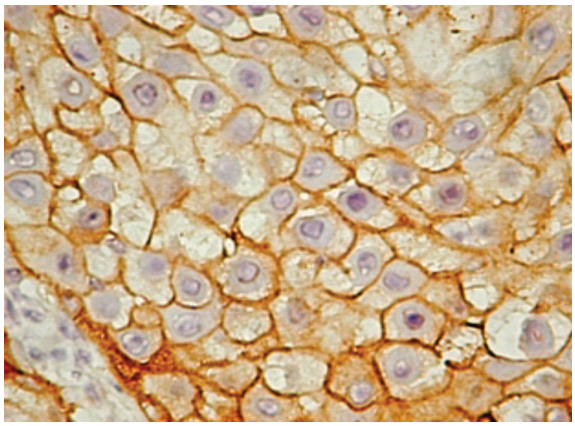


Fig. 8 EGFR, score 3, squamous cell carcinoma in the area of the tongue, membrane pattern, X400

In the case of squamous cell carcinoma in the area of the lip we noticed in one of the situations, a score 2, and for the other one, score 3 (figure 9).

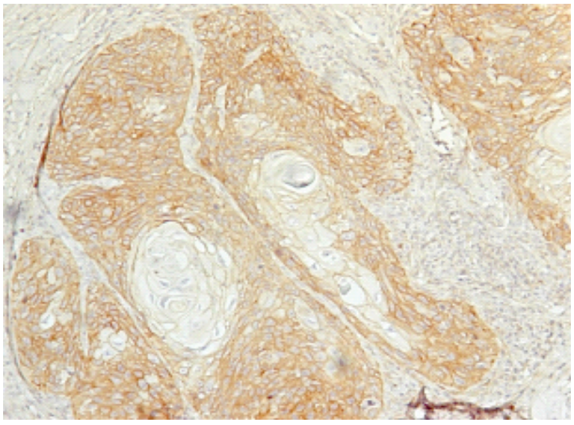


Fig. 9 EGFR, score 3, squamous cell carcinoma in the area of the lip, X100

Besides the squamous cell carcinoma in the area of the tongue we noticed the presence of score 1 in the cases with origin in the submandibulary (figure 10) and maxilla (figure 11) area.

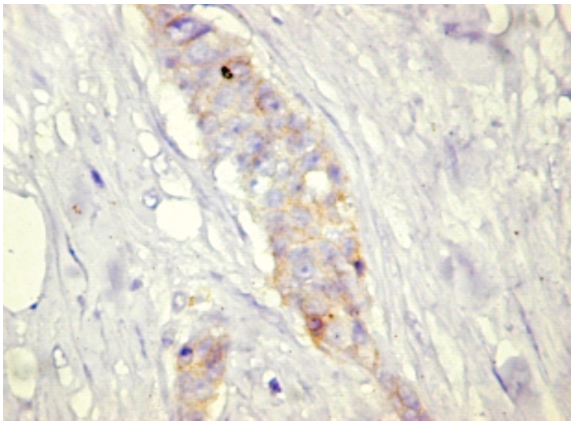


Fig. 10 EGFR, score 1, submandibulary squamous cell carcinoma, X200

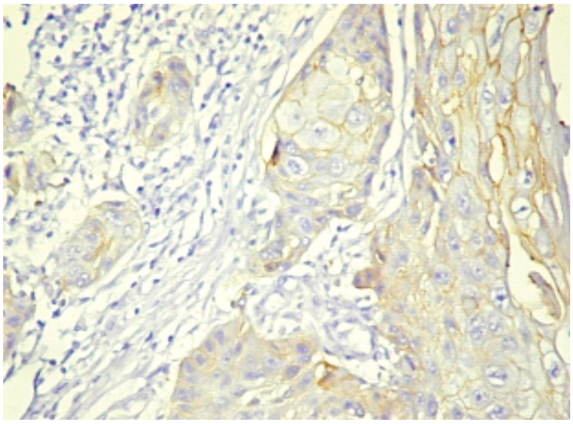


Fig. 11 EGFR, score 1, squamous cell carcinoma, upper maxilla, X400

For the cases of squamous cell carcinoma in the area of the nasal cavity (figure 12) and of the gingival sulcus, we noticed a maximum score of 3, over 30% of the tumoral cells expressed EGFR.

