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Epidermal growth factor receptor (EGFR) expression and mutations in the EGFR signaling pathway in correlation with anti-EGFR therapy in head and neck squamous cell carcinomas

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Epidermal growth factor receptor (*EGFR*) is an important therapeutic target and a poor prognosis factor in head and neck squamous cell carcinoma (HNSCC). The aim of the study was to analyze *EGFR* expression and *KRAS* and *EGFR* mutational status and to correlate it with treatment response to anti-EGFR therapy combined with radiotherapy in 29 patients with advanced head and neck squamous cell carcinomas (HNSCC).

EGFR gene expression normalized to GAPDH and EGFR variant type III (EGFRvIII) was detected in tumor tissue using real time reverse transcription –PCR. The mutational status of the *EGFR* and *KRAS* genes was investigated by real time PCR with sequence specific primers.

Gene expression median values were $3.1x10^8$ GAPDH gene copies per μg of RNA, and $8x10^6$ EGFR gene copies per μg of RNA. The median EGFR/GADPH ratio reached 0.14. Patients, who achieved complete response after Cetuximab combined with radiotherapy, had significantly higher expression of the *EGFR* gene in tumors than patients with partial remission or patient without treatment response. An EGFRvIII mutation was found in 20.7 % of patients and no association was found between this mutation and treatment response. 27 patients (93.1 %) had an *EGFR* gene wild type tumor, and deletion in exon 19 was found in two patients with a poor clinical outcome. Most of the patients (82.8%) had a *KRAS* wild type tumor; a p.Gly12Cys was found in three patients and a p.Gly12Val mutation in one. Presence of a p.Gly12Val mutation in the *KRAS* gene was associated with an absence of response to treatment.

Conclusion: Our data suggest that *KRAS* mutation (p.Gly12Val) and somatic *EGFR* mutation located in exon 19 may contribute to the limited clinical response to therapy with cetuximab + radiotherapy. Higher *EGFR* gene expression serves as an independent indicator of good clinical response to EGFR-targeted therapy + radiotherapy.

Key words: HNSCC, EGFR, KRAS, EGFR gene expression

In the treatment of head and neck squamous cell carcinomas nowadays we can see a worldwide effort to prolong survival with a satisfactory quality of life. This requires earlier detection of disease and also new treatment strategies – new chemotherapeutic regimens, intensity-modulated radiotherapy, and targeted treatment. The latter is especially promising when combined with radiotherapy. Monoclonal antibody against epidermal growth factor receptor (EGFR) demonstrated good antitumor activity in the first line treatment of locally advanced head and neck cancers[1]. EGFR (HER1, *c-erb B1*) is a member of the receptors family. It is a well -characterizedproto-oncogene that is present in many cancer site where itpromotes tumor progression. It comprises an extracellular ligand-binding domain, a transmembrane

Abbreviations: HNSCC – head and neck squamous cell cancer; EGFR (HER1, *c-erb B1)* – epidermal growth factor receptor; RT-PCR– reverse transcriptase polymerase chain reaction; KRAS – oncoprotein, encoded by the *KRAS* gene, intracellular transducer in the EGFR signal transduction pathway; OS – overall survival; EFS– event-free survival (time to recurrence or progression or death); FISH - fluorescent in situ hybridization; GAPDH – housekeeping gene (Glyceraldehyde 3-phosphate dehydrogenase) is control gen serving for normalization of expression.

