Initial evidence for Sec62 as a prognostic marker in advanced head and neck squamous cell carcinoma

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Abstract. Head and neck squamous cell carcinoma (HNSCC) is a malignancy with an increasing incidence. To aid with the selection of the most appropriate therapy, biomarkers have become a specific research focus. Sec62 is involved in endoplasmic reticulum stress tolerance and cell migration, and has been identified as a novel prognostic marker for non-small cell lung cancer. In addition, Sec62 may be a promising candidate in HNSCC. Pretreatment biopsies of 35 patients with locally advanced HNSCC, who were treated with definitive chemoradiation therapy without prior surgery, were examined for the expression of Sec62 protein, as well as the expression of epidermal growth factor receptor (EGFR), p16 and survivin proteins. Immunohistological results were correlated with patient overall survival (OS) and progression-free survival (PFS) times. In the present patient cohort, 12/35 cases (34%) demonstrated strong and 8/35 cases (23%) moderate Sec62 staining intensity. Additionally, in 11/35 cases (31%), weak staining was observed, and only 4/35 cases (11%) were Sec62-negative. Notably, a high Sec62 protein level was associated with a significantly poorer OS and PFS (P=0.020 and P=0.028, respectively). Furthermore, higher nuclear survivin expression showed a weak trend for poorer OS rate (P=0.079), whilst neither cytoplasmic survivin, EGFR nor p16 influenced OS or PFS significantly. The present study indicated that Sec62 is a promising prognostic marker for HNSCC. Increased Sec62 protein expression may indicate a poorer prognosis in advanced HNSCC. As the present study was focused on patients treated by chemoradiation therapy, further studies with larger patient cohorts and alternative treatment approaches are required in order to define the prognostic value of Sec62 in HNSCC.

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Introduction

Head and neck squamous cell carcinoma (HNSCC) accounts for >500,000 newly diagnosed cases annually worldwide (1). The primary risk factors are tobacco use and alcohol consumption, which appear to exert a synergistic effect (2). Patient prognosis is largely determined by the extent of the tumor, as well as the presence of lymph node and distant metastases (1,2). Furthermore, a unique subset of HNSCCs, particularly oropharyngeal tumors, are associated with human papillomavirus (HPV) infection, which has been demonstrated to be of significant prognostic value (3,4). HPV-positive tumors are characterized by increased expression of p16 due to functional inactivation of retinoblastoma protein by the viral oncoprotein E7. Therefore detection of p16 by immunohistochemistry (IHC) is considered to be a robust marker of HPV infection in these tumors (5,6).

In locally advanced HNSCC, concomitant chemoradiation therapy (CRT) has emerged as a useful treatment modality for organ preservation, treatment of unresectable disease and as an adjuvant therapy in resected high-risk disease (7). However, CRT is associated with significant short- and long-term treatment-associated morbidities and complications. Furthermore, histologically similar tumors receiving identical therapies may exhibit varying clinical outcomes, and a proportion of patients will not respond to CRT (3-5,7). Therefore, identification of markers that predict the prognosis of advanced HNSCC treated by CRT will be significant for the improvement of clinical treatment decisions.

HNSCC is a heterogeneous disease with respect to its complex molecular abnormalities and tumor-associated proteome. Over 20 recurrent chromosomal alterations have been identified in invasive HNSCC, and gains at 3q, 11q and 12q, as well as losses at 5q, 6q, 8p, 21q and 22q have been associated with poor prognosis (8-10). A number of tumor-associated markers have been identified for HNSCC carcinogenesis, and have been considered as potential reasons for various responses to therapy. They may also have value as prognostic markers in patients with HNSCC. Epidermal growth factor receptor (EGFR) and survivin are two candidates that have been investigated as promising prognostic biomarkers for HNSCC (11-17).

EGFR, a transmembrane glycoprotein receptor, is encoded by the human epidermal growth factor receptor 1/*c-ERBB1* proto-oncogene located on chromosome 7p12. Ligand binding causes activation of the intracellular tyrosine kinase domain, resulting in signal transduction involving proliferation, cell division and differentiation (18). EGFR is overexpressed in the majority of epithelial malignancies and a prognostic impact of EGFR overexpression has been identified in a number of types of human cancer, including ovarian, bladder, cervical, gastric, esophageal, breast, endometrial and colorectal cancer (19). Overexpression of EGFR has additionally been observed in >80% of HNSCC cases and occurs early during head-neck carcinogenesis (20,21). However, conflicting results have been reported from certain laboratories. Meta-analyses have discussed the effects of various clinicopathological parameters and evaluation methods for the prognostic value of EGFR (11,12).

