The expression of EGFR, HER2 and EpCam in Head and Neck squamous cell carcinomas

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Purpose: Head and Neck squamous cell carcinomas show an increasing incidence and make up to 5% of all malignancies. In this work three cell surface proteins are investigated, which could be possible targets in currently arising tailored cancer treatment: Epithelial cell adhesion molecule (EpCam), the epidermal growth factor receptor (EGFR, c-erbB-1 or HER1) and HER2/neu (c-erbB-2).

Materials and methods: In this study, specimens from 114 histologically verified squamous cell carcinoma of pharynx and oral cavity were analysed. The surface proteins were tested with pharmacodiagnostic kits.

Results: With 44.7% (51/114) EGFR was found to be overexpressed most frequently in this cohort. A usable distribution for EpCam and nearly lacking of HER2 was shown in 22.8% (26/114) and 3.5% (4/114), respectively.

Conclusion: These data show that EGFR and EpCam might have a relevant role for the biology of this disease. Its relevance for a therapeutic application cannot yet be concluded.

Keywords: EpCam, EGFR, HER2, overexpression, head and neck cancer

Introduction

Head and neck squamous cell carcinoma (HNSCC) is on increasing incidence and makes up about 5% of all malignancies worldwide. Despite the advances in oncology of HNSCC overall survival did not improve over the past 20 years. In addition to that no reliable prognostic factors could be established for advanced disease [1, 2].

In this work we investigated three cell surface proteins, which are possible targets in tailored cancer treatment: Epithelial cell adhesion molecule (EpCam), the epidermal growth factor (EGFR, c-erbB-1 or HER1) and HER2/neu (c-erbB-2).

Together with c-erbB-3 (HER3) and c-erbB-4 (HER4) the two last-mentioned receptors represent the first group of tyrosin-kinase receptors. They have an extra cellular ligand-binding domain, a transmembranal part and an intracellular domain with tyrosin kinase activity [3]. Receptor activation leads to phosphorylation of tyrosin-kinase residues in the cytoplasmic region, so cellular proteins can bind with their Src homology 2-(SH2) or phosphotyrosin-binding-domain (PTB) and start complex signalling pathways. Mitogen-activated protein kinases (MAPKs), phospholipase C-γ (PLC-γ) or phosphatidylinositol-3-kinase (PI3K) are cascades which finally lead to an enhancement of cell growth, motility, invasion and angiogenesis [1, 4].

EGFR is a 170k-Da protein localised on chromosome 7p14-12 and is expressed at low levels in almost all normal adult tissues, without the haematopoietic [5] tissue. Besides its physiological function, EGFR is associated with tumourigenesis, and an overexpression is found in a wide variance of malignancies including breast, ovarian, prostate, bladder, lung, brain, pancreas and HNSCC [6].

The 185k-Da HER2 shows high similarity to the EGF-Receptor and is encoded on chromosome 17q21 [7]. The HER2/neu oncoprotein is primarily associated with breast cancer, but furthermore overexpression was detected in human ovarian, gastric, lung and head and neck carcinoma [1, 8–11].

The epithelial cell adhesion molecule (EpCam) is a transmembranal 40-kDa glycoprotein encoded on chromosome 4q and does not structurally belong to one of the four major families of cell adhesion molecules. EpCam mediates Ca²⁺-independent cell–cell adhesions and is expressed during embryogenesis in lung, kidney, liver, pancreas, skin and germ cells. In normal adult tissues EpCam is positive in all simple pseudo-stratified transitional epithelia, whereas squamous epithelia including head and neck is clearly negative. Tumourigenesis is associated with enhanced or de-novo expression of EpCam, such as esophagus, gastric, colon, liver, pancreas, lung, mammary gland and head and neck [12–14].

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The critical role that predescribed molecules play in cancer has led to an extensive search for selective inhibitors of their signalling pathways. Optimal use of tumour-tailored therapy, however, requires determination of the expression status, since the presence of receptor overexpression is the sole eligibility criterion for treatment with target-specific therapeutic agents. To identify those patients who may benefit from targeted cancer therapy, we determined the expression status in tissue specimens from 114 patients with HNSCC by semiquantitative immunohistochemistry.

Materials and methods

In this study, specimens from 114 histologically verified squamous cell carcinoma of pharynx and oral cavity were analysed. Patients received therapy at the Department of Otorhinolaryngology of the University Hospital Innsbruck between 1997 and 2002. The following features of the patients were recorded: gender, age at tumour resection, site, staging, grading, therapy and day of death. The bigger part of the survival data was provided from the local cancer registry of the Federal State Tirol.

Formalin-fixed, paraffin-embedded tissue blocks containing sections of primary tumour had been stored at the Department of Pathology. Recutted 5 µm slices were prepared for immunohistochemistry by deparaffinization in two sequential xylene baths and rehydration in downgraded ethanol series.

