Expression of the Epithelial Cell Adhesion Molecule and Cytokeratin 8 in Head and Neck Squamous Cell Cancer: A Comparative Study

MICHAELA ANDRATSCHKE[1,2], HJALMAR HAGEDORN[1] and ANDREAS NERLICH[3]

[1]*Department of Otorhinolaryngology, Head and Neck Surgery, Helios Medical Center Dachau, Teaching Hospital of the Ludwig Maximilian University of Munich, Dachau, Germany;*
[2]*Department of Otorhinolaryngology, Head and Neck Surgery, University of Schleswig-Holstein, Campus Luebeck, Luebeck, Germany;*
[3]*Department of Pathology, Bogenhausen Medical Center, Munich, Germany*

Abstract. *The epithelial cell adhesion molecule (EpCAM) is a well-known and widely accepted tumor-associated antigen in head and neck squamous cell carcinoma (HNSCC). In contrast, little is known about cytokeratin 8 (CK8), an intermediary filament protein, recently associated with HNSCC. Studies demonstrated an aberrant expression on the cell surface of different carcinomas of both antigens. We performed an immunohistochemical study on the expression pattern of CK8 in comparison to EpCAM on cryosections, followed by microscopic quantitative and semi-qualitative analyses. Both antigens showed heterogenous expression both in individual carcinomas and between different carcinoma types. Furthermore, the expression of CK8 is clearly dependent on the degree of histological tumor cell differentiation. With increasing de-differentiation, the amount of CK8 expression increased, which was not seen for EpCAM. The expression of EpCAM was high on all carcinomas independent of their anatomical localization. Regarding CK8, there seems to be a correlation between the expression grade and the anatomical site. The application of CK8 may provide additional supplementary information on HNSCC.*

The prognosis of head and neck squamous cell carcinoma (HNSCC) is still poor. Therefore, many efforts focus on new therapeutic options in order to improve the clinical situation. Tumor-associated antigens (TAA) seem to be interesting tools for optimizing diagnosis and therapy of HNSCC.

One well-known TAA is the epithelial cell adhesion molecule (EpCAM), a transmembrane glycoprotein which is *de novo* expressed in many squamous cell carcinomas of the cervix, lung, and head and neck (1-3). There is extensive knowledge on this glycoprotein and its role in cancer (4-11).

Another new promising TAA for diagnosis and therapy of head and neck cancer seems to be cytokeratin 8 (CK8). It is found on simple epithelia and in various carcinoma types (12, 13). CK8 is a columnar filament, located mainly intracellularly. In addition, a soluble form of CK8 exists in the serum (14). It is also expressed on the cell surface of breast carcinoma cells (15). Hembrough *et al.* verified CK8 expression on the cell surface of both, hepatocytes and hepatocellular carcinomas (16). Many studies have already provided evidence of high levels of CK8 on the surface of malignant cells. Likewise, increased levels of CK8 were found on squamous cell carcinomas by immunohistochemistry (17). Xu *et al.* showed an increased expression of CK19 and CK8 already in dysplastic and pre-malignant tissues (18). Using the technology of autoantibody-mediated identification of antigens (AMIDA) antibodies against CK8 have already been detected in the serum of patients with HNSCC (19, 20). In both different cell lines of HNSCC and biopsies of HNSCC, an aberrant expression of CK8 was shown (19, 21). However it is still not known why CK8 is expressed on the cell surface. Some studies have shown that CK8 acts as a receptor for plasminogen, promoting its activation (22-25).

The aim of the present study was to investigate the expression pattern of CK8 in healthy tissues and in HNSCC in comparison to EpCAM. We also evaluated the suitability of CK8 as a TAA in future antibody therapy of HNSCC.

Correspondence to: Professor Andreas Nerlich, Institut für Pathologie, Klinikum München-Bogenhausen, Englschalkingerstrasse 77, D-81925 München, Germany. Tel: +49 8992702310, Fax: +49 8992702067, e-mail: Andreas.Nerlich@klinikum-muenchen.de

Key Words: Head and neck cancer, squamous cell carcinoma, cytokeratin 8, CK8, EpCAM.

Materials and Methods

Patient tissue samples. Specimens of 46 patients with primary HNSCC were taken intraoperatively, snap-frozen in liquid nitrogen and stored at −80˚C. The group consisted of three females aged between 65 and 81 years and 43 males aged between 37 and 80 years. The median age was 73.3 years for the females and 58.3 years for the males. Six carcinomas were located in the oral cavity, 17 carcinomas in the oropharynx, seven carcinomas in the hypopharynx and the remaining 16 tumors were located in the larynx. Histology confirmed malignant growth in all cases. The grade of tumor cell differentiation was in one well-differentiated (G1) carcinoma, 17 carcinomas were moderately differentiated (G2) and the remaining 28 tumors were poorly differentiated (G3). The tumors were divided into different groups according to the classification of the International Union against Cancer (26) (Table I).

For control purposes, healthy epithelium from the oropharyngeal mucosa was obtained from 25 patients who had underwent tonsillectomy due to chronic tonsillitis or tonsillar hyperplasia. None of them presented dysplasia or malignant growth histologically. The control group consisted of 14 males and 11 females; the median age was 18.5 years (range=4-55 years). The group comprised 10 smokers and 15 non-smokers.