Survivin, a member of the apoptosis inhibitor family located on chromosome 17q25.3, serves a significant role in cell cycle progression and is expressed in fetal tissues, as well as in a wide range of cancer tissues; however, it is not expressed in normal adult differentiated tissues (22,23). Notably, survivin is detected as a cytoplasmic and nuclear protein in cancer patients. Nuclear survivin is suspected to control cell division, whereas cytoplasmic/mitochondrial survivin is considered to be cytoprotective (13). Consequently, intracellular localization of survivin in tumor cells has been analyzed as a prognostic marker in a number of patient-based studies, achieving differing results (13).

Gain of 3q is the most frequently detected chromosomal alteration in HNSCC and has additionally been reported in numerous other tumor entities, including prostate, esophageal squamous cell, cervical squamous cell and non-small cell lung cancer (NSCLC) (24-27). Furthermore, an amplification of the chromosomal region 3q was identified as the most frequent genomic alteration in oropharyngeal squamous cell carcinoma (OSCC), independent of HPV status, and was significantly associated with advanced tumor stage in the total patient group, as well as in the non-HPV-associated subgroup (28). Notably, studies have identified *SEC62* as the most commonly overexpressed gene within this 3q region, based on increased messenger RNA and protein levels in prostate and thyroid cancer, as well as NSCLC (24,29-31). *SEC62*, a gene that encodes an endoplasmic reticulum (ER) transmembrane protein, has an essential function in cell migration (29) and ER stress tolerance (30). Furthermore, Sec62 overexpression has been demonstrated to be correlated with the presence of lymph node metastases (increased Sec62 levels in node-positive tumors compared with node-negative tumors) and tumor cell dedifferentiation (increased Sec62 levels in poorly-differentiated grade 3 tumors compared with grade 2 tumors) in NSCLC patients (31). Furthermore, it is reported to be associated with significantly poorer overall survival (OS), particularly in NSCLC with squamous differentiation (32).

In the present study, Sec62 expression was analyzed for the first time in HNSCC by IHC, and the results were correlated with the clinicopathological data of a cohort of 35 patients with advanced HNSCC treated by CRT. In addition, the immunohistological expression of EGFR, survivin and p16 as biomarkers of interest in HNSCC were investigated and included in a multiple regression analysis.

Materials and methods

Patient characteristics. The records of 35 patients treated at Saarland University Hospital (Homburg, Germany) between August 1996 and May 2006 were analyzed retrospectively. All patients exhibited newly diagnosed squamous cell carcinoma of the head and neck region. Informed consent was obtained from the patients and the study design was approved by the local ethics committee (reference #218/10). The primary tumors were located as follows: Oropharynx, 24 cases (69%); hypopharynx, 6 cases (17%); larynx, 3 cases (9%); and nasopharynx, 2 cases (6%). The median age at diagnosis was 55 years (range, 43-74 years), and the mean Karnofsky performance index amounted to 8 (6-10). The cohort included 33 male patients (94%) and 2 female patients (6%). Smoking history data was not available. All tumors were classified as stage IV according to the International Union Against Cancer system (33), and distant metastases had been excluded.

All patients underwent simultaneous definitive CRT without prior surgery. A total dose of 72 Gy in single fractions of 1.2 Gy was administered 10 times/week and twice daily in a hyperfractionated manner (inter-fraction interval, ≥6 h). A linear accelerator was used to apply 6 MV photons and electrons. Chemotherapy was administered to all patients, and consisted of two cycles of cisplatin (20 mg/m² for 5 days, for a total dose of 60 mg/m²) and fluorouracil (5-FU; continuous infusion of 600 mg/m²/day on days 1-5) in 34 patients, or mitomycin and 5-FU in the remaining patient (continuous infusion of 600 mg/m²/day 5-FU on days 1-5; single intravenous bolus injection of 10 mg/m² mitomycin C on days 5 and 36). Follow-up time was defined from the date of diagnosis to the date of the final visit or date of mortality. The mean follow-up time was 52.2 months, with a minimum of 1.6 and a maximum of 203 months (median, 24 months). The clinicopathological characteristics of the patient group are presented in Table I.

Immunohistochemical staining. Slides from all biopsies were reviewed by one pathologist in order to select representative areas of the tumors for additional IHC analysis. The criteria were vital tumor tissue without necrosis and an estimate of ≥70% tumor cells in the analyzed tissue area.

Selected sections were deparaffinized and rehydrated using repeated xylene and graded alcohol washes. For antigen retrieval, slides were incubated with citrate buffer (for Sec62; pH 6.0 for 1 h at 90°C), Tris-HCl (for survivin; pH 9.0 for 1 h at 95°C) or Protease Type XIV (for EGFR; 2 min at room temperature; Sigma-Aldrich Chemie GmbH, Munich, Germany).

