Journal of Cancer Research and Clinical Oncology (2019) 145:2039–2044
https://doi.org/10.1007/s00432-019-02955-7

ORIGINAL ARTICLE – CANCER RESEARCH

Aberrant expression of Sec61α in esophageal cancers

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Received: 8 May 2019 / Accepted: 11 June 2019 / Published online: 13 June 2019

Abstract

Introduction The heterotrimeric Sec61α translocon complex is topological located in the membrane of the endoplasmic reticulum (ER) and allows protein transport and calcium across the membrane. Recently, aberrant expression of Sec proteins was linked to carcinogenesis and prognosis of patients.

Materials and methods Here, we analysed the role of Sec61α in esophageal cancer, and we analysed Sec61α staining on a tissue microarray containing more than 600 esophageal cancer specimens by immunohistochemistry.

Results Sec61α staining was always strong in benign esophagus, but was only found in 5% of interpretable esophageal adenocarcinomas (EACs) and 14.5% of squamous cell carcinomas (ESCCs). Reduced Sec61α staining was not strongly linked to tumor phenotype in both subgroups of esophageal cancers and was unrelated to clinical outcome of patients (EACs: $p = 0.8051$ and ESCCs: $p = 0.2751$).

Conclusions Thus, Sec61α measurement has not an additional prognostic benefit for the patients.

Keywords Sec61α · Esophageal cancer · Tissue microarray

Introduction

Esophageal cancer is one of the most aggressive cancers and the sixth leading cause of cancer death worldwide (Song et al. 2014). Currently, there are limited clinical approaches for the early diagnosis and treatment of esophageal cancer, resulting in a 10% 5-year survival rate for patients (Song et al. 2014). It can be hoped that the identification of novel biological markers and tumorigenic pathways will improve diagnosis and therapeutic strategies for esophageal cancer.

The biogenesis of secretory and transmembrane proteins requires the activity of the universally conserved protein-conducting channel Sec61 complex consisting of three subunits (Sec61α, β, and γ) (Linxweiler et al. 2017; Görlich et al. 1992; Wirth et al. 2003; Van den Berg et al. 2004; Pfeffer et al. 2014, 2015; Becker et al. 2009). The polypeptide conducting Sec61 channel is located in the membrane of the ER, where it can bind to translating ribosomes for co-translational protein transport (Conti et al. 2015; Linxweiler et al. 2017; Nyathi et al. 2013; Park and Rapoport 2012; Shao and Hegde 2011; Skach 2009). Besides its role in the intracellular transport of polypeptides, Sec61 complex is involved in endoplasmic reticulum associated degradation, responsible for the retro-translocation of misfolded proteins to the cytosol for degradation (Kaiser and Römisch 2015; Smith et al. 2011; Kim et al. 2015). Moreover, Sec61 channel acts as a passive ER calcium leak channel (Simon and Blobel 1991; Ong et al. 2007; Lomax et al. 2002; Roy and Wonderlin 2003; van Coppenolle et al. 2004; Flourakis et al. 2006; Giunti et al. 2007; Wonderlin 2009).

For cancer, Sec61γ was amplified and overexpressed in glioblastomas and silencing of Sec61γ suppressed cell growth and induced apoptosis (Lu et al. 2009). To get insights in the prevalence and clinical significance of Sec61α in esophageal cancers, we took advantage of our esophageal cancer TMA containing more than 600 esophageal cancer specimens. The results of our study exclude Sec61α as a prognostic marker in esophageal cancer.

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Materials and methods

Clinical data and tissue samples

A TMA was constructed from cancer tissues from 359 EAC and 254 ESCC patients who underwent surgery at the University Medical Center Hamburg-Eppendorf. Follow-up data were available of 359 EAC and 254 ESCC patients with a median follow-up of 17.3 and 12.2 months (range 0–208 and 0–191 months). All esophageal specimens were analysed according to a standard procedure, including complete embedding of the entire esophagus for histological analysis. The TMA-manufacturing process was described earlier in detail (Mirlacher and Simon 2010). In short, one 0.6 mm core was taken from a representative tissue block from each patient. The tissues were distributed among 2 TMA blocks. For internal controls, each TMA block also contained various control tissues, including normal esophageal tissue.