Immunohistochemistry. Immunohistochemical analysis of the expression of EpCAM and CK8 was performed on cryosections applying the avidin-biotin complex immunperoxidase technique (Dianova, Hamburg, Germany). To block non-specific binding, sections were incubated with goat serum for 25 min (Dako, Glostrup, Denmark). After removing excess fluid, the slides were incubated with monoclonal EpCAM-specific antibody C215 (Trion Pharma Inc., Munich, Germany) and monoclonal CK8-specific antibody HK-8 (Covance Research Products, Berkeley, California, USA) for 60 min. The alkaline phosphatase-antialkaline phosphatase system (Dako, Glostrup, Denmark) was used for detecting the primary antibody. The specifically bound primary antibody was visualized by staining with 3-amino-9-ethylcarbazol (AEC). Cell nuclei were counterstained with haemalaun (Merck, Darmstadt, Germany). All experiments were performed in triplicate on at least two different slides.

Data evaluation and morphometric analysis. The microscopic analysis was performed in two steps. Firstly, a qualitative analysis was performed, followed by a semiquantitative analysis.

Regarding the qualitative analysis, the specific staining of the antibodies HK-8 and C215 were confirmed, especially if there was a cytoplasmatic or membranous staining. Additionally, the distribution within the various tissue compartments, such as tumor center or tumor margins, was analyzed.

For the semiquantitative morphometric analysis, the absolute count of tumor cells was determined in five randomly chosen visual fields (0.041 cm^2). In a second step, the number of positively labeled cells was related to the total cell count (expression index). The percentage expression was divided into five different groups (Table II). Group 1 contained carcinomas with a low proportion of HK-8- and C215- positive cells (0-25%); group 2 consisted of carcinomas with a moderate proportion of HK-8- and C215-positive cells (26-50%); group 3 comprised those carcinomas with a high proportion (51-75%) and group 4 contained carcinomas with a very high proportion of HK-8 and C215 stained cells (76-90%). Carcinomas expressing HK-8 and C215 in nearly all cells comprised group 5 (91-100%).

The examination was performed by two independent observers. The aberrance between the results of these two examiners was less than 10%.

All data were further evaluated by statistical analysis. In order to analyze the correlation between the expression pattern of CK8 and EpCAM and the biological behavior of the tumor, we used the Spearman test to determine the correlation. A p-value<0.05 was determined to be statistically significant.

Results

Expression of CK8 and EpCAM in healthy mucosa. All specimens of normal healthy mucosa exhibited specific staining with the antibody against CK8 present only in single basal epithelial cells. Only 5% of all evaluated cells were labeled. All stroma cells were negative for CK8. The glands showed strong positive staining and were used as an internal positive control for the specificity of staining (Figure 1). For EpCAM, none of the normal mucosal specimens revealed any expression of EpCAM. Again, the stromal cells were negative. The glands exhibited a strong positive staining for EpCAM and were again used as the positive control for the specificity of staining (Figure 2).

Expression of CK8 in HNSCC. A total of 44 patients were evaluated. Two data sets had to be omitted due to minor quality of the tissue sections. Both were poorly-differentiated tumors.

Except for four carcinomas, all tumors exhibited both specific and clearly cytoplasmatic and membranous staining for CK8 in the tumor cells (Figure 3).

The proportion of the labeled tumor cells was heterogeneously-distributed between the different carcinomas. The expression percentage ranged between 0% and 100% of examined carcinomas. Similarly, the expression was statistically significantly different with respect to grade of differentiation. Thus, the single case of well-differentiated carcinoma (G1) had an expression of category 1 (0-25%). Carcinomas of medium differentiation (G2) exhibited maximal expression in up to 50% of the tumor cells in 88% of the cases. Out of the moderately differentiated carcinomas, 47% had an expression of category 1 (0-25%) and 41% belonged to category 2 (26-50%). Only 12% of the carcinomas of the G2 group exhibited expression in more than 50% of the tumor cells; 6% of them had category 3 (51-75%) expression, the other 6% belonged to category 5 (91-100%). In contrast, 23% of the poorly-differentiated carcinomas had an expression index belonging to category 3 (51-75%), 15% belonged to category 4 (76-90%) and 39% to category 5 (91-100%). Thus, the 77% of the poorly-differentiated carcinomas

Table I. *Distribution of study patients with head and neck squamous cell cancer by tumor stage and grade.*

	Number
T-Stage	
T1	2
T2	20
T3	12
T4	12
Grade	
G1	1
G2	17
G3	28

Table II. *Different categories representing the different degree of expression according to staining with cytokeratin 8 (HK8) and EpCAM (C215).*

Group	Percentage of positively-stained cells
1	0-25%
2	26-50%
3	51-75%
4	76-90%
5	91-100%

exhibited expression of CK8 in more than 50% of the tumor cells. A quantitative differentiation between cytoplasmatic and membranous expression was limited due to expression in both cell compartments. Overall, there were no statistically significant differences. The correlation between the expression and the histological differentiation was statistically significant with $p<0.05$.

With respect to the intra-tumoral distribution of CK8 staining, heterogenous expression of CK8 was seen within the carcinomas. Besides strongly CK8-positive areas, occasionally adjacent tumor areas remained completely negative for CK8. However, the expression at the invasive front of the tumor was much higher than in other areas of the tumor (Figure 4). In addition, the extent of CK8 expression seems to be dependent on the anatomical localization of the carcinoma. Carcinomas of the oral cavity exhibited mainly expression of category 1. Only one carcinoma belonged to category 2. Half of the laryngeal carcinomas had category 4 or 5 expression. Carcinomas of the hypopharynx mostly belonged to category 2 and 3. Oropharyngeal carcinomas were ambiguous.