Primary antibody incubations for EGFR (clone E30; monoclonal mouse anti-human; #M7239; Dako, Glostrup, Denmark; dilution, 1:50) and survivin (polyclonal rabbit anti-human; #ab469; Abcam, Cambridge, UK; dilution, 1:250) were performed overnight at 4°C. For Sec62, primary antibody incubation was conducted for 1 h at room temperature (affinity-purified, polyclonal rabbit antibody directed against the COOH-terminal undecapeptide of human Sec62 protein; dilution, 1:200. This antibody was kindly provided by Dr Markus Greiner, Department of Medical Biochemistry and Molecular Biology, Saarland University, (Homburg/Saar, Germany) and was previously demonstrated to be of excellent sensitivity and specificity under denaturing and native

Table I. Clinicopathological features of head and neck squamous cell carcinoma patients (n=35) and influence on survival time.

Clinicopathological feature	Patients		P-value	
	Value	%	Overall survival	Progression-free survival
Gender, n			0.683	0.004
Male	33	94		
Female	2	6		
Age, years			0.621[a]	0.594[a]
Median	55			
Range	43-77			
Overall survival time, months				
Mean/median	54/29			
Range	2-203			
Localization, n			0.338	0.232
Oropharynx	24	69		
Hypopharynx	6	17		
Nasopharynx	2	6		
Larynx	3	9		
Karnofsky score, n			0.053	0.216
6	2	6		
7	8	23		
8	13	37		
9	11	31		
10	1	3		
Tumor stage, n			0.725	0.850
2	3	9		
3	7	20		
4	25	71		
Node stage, n			0.213	0.245
0	2	6		
1	4	12		
2	26	74		
3	3	9		

[a]Cox regression analysis.

conditions (30) Visualization of bound primary antibodies was performed with the Real Detection System (Dako) according to the manufacturer's protocol. p16 staining was performed with the CINtec® Histology kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's protocol. The slides were counterstained using hematoxylin (Merck Millipore, Darmstadt, Germany) and each analysis included positive and negative controls.

Staining intensity of EGFR and cytoplasmic survivin was scored as 0 (none), 1 (weak), 2 (moderate) or 3 (strong), and was considered to be high when the fraction of moderately and strongly stained cells within the tumor cell population was >10%. Furthermore, slides were considered to be survivin nuclear-positive when >10% of all tumor cell nuclei were stained, regardless of the cytoplasmic staining level. p16 expression was dichotomized as p16-positive (strong nuclear or cytoplasmic staining in ≥70% of tumor cells) or p16-negative.

For Sec62, the cytoplasmic Sec62 staining intensity was scored as 0 (none), 1 (weak), 2 (moderate) or 3 (strong). The product of the scores for staining intensity and number of stained cells (0, no stained cells; 1, <10%; 2, 10-50%; 3, 51-80%; or 4, >80%), defined as the immunoreactive score (IRS) according to Remmele and Stegner (34), was used. IRS scores from 0-2 were valued as negative, 3-4 as weak, 6-8 as moderate and 9-12 as strong; subsequently, these were dichotomized into low (IRS, <9) and high (IRS, 9-12) expression.

Statistical analysis. Statistical analysis was performed with SPSS version 20 (IBM SPSS, Armonk, NY, USA). OS was calculated as the time from diagnosis date to mortality date or the final documented follow-up date. Progression-free survival (PFS) was defined as the time from diagnosis date to the date of disease progression, the final documented follow-up date or mortality date from any cause. Survival curves were

Table II. Immunohistochemical expression analysis according to various head and neck squamous cell carcinoma localization sites.

Protein	Expression level (high/low, n)			
	Oropharynx (n=24)	Hypopharynx (n=6)	Nasopharynx (n=2)	Larynx (n=3)
Sec62	10/14	2/4	0/2	0/3
EGFR	11/13	2/4	2/0	2/1
p16	5/19	0/6	0/2	0/3
Survivin				
Nuc	15/9	3/3	1/1	0/3
Cyt	15/9	3/3	0/2	2/1
Nuc+cyt	20/4	1/1	5/1	2/1

EGFR, epidermal growth factor receptor; nuc, nuclear; cyt, cytoplasmic.

Table III. Immunohistochemical expression of Sec62 in the cytoplasm on the basis of the IRS according to HNSCC site.

Sec62 expression level	HNSCC localization site			
	Oropharynx	Hypopharynx	Nasopharynx	Larynx
Strong (IRS 9-12)	10	2	-	-
Moderate (IRS 6-8)	4	3	-	1
Weak (IRS 3-4)	7	1	1	2
Negative (IRS 0-2)	3	-	1	-

-, not available. IRS, immunoreactive score; HNSCC, head and neck squamous cell carcinoma.

calculated by the Kaplan-Meier method and compared by the log-rank test and univariate Cox regression. The χ^2 test was utilized to determine the association and differences between dichotomized variables, encompassing IHC expression and clinicopathological characteristics. P<0.05 (two-sided) was considered to indicate a statistically significant difference. Reported P-values are unadjusted for the issue of multiple testing due to the screening nature of the present study.

