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Mutations In EGFR Signal Pathway In Correlation With Response To Treatment Of Head And Neck Cancers

J. Neuwirthová[1], S. Pavel[1], J. Rottenberg[1], R. Kostřica[1], M. Zdeněk[1], M. Hajdúch[2], J. Drábek[2], J. Srovnal[2], J. Berkovcová[2]

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Abstract

The prognostic and predictive value of the epidermal growth factor receptor (EGFR) expression and some genetic alterations in an EGFR signal pathway, such as the EGFR amplification, the EGFR activating tyrosine kinase domain mutations or the k-ras gene mutation were investigated in our study. The aim of the research was to evaluate the occurrence of the above-mentioned biomarkers in correlation with a therapeutic response and survival in patients with locoregionally advanced spinocellular head and neck cancers.

Keywords

Head and neck cancer, EGFR, predictive marker, k-ras, EGFR amplification, EGFR tyrosine kinase domain mutation

Introduction

A surgical resection is standard therapy of an advanced head and neck squamous cell carcinoma (HNSCC). Radiotherapy or chemoradiotherapy is frequently added to the treatment scheme to target remaining tumor cells and to induce programmed cell death (apoptosis). However, HNSCC tumors regularly exhibit resistance to the apoptosis induction. Some genetic alterations detected from tumor cells could predict a response to treatment or could be helpful in deciding which type and how aggressive therapy should be selected for a patient. The aim of investigating predictive factors is to individualise therapy.

The epidermal growth factor receptor (EGFR) is a well-characterized proto-oncogene that is present in multiple cancers where – as being shown - it promotes tumor progression. The EGFR (HER1) is a member of the family of transmembrane receptors which also include the HER2/neu (ErbB-2), the Her3 (ErbB-3) and the Her4 (ErbB-4). The EGFR has an extracellular ligand-binding domain, a transmembrane region and an intracellular domain which includes a kinase domain and autophosphorylation sites. The EGFR is ubiquitously distributed on normal epithelial tissues and is over-expressed in several cancers. The EGFR plays a critical role in the control of cellular proliferation, differentiation and survival. A series of EGFR ligands has been identified, with most studies implicating the transforming growth factor alpha as a predominant autocrine growth factor in head and neck cancers. The binding ligand to the EGFR triggers homodimerisation with the EGFR or heterodimerisation of the EGFR with another receptor from its family, resulting in autophosphorylation and downstream signalling. Abnormalities in the signalling of the EGFR pathway are found in a wide range of cancers[1,2]. High EGFR gene copy number by FISH is a poor prognostic indicator[3,4].

Targeted therapy directed against the EGFR represents a new approach in oncology. Monoclonal antibodies and tyrosine kinase inhibitors, acting extracellularly and intracellularly, respectively, comprise two

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classes of agents being currently available for use. Various genetic alterations predict the response to EGFR inhibitors activating especially mutations of intracellular transducers in the EGFR signal pathway (Fig. 1). Some of these mutations are clinically used before the selection of patients convenient for this treatment in some types of tumors[5] but not all of them were studied in HNSCC. Activating mutations in the

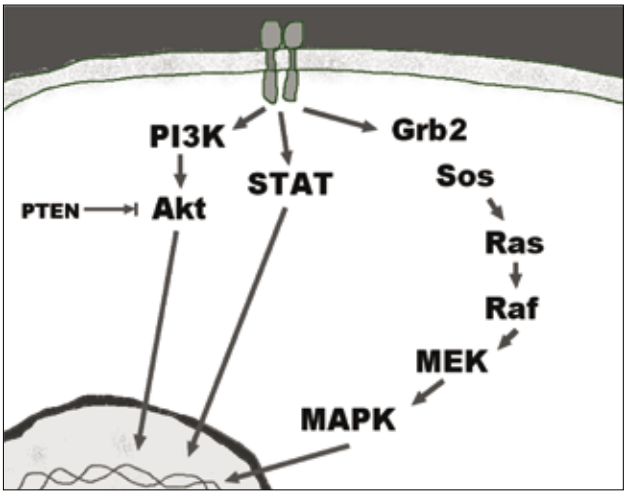

Fig. 1 : Signal transducers in EGFR signal pathway

EGFR signal pathway cause a more aggressive phenotype which could influence the response to EGFR targeted therapy, chemotherapy or radiotherapy. However, indicating these mutations as predictive markers for clinical use requires strong association with the therapeutic response and enough representation in specific tumors.