region, and an intracellular domain which includes a kinase domain and autophosphorylation sites. EGFR is ubiquitously-distributed in normal epithelial tissues and is over-expressed in a wide range of cancers; in HNSCC approximately in 80 % [2, 3]. EGFR plays a critical role in the control of cellular proliferation, differentiation and survival. The binding of ligand to the EGFR triggers EGFR homodimerisation or heterodimerisation of the EGFR with another receptor from its family, results in autophosphorylation and downstream signaling [4]. Genetic abnormalities in the EGFR signaling pathway in HNSCC were not yet sufficiently described. The effect of EGFR inhibitors depends on the presence of genetic alteration in the EGFR signaling pathway [5-7]. Therefore it is necessary to seek markers of response to this therapy at the level of an individual genetic profile. Predicting the outcome in EGFR-targeted therapies is complex and involves genetic and clinical characteristics. In addition to the predictive value of non-smoking, and the higher intensity of skin reaction [8], predictive markers appear to exist at the level of specific genetic aberrations causing deregulation of the cell cycle. The influence of the tyrosinkinase domain of EGFR on positive response to the treatment with extracellular inhibitors has not been elucidated yet [9, 10]. Constitutive activation of intracellular transducers in the EGFR signaling pathway (*KRAS* mutation or STAT-3 mutation), loss of the extracellular EGFR inhibitor binding site (EGFRvIII mutation), activation of EGFR-independent tumor angiogenesis (overexpression of VEGF) or altered activation of alternative tyrosinkinase receptors are among promising negative predictors [11]. In many studies, resistance to extra- and intracellular inhibitors was proven during continuous activation of the MAPK pathway [11-13]. In this context, a presence of activating *KRAS* mutation, which is typical of smokers, has a negative predictive value in various types of tumors, e.g. colorectal cancer[14, 15, 18]. EGFRvIII mutation (EGFR variant with deletion in exons 2-7) is found only in tumor cells. Such a mutated receptor lacks an extracellular domain; therefore extracellular inhibitors cannot bind to it. However, EGFRvIII is able to dimerize even without constitutive activation by ligand binding, and its moderate degree of auto-phosphorylation is sufficient for activation of the signaling pathway but insufficient for induction of its internalization (and down-regulation).

The aim of the present study was to analyze *EGFR* gene expression, and *EGFR* and *KRAS* mutational status in the correlation with treatment response to targeted therapy combined with radiotherapy in patients with advanced HNSCC.

Patients and methods

Patients.A prospective clinical study include 83 patients with verified locoregional advanced HNSCC. 29 patients were Cetuximab-treated in two tertiary centers. Remaining 54 patients were screened, and the sample for genetic analysis was taken, but Cetuximab treatment was not started due to not fulfilling inclusion criteria (n=36) or noncompliance (n=4) or for organizational reasons (n=10) or for allergic reaction (n=4). Inclusion criteria comprised a good general condition without allergy (ECOG 0,1), age up to 65 years, absence of serious