Results

Immunohistological staining of EGFR, survivin, p16 and Sec62. Immunohistochemical EGFR staining revealed strong membranous and cytoplasmic signals in 17/35 cases (49%). Cytoplasmic survivin staining was observed in 20 cases (57%). Using a cut-off value of ≥10% of tumor cells demonstrating positive nuclear staining signals, 19/35 cases (54%) were classified as nuclear survivin-positive. Furthermore, in 28 cases (80%) survivin was detected in the nucleus and/or cytoplasm of tumor cells. p16 expression was observed either to be generally absent, or present as strong and diffuse nuclear and cytoplasmic staining. Overexpression of p16 was observed in 5 tumors (14%) and was restricted to OSCCs (5/24 tumors; 21%). A summary of the observed staining levels for each candidate marker according to the tumor site is presented in Table II.

Strong Sec62 staining intensity was observed in 12/35 cases (34%) and was primarily identified in OSCC (10/24 tumors). Furthermore, in 8 (23%) and 11 HNSCC cases (31%), moderate

and weak staining was observed, respectively, whereas only 4 cases (11%) were Sec62-negative in the analyzed representative tumor tissue samples (Table II; Fig. 1). The detailed results of Sec62 immunostaining with regard to IRS and tumor site are presented in Table III and Fig. 2. There was no association between tumor site and Sec62 expression level (χ^2, P=0.458). Normal squamous epithelium demonstrated weak Sec62 staining in regenerating basal cells (stratum germinativum), while no immunoreactivity was observed in the more superficial cell layers (Fig. 1). As described previously (31), plasma cells within inflammatory infiltrates demonstrated strong Sec62 staining in all samples, irrespective of the Sec62 signals of adjacent tumor cells, due to their large amount of rough ER, thereby serving as internal positive controls (Fig. 1).

Correlation of protein expression with clinicopathological parameters. Analysis of clinicopathological parameters in the CRT-treated patient cohort revealed no statistically significant differences in OS with regard to gender, tumor localization, Karnofsky score, tumor grading and lymph node metastases (Table I).

