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Expression and prognostic value of glucose transporters and hexokinases in tonsil and mobile tongue squamous cell carcinoma

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Summary. The aim of this study was to assess the expression pattern and prognostic value of the high affinity glucose transporters GLUT-1, 3, 4, 8 and 9, SGLT-1 and of hexokinases (HK) I, II and III in squamous cell carcinoma of the tonsil and mobile tongue (TTSCC) by means of immunohistochemistry.

Seventy-one consecutive patients suffering from TTSCC were included. The intensity and amount of positive tumour cells in the immunoreaction (histology score (H-score)) for GLUT-1, 3, 4, 8 and 9 as well as for HK-I, II and III were assessed independently by two experienced observers, blinded to the clinical results. H-scores as well as clinical variables were related to patient outcome. Median follow-up time was 49 months (range 1-123 months).

Mean H-scores for GLUT expression in decreasing order of magnitude were respectively 10.99 for GLUT-1 (sd 3.9), 5.7 for GLUT-8 (sd 4.0), 5.4 for GLUT-3 (sd 3.7), 1.0 for GLUT-4 (sd 2.0), 1.1 (sd 1.3) for SGLT-1, and 0.4 for GLUT-9 (sd 0.6); GLUT-1 > GLUT-8 = GLUT-3 > GLUT-4 = GLUT-9 = SGLT-1 (with > meaning significantly ($p<0.05$ on ANOVA + posthoc Bonferroni correction) higher than and =, meaning not significantly different from). Mean H-scores for hexokinase expression were respectively 5.8 for HK-I (sd 3.5), 4.6 for HK-II (sd 3.0) and 2.0 for HK-III (sd 2.0); HK-I > HK-II > HK-III. Finally high H-scores for GLUT-4 were favourably related to disease-free and overall survival on multivariate analysis.

To conclude, TTSCC expresses a wide variety of glucose transporter systems and hexokinase enzymes with the "housekeeping" GLUT-1 and HK-I being the most intensely expressed. GLUT-4 over-expression appears to confer a favourable prognosis in squamous cell carcinoma of the tonsil and mobile tongue. Additional studies confirming this finding in larger cohorts of patients are mandatory.

Key words: Tonsil and mobile tongue, Squamous cell carcinoma, Glucose transporter, Hexokinase, Prognosis

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Introduction

Glucose is the major substrate for energy production of mammalian cells (Joost and Thorens, 2001; Wood and Trayhorn, 2003, Macheda et al., 2005). Its uptake by cells is mediated by two different processes, via satiable non-energy dependent glucose transporter proteins (GLUT 1 - 14) and via active energy- and sodium-dependent glucose transporters (SGLT-1 and 2). Following transport of glucose into human cells, it is phosphorylated by the first glycolysis rate-limiting enzyme, hexokinase (HK-I -III). Increased glycolytic metabolism is an established feature of malignant cells that is characterized by an altered expression pattern of non-energy dependent glucose transporters and hexokinases (Warburg, 1956). Over-expression of glucose transporters and hexokinases has been reported for a large variety of tumours, including squamous cell carcinoma of the head and neck (SCCHN) which is the sixth most prevalent cancer in the world with an incidence of 700000 cases annually (Hoogsteen et al., 2007). Available studies on the over-expression of glucose transporters and hexokinase expression in SCCHN and their clinical, prognostic value are limited to GLUT-1 - 4 and SGLT-1 or HK-II and most studies reported on the expression of only one GLUT or

hexokinase (Hoogsteen et al., 2007). In addition, in most studies tumours of various locations in the head and neck were included that differ in terms of incidence, aetiology, therapeutic strategy and biological and clinical behaviour. In order to limit these variables we selected 2 clearly defined subregions of the head and neck (tongue and tonsil) with a known and different clinical and biological behaviour, in that tonsil carcinoma tends to be more aggressive and presents a less favourable outcome than tongue carcinoma.