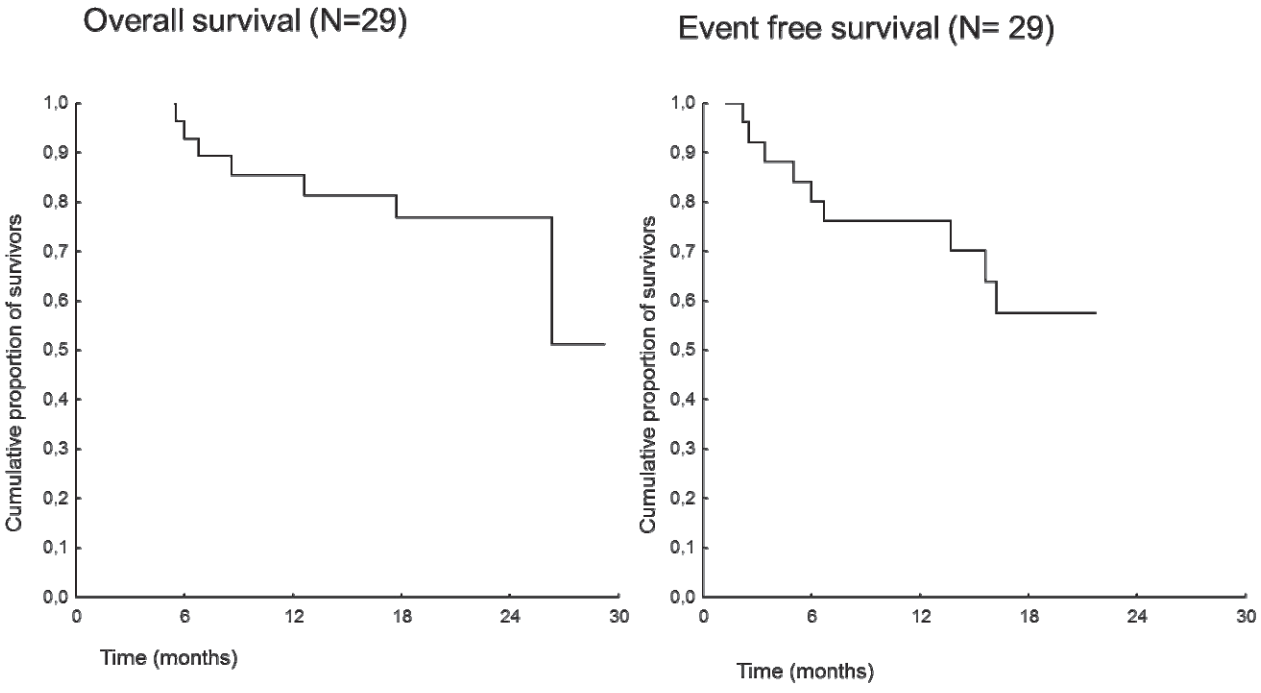

Figure 1. Survival end-points in Kaplan-Meier analysis (patients in stage 3+4, N=29)

Table 1 Characteristics of the patient population

		Description
Sex	Male	24 (82.8%)
	Female	5 (17.2%)
Age	55 years ≤	14 (48.3%)
	55 years >	15 (51.7%)
Site		
	Oral cavity	2 (6.9%)
	Oropharynx	19 (65.5%)
	Hypopharynx	5(17.3%)
	Larynx	3 (10.3%)
T	2	5 (17.2%)
	3	7 (24.1%)
	4	17 (58.6%)
N	0	6 (20.7%)
	1	2 (6.9%)
	2	21 (72.4%)
M	0	29 (100%)
Stage	III	5 (17.2%)
	IV	24 (82.8%)
Grade	1	3 (10.3%)
	2	17 (58.6%)
	3	9 (31.0%)
Toxicity – skin	1	7 (24.1%)
	2	9 (31.0%)
	3	12 (41.4%)
	4	1 (3.4%)
Toxicity – mucous membrane	1	8 (27.6%)
	2	11 (37.9%)
	3	10 (34.5%)
GAPDH		309×10^6 (91×10^6; 918×10^6)
EGFR1		48×10^6 (6×10^6; 339×10^6)
EGFR1/GADPH		0.14 (0.4 – 0.57)
EGFRvIII	0	22 (75.9%)
	1	6 (20.7%)
	Not done	1 (3.4%)
k-ras	12Cys	3 (10.3%)
	12Val	1 (3.4%)
	wt	24 (82.8%)
	Not done	1 (3.4%)
EGFR	Deletion in exon 19	2 (6.9%)
	wt	27 (93.1%)
Any mutation	No	22 (78.6%)
	Yes	6 (21.4%)

Legend to Table 1.Absolute and relative frequencies were used to describe categorical data; a median with the 5th–95th percentile range was adopted for continuous parameters.

GAPDH (housekeeping gene Glyceraldehyde 3-phosphate dehydrogenase) is control gen serving for normalization of expression. Number in GAPDH and EGFR mean degree of expression;it means how many copies are present in 1 μg mRNA acquired from tumor sample.

intercurrent diseases, signing of the informed consent form, and weight loss under 10%. The study was approved by the Ethics committee of the St. Anne Faculty Hospital. The patients were treated with radiotherapy (70 Gy) with concomitant 8-week intravenous administration of Cetuximab. The patients characteristics are presented in Table 1.

Methods. The recorded data were age, gender, tumor location, TNM classification, histopathological grading, therapeutic response, toxicity according to WHO, event-free survival (EFS – interval between treatment and relapse or progression of the disease) and overall survival (OS). Tumor samples were taken before treatment mostly from peripheral active parts of tumors and were fixed in RNA Later (Qiagen) and frozen. *EGFR* and *GAPDH* gene expressions and EGFRvIII mutations were detected in RNA by the RT-PCR method [16, 17]. Total RNA from RNA Later (Qiagen) fixed tissues were purified by the phenol-chloroform method using TRI Reagent (Molecular Research Center) according to the manufacturer's instructions. For reverse transcription, 3 μg of total RNA was pre-incubated with Random Primers (Promega) and then reverse transcribed using RevertAid Moloney Murine Leukemia Virus reverse transcriptase (Fermentas). To amplify cDNA of the epidermal growth factor receptor 1 (EGFR1, NM_005228), epidermal growth factor receptor vIII (EGFRvIII, NM_005228) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NM_002046) (10), the following set of primers and probes were used: EGFR-fw 5'-ACTTCAAAAACTGCACCTCCAT-3', EGFR-rev 5'-AATCAGCAAAAACCCTGTGATT-3' and EGFR-probe 5'-ACATCCTGCCGGTGGCATTTAGG-3' BHQ1-HEX (149 bp); EGFRvIII-fw 5'-AGTCGGGCTCTGGAGGAA-3', EGFRvIII-rev 5'-GCCGTCTTCCTCCATCTCATA-3' and EGFRvIII-probe 5'-ATCACGGCTCGTGCGTCCG-3' BHQ1-HEX (102 bp); GAPDH-fw 5'- GAAGATGGTGAT-GGGATTTC-3',GAPDH-rev5'-AGTCGGGCTCTGGAGGAA -3' and GAPDH-probe 5'- CAAGCTTCCCGTTCTCAGCC -3' BHQ1-FAM (226 bp) (Generi-Biotech).