A detailed analysis of potential correlations between the analyzed protein expression scores and the clinicopathological parameters of the patients was performed. With regard to tumor localization, only p16 overexpression was restricted to OSCCs (5/24 tumors; χ^2, P=0.593; Table II). Regarding survival, the expression levels of EGFR, cytoplasmic survivin or p16 were not significantly associated with OS (P=0.585, P=0.785 and
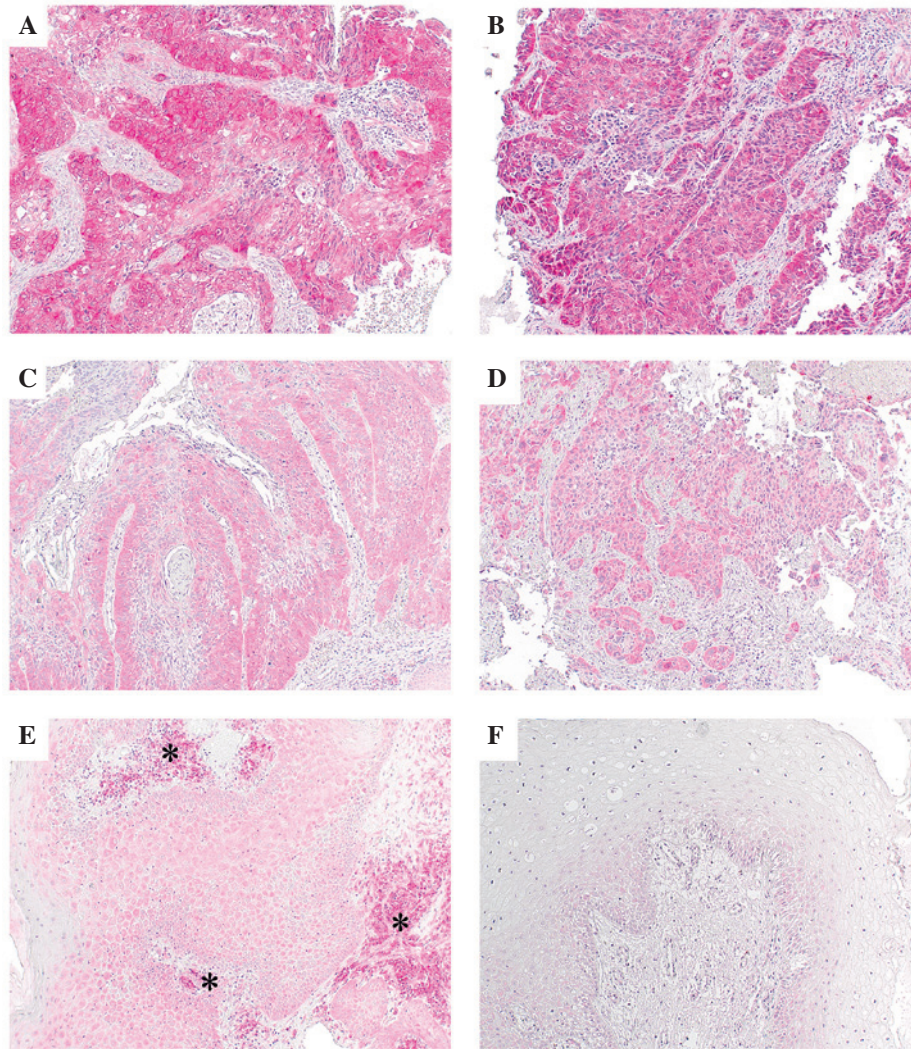
Figure 1. Sec62 immunostaining in head and neck squamous cell carcinoma. Representative microphotographs of strong Sec62-immunoreactivity in (A) hypopharyngeal and (B) oropharyngeal tumor site, moderate Sec62-immunoreactivity in (C) hypopharyngeal tumor sites, and weak Sec62-immunoreactivity in (D) hypopharyngeal and (E) oropharyngeal tumor sites. Plasma cells within inflammatory infiltrates (marked with asterisks) serve as internal positive controls due to their excessive amount of rough endoplasmic reticulum. (F) Normal epithelial tissue (oropharyngeal squamous epithelium) did not exhibit Sec62-immunoreactivity (magnification, x10). All sections were counterstained with hematoxylin.
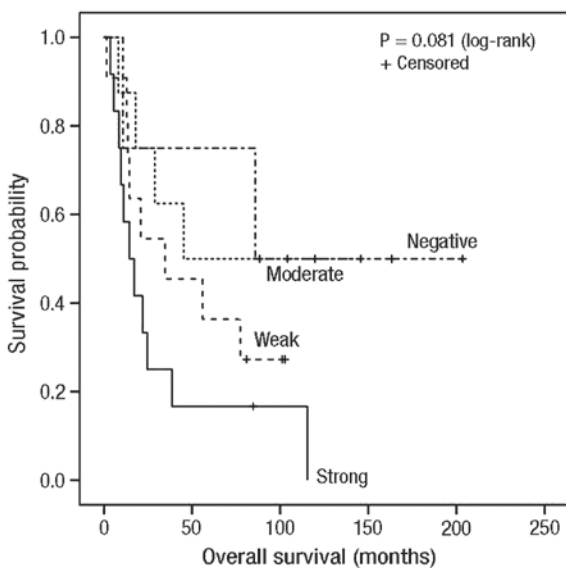

Figure 2. Overall survival of patients according to Sec62 staining proportion (P=0.081). Patients alive at final follow-up were censored.

P=0.557, respectively) or PFS (P=0.847, P=0.943 and P=0.560, respectively). However, strong nuclear expression of survivin showed a weak trend for poorer OS (P=0.079; Fig. 3), but not PFS (P=0.103). Notably, high Sec62 IRS was significantly associated with poorer OS and PFS rates in the present patient cohort (P=0.020 and P=0.028, respectively; Table IV; Fig. 4).

Subsequently, whether the expression levels of a combination of these markers were associated with a greater difference in PFS and OS compared with the expression of each single protein was analyzed. Due to the relatively small number of clinical samples, only combinations of two different proteins were analyzed, and not combinations of three or more. Initially, the combination of high Sec62 and high nuclear survivin expression appeared to indicate a poorer prognosis for HNSCC patients (PFS, P=0.071; OS, P=0.071; Fig. 5); however, statistical significance was achieved only when considering patients with low Sec62 and low nuclear survivin expression vs. high Sec62 and high nuclear survivin expression (PFS, P=0.006; OS, P=0.009). No combinatorial effect was observed for Sec62 and EGFR (PFS, P=0.091; OS, P=0.097), Sec62 and p16 (PFS,

Table IV. Summary of immunohistochemical expression analysis and influence on survival time in head and neck squamous cell carcinoma patients.