This study aimed at assessing the over-expression and prognostic value of most of the currently identified glucose transporters that are of relevance for glucose transport by human cells, respectively GLUT-1, 3, 4, 8, 9 and SGLT-1, in addition to the expression of the rate-limiting hexokinase enzymes, HK-I, II, III, in a well defined series of patients suffering from SCCHN limited to the tonsil and mobile tongue. The over-expression and prognostic value of GLUT-8, 9, HK-I and III, as well as the prognostic value of SGLT-1, has not been addressed previously in patients suffering from SCCHN.

Materials and methods

Patients

Seventy-one consecutive patients suffering from carcinoma of the tonsil or the mobile tongue presenting at the department of Head and Neck Surgery from June 1997 till December 2002 were included in the study. Diagnosis and staging of tonsillar or lingual carcinoma was based on clinical examination, endoscopy, biopsy and CT-scans of the head and neck and thorax.

Immunohistochemistry

Routinely processed, formalin fixed, paraffin embedded pathology specimens (62 surgical and 9 biopsy) from the tonsillar and mobile tongue squamous cell carcinomas were examined. Sections of 4μm thick were mounted on Superfrost® Microscope slides (Menzel-Glaser, Braunschweig, Germany), which were deparaffinised in xylene and rehydrated in a downgraded series of ethanol. After flushing in water, heat induced antigen retrieval was performed for 20 minutes with the following buffer (EDTA pH=8.0 or CIT pH=6.0), cooled down for 20 minutes and then flushed in water for 10 minutes. The endogenous peroxidase present in tissue was blocked for 5 minutes with H_2O_2 (DAKO, Glostrup, Denmark) on each tissue slide. GLUT and hexokinase targeting antibodies were then incubated for 1 hour; the corresponding dilution factors (primary antibody diluted in 1%BSA/TBS) are indicated in Table 1. After washing, the sections were then incubated for 30 minutes with a labelled polymer-HRP anti-rabbit secondary antibody (DAKO, Glostrup, Denmark). We used the chromogen 3,3-diamonibenzidine+ (DAKO, Glostrup, Denmark) for 10 minutes to visualise the signal into brown. After washing, the sections were counterstained with haematoxylin.

TRIS-buffered saline instead of the primary antibody was used as negative control on each slide in order to exclude false positive responses from non-specific binding of the secondary antibody. Prior to staining the specimens, an isotype control was performed to estimate the non-specific binding of target primary antibodies to cell surface antigens. Non-specific binding is due to Fc receptor binding or other protein-protein interactions. Positive control tissues were stained to optimize the immunostainings as listed in Table 1. For each GLUT and hexokinase a positive control was included as listed in table 1. The entire technique as described above, including antigen retrieval and incubation time of the antibodies has been described and published by Casneuf et al. (2008) and Fonteyne et al. (2009). Sections were taken in a serial manner.

Immunohistochemical analysis

Two experienced observers, blinded to the clinical results, scored the intensity and amount of positive cells in the immune reaction independently. The percentage of tumour cells that were positive on the immune-reaction were scored as follows: 0% (score 0), 0-20% (score 1),

Table 1. The different antibody dilutions and heat induced pretreatment methods needed for the immunostainings.

Antibody	Company	Pre-treatment	Dilution	+ Control Tissue
GLUT1	DAKO	EDTA	1/100	internal RBC
GLUT3	santa cruz biotechnology	CIT	1/50	Testis
GLUT4	santa cruz biotechnology	EDTA	1/100	Placenta
GLUT8	α-diagnostics international	CIT	1/10	Testis
GLUT9	α-diagnostics international	EDTA	1/25	Pancreas CA
SGLUT1	fitzgerald	EDTA	1/20	Oesophagus
HK I	santa cruz biotechnology	EDTA	1/500	Liver
HK II	santa cruz biotechnology	EDTA	1/100	Liver
HK III	santa cruz biotechnology	EDTA	1/400	Liver

For each antibody the correct positive control tissues were used to optimize the immunostainings. These tissues were stained together with the squamous cell carcinomas to verify the staining procedure. EDTA: ethylene diamine tetra acetaat, CIT: citrate, RBC: red blood cells.