To analyze the EGFR1, GAPDH and EGFRvIII mRNA copies, a quantitative real-time polymerase chain reaction (qPCR) was performed in three 25 μl reactions containing 1 U of HotStart Taq Polymerase, 3 mM $MgCl_2$, 10x PCR buffer (AB Gene), 200 μM dNTPs (Promega), 100 ng of cDNA, and either 400 nM EGFR-fw, 400 nM EGFR-rev, 200 nM EGFR-probe, or 300 nM GAPDH-fw, 300 nM GAPDH-rev, 200 nM GAPDH-probe, or 400 nM EGFRvIII-fw, 400 nM EGFRvIII-rev, 200 nM EGFRvIII-probe and using Rotor Gene 3000 (Corbett Research). The optimized thermal profile for EGFR1 amplification was initiated with 15-min. polymerase activation at 96°C, followed by 50 cycles of 95°C for 15 s and 62°C for 15 s, for GAPDH amplification 50 cycles of 95°C for 1 5s and 60°C for 30 s and for EGFRvIII 50 cycles of 95°C for 15 s and 61°C for 12 s. The presence of the truncated EGFRvIII gene was subsequently verified by post-PCR electrophoresis using Agilent 2100 Bioanalyzer DNA chips (Agilent).

Tumor wild type DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen) from RNA Later (Qiagen) fixed tissues. *KRAS* mutations were detected by the quantitative real-time PCR method using the Amplification Refractory Mutation (ARMS) principle for discrimination and Scorpion primers for visualization: eight PCR reactions from a TheraScreen DxS KRAS kit (QiaGen) were used for detection of the seven most frequent mutations of *KRAS* gene (codon 12: Ala, Asp, Arg, Cys, Ser, Val and codon 13: Asp). Similarly, EGFR mutations were detected by the quantitative real-time PCR method using a combination of the ARMS principle and PNA clamping for discrimination and an SYBR Green intercalator for visualization: eight PCR reactions from Panagene PNAClamp kit (PentaGen) were used for detection of substitutions G719X in exon 18, 20 types of deletions in exon 19, substitutions S768I and T790M in exon 20, 2 types of insertions in exon 20, and substitutions L858R and L861Q in exon 21. The sensitivity of both kits is 1 % of mutant DNA in background of wild type DNA.

Data analysis. Standard descriptive statistics were used to summarize primary data, i.e. frequency analysis for categorical variables and a median supplied with the 5^{th}– 95^{th} percentile range for continuous variables. Statistical comparison of variants was based on Mann-Whitney U test in continuous variables and on an ML-c^2 test for categorical variables. Standard Kaplan-Meier analysis was used to estimate profiles of survival. Differences among groups of patients in stratified survival analysis were tested using log-rank test. A value a=0.05 was accepted as the cut-off for a statistically significant outcome in all applied tests. Analyses were realized using the software SPSS 19.0.1 (IBM Corporation, 2010).