There was no statistical correlation between the tumor stage and the expression index.

Expression of EpCAM in HNSCC. In the same study population as described before, one of the carcinomas was highly differentiated, 15 carcinomas were moderately differentiated carcinomas and the remaining 28 carcinomas were low-grade carcinomas. Except for one tumor, all HNSCC exhibited both cytoplasmatic and membranous staining for EpCAM (Figure 5). The glands also exhibited a strongly positive staining for EpCAM. The stromal cells were completely negative. In contrast to CK8, there was minor heterogenity in EpCAM expression between the carcinomas. Although the expression index ranged between 0% and 100%, 86% of the HNSCC showed an expression index of category 3 or higher; 14% of the carcinomas had an expression index of category 1 to 3. There was no correlation between histological grading and the expression index. The highly differentiated carcinoma had an expression index of category 5; 13% of the medium differentiated carcinomas had an index of category 1, 27% belonged to category 3, 40% to category 4 and the remaining 20% to category 5. Thus, 87% of the HNSCC had positive staining for EpCAM in more than 50% of the tumor cells. 84% of the low-grade carcinomas had an expression index of category 3 and higher. A total of 16% of carcinomas had expression in fewer than 50% of the tumor cells. Therefore, there was no correlation between the expression index and the histological grading.

Due to both a cytoplasmatic and membranous expression pattern, no statistically significant difference was seen for cellular sub-groups.

Regarding the expression index, it was clearly shown that the expression within an individual tumor was heterogeneous. Besides strongly positively stained tumor cells, there were negative tumor cells. Increased expression at the invasive front was not identified for EpCAM.

The statistical analysis showed that the expression of EpCAM is independent of both the tumor stage and the origin of the tumor.

Expression of both EpCAM and CK8 in HNSCC. The expression of EpCAM and CK8 in HNSCC showed distinct differences when compared to each other. Both antigens were heterogeneously expressed both within individual carcinomas and between different carcinomas. This heterogeneous expression was much stronger for CK8 than EpCAM. Furthermore, the expression of CK8 was clearly dependent on the histological grading of the carcinoma. With increasing de-differentiation, positive association of tumor cell staining with expression grade was seen. This was not the case for EpCAM. The highly differentiated carcinoma had an expression index of CK8

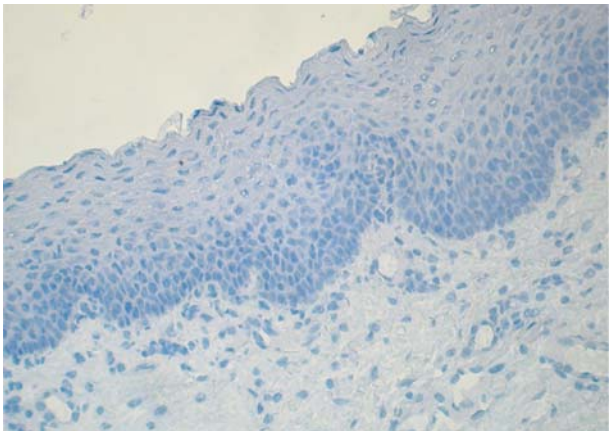

Figure 1. *No expression of cytokeratin 8 (CK8) in normal healthy mucosa of the oropharynx. There is no specific staining for CK8 in the epithelial cells. The stromal cells are also negative (magnification ×200).*

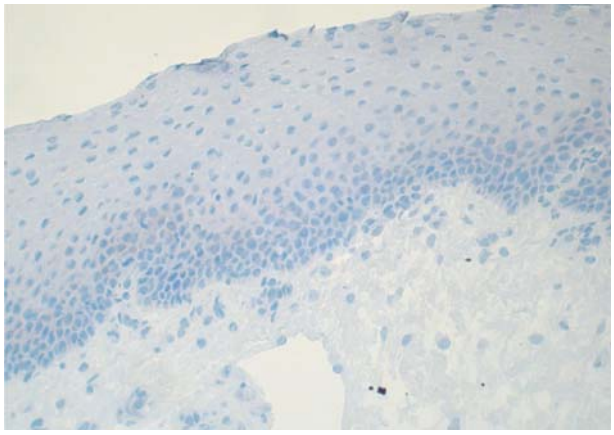

Figure 2. *Normal healthy oropharyngeal mucosa showing no expression of the epithelial cell adhesion molecule (magnification ×200).*