20-40% (score 2), 40-60% (score 3), 60-80% (score 4) and 80-100% (score 5). Intensities of staining were categorised as absent (score 0), faint (score 1), average (score 2) or strong (score 3). Positive tumour cells were counted per high-power field (final magnification, 400X). An estimation of intensity and % positive tumour cells was made after counting ten high-power fields. A final score was calculated as follows: H-score = [(a1 x i1) + (a2 x i2)]/2, where a = the score of amount of tumour cells that stained positive, i = the score of intensity and 1 and 2 refer to the scores of the 2 observers. The use and validation (intra- and interobserver variability, Kappa-statistics) of the H-score has been described previously (Casneuf et al. (2008) and Fonteyne P et al. (2009)).

Statistical analysis

SPSS version 15.0 for Windows was used for statistical analysis.

The Kolmogorov-Smirnov test was used to check the normality of data distribution. Correlation analysis was performed using the two-tailed Pearson test in the case of normally distributed data or the Spearman-rank test in the case of abnormally distributed data.

Potential differences in mean H-scores between different glucose-transporters and hexokinases were assessed using ANOVA and post-hoc Bonferroni correction.

The Chi-square and Fisher's exact test were used to determine differences in proportions when appropriate.

Disease free survival times were calculated from the date of definitive radio(chemo)therapy completion or the date of definitive surgery. Persistent or recurrent disease at a presenting primary site was scored as local disease failure. Disease free survival took into account all disease events, including local, regional and distant failures. Patients with persistent disease at the end of treatment were considered to have experienced failure at time zero. Patients with no signs of relapse were censored at the time of last follow-up or death. Overall survival was defined as the time from initial diagnosis until death or until last follow-up (right censored data). Overall survival took all deaths into account. For univariate and regression analysis, the distribution (histogram) of obtained values of the different variables studied was checked. Variable-distributions were classified as skewed or non-skewed. In non-skewed distributions we dichotomized values according to the median values of the study cohort. In case of skewed distributions, stratification was performed using the 80th percentile. We also dichotomized the following clinical covariates: Gender (men versus women), T-stage (T0-T2 vs. T3-T4), N stage (N0 vs. N1-N2-N3) and the American Joint Committee on Cancer (AJCC) overall stage (stages I-II vs. III-IV). Local recurrence free as well as overall survival times were estimated by the Kaplan-Meier method and log rank testing to examine the predictive value of dichotomized/stratified values and other clinical risk factors for local disease control and overall survival. Multivariate analysis was performed using Cox regression analysis, including in sequential order of statistical significance those variables that were found to be significant in univariate analysis followed by the interactive terms.

Results

Patients

Mean age of patients at diagnosis was 59.2 years (range 37.2–86.6); there were 62 men and 9 women (Table 2). All patients suffered from squamous cell carcinoma of the head and neck, 35 patients of the mobile tongue and 36 patients of the tonsil. Nineteen carcinomas were well differentiated, 42 moderately differentiated and 10 poorly differentiated. The International Union Against Cancer 6th edition TNM criteria were used for TNM-classification and UICC stage grouping. Fourteen patients were T1, 34 were T2, 14 were T3, 8 were T4a and 1 was T4b. Thirty-seven patients were N0, 10 were N1, 3 were N2a, 13 were N2b, 3 were N2c and 5 were N3. So eight patients were

Table 2. Description of the clinicopathological findings of the study population.

Clinicopathological findings	Cases
Gender	
Men	62
Women	9
Age	
Mean (years)	59.2
Range (years)	37.2-86.6
Tumour location	
Lingual	35
Tonsillar	36
Differentiation	
Good	19
Moderate	42
Poor	10
T-stage	
T1	14
T2	34
T3	14
T4a	8
T4b	1
N-stage	
N0	37
N1	10
N2a	3
N2b	13
N2c	3
N3	5
UICC stage grouping	
I	8
II	22
III	12
IVA	23
IVB	6
Survival	
Median (months)	49
Range (months)	1-123