	Patients		P-value	
Expression level	n	%	Overall survival	Progression-free survival
Sec62			0.020	0.028
High	12	34		
Low	23	66		
Survivin nuc			0.079	0.103
High	19	54		
Low	16	46		
Survivin cyt			0.785	0.943
High	20	57		
Low	15	43		
Survivin nuc+cyt			0.338	0.270
High	28	80		
Low	7	20		
p16				
Oropharynx, high/low	5/19	21	0.248	0.245
All cases, high/low	5/30	14	0.557	0.560
EGFR			0.585	0.847
High	17	49		
Low	18	51		

Nuc, nuclear; cyt, cytoplasmic; EGFR, epidermal growth factor receptor.
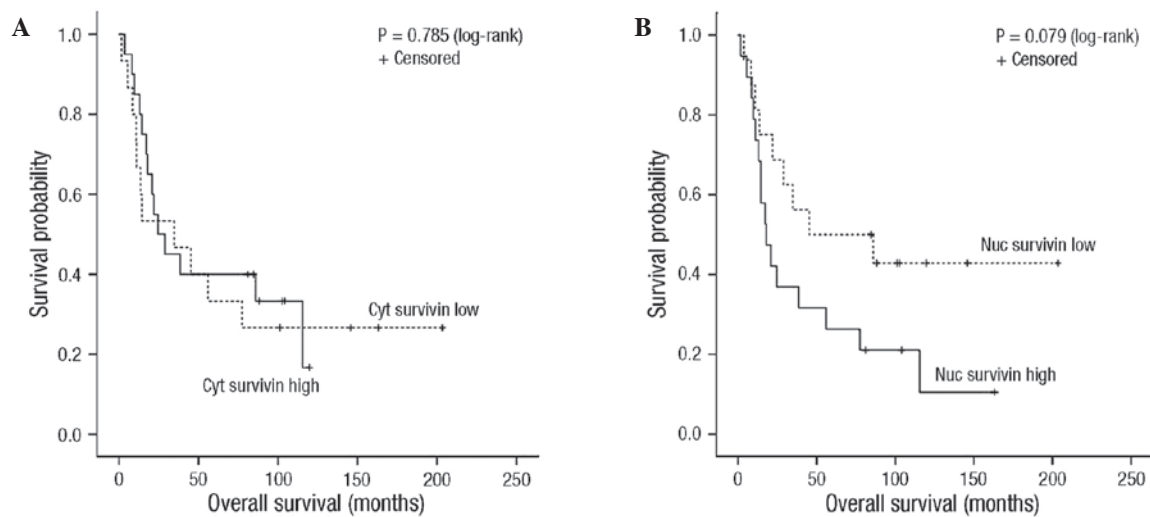

Figure 3. Kaplan-Meier estimates of the probability of overall survival according to (A) cytoplasmic (cyt) survivin and (B) nuclear (nuc) survivin immunostaining. Overexpression of nuclear survivin predicted a weak trend of poorer overall survival in head and neck squamous cell carcinoma patients (P=0.079). Patients alive at final follow-up were censored.

P=0.133; OS, P=0.095), EGFR and p16 (PFS, P=0.928; OS, P=0.841) or EGFR and survivin (PFS, P=0.297; OS, P=0.431).

Discussion

The purpose of the present study was to characterize the role of Sec62 as a novel potential biomarker for HNSCC, as well

as expression of the already extensively discussed biomarkers EGFR, p16 and survivin in a cohort of patients with locally advanced HNSCC treated with CRT.