Immunohistochemical staining and evaluation

Freshly cut TMA sections were immunostained in 1 day and in one experiment. Slides were deparaffinized and exposed to heat-induced antigen retrieval for 5 min in an autoclave at 121 C in pH 7.8 Tris–ethylenediaminetetraacetic acid–citrate buffer. Primary antibody specific for Sec61A1 polyclonal rabbit, 15512-1-AP; Acris; at 1/150 dilution) was applied at 37 °C for 60 min. Bound antibody was then visualized using the EnVision Kit (Dako, Glostrup, Denmark) according to the manufacturer instructions. Sec61A1 staining was analysed by one person (KG) experienced in immunohistochemisty. Bound antibody was then visualized using the EnVision Kit (Dako, Glostrup, Denmark). Sec61α staining was homogenous in the analysed tumor samples and staining intensity of all cases was thus assessed in four categories: negative, weak, moderate, and strong immunostaining.

Statistical analysis

Statistical calculations were performed with the JPM 9 software (SAS Institute Inc., NC, USA). Contingency tables and the Chi-square test were performed to search for associations between molecular parameters and tumor phenotype. Survival curves were calculated according to Kaplan–Meier. The log-rank test was applied to detect significant survival differences between groups. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular and clinical variables. Logistic regression was used to quantify the area under receiver–operator curve (ROC).

Results

Technical aspects

A total of 24.9% of 613 tissue cores were non-informative for Sec61α immunohistochemistry due to the complete lack of tissue or absence of unequivocal cancer cells on individual TMA spots.

Sec61α protein expression in tissue arrays of esophageal cancer patients

Sec61α expression was localized in the cytoplasm of the cells and was generally detectable in strong intensities in benign esophagus. Positive Sec61α expression was only seen in 57.9% and 71% of interpretable EACs and ESCCs, including 36.4% and 40.5% with weak, 16.5% and 16% with moderate, and 5% and 14.5% with strong expression. Representative images of Sec61α immunoreactivity in cancerous esophageal tissue are given in Fig. 1.

Association of Sec61α staining with clinicopathological parameters and survival of patients

Sec61α staining was not strongly associated with tumor phenotype in both subgroups of esophageal cancers. The associations of Sec61α staining with esophageal cancer phenotype are demonstrated in Tables 1 and 2.

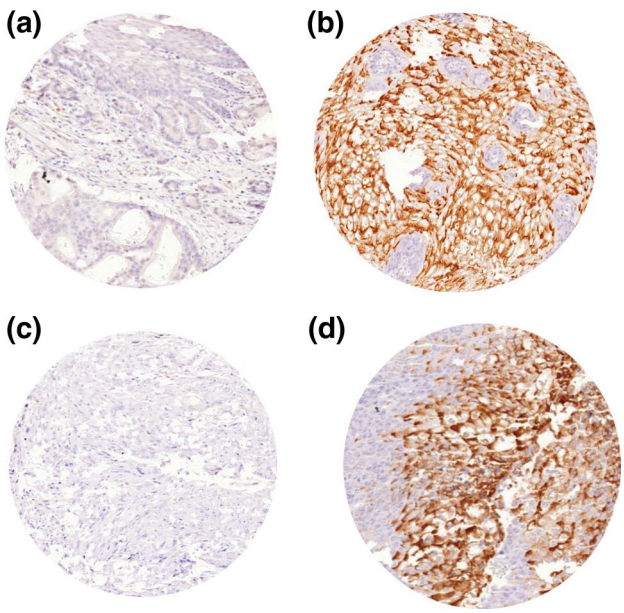

Fig. 1 Sec61α immunostaining in esophageal cancers. Negative and strong expression of Sec61α in EACs (**a**, **b**) and ESCCs (**c**, **d**)

Table 1 Clinico-pathological parameters relative to Sec61α IHC results in esophageal adenocarcinoma

Parameter	Immunostaining					
	Evaluable (*n*)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	*p* value
Tumors	261	42.15	36.40	16.48	4.98	
Age group						
< 65 years	88	44.32	32.95	14.77	7.95	0.3862
> 65 years	173	41.04	38.15	17.34	3.47	
Sex						
Male	225	42.67	36	17.33	4	0.3007
Female	35	37.14	40	11.43	11.43	
Tumor stage						
pT1	41	31.71	53.66	12.2	2.44	0.5203
pT2	29	37.93	34.48	20.69	6.9	
pT3	172	44.19	33.14	17.44	5.23	
pT4	18	55.56	27.78	11.11	5.56	
UICC stage						
I	43	32.56	51.16	11.63	4.65	0.2223
II	32	50	37.5	12.5	0	
III	160	41.88	34.38	18.13	5.63	
IV	25	52	20	20	8	
Grading						
G1	13	23.08	61.54	15.38	0	0.093
G2	93	43.01	40.86	12.9	3.23	
G3	148	44.59	31.76	17.57	6.08	
G4	4	0	25	50	25	
R status						
R0	184	42.39	34.24	17.39	5.98	0.6704
R1	71	42.25	40.85	14.08	2.82	
R2	3	33.33	66.67	0	0	
pN category						
N0	71	35.21	49.3	12.68	2.82	0.0385
N1	41	53.66	26.83	14.63	4.88	
N2	65	30.77	36.92	27.69	4.62	
N3	82	50	30.49	12.2	7.32	
M status						
M0	236	41.1	38.14	16.1	4.66	0.3039
M1	25	52	20	20	8	