Results

Patient characteristics. Twenty-nine patients were included in the study – 24 men and 5 women. Half of the cohort (14 patients) were under 55 years of age, while 15 patients were over 55 years old. All patients had advanced disease (clinical stage III and IV). Almost 60% of patients had grade 2 tumor, 31% grade 3 and 10% grade 1 (Table 1). Tumors were located in the oral cavity (6.9%), oropharynx (65.5%), hypopharynx (17.3%) and larynx (10.3%). Five (17.2%) patients were treated in stage III and 24 (82.8 %) in stage IV. Overall survival (OS) and event-free survival (EFS) were assessed: the median follow-up was 21.7 months, the 2-year OS was 75%, and seven patients died (Graph 1). Both survival parameters were also assessed for various types of patient stratification (gender, age, clinical stage, grade, genes activity, and mutations). No statistically significant differences were found however in this analysis. Overall, a good treatment response was recorded during the study. Complete remission was achieved in 80% of patients, another 10% had partial remission, and only 10% of patients did not respond to the treatment. Serious adverse effects (grades 3 and 4) associated with toxicity on the skin occurred in 44.8% of patients, while serious mucosal toxicity was recorded in 34.5% of patients (Table 1). *EGFR* expression and *KRAS* and *EGFR* mutational status were correlated with treatment response to anti-EGFR therapy combined with radiotherapy (Table 2).

EGFR Gene expression.*GAPDH* and *EGFR* gene levels were assessed. Median values were $3.1x10^8$*GAPDH* gene copies per μg of RNA, $8x10^6$EGFR gene copies per μg of RNA and 0.14 for the EGFR/GADPH ratio. Treatment results were analyzed in relation to molecular characteristics of patients. Patients who achieved a complete response had significantly higher expression of the *EGFR* gene than patients with a partial response or without a response (Table 2, p=0.02). The relation between treatment result and *EGFR* gene expression normalized to *GAPDH* was not statistically confirmed. A statistically significant relationship ($p<0.05$) of EGFR/GADPH for mucosal toxicity was found (data not shown).

EGFRvIII mutation analysis.EGFRvIII mutation was found in 20.7% (6 out of 29) patients. The expected significant association between this mutation and treatment response to anti-EGFR therapy (Cetuximab) was not found. No significant difference was found between different clinical characteristics (age, clinical stage, grade, toxicity) and EGFRvIII mutation with the exception of gender (EGFRvIII was not found in women).

EGFR mutation analysis.27 patients (93.1 %) had wild-type *EGFR* gene; deletion in exon 19 was found in two patients. Presence of *EGFR* mutation (deletion in exon 19) was associated with a worse prognosis – presence of recurrence and/or progression and/or death (Table 2, p=0.033) and with no response to treatment (Table 2, p=0.042).

***KRAS* mutation analysis.**Most of the patients (82.8%) had wild-type *KRAS*. P.Gly12Cys and p.Gly12Val mutation was found in three patients and one patient, respectively. Presence of p.Gly12Val mutation in the *KRAS* gene was associated with no response to treatment; this statement is, nevertheless, based on experience with one patient only (Table 2, p=0.027). In total, one of two mutations tested (deletion in exon 19 and KRAS mutation) was found in 6 patients (Table 1).

No significant difference was found between different clinical characteristics (gender, age, clinical stage, grade, toxicity) and any of these mutations.

Discussion

The treatment response (CR 80 %) of radiotherapy combined with Cetuximab in our set of patients is consistent with Bonner´s et al. report in which patients have good therapeutical results[1] and with experimental data of Gurtner et al. [18]. The knowledge of *EGFR* expression and *EGFR* and *KRAS* mutational status becomes clinically important with the wider use of anti-EGFR therapies in HNSCC. In our study, patients who achieved a complete response had significantly higher number of *EGFR* gene mRNA copies than patients wit partial remission only or patients without a treatment response.

Table 2. Association of molecular characteristics of patients with survival endpoint and treatment response