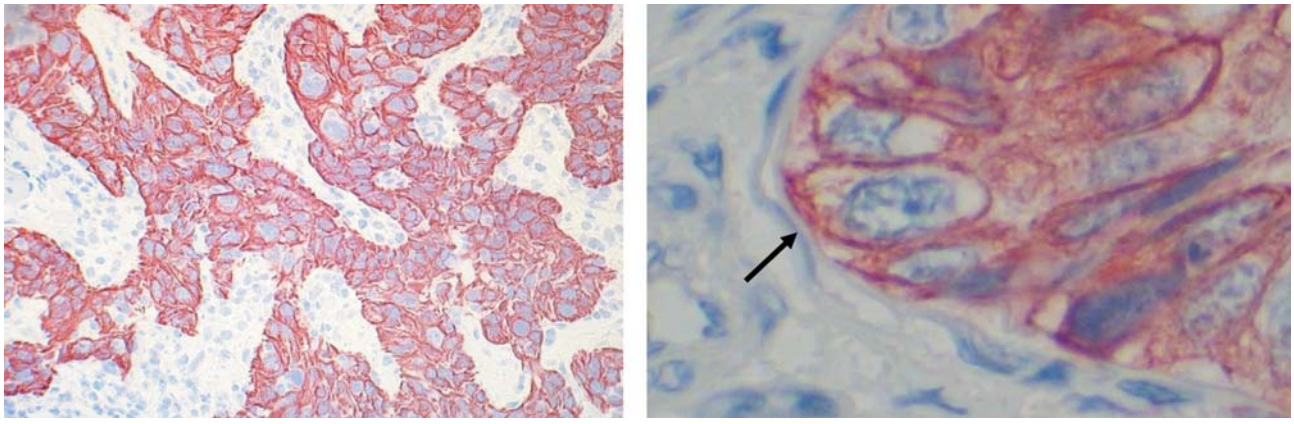

Figure 3. *Expression of cytokeratin 8 in a carcinoma of the hypopharynx. Left: There is a specific staining of the tumor cells. Almost a homogeneous staining of the tumor cells can be seen. Some cells exhibit cytoplasmatic staining, others both cytoplasmatic and membranous staining (magnification ×400). Right: There is a distinct staining of the cell membrane (arrow) (magnification ×800).*

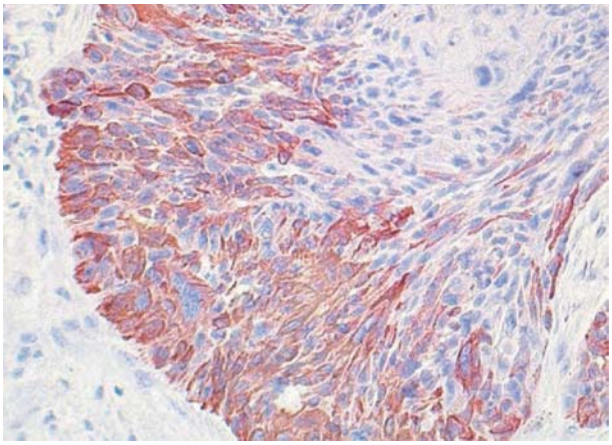

Figure 4. *The percentage expression of cytokeratin 8 is much higher at the invasive front of the hypopharynx carcinoma than in other areas (magnification ×400).*

belonging to category 1 (0-25%), for EpCAM an expression of category 5 (91-100%) was found. EpCAM seems to be much more strongly expressed in HNSCC than CK8. Regarding CK8, most of the moderately differentiated carcinomas had a maximal expression rate of 50%. Regarding EpCAM, most carcinomas had an expression rate of more than 50%. There were carcinomas which had a strong expression of EpCAM, but no or only low expression of CK8 (Figure 6).

At the invasive front, there was an enhanced expression of CK8, but not of EpCAM. The expression of EpCAM was high in all carcinomas, independent of their anatomical localization. Regarding CK8, there seems to be a correlation between the expression grade and the anatomical site. Carcinomas of the oral cavity had a low expression of CK8, whereas the laryngeal and the hypopharyngeal carcinomas had high expression.

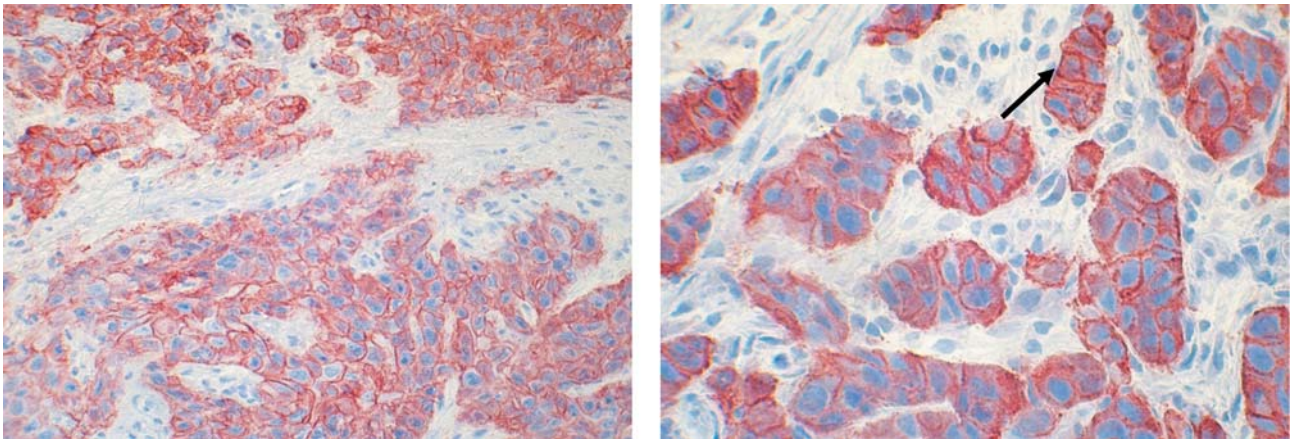

Figure 5. *Expression of the epithelial cell adhesion molecule in an oral mucosa carcinoma. Left: There is specific staining of the tumor cells. The overview shows almost uniform staining of single tumor cells. Thereby, some cells exhibit cytoplasmatic staining, other cells exist staining of both the cell membrane and the cytoplasm (magnification ×200). Right: The detailed image shows the partial specific membranous staining of the tumor cells (arrow) (magnification × 400).*