Material and Methods

Forty-five tumor samples from patients with HNSCC of oropharynx, hypopharynx, oral cavity or larynx were evaluated. The patients were treated by chemoradiotherapy/ radiotherapy as a primary definitive treatment (20 patients) or as an adjuvant treatment after the surgical resection of tumor (25 patients). The surgical resection was preferred in resectable cases while definitive chemoradiotherapy or radiotherapy was selected in unresecable cases or if the organ preservation was preferred. Dividing patients into two groups was caused by a well-known reason: patients with the primary surgical resection have better therapeutical results and a better chance of surviving than patients with definitive chemoradiotherapy. All clinical stages were represented in these 2 groups of patients, with the stage IVA being represented mostly. The following genetic alterations of tumor cells from paraffin blocks were evaluated: the EGFR expression, the ras gene mutation, the EGFR gene amplification, and the activating mutations of an EGFR gene in tyrosine kinase domain. The EGFR expression was evaluated by an international scoring system 0, 1+, 2+, 3+ (Novocastra). The activating mutation of the k-ras gene was detected in an exon 1, other cases were non-mutated (a wild type). Activating mutations of the EGFR tyrosine kinase domain were detected in exons 19 and 21. The gene amplification was defined as a ratio of EGFR signals to the chromosome 7 copy number of 1.5 or more.

Tumor samples were taken mostly from peripheral active parts of tumors. The receptor expression was detected by imunohistochemical staining. The paraffin sections were assessed by the laser capture microdissection. The polymerase chain reaction (PCR) was used to identify mutations; the fluorescence in situ hybridisation (FISH) was used for the EGFR gene amplification.

Kaplan-Meier analysis and Gehan-Wilcoxon test were used for the survival evaluation; χ^2 test a Fischer test were used for the evaluation of the EGFR expression dependence on clinical stages; and Mann-Whitney test was used for the relation between the EGFR expression and the gene amplification. A statistical analysis was carried out with the help of Statistica for Windows 6.0 software.

Results

In our study, 91% of patients achieved complete remission; 5-year survival was recorded in 70%. These results were influenced by patients with the first stage of glottic cancer.

- The EGFR expression was positive in 72% and negative in 28% of patients.
- The EGFR amplification was present in 15% (Fig. 2).

Four mutations in the tyrosine kinase domain in an exon 21 were found; however, no mutation in an exon 19 was discovered. No significant

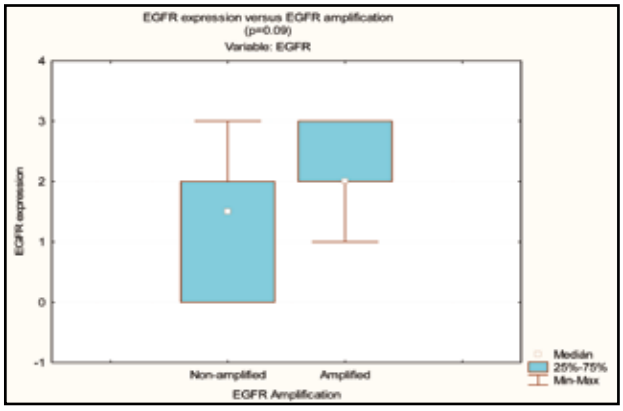

Fig. 2 : Correlation between EGFR gene amplification and protein expression (confidence interval 91%)

correlation between mutations or amplification of the EGFR gene and patients' survival was proven in spite of that only data of patients with definitive chemo/radiotherapy (organ preservation protocols) (Fig. 3, 4) were analysed. The k-ras gene mutation, which is known as a negative predictive and prognostic marker, was only seen in 1 patient (laryngeal cancer of glottis

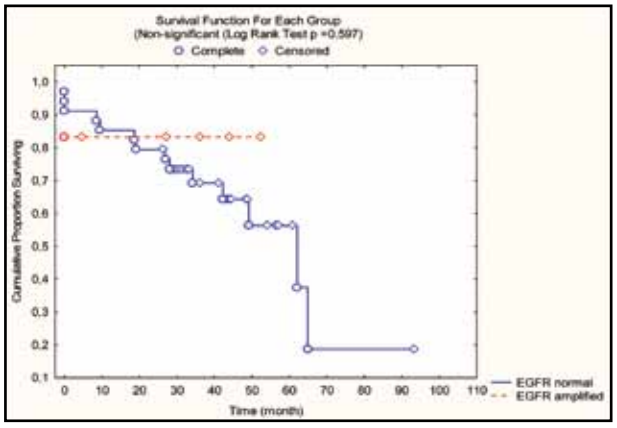

Fig. 3 : Survival of EGFR non-amplified vs. amplified group (non-significant)