Expression of EGFR and HER2 was detected with two pharmacodiagnostic kits according to manufacturers' guidelines. EGFR pharmDx™ test (DakoCytomation, Glostrup, DK) staining protocol works as follows: 5 min wash using wash buffer, 5 min proteinase-K incubation, 5 min wash, 5 min peroxidase blocking agent incubation, 5 min wash, 30 min incubation with primary anti-EGFR mAb, clone 2-18C9, 5 min wash, 30 min incubation with HRP-labelled goat anti-mouse Ig polymer, 2×5 min wash, 10 min incubation using DAB substrate, 5 min wash, 1 min counterstaining with haematoxylin, 5 min gently rinse in water.

HercepTest™ (DakoCytomation, Glostrup, DK) was used to identify tumours that overexpress HER2. Briefly, the sequence: 60 min incubation at 99°C in epitope retrieval solution, 20 min cooling down at RT, 5 min wash in wash buffer, 5 min peroxidase blocking agent incubation, 5 min wash, 30 min incubation with primary anti-HER2 mAb, 5 min wash, 30 min incubation with HRP-labelled goat anti-rabbit Ig polymer, 2×5 min wash, 10 min incubation using DAB substrate, 5 min wash, 1 min counterstaining with haematoxylin, 5 min gently rinse in water.

After 15 min antigen retrieval with Accutase and 5 min wash, the central steps of EpCam detection were a 30 min incubation with primary mAb VU1D9 (Novocasta Ltd.; 1:100), followed by 5 min wash and the visualization reaction with the DAKO EnVision System based on alkaline phosphatase. Five min wash, 1 min counterstaining with haematoxylin, 5 min gently rinse in water.

Slides were classified by one author (V.S.) and by a pathologist without knowledge of the patient's outcome. For EGFR pharmDx™ and EpCam the intensity of immunohistochemical staining was scored using four-tier system (0, no staining; 1, weak; 2, moderate; 3, strong). Percentage of positive-stained tumour cells represented the proportion score (0, none; 1, <10%; 2, 10–50%; 3, 50–80%; 4, >80%). The total score was calculated by the multiplication of the intensity with proportion score. A total score greater than 4 was defined as overexpression. The staining pattern achieved with HercepTest™ was categorised as 0 (none or staining in <10% of tumour cells), 1+ (weak membrane staining in >10% of tumour cells), 2+ (moderate >10%) and 3+ (strong in >10%), whereas 2+ and 3+ samples have been classified as overexpression in accordance with manufacturers' guidelines.

Statistical analyses were accomplished using SPSS® Software. The relationship between the overexpressing tumours and the clinicopathological parameters has been calculated with the χ² test. Survival rates were done with the method of Kaplan and Meier, and analysed with the log rank test.

Results

The 114 patients tested for overexpression with a median age of 56:9 (37–92) were split in 4 groups according to the UICC classification guidelines: ten in stage I, eight in stage II, ten in stage III and 86 cases in stage IV. Patients’ characteristics are detailed in Tab. 1.

101 of them had the first, nine patients the second and four the third head and neck carcinoma. Tumours were either located in pharynx (86 specimens) or in the oral cavity (28 samples). 98 men versus 16 women show the disposition of male gender.

All patients except one underwent consecutively a surgical tumour resection, combined radiochemotherapeutical treatment or both depending on their staging and compliance at the local Dept. of ORL. Eighty-six patients completed suggested therapy down to the present day (Tab. 2), while twenty-eight are still in therapy or broke up.

All the sections remained adherent and background staining or artifacts were not observed. The results of this immunohistochemical examination are summarised in Tab. 3.

An analysis showed that 55 cases were positive for EpCam, among them strong and complete membrane staining with occasional cytoplasmic reactivity considered as 3+ score was found in 24 samples. A moderate, complete or incomplete membrane staining considered as 2+ score was observed in 22 samples, weak 1+ intensity in 9 and no reaction in 59 cases. With simultaneous consideration of cell proportion of positive tumour cells, a total score greater than 4 was observed in 26 specimens (22.8%).

For EGFR we used the same evaluation criteria and found an expression in 81 specimen. A 3+ reaction was achieved in 49, 2+ and 1+ staining in respectively 16 and 12 negative results in 33 cases. The criteria defined as overexpression were fulfilled in 51 (44.7%) cases.

HER2 reactivity was determined in all 114 specimens using evaluation criteria recommended by the manufacturer of HercepTest™. A weak (1+) reaction was observed in 17, a moderate (2+) in 1 and a strong (3+) in 3 cases, whereas no signal was detected in the remaining 93 cases. Only 2+ and 3+ signals are rated as overexpression what is equivalent to 3.5% in our cohort.

Another field of interest were possible correlations of positive assessed tumours and clinicopathological param-