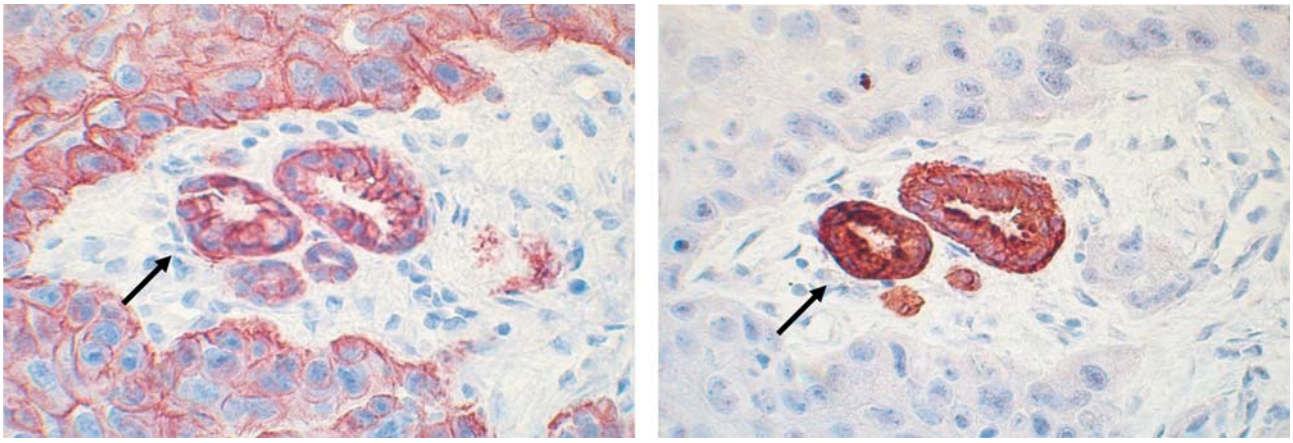

Figure 6. *Head and neck squamous cell carcinoma exhibited different expression grade regarding the epithelial cell adhesion molecule (EpCAM) and cytokeratin 8 (CK8). A section from laryngeal carcinoma is shown stained using antibodies for both. Left: There is strong staining for EpCAM (magnification ×600). Right: No expression of CK8 is apparent. The positively-stained glands were used as positive control (arrow) (magnification ×800).*

Discussion

There exist several studies investigating the expression of various cytokines in HNSCC. Vaidya *et al*. demonstrated an aberrant expression of different cytokines with malignant progression to carcinoma of the tongue (27). Moll *et al*. verified the expression of CK5/6, CK10, CK13, CK17 and CK19 in carcinomas of the pharynx and the oral cavity (12). Sesterhenn *et al*. identified a strong expression of CK6 and CK16 by both immunohistochemistry and reverse transcription polymerase chain reaction in head and neck cancer (28).

Bongers *et al*. described that there are statistically significant differences in the expression of CK16 and CK19 between the normal mucosa of patients with HNSCC and healthy donors (29). Therefore they postulated these two cytokines as a marker of field cancerization. From the immunohistochemical findings of Balm *et al*. the aberrant expression of several cytokines apparently depends on the anatomical localization of the carcinoma (30). The Authors demonstrated expression of CK18 in carcinomas of the larynx and the hypopharynx but not in carcinomas of the oral cavity. Our data accord well with their findings. Cytokeratin 8 is strongly expressed by carcinomas of the

larynx and the hypopharynx, but not by carcinomas of the oral cavity.

A retrospective study of van der Velden *et al*. showed that the expression of CK17 in dysplastic tissue and in carcinomas is clearly increased compared to its expression in healthy laryngeal tissue (31). Cohen-Kerem *et al*. also demonstrated an increased expression of CK17 in laryngeal carcinomas compared to healthy laryngeal tissue (32). Therefore, both groups postulated CK17 as a marker for the early detection of malignant transformation of the larynx. The intensity and the proportion of cells positively labeled for CKI7 are significant higher in healthy mucosa next to the tumor than that far distant from the tumor. However, there is also an increased expression of other cytokines such as CK10, CK13, CK16, CK18, CK19 and CK20 in squamous cell carcinomas of the larynx compared to healthy tissue (17, 29, 30).

Other squamous cell carcinomas such as carcinoma of the uterine cervix also exhibited a modified expression of CK8, CK10, CK13 and CK17 during malignant transformation. Thereby, the expression of CK8 and CK17 increases in invasive carcinomas, whereas the expression of CK10 and CK13 decreases (33, 34).

Despite these intriguing observations, surprisingly little is known regarding the expression of CK8 in HNSCC. CK8 is normally expressed in single-layer epithelium except squamous epithelium. As an intermediary filament protein, it is generally located intracellularly. However, we demonstrated aberrant membranous expression of CK8 for different cell lines and biopsies of HNSCC (21). The membranous expression of CK8 in HNSCC cell lines differed in its intensity. FaDu cells (hypopharyngeal carcinoma) exhibited the strongest expression. We performed immunohistochemical examinations for CK8 on primary HNSCC. Nearly all carcinomas were positive for CK8. The stromal cells were negative for CK8. The extent of the positively labeled cells showed a clear heterogeneity between the various carcinomas varying between 0% and 100% of the tumor cells. Poorly-differentiated carcinomas had the highest expression of CK8. Thus, the expression rises with increasing de-differentiation of the carcinoma. Similarly, in their study on carcinomas of the larynx van der Velden *et al*. described an increased expression of CK8 with decreasing differentiation (31). Schaafsma *et al*. demonstrated an increased expression of CK8 at the tumor invasion front of HNSCC according to their findings in genitourinary carcinomas (17) and named it an 'interface phenomenon' (35). Our data also showed an increased CK8 expression at the invasive front of the carcinomas. We also demonstrated that there is a heterogenity within a carcinoma.