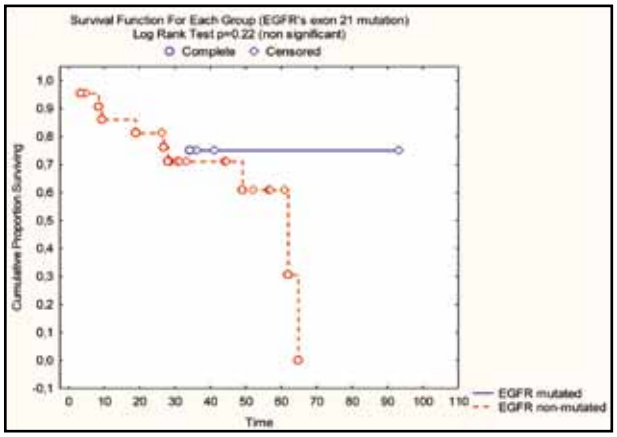

Fig. 4 : Survival of EGFR non-mutated vs. mutated in exon 21 group (non-significant)

T3N0M0) and this case was associated with a negative prognosis.

Discussion

It has been observed that there are discrepancies between the results of studies comparing the EGFR amplification and the EGFR expression; some authors have reported the correlation while the others have not. The detection of the EGFR expression depends partly on specificity and sensitivity of available immunohistochemical sets[6]. Furthermore, there are differences of the EGFR expression inside a tumor; the highest expression, e.g. the most active parts, can be found in periphery whereas in central parts, the expression could be very low or negative. The FISH has been used as a reference method for the assessment of the gene amplification for many years; however, there are several ways how to analyse the gene expression by the FISH and not all authors differ true gene amplifications from chromosomal polysomy. This could be a reason why in literature there are studies with low percentage of FISH positivity, e.g. 17%[7], whereas other studies present 58%[8] or 63%[9] in HNSCC. In our study, the true EGFR amplification was present in 15% and correlation with the EGFR protein expression was found with a confidence interval of 91% (p value 0,09).

The mutations of the tyrosine kinase domain of the EGFR gene in the exons 19 and 21 are known to affect sensitivity to the EGFR inhibitors; at the same time, they can be used as positive predictive markers during therapy with intracellular inhibitors[10,11]. In our group of patients, several cases of the EGFRex21 have been found.

K-ras gene mutation is a negative predictive and prognostic marker. This mutation is strongly associated with a negative response to the EGFR inhibitors and it is used as a negative predictive marker in some types of tumors[5]. The ras gene mutation is rare in HNSCC[12]. Only 1 ras gene mutation was found in our group of patients and this case had a highly negative prognosis.

Conclusions

The aim of the present study was to map out the occurrence of some clinically important genetic alterations in EGFR signal pathway in head and neck squamous cell cancers and to analyse possible correlation between these genetic alterations and the response to chemo/radiotherapy and survival. The study detected EGFR gene amplification, k-ras and tyrosine kinase domain mutations; however, not enough cases of the EGFR genetic alterations for analysing their importance as predictive factors in clinical use have been found. K-ras mutation, which is known as a negative predictive and prognostic marker, occurs only very rarely in head and neck cancers.

For the reason that the above mentioned mutations do not seem to be as useful for the prediction of a response to chemo/radiotherapy as they are to the EGFR inhibitors, we continue with monitoring predictive factors of targeted therapy in combination with radiotherapy, which are indicated for locoregionally advanced tumours (clinical stage III-IVb). The aim of our further study is to identify molecular predictive markers of response to cetuximab which may improve therapeutic results. At present, adequate biomarkers for defining which patients with head and neck cancers are most likely to respond to anti EGFR-based therapy, are lacking. Currently we are mainly focusing on a constitutively active K-ras mutation, EGFRvIII and EGFR tyrosine kinase domain mutations in exon 19 and 21.

Acknowledgement

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