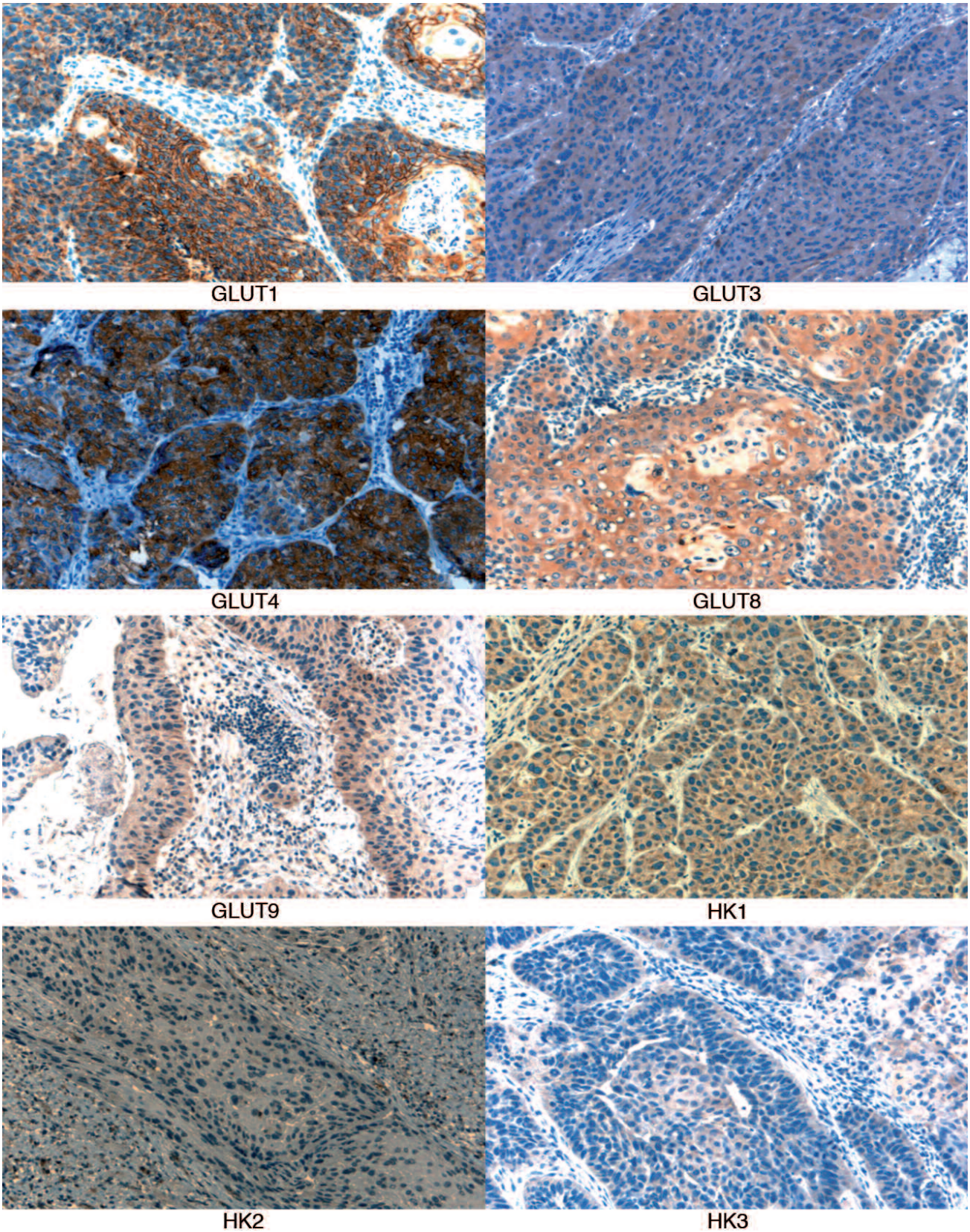

Fig. 1. GLUT and HK positive tumour cells. GLUT 1, GLUT3, GLUT4, GLUT8, GLUT9 and HK1 with high intensity and high number of positive cells; HK2 and HK3 with low intensity and low number of positive cells. x 200.

UICC stage I, 22 were stage II, 12 were stage III, 23 were stage IVA and 6 were stage IVB.