Immunohistochemical studies on leukoplakia of the head and neck region showed that CK8 is expressed *de novo* in dysplastic areas. In contrast, hyperplastic areas showed no expression of CK8 (36, 37). An increased expression of CK8 changes the phenotypic characteristics of epithelial cells, which leads to malignant transformation (38). In parallel with the findings of Gires *et al*. (36), our data confirm the absence of CK8 in healthy epithelium (only isolated cases revealed expression of CK8 in the basal layer of few tumor cells). These findings are in accordance with the data in the literature that CK8 is not expressed by healthy squamous epithelium. Similar results have been shown by Ram Prassad *et al*. concerning the expression of CK19 on leukoplakias and squamous cell carcinomas of the oral cavity (39). Healthy mucosa exhibited expression of CK19 only in the basal layers, whereas in dysplastic tissue expression of CK19 was found in both the basal and the suprabasal layers. Invasive carcinomas demonstrated an increasing proportion of CK19-positive cells with increasing dedifferentiation.

Although EpCAM is *de novo* expressed in HNSCC, no expression is found on healthy epithelium (1, 3). Therefore EpCAM attracted interest in the diagnosis of HNSCC a long time ago – with a potential as a therapy target.

Most squamous cell carcinomas have been described as EpCAM-positive (1, 40, 41). Studies on biopsies of patients with primary HNSCC showed that most tumor cells were positive for EpCAM (42, 43). There is evidence that this is independent both of the origin of the carcinoma, the degree of differentiation and the tumor stage (3, 44, 45). In contrast, Yanamoto *et al*. showed a significant association between the expression of EpCAM and tumor size, regional lymph node metastases and histological differentiation (46). In ovarian cancer, the expression of EpCAM is high in both metastastatic and recurrent ovarian cancer (47).

Our data show an inverse correlation between the expression of EpCAM and the distance from the tumor border, whereas healthy mucosa of the upper aerodigestive tract showed no expression of EpCAM. This is in accordance with data in the literature, which highlighted an increase of the frequency and the expression of EpCAM regarding the changes in the tissue from healthy through hyperplastic and dysplastic epithelium, to finally manifest a carcinoma (2).

We previously had initial promising results using monoclonal antibody to EpCAM for an antibody therapy of HNSCC *in vivo* in a mouse model (48). Because of both *de novo* expression and the aberrant membranous expression in HNSCC, CK8 is a potential new target for an antibody therapy for HNSCC. We already demonstrated this *in vivo* in a mouse model (49).

Acknowledgements

The monoclonal antibody C215 was kindly provided by Trion Pharma Inc., Munich. This work was supported by a grant of the Friedrich-Baur-Stiftung/ Munich. The laboratory work was carried out by Brigitte Mack and Baerbel Schmitt, Department of Otorhinolaryngology, Head and Neck Surgery, Grosshadern Medical Center, Munich, Germany.

Rerences

1 Quak JJ, Balm AJ, van Dongen GA, Brakkee JG, Scheper RJ, Snow GB and Meijer CJ: A 22-kDa surface antigen detected by monoclonal antibody E 48 is exclusively expressed in stratified squamous and transitional epithelia. Am J Pathol *136(1)*: 191-197, 1990.

2 Litvinov SV, van Driel W, van Rhijn CM, Bakker HA, van Krieken H, Fleuren GJ and Warnaar SO: Expression of Ep-CAM in cervical squamous epithelia correlates with an increased proliferation and the disappearance of markers for terminal differentiation. Am J Pathol *148(3)*: 865-875, 1996.

3 Andratschke M, Hagedorn H, Luebbers CW, Schmitt B, Lang S, Zeidler R and Wollenberg B: Limited suitability of EpCAM for molecular staging of tumor borders in head and neck cancer. Anticancer Res *26(1A)*: 153-158, 2006.

4 Trazpiz M, McLaughlin P, de Leij L and Harmsen M: Epithelial cell adhesion molecule. More than a carcinoma marker and adhesion molecule. Am J Pathol *171(2)*: 386-395, 2007.

5 Munz M, Baeurle PA and Gires O: The emerging role of EpCAM in cancer and stem cell signalling. Cancer Res *69(14)*: 5627-5629, 2009.

6 Maetzel D, Denzel S, Mack B, Canis M, Went P, Benk M, Kieu C, Papior P, Baeuerle PA, Munz M and Gires O: Nuclear signalling by tumour-associated antigen EpCAM. Nat Cell Biol *11(2)*: 162-171, 2009.

7 Inoue H, Ohnishi Y, Nakajiama M, Kakudo K and Nozaki M: A novel function of EpCAM in oral squamous cell carcinoma cells under anchorage-independant conditions. Int J Oncol *39(6)*: 1401-1405, 2011.

8 Chaves-Pérez A, Mack B, Maetzel D, Kremling H, Eggert C, Harréus U and Gires O: EpCAM regulates cell cycle progression *via* control of cyclin D1 expression. Oncogene *32(5)*: 641-650, 2013.

9 Patriarca C, Macchi RM, Marschner AK and Mellstedt H: Epithelial cell adhesion molecule expression (CD326) in cancer: A short review. Cancer Treatment Reviews *38*: 68-75, 2012.