		Survival/event/combined endpoint								
		Survived (N=22)	Exitus (N=7)	p	Without event (N=19)	With event (N=10)	p	Exitus, event or no CR (N=11)	CR, without event (N=17)	
GAPDH[1]		363 (112; 918)	206 (74; 550)	**0.027**	317 (102; 1 717)	257 (74; 725)	0.408	270 (74; 725)	326 (102; 1 717)	0.378
EGFR[1]		51 (16; 339)	18 (6; 174)	0.208	51 (16; 538)	30 (6; 72)	0.089	42 (6; 174)	51 (16; 538)	0.264
EGFR/GAPDH		0.12 (0.04; 0.44)	0.14 (0.03; 0.57)	0.678	0.15 (0.04; 0.85)	0.11 (0.03; 0.20)	0.208	0.12 (0.03; 0.57)	0.14 (0.04; 0.85)	0.517
EGFR vIII[2]	No	16 (72.7%)	6 (85.7%)	0.466	15 (78.9%)	7 (70.0%)	0.596	8 (72.7%)	14 (77.8%)	0.759
	Yes	5 (22.7%)	1 (14.3%)	0.620	3 (15.8%)	3 (30.0%)	0.378	3 (27.3%)	3 (16.7%)	0.498
KRAS[2]	12Cys	3 (13.6%)	0 (0.0%)	0.184	3 (15.8%)	0 (0.0%)	0.099	0 (0.0%)	3 (16.7%)	0.080
	12Val	0 (0.0%)	1 (14.3%)	0.085	0 (0.0%)	1 (10.0%)	0.138	1 (9.1%)	0 (0.0%)	0.158
	wt	19 (86.4%)	5 (71.4%)	0.383	16 (84.2%)	8 (80.0%)	0.777	9 (81.8%)	15 (83.3%)	0.917
EGFR[2]	Deletion exon 19	2 (9.1%)	0 (0.0%)	0.283	0 (0.0%)	2 (20.0%)	**0.033**	2 (18.2%)	0 (0.0%)	**0.042**
	wt	20 (90.9%)	7 (100.0%)		19 (100.0%)	8 (80.0%)		9 (81.8%)	18 (100.0%)	
Any mutation[2]	No	16 (76.2%)	6 (85.7%)	0.583	15 (83.3%)	7 (70.0%)	0.417	8 (72.7%)	14 (82.4%)	0.548
Yes		5 (23.8%)	1 (14.3%)		3 (16.7%)	3 (30.0%)		3 (27.3%)	3 (17.6%)	
		Treatment response								
		CR (N=23)	NR/PR (N=6)	p	NR (N=3)	PR/CR (N=26)	p			
GAPDH		344 (102; 918)	225 (74; 550)	0.078	206 (101; 244)	326 (91; 918)	0.090			
EGFR		51 (16; 339)	13 (6; 72)	**0.020**	14 (6; 42)	51 (11; 339)	0.062			
EGFR/GAPDH		0.14 (0.05 - 0.57)	0.11 (0.03 - 0.17)	0.214	0.14 (0.03 - 0.17)	0.13 (0.04 - 0.57)	0.726			
EGFR vIII[2]	No	17 (77.3%)	5 (83.3%)	0.620	3 (100.0%)	19 (73.1%)	0.184			
KRAS[2]	Yes	5 (22.7%)	1 (16.7%)	0.781	0 (0.0%)	6 (23.1%)	0.224			
	12Cys	3 (13.6%)	0 (0.0%)	0.224	0 (0.0%)	3 (11.5%)	0.405			
	12Val	0 (0.0%)	1 (16.7%)	0.070	1 (33.3%)	0 (0.0%)	**0.027**			
	wt	19 (86.4%)	5 (83.3%)	0.967	2 (66.7%)	22 (84.6%)	0.472			
EGFR[2]	Deletion exon 19	2 (8.7%)	0 (0.0%)	0.326	0 (0.0%)	2 (7.7%)	0.501			
	wt	21 (91.3%)	6 (100.0%)		3 (100.0%)	24 (92.3%)				
Any mutation[2]	No	17 (77.3%)	5 (0.8%)	0.743	2 (66.7%)	20 (0.8%)	0.612			
	Yes	5 (22.7%)	1 (0.2%)		1 (33.3%)	5 (0.2%)				

Legend to Table 2. Absolute and relative frequencies were used to describe categorical data; median with the 5th–95th percentile range was adopted for continuous parameters. The statistical significance of differences between groups of patients in continuous parameters was tested using the Mann–Whitney U test. Maximum likelihood chi square test was applied for the analyses of relationship between categorical variables.