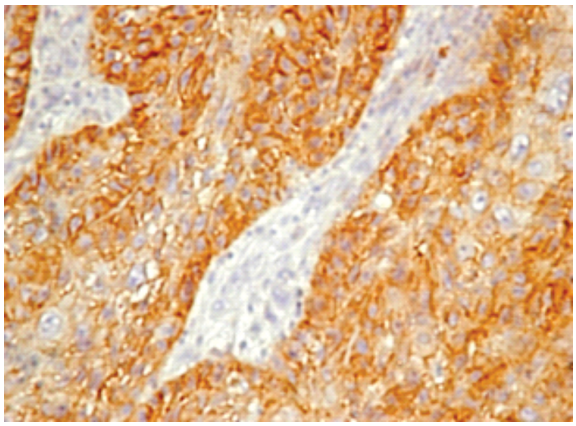
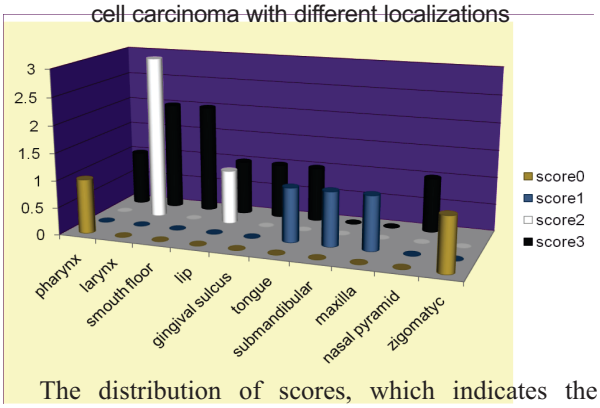


Fig. 12 EGFR, score 3, squamous cell carcinoma in the area of the nasal cavity, X 200

Variation of the EGFR expression, quantified in accordance with the score previously described, in the squamous cell carcinoma from the different areas of the head and neck is presented in the table below:

Table 1

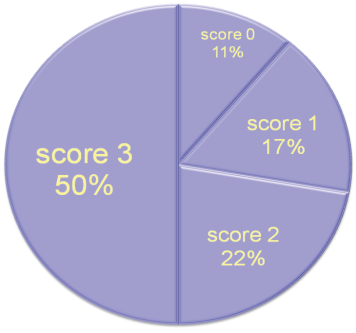
The rapport between the score of the EGFR expression and the number of cases in the head and neck squamous cell carcinoma with different localizations



The distribution of scores, which indicates the percentage of the positive EGFR tumoral cells, related to the number of cases inculded in the study is rendered in the graphic below.

Graph 1

The percentual distribution of the EGFR expression scores



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DISCUSSIONS

In most of the studies related to the chromosomal alterations or to the proteic expression in the head and neck squamous cell carcinoma there has been no distinction performed between the different surfaces of this area. The tumoral behaviour of the carcinioma coming from different areas, varies significantly, suggesting different intrinsic tumoral particularities. These may have a clinical and prognosis importance.

If analysing, from an immunohistochemical point of view, the EGFR bcl2, Rb, cyclin D1 myc, E-cadherin expression in the area of the larynx, pharynx and the oral cavity, Takes et al., 1998, noticed in the EGFR case, a very low expression in the area of the larynx and rather increased in the area of the pharynx. Pectasides and collaborators, noticed the association of the EGFR VIII expression, a common form of the mutant EGFR, in the area of the larynx. In our study, in the case of squamous cell carcinoma in the area of the larynx, we noted the presence of scores 2 and 3. A particular aspect was represented by the heterogeneous distribution in the area of the tumoral surfaces, with a higher intensity at the periphery for the cases with score 2 and homogeneous for the cases with score 3. The 0 and 1 scores, were noticed in the case of one of the carcinoms with pharyngeal origin, one of the cases localized in the area of the tongue, those with a zygomatic, submandibular and sublingual origin. Relating to the carcinoma locatized in the pharynx area, one of the cases has a maximum score of the EGFR expression.

The signaling ways activated by EGFR are involved in the pathonegesis of the squamous cell carcinoma. A monoclonal antibody which determines the EGFR blocking, ensuring the blocking of the activity of the receptor (cetuximab, Erbitux, C225) has been used in therapy beginning with the year 2006.

However, the mechanisms of the EGFR suppression have not been fully understood. The adjustment methods, structural alterations of gene EGFR or of its amplification may be involved. Mrhalova et al., 2005 noticed from the point of view of the immunohistochemical expression, three types of expression for EGFR: homogeneous, maximum intensity, membrane pattern, intensity ranging between 1 and 3, in a proportion that is close to the first type; maximum intensity, membrane pattern, at the periphery of the tumoral cells surface. In our study, we noticed a heterogenous distribution, prevailing in the cells from the periphery of the tumoral surfaces in the squamous cell carcinoma with origin in the area of the larynx, lips, nasal pyramid.

CONCLUSIONS

In each type the maximum value 3 of score was present, excepted the squamous cell carcinoma from the submandibulary, maxillary and zygomatic areas. Heterogeneous distribution, more intense in the cells from the periphery of the tumoral areas were noticed in the case of the squamous cell carcinoma from the areas of the larynx, lip, nasal pyramid. A new classification of the squamous cell carcinoma, based on the molecular characteristics of each tumoral subtype, may determine a better stratification of the patients and several terapeutical specific schemes.

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