10 Chen X, Pang B, Liang Y, Xu SC, Xin T, an HT, Yu YB and Pang Q: Overexpression of EpCAM and Trop2 in pituitary adenomas. Int J CLin Exp Pathol *7(11)*: 7907-7914, 2014.

11 Gires O and Stoecklein NH: Dynamic EpCAM expression on circulating and disseminating tumor cells: causes and consequences. Cell Mol Life Sci *71(22)*: 4393-4402, 2014.

12 Moll R, Franke WW, Schiller DL, Geiger B and Krepler R: The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell *31(1)*: 11-24, 1982.

13 Chu PG and Weiss LM: Keratin expression in human tissues and neoplasms. Histopathology *40(5)*: 403-439, 2002.

14 Bjorklund B and Bjorklund V: Specificity and basis of the tissue polypeptide antigen. Cancer Detect Prev *6(1-2)*: 41-50, 1983.

15 Godfroid E, Geuskens M, Dupressoir T, Parent I and Szpirer C: Cytokeratins are exposed on the outer surface of established human mammary carcinoma cells. J Cell Sci *99*: 595-607, 1991.

16 Hembrough TA, Vasudevan J, Allietta MM, Glass WF II and Gonias SL: A cytokeratin 8-like protein with plasminogen-binding activity is present on the external surfaces of hepatocytes, HepG2 cells and breast carcinoma cell lines. J Cell Sci *108(Pt 3)*: 1071-1082, 1995.

17 Schaafsma HE, van der Velden LA and Manni JJ: Increased expression of cytokeratins 8, 18 and vimentin in the invasion front of mucosal squamous cell carcinoma. J Pathol *170*: 77-86, 1993.

18 Xu XC, Lee JS, Lippman SM, Ro JY, Hong WK and Lotan R: Increased expression of cytokeratins CK8 and CK19 is associated with head and neck carcinogenesis. Cancer Epidemiol Biomarkers Prev *4*: 871-876, 1995.

19 Gires O, Munz M, Schaffrik M, Kieu C, Rauch J, Ahlemann M, Mack B, Wollenberg B, Lang S, Hofmann T, Hammerschmidt W and Zeidler R: Profile identification of disease-associated humoral antigens by AMIDA, a novel proteomics-based technology. Cell Mol Life *61(10)*: 1198-1207, 2004.

20 Rauch J, Ahlemann M, Schaffrik M, Mack B, Ertongur S, Andratschke M, Zeidler R, Lang S and Gires O: Allogenic antibody-mediated identification of head and neck cancer antigens. Biochem Biophys Res Commun *323(1)*: 156-162, 2004.

21 Gires O, Andratschke M, Schmitt B, Mack B and Schaffrik M: Cytokeratin 8 associates with the external leaflet of plasma membranes in tumour cells. Biochem Biophys Res Commun *328(4)*: 1154-1162, 2005.

22 Hembrough TA, Li L and Gonias SL: Cell-surface cytokeratin 8 is the major plasminogen receptor on breast cancer cells and is required for the accelerated activation of cell-associated plasminogen by tissue-type plasminogen activator. J Biol Chem *271(41)*: 25684-25691, 1996.

23 Hembrough TA, Kralovich KR, Li L and Gonias SL: Cytokeratin 8 released by breast carcinoma cells *in vitro* binds plasminogen and tissue-type plasminogen activator and promotes plasminogen activation. Biochem J *317(Pt 3)*: 763-769, 1996.

24 Kralovich KR, Li L, Hembrough TA, Webb DJ, Karns LR and Gonias SL: Characterization of the binding sites for plasminogen and tissue-type plasminogen activator in cytokeratin 8 and cytokeratin 18. J Protein Chem *17(8)*: 845-854, 1998.

25 Gonias SL, Hembrough TA and Sankovic M: Cytokeratin 8 as a major plasminogen receptor in select epithelial and carcinoma cells. Front Biosci *6*: 1403-1411, 2001.

26 Paleri V, Mehanna H and Wight RG: TNM classification of malignant tumours 7th edition: what`s new for head and neck? Clin Otolaryngol *35(4)*: 270-272, 2010.

27 Vaidya MM, Borges AM, Pradhan SA and Bhisey AN: Cytokeratin expression in squamous cell carcinomas of the tongue and alveolar mucosa. Oral Oncol Eur J Cancer *32*: 333-336, 1996.

28 Sesterhenn AM, Mandic R, Dünne AA and Werner JA: Cytokeratins 6 and 16 are frequently expressed in head and neck squamous cell carcinoma cell lines and fresh biopsies. Anticancer Res *25(4)*: 2675-2680.

29 Bongers V, Snow GB, de Vries N and Braakhuis BJ: Potential early markers of carcinogenesis in the mucosa of the head and neck using exfoliative cytology. J Pathol *178*: 284-289, 1996.

30 Balm AJ, Hageman PC, van Doornewaard MH, Groeneveld EM and Ivanyi D: Cytokeratin 18 expression in squamous cell carcinoma of the head and neck. Eur Arch Otorhinolaryngol *253*: 227-233, 1996.

31 van der Velden LA, Schaafsma HE, Manni JJ, Ruiter DJ, Ramaekers CS and Kuijpers W: Cytokeratin and vimentin expression in normal epithelium and squamous cell carcinomas of the larynx. Eur Arch Otorhinolaryngol *254(8)*: 376-383, 1997.