[1]Number shows how many copies are present in 1 μg mRNA acquired from tumor sample. [2]Binary/categorical variables expressed in % (counts in parenthesis)

There is experimental evidence that *EGFR* amplification and elevated EGFR expression levels in HNSCC may result in a less favorable prognosis [18, 20], but clinical studies are inconclusive [21]. A high *EGFR* gene copy number could be a positive predictive marker for treatment response[2, 19]. There are discrepancies between the results of studies comparing the *EGFR* amplification and the *EGFR* expression in various tumors: some authors have reported the correlation [22, 23] while others have not [24]. The detection of the *EGFR* expression depends partly on the specificity and sensitivity of available immunohistochemical sets [24]. Furthermore, there are differences of the *EGFR* expression within a tumor: the highest expression, e.g. the most active parts, can be found in the periphery, whereas in central parts the expression could be very low or negative. FISH has been used as a reference method for the assessment of gene amplification for many years; however, there are several ways to analyze gene expression by FISH [2, 25, 26] and not all authors distinguish true gene amplifications from chromosomal polysomy. This could be a reason why there are studies with a low percentage of FISH positivity, e.g. 17% [2], whereas other studies present 58% [27] or 63% [19] in HNSCC. In chemotherapy-treated non-small-cell lung cancer patients, the *EGFR* gene copy number was positively associated with protein level but none of the features were predictive for either treatment response or survival[28]. The amplification of the *EGFR* gene is the most frequent alteration of glioblastoma multiforme and confers advantages of growth and invasiveness and radio- and chemo resistance on tumor cells. The FISH method is commonly used to determine gene copy numbers, but the quantitative real-time polymerase chain reaction (qPCR) used in our study can obtain a gene copy number, is less expensive, and makes data interpretation more reliable because of use of a large area of tumors [2]. 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 [6, 29, 30]. Some data suggest that *EGFR* mutations do not confer sensitivity to the monoclonal antibody Cetuximab. Cetuximab binds to the cell-surface receptor, whereas the kinase inhibitors bind to the intracellular tyrosine kinase domain; therefore in EGFR mutant cell lines Cetuximab has relatively little effect [11].

KRAS mutation (p.Gly12Cys) was found in our study in 3 patients out of 29 (10.3%) and *KRAS* mutation (p.Gly12Val) was found in one patient out of 29 (3.4%). *KRAS* mutation was connected with an absence of response to Cetuximab therapy combined with radiotherapy. *KRAS* gene mutation is known as a negative prognostic marker [18]. 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 [14, 31]. The *KRAS* gene mutation is rare in HNSCC [32]. Van Damme et al. did not find any *KRAS* mutation by sequencing in a set of 24 patients with tonsil squamous cell carcinoma. The authors conclude that *KRAS* mutation analysis is not useful as a screening test for sensitivity to anti-EGFR therapy in tonsil squamous cell carcinoma [26]. Hoa et al. supposed that amplification of wild-type *KRAS* promotes growth of HNSCC [33].

EGFRvIII has been examined only in a small amount of HNSCC tumors[20]. In our study the rate of EGFRvIII detected at the mRNA level by RT-PCR was 20.7 % (6 out of 29). Sok et al. found EGFRvIII expression established by immunohistochemistry in 42 % of HNSCC tumors (14 out of 33), and in nearly half of HNSCC tumors established by RT-PCR [20]. Ji et al. [34] found EGFRvIII mutation in 5% (3 out of 56) in squamocellular cancer and not in lung adenocarcinomas. Chau et al.[35] found EGFRvIII mutation in 40 % (22 out of 53) in HNSCC and it appeard to be biomarker associated with better disease control in recurrent or metastatic disease, but no difference was seen between erlotinib-treated versus non-erlotinib treated patients. In our study we could not prove an influence of EGFRvIII on treatment response to EGFR targeted therapy. In HNSCC, resistance to monoclonal antibodies and decreased response to cisplatin was demonstrated in the presence of EGFRvIII [36]. Sok et al. proved that EGFRvIII contributes to enhanced growth and resistance to targeting wild-type EGFR [20].

Conclusions

Our data suggest that *KRAS* mutation (p.Gly12Val) and somatic *EGFR* mutation located in exon 19 may contribute to the limited clinical response to therapy with Cetuximab + radiotherapy. Higher *EGFR* gene expression serves as an independent indicator of good clinical response to Cetuximab therapy combined with radiotherapy. A correlation between EGFRvIII and clinical response to EGFR targeted treatment was not proven. Our results are limited by the size of the evaluated sample and should be verified on a larger cohort of HNSCC patients.

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