In 33 patients, treatment consisted of surgery alone (resection of the primary tumour, with or without lymph node dissection). In 29 patients, treatment consisted of surgery, followed by adjuvant radiotherapy (cumulative dose: 60-70 Gy). Two patients were treated by means of primary radiotherapy (cumulative dose: 70 Gy) after lymph node dissection. Finally, seven patients were treated by means of radiochemotherapy (cumulative dose of 70 Gy combined with cisplatin at a dose of 100 mg/m^2 on day 1, 22 and 43). All patient files were available for follow-up. Median follow-up time was 49 months (range 1-123 months).

Histology

Results of the entire group

Mean H-scores for GLUT expression in decreasing order of magnitude were respectively 10.99 for GLUT-1 (range 15-2.5, sd: 3.9, 71/71 tumours staining positively), 5.7 for GLUT-8 (range 15-0.5, sd: 4.0, 71/71 tumours staining positively), 5.4 for GLUT-3 (range 13.5-0, sd: 3.7, 69/71 tumours staining positively), 1 for GLUT-4 (range 14-0, sd: 2.0, 47/71 tumours staining positively), 1.1 (range 5-0, sd: 1.3, 44/71 tumours staining positively) for SGLT-1 and 0.4 for GLUT-9 (range 2.5-0, sd: 0.6, 33/71 tumours staining positively). Based on analysis of variance, the following differences in H-scores were noted GLUT-1> GLUT-8 = GLUT-3 > GLUT-4 = GLUT-9 = SGLT-1 (with > meaning significantly higher than and = meaning not significantly different from (Fig. 1). SGLT-1 expression proved more pronounced in the more differentiated parts of tumours.

Mean H-scores for hexokinase expression were 5.8 for HK-I (range 13.5-0, sd: 3.5, 69/71 tumours staining positively), 4.6 for HK-II (range 11.5-0, sd: 3.0, 70/71 tumours staining positively) and 2.0 for HK-III (range 9-0, sd: 2.2, 66/71 tumours staining positively). Based on analysis of variance, the following differences in H-scores were noted HK-1 > HK-2 > HK-3 (with > meaning significantly higher than).

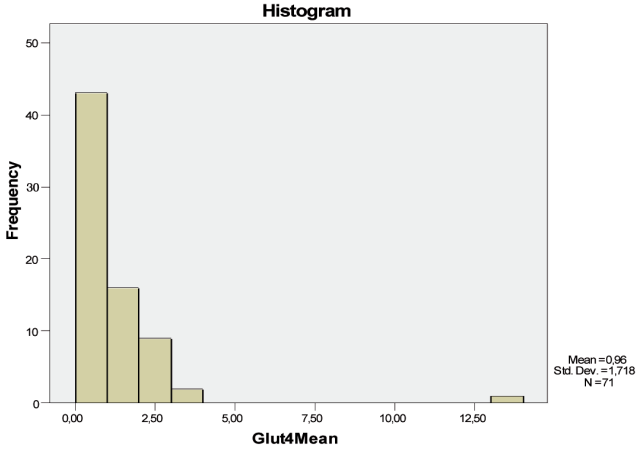

Fig. 2. Frequency distribution of the GLUT4 mean H-scores.

Differences between tonsil and mobile tongue carcinoma

GLUT-1, GLUT-8 and SGLT-1 expression proved significantly more pronounced in patients suffering from tonsil carcinoma, and mean values for GLUT-1 obtained were 9.5 (sd: 4.0) in mobile tongue- versus 12.4 (sd: 3.2) in tonsil carcinoma (p=0.001), 4.5 (sd: 3.7) in mobile tongue- versus 6.9 (sd: 4.0) in tonsil carcinoma for GLUT-8 (p=0.012) and 0.6 (sd: 0.8) in mobile tongue- versus 1.6 (sd: 1.4) in tonsil carcinoma for SGLT-1 (p=0.002). These differences were unrelated to differences in clinical parameters (gender, T-stage, N-stage or disease stage, p>0.3). The sequence of expression based on the order of magnitude in both groups remained identical to the one obtained in the entire group.