EGFR is expressed in normal epithelium and has been observed to be upregulated in a number of types of carcinoma (19). High expression levels of EGFR are also a common feature of HNSCC across various anatomical sites to differing
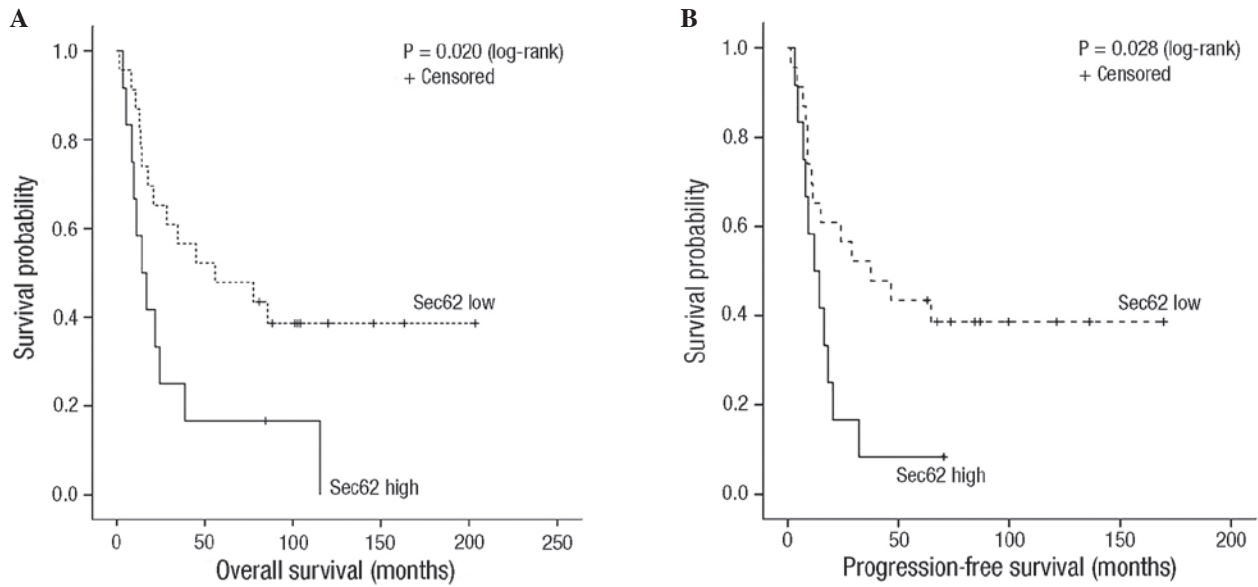
Figure 4. Kaplan-Meier estimates of the probability of (A) overall survival and (B) progression-free survival according to Sec62 immunostaining. Overexpression of Sec62 predicted a poorer overall and progression-free survival in head and neck squamous cell carcinoma patients (P=0.020 and P=0.028, respectively). Patients alive at final follow-up were censored.
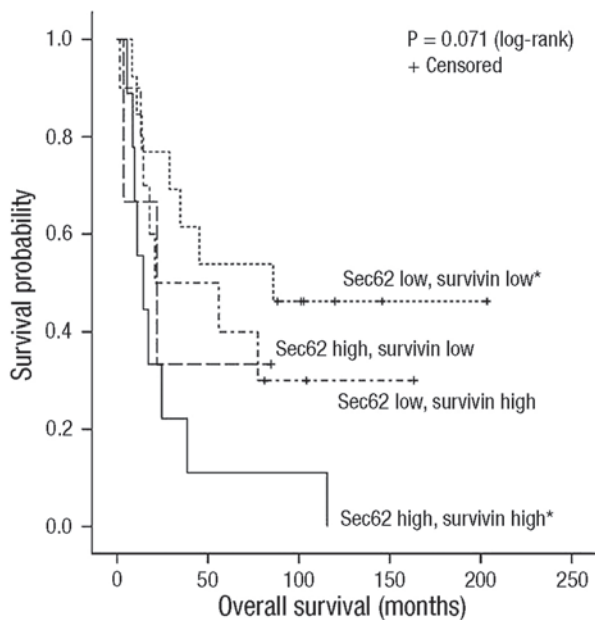

Figure 5. Kaplan-Meier estimates of the probability of overall survival in patients according to the combination of Sec62 and survivin overexpression (P=0.071). *Patients with high Sec62 and high nuclear survivin demonstrated a poorer prognosis compared with patients exhibiting low Sec62 and low nuclear survivin expression (P=0.009). Patients alive at final follow-up were censored.

degrees (21,35). However, previous studies focusing on the prognostic impact of EGFR expression levels in HNSCC reported varying results (11,12). A number of reasons may account for the discrepant results in terms of the prognostic impact of EGFR expression in HNSCC. These include differences in the anatomical sub-sites of HNSCC, varying sample compositions (biopsies and whole tumor sections) and sample sizes, differing EGFR expression measurement methods, different EGFR immunohistochemical staining protocols

and varying scoring methods (36,37). In the present study, no significant correlation between EGFR expression and OS time was observed, which contrasted with the results of the majority of studies that have analyzed and compared EGFR expression in HNSCC (11,12). However, there are results that support the present observations, particularly studies that identified no prognostic significance of EGFR expression when comparing the treatment of HNSCC with radiotherapy or CRT (38-40).

Previous studies have revealed that patients exhibiting HPV-positive HNSCC demonstrate an improved prognosis compared with patients exhibiting virus-negative tumors (3,28,41-43). Previous work by a number of groups has demonstrated that significantly more tumors originating in the oropharynx express p16, a surrogate marker for high-risk HPV infection, compared with non-oropharyngeal sites (3,41,43). Furthermore, previous studies have revealed the prognostic impact of p16 positivity for tumor collectives treated with various treatment modalities, including surgery, radiotherapy and CRT (3,4,41,43), or OSCC treated by various modalities (that were not used to distinguish patients) (28,42,44). This favorable prognosis is hypothesized to be due to a combination of tumor biology, favorable patient characteristics (younger patients with limited comorbidity and positive performance status, and reduced likelihood of tobacco and alcohol abuse in these patients) and an apparent reduced risk of experiencing secondary primary tumors (28,45).