32 Cohen-Kerem R, Madah W, Sabo E, Rahat MA, Greenberg E and Elmalah I: Cytokeratin-17 as apotential marker for squamous cell carcinoma of the larynx. Ann Otol Rhinol Laryngol *113*: 821-827, 2004.

33 Maddox P, Sasieni P, Szarewski A, Anderson M and Hanby A: Differential expression of keratins 10, 17 and 19 in normal cervical epithelium, cervical intraepithelial neoplasia, and cervical carcinoma. J Clin Pathol *52(1)*: 41-46, 1999.

34 Carrilho C, Alberto M, Buane L and David L: Keratins 8, 10, 13, and 17 are useful markers in the diagnosis of human cervix carcinomas. Hum Pathol *35(5)*: 546-551, 2004.

35 Schaafsma HE, Ramaekers FC, van Muijen GN, Robben H, Lane EB, Leigh IM, Ooms EC, Schalken JA, van Moorselaar RJ and Ruiter DJ: Cytokeratin expression patterns in metastatic transitional cell carcinoma of the urinary tract. An immunohistochemical study comparing local tumor and autologous metastases. Am J Pathol *139(6)*: 1389-400, 1991.

36 Gires O, Mack B, Rauch J and Matthias C: CK8 correlates with malignancy in leukoplakia and carcinoma of the head and neck. Biochem Biophys Res Commun *343(1)*: 252-259, 2006.

37 Matthias C, Mack B, Berghaus A and Gires O: Keratin 8 expression in head and neck epithelia. BMC Cancer *22(8)*: 267, 2008.

38 Raul U, Sawant S, Dange P, Kalraiya R, Ingle A and Vaidya M: Implications of cytokeratin 8/18 filament formation in stratified epithelial cells: induction of transformed phenotype. Int J Cancer *111(5)*: 662-668, 2004.

39 Ram Prassad VV, Nirmala NR and Kotian MS: Immunhistochemical evaluation of expression of cytokeratin 19 in different histological grades of leukoplakia and oral squamous cell carcinoma. Indian J Dent Res *16(1)*: 6-11, 2005.

40 Spurr N, Durbin H, Sheer D, Parkar M, Bobrow L and Bodmer W: Characterization and chromosomal assignment of a human cell surface antigen defined by the monoclonal antibody AUAI. Int J Cancer *38*: 631-636, 1986.

41 Went PT, Lugli A, Meier S, Bundi M, Mirlacher M, Sauter G and Dirnhofer S: Frequent EpCam protein expression in human carcinomas. Hum Pathol *35(1)*: 122-128, 2004.

42 Went P, Vasei M, Bubendorf L, Terracciano L, Tornillo L, Riede U, Kononen J, Simon R, Sauter G and Baeuerle PA: Frequent high-level expression of the immunotherapeutic target Ep-CAM in colon, stomach, prostate and lung cancers. Br J Cancer *94*: 128-135, 2006.

43 Spizzo G, Went P, Dirnhofer S, Obrist P, Simon R, Spichtin H, Maurer U, Metzger U, von Castelberg B, Bart R, Stopatschinskaya S, Köchli OR, Haas P, Mross F, Zuber M, Dietrich H, Bischoff S, Mirlacher M, Sauter G and Gastl G: High Ep-CAM expression is associated with poor prognosis in node-positive breast cancer. Breast Cancer Res Treat *86*: 207-213, 2004.

44 Moldenhauer G, Momburg F, Moller P, Schwartz R and Hammerling GJ: Epithelium-specific surface glycoprotein of Mr 34,000 is a widely distributed human carcinoma marker. Br J Cancer *56(6)*: 714-721, 1987.

45 Momburg F, Moldenhauer G, Hämmerling G and Möller P: Immunhistochemical study of the expression of a Mr 34,000 human epithelium-specific surface glycoprotein in normal and malignant tissues. Cancer Res *47*: 2883-2891, 1987.

46 Yanamoto S, Kawasaki G, Yoshitomi I, Iwamoto T, Hirata K and Mizuno A: Clinicopathologic significance of EpCAM expression in squamous cell carcinoma of the tonhue and its possibility as a potential target for tongue cancer gene therapy. Oral Oncol *43(9)*: 869-877, 2007.

47 Bellone S, Siegel ER, Cocco E, Cargnelutti M, Silasi DA, Azodi M, Schwartz PE, Rutherford TJ, Pecorelli S and Santin AD: Overexpression of epithelial cell adhesion molecule in primary, metastatic, and recurrent/chemotherapy-resistent epithelial ovarian cancer: implications for epithelial cell adhesion molecule-specific immunotherapy. Int J Gynecol Cancer *19*: 860-866, 2009.

48 Andratschke M, Gildehaus FJ, Johannson V, Schmitt B, Mack B, Reisbach G, Lang S, Lindhofer H, Zeidler R, Wollenberg B and Luebbers CW: Biodistribution and radioimmunotherapy of SCCHN in xenotransplantated SCID mice with a 131I-labelled anti-EpCAM monoclonal antibody. Anticancer Res *27(1A)*: 431-436, 2007.

49 Andratschke M, Luebbers CW, Johannson V, Schmitt B, Mack B, Zeidler R, Lang S, Wollenberg B and Gildehaus FJ: Biodistribution of 131I-labeled anti-CK8 monoclonal antibody in HNSCC in xenotransplantated SCID mice. Anticancer Res *10*: 3315-3321, 2011.

Received March 26, 2015
Revised April 27, 2015
Accepted May 1, 2015