Survival analysis

Disease Free Survival

None of the patients presented with persistent or

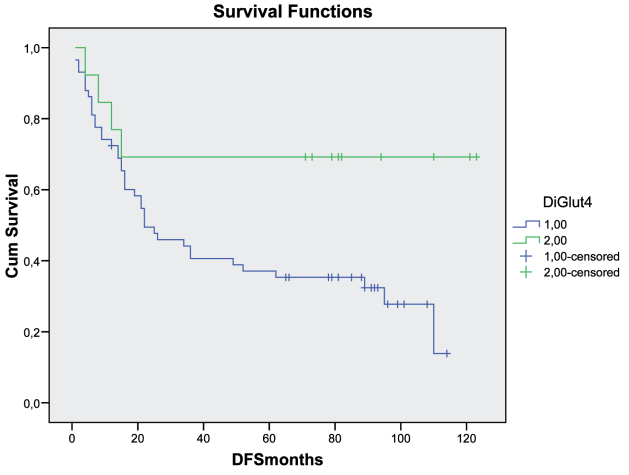

Fig. 3. Kaplan-Meier Curve of the disease-free survival of patients with a GLUT-4 H-score ≥ 2 (group 2) versus those with an H-score < 2 (group 1).

Table 3. Significant results of the univariate and multivariate analysis for disease free survival.

Dichotomized variable	Univariate analysis (p-values)	Multivariate analysis (p-values)
N-status	0.015	0.01
Disease Stage	0.0004	0.015
GLUT-4	0.03	0.03

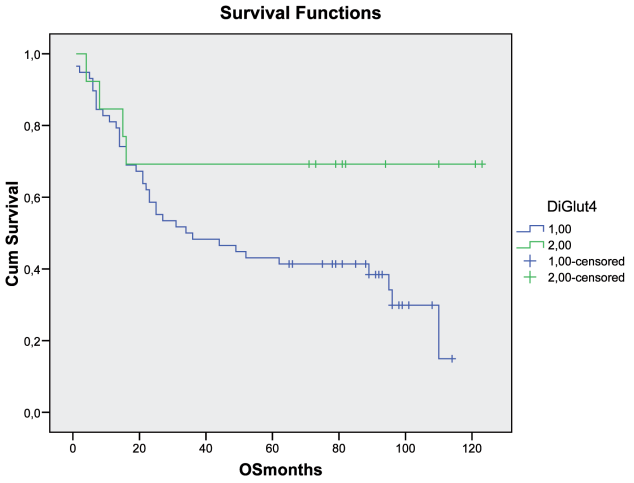

Fig. 4. Kaplan-Meier Curve of the overall survival of patients with a GLUT-4 H-score ≥ 2 (group 2) versus those with an H-score < 2 (group 1).

recurrent disease at the primary site or in the cervical node region at the end of treatment. Median disease free survival in the patients included in this study was 26 months (range 1-123 months). On univariate analysis, N-status, disease stage and GLUT-4 H-score were predictive of recurrence-free survival (p≤0.03) (Table 3). The median disease free survival for patients with a GLUT-4 H-score ratio >2 was 79 months, versus 22 months for patients with a GLUT-4 H-score <2 (Figs. 2, 3). When included in the multivariate model together with age and gender as covariates and considering the interaction between N-stage and disease stage, GLUT-4 H-score, dichotomized as described above, retained its prognostic significance (p=0.03) (Table 3).

Overall survival

At the time of the study 29 patients were alive without evidence of disease. Twenty-four had died from their cancer and 18 died of inter-current disease (Table 2). On univariate analysis, N-status, disease stage and GLUT-4 H-score were predictive of overall survival (p<0.05) (see Table 4). The median overall survival for patients with a GLUT-4 H-score ratio >2 was 79 months, versus 35 months for patients with a GLUT-4 <2 (Fig. 4). When included in the multivariate model together with age and gender as covariates and considering the interaction between N-stage and disease stage, GLUT-4 H-score, dichotomized as described above, retained its prognostic significance (p=0.05) (Table 4).