In the present study, p16 expression was restricted to the oropharyngeal sub-site; however, a prognostic influence on survival was not observed. One of the limitations of the present study was the small number of p16-positive tumors (n=5), which may explain the non-significant difference in survival time. The slightly reduced rate as compared with other studies may additionally be the result of varying socioeconomic profiles and risk factors of the patients. Furthermore, previous studies have suggested that the biological behavior of HPV-positive tumors may be altered by tobacco use and may render positive

tumors less responsive to therapy (44,45). As smoking status was inconsistently recorded in the current patient cohort, this influence could not be analyzed. However, the importance of determining the HPV status in HNSCC, particularly in oropharyngeal cancer, has been extensively demonstrated in the relevant literature and should be considered in additional studies (3-5,28,41-45).

As previously mentioned, survivin is overexpressed in the majority of human tumors; however, is undetectable or present at very low levels in the majority of normal adult tissues (23). Survivin expression has been associated with poor prognosis, disease progression and drug resistance in various types of malignancy, including HNSCC, and particularly in those exhibiting high nuclear survivin expression (14,17,46). However, a number of reports have revealed survivin expression to be associated with favorable prognosis, including in gastric (47) and breast cancer (48), as well as in HNSCC, regardless of the various nuclear and cytoplasmic staining patterns of survivin (15,16). In concordance with the results of several previous studies (13-15,17,49), the present study identified that survivin exists in two subcellular pools, cytoplasmic and nuclear. Studies considering these differences reported a consistent association between high levels of nuclear survivin expression and poor prognosis (17,49). Due potentially to the limited number of patients investigated, the present study could demonstrate only a weak trend between nuclear survivin expression and a reduced OS time, whereas cytoplasmic survivin expression had no prognostic influence.

Multiple reports have described 3q amplification, particularly within the region from 3q22 to 3qter, as one of the most common genomic alterations in HNSCC (8,10), and this amplification has been demonstrated to be associated with more aggressive tumor behaviors and to be predictive of a poorer prognosis (9,10). In NSCLC, the clinical relevance of Sec62, another tumor entity characterized by 3q amplification, has already been demonstrated. A significantly increased Sec62 content was observed in tumors exhibiting lymph node metastases compared with non-metastasized tumors, as well as in poorly differentiated (grade 3) compared with moderately differentiated (grade 2) tumors (31). Furthermore, *SEC62* overexpression was associated with significantly reduced OS time in NSCLC patients who underwent curative surgery, particularly in patients exhibiting squamous cell lung carcinoma (32).

In addition to its correlation with a cancerous phenotype and adverse clinical parameters, *SEC62* overexpression has been demonstrated to serve a significant functional role in molecular carcinogenesis *in vitro*; *SEC62* silencing by small interfering RNA transfection resulted in a markedly decreased migration capability, as well as increased sensitivity towards ER stress in various cell lines (29,31). Furthermore, artificial *SEC62* overexpression in HEK293 cells induced a potential to migrate in this otherwise scarcely migrating cell line (32). Thus, based on current knowledge regarding Sec62 biology in cancer, tumor cells may benefit from an increased Sec62 level with regard to an increased migration potential, as well as an enhanced ability to cope with ER stress (50,51). However, the exact molecular mechanisms by which Sec62 is able to influence the processes of cell migration and ER stress tolerance remain to be elucidated. As Sec62 is known to have a role in protein translocation into the ER, and thereby in the biosynthesis of numerous secretory and membrane proteins (52-54), it may be speculated that the inhibition of cell migration associated with decreased Sec62 protein content may be due to a reduced biogenesis of specific membrane or secretory proteins that are involved in cell migration and ER stress-associated mechanisms. Furthermore, the tumor-promoting effects of Sec62 in cancer cells appear to be mediated, at least in part, by Ca^{2+}-dependent signaling mechanisms (32). A previous study examining various putative oncogenes within 3q26 by systematic loss- and gain-of-function experiments was able to identify *SEC62/TLOC1* as a major cancer driver within this frequently amplified region (55).

Notably, in the present study, patients with tumors exhibiting an elevated level of Sec62 and nuclear survivin demonstrated the poorest prognosis. The interaction of survivin with other molecules, such as X-linked inhibitor of apoptosis protein, is known to inhibit the catalytic activity of caspase-9, thereby preventing apoptosis (46). Caspase-9 activation represents a major component of ER stress-induced apoptosis (56), providing an association with Sec62, which is thought to have a significant role in the regulation of Ca^{2+} leakage from the ER, via indirectly mediating the interaction of the Sec61-channel in the ER membrane with calmodulin on the cytoplasmic side and binding immunoglobulin protein on the ER-luminal side (32). Thus, survivin and Sec62 may synergistically act as potent apoptosis inhibitors, conferring a proliferation advantage to cancer cells. Based on the results of the present study, the simultaneous therapeutic targeting of these two cancer drivers in HNSCC may be suggested to provide a potentially promising therapeutic approach.

The conclusions of the present study indicate that Sec62 is a promising novel candidate for a prognostic marker in HNSCC patients, and additional studies should include larger populations, as well as a comparison of various treatment strategies.

Acknowledgements

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