Discussion

This study aimed at assessing the over-expression and prognostic value of most of the currently identified glucose transporters that are of relevance for glucose transport by human cells, respectively GLUT-1, 3, 4, 8, 9 and SGLT-1, in addition to the expression of the rate-limiting hexokinase enzymes in a well-defined series of patients suffering from SCCHN limited to the mobile tongue and tonsil. In decreasing order of magnitude and frequency, the following transporters were found to be over-expressed in SCCHN: GLUT-1, GLUT-8, GLUT-3, GLUT-4, SGLT-1 and GLUT-9. With regard to hexokinase expression, the sequence was as follows: HK-I followed by HK-II and HK-III.

Table 4. Significant results of the univariate and multivariate analysis for overall survival.

Dichotomized variable	Univariate analysis (p-values)	Multivariate analysis (p-values)
N-status	0.005	0.005
Disease Stage	0.001	0.003
GLUT-4	0.05	0.05

In both preclinical and clinical studies it has been previously suggested that GLUT-1 mediates basal glucose transport in cancer cells and that over-expression of GLUT-1 may be involved in mechanisms that favour tumour growth at the expense of the host tissue (Kallinowski et al., 1989; Heber et al., 1992). In SCCHN, GLUT-1 was reported to be nearly homogenously over-expressed on epithelial cells of SCCHN and their metastases, whereas expression of GLUT-1 in normal mucosa was confined exclusively to the basal compartment (Reisser et al., 1999; Burstein et al., 2006; Li et al., 2008). In line with these findings, GLUT-1 expression was found in all cancers under study in our series. In addition, mean GLUT-1 expression as assessed by H-scores was significantly higher when compared to all other glucose transporter systems under study, providing direct support for the thus-far assumed critical role of GLUT-1 in SCCHN glucose metabolism. Available data on the expression of glucose transporters other than GLUT-1 in SCCHN are limited and conflicting. Mellanen et al. (1994) studied mRNA expression of GLUT-2 - 4 in addition to GLUT-1 in SCCHN. While detectable levels of GLUT-2 or GLUT-4 mRNA were not observed, GLUT-3 mRNA expression was present but low in several SSCHN. Similarly, in a study by Zhou et al. (2008) including 38 primary SCCHN, mRNA levels of GLUT-3 were also assessed and found to be positive in a large part of the patients under study. However, GLUT-3 protein expression on tumour cells could not be documented on any of the samples under study using immunohistochemistry. Likewise, Reisser et al. (1999) were unable to document GLUT-3 expression by means of immunohistochemistry in any of the 13 SCCHN tumours studied by them. As mRNA is measured in whole tissue samples and GLUT-3 was previously reported to be ubiquitously expressed in inflammatory cells, the difference between results obtained using mRNA analysis and IHC was hypothetically attributed to the presence of inflammatory cells in SCCHN by these authors (Fu et al., 2004). On the other hand, in a series by Jonathan et al. (2006) GLUT-3 expression on cancer cells was evidenced in

most of the 58 SCCHN tumours under study, although largely heterogeneous. Comparable results to those obtained by Jonathan et al. (2006) were also observed in the current study. Thus, patient-related and methodological differences, e.g. the antibodies used for staining should also be considered as possible explanatory factors for the differences of GLUT-3 expression levels observed between available studies. The over-expression of GLUT-3 as identified in our study and the study by Jonathan et al. (2006) is of interest as GLUT-3 is also capable of transporting galactose, mannose, xylose and maltose, which theoretically may confer a survival-advantage to cells that do over-express GLUT-3 versus cells that don't. In addition to over-expression of GLUT-1 and GLUT-3, this study is the first to demonstrate over-expression of GLUT-4, GLUT-8 and GLUT-9 on SCCHN tumours. In normal cells, the presence of GLUT-4 and GLUT-8 at the cell surface is usually limited, and co-localization with both the endoplasmic reticulum and late endosomes/lysosomes have been reported for both (Scheepers et al., 2004). The underlying mechanism for selective translocation of both transporters to the cell-surface of a subset (for GLUT-4) or all (for GLUT-8) of SCCHN tumours under study warrants further elucidation. Of interest, GLUT-1, GLUT-8 and SGLT-1 expression proved significantly more pronounced in patients suffering from tonsil carcinoma when compared to mobile tongue carcinoma, in line with its more aggressive nature. Finally, in analogy with the study by Helmke et al. (2003), we also identified over-expression of SGLT-1 in a subset of SCCHN tumours, confined to more differentiated tumour-compartments.

After rapid entry of glucose into cancer cells on the glucose transporter, the highly glycolytic phenotype of human cancers is supported by three hexokinases, HK-I - III (Smith, 2000). As opposed to HK-I and -III, HK-II binds to the outer mitochondrial membrane of cancer cells and is directly coupled to ATP synthesis on the inner membrane providing high levels of glucose-6-phosphate that "jump start" the glycolytic pathway (Pedersen et al., 2002). Because of this feature, it is generally believed to be the most important HK in human malignancies, and available studies on HK-expression in human malignancies almost exclusively report on HK-II expression. Specifically in SCCHN, to the best of our knowledge, only one study by Tian et al. (2005) reported on HK-II expression, not HK-I or HK-III. In this series of 19 patients/tumours HK-II expression as assessed by means of IHC proved strong in 11 patients, moderate in 6 and weak in 2. Similar results were observed in our study. However, HK-I expression proved significantly more pronounced when compared to HK-II expression, whereas HK-III expression proved the least pronounced. As all three HKs studied are "low-Km" isozymes because of their high affinity for glucose even at low concentrations (below 1 mM), and our findings suggest that the housekeeping HK-I is likely the most relevant in SCCHN cells.

Based on the above findings, selective inhibition of GLUT1, GLUT3 and/or HKI in SCCHN might prove a potential strategy to reduce SCCHN growth, as such an approach would prevent ATP production in SCCHN cancer cells and thus interfere with SCCHN growth through energy depletion.

In spite of advances in surgery and radio(chemo)therapy, no significant increase in overall survival and disease-free survival has been observed for SCCHN over the past two decades (De Vicente et al., 2001; Lo et al., 2003). While molecular markers are often linked to the occurrence and progression of malignancies or are used for molecular targeting of cancers, little is known regarding the prognostic role of biomarkers in SCCHN. While several studies have been published to date regarding the prognostic value of GLUTs in SCCHN, nearly all of these studies are limited to GLUT-1. In most of these studies, tumours of various locations, e.g. oral cavity, nasopharynx etc. were included, which differ in terms of incidence, aetiology, therapeutic strategy and biological and clinical behaviour (Kunkel et al., 2003; Jonathan et al., 2006; Choi et al., 2007; Kunkel et al., 2007; Eckert et al., 2008; Schrijvers et al., 2008; Roh et al., 2009). Overall, the results described in these studies are conflicting with some studies suggesting that high expression of GLUT-1 is related to poor survival in multivariate analysis and others not. In the series presented, limited to SCCHN of the tonsil and mobile tongue, expression of GLUT-1 in addition to the expression of all other variables under study, with the exception of GLUT-4, proved unrelated to overall and disease-free survival. As already addressed above, various factors might account for the disparity between this finding and those by other authors, including differences in tumour location, therapeutic strategy and methodology used to assess GLUT-1 expression. The expression of GLUT-4 proved to be highly skewed and the limited number of patients presenting with a high tumour-expression of GLUT-4 (above the 80th percentile) proved to have a better outcome both in terms of disease-free and overall-survival when compared to those with poor tumour-expression of GLUT-4. If confirmed in larger cohorts of patients, the underlying mechanism that links high GLUT-4 expression in SCCHN to a favourable prognosis warrants further exploration. As shown in healthy human tissues, over-expression of GLUT-4 may not only result in increased glycolysis but also in glucose-storage and modification of substrate-utilization, suggesting that additional factors are implicated (Tsao et al., 2001). Thus, hypothetically, it may well be that in SCCHN GLUT-4 over-expression decreases rather than augments the availability of glucose to tumour cells.

To conclude, SCCHN of the tonsil and mobile tongue express a wide variety of glucose transporter systems and hexokinase enzymes, with the "housekeeping" GLUT-1 and HK-I being the most intensely expressed. GLUT-4 over-expression appears to confer a favourable prognosis in SCCHN of the mobile tongue and tonsil. Additional studies confirming this

finding in larger cohorts of patients are mandatory.

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