Prognostic role of Sec61α immunostaining

Follow-up data were available for 261 and 200 patients with informative Sec61α data in adenocarcinoma and squamous cell carcinoma of the esophagus. Statistical analysis revealed that there were no significant associations between Sec61α immunostaining and clinical outcome of the patients (EACs: $p = 0.8051$ and ESCCs: $p = 0.2751$; Fig. 2a, b).

Discussion

The results of our study demonstrate that Sec61α expression is decreased in malignant as compared to benign esophageal tissue. However, Sec61α expression was unrelated to esophageal tumor phenotype and prognosis of patients.

This is the first study analyzing the prevalence and clinical significance of Sec61α expression in esophageal cancers.

Table 2 Clinico-pathological parameters relative to Sec61α IHC results in esophageal squamous cell carcinoma

Parameter	Immunostaining					
	Evaluable (*n*)	Negative (%)	Weak (%)	Moderate (%)	Strong (%)	*p* value
Tumors	200	29.00	40.50	16.00	14.50	
Age group						
< 65 years	70	28.57	42.86	12.86	15.71	0.8133
> 65 years	130	29.23	39.23	17.69	13.85	
Sex						
Male	146	30.14	41.1	13.7	15.07	0.5539
Female	54	25.93	38.89	22.22	12.96	
Tumor stage						
pT1	35	8.57	40	14.29	37.14	0.0007
pT2	39	33.33	33.33	15.38	17.95	
pT3	114	35.96	42.11	15.79	6.14	
pT4	12	8.33	50	25	16.67	
UICC stage						
I	49	14.29	44.9	14.29	26.53	0.0405
II	49	30.61	44.9	18.37	6.12	
III	93	34.41	38.71	15.05	11.83	
IV	9	44.44	11.11	22.22	22.22	
Grading						
G1	3	66.67	33.33	0	0	0.6871
G2	125	27.2	41.6	17.6	13.6	
G3	72	30.56	38.89	13.89	16.67	
G4	0	0	0	0	0	
R status						
R0	150	28.67	42	14	15.33	0.6158
R1	41	34.15	34.15	19.51	12.2	
R2	8	12.5	37.5	37.5	12.5	
pN category						
N0	88	20.45	46.59	15.91	17.05	0.1084
N1	46	28.26	47.83	15.22	8.7	
N2	41	46.34	26.83	17.07	9.76	
N3	25	32	28	16	24	
M status						
M0	192	28.13	41.67	16.15	14.06	0.2798
M1	8	50	12.5	12.5	25	

In our study, Sec61α expression was decreased in malignant relative to benign esophageal epithelium. The reduction of Sec61α expression might be due to differences in the activity of regulatory transcription factors involved in Sec61A1 gene transcription operating in malignant tissue. For example, one of the regulatory transcription factors binding to Sec61A1 gene promoter is STAT1, which has been shown to be lost during esophageal carcinogenesis in a considerable fraction of carcinomas (Watanabe et al. 2001).

For cancer, Sec61γ has been amplified and overexpressed in glioblastomas and silencing of Sec61γ suppressed cell growth and induced apoptosis (Lu et al. 2009). In this study, high resolution of digital karyotyping and single-nucleotide polymorphism arrays identified the minimal region of amplification of the chromosome 7p11.2, which contains amongst other the Sec61γ gene (Lu et al. 2009). Moreover, the authors showed that Sec61γ was overexpressed in 77% of glioblastoma multiforme and that knockdown of Sec61γ resulted in growth suppression and apoptosis of the cells (Lu et al. 2009). Furthermore, pharmacologic-induced ER stress resulted in induced SEC61γ expression in glioblastoma multiforme cells (Lu et al. 2009). Taken together, the authors conducted that SEC61γ might play an important role in glioblastoma multiforme cell survival likely due to a mechanism that is involved in the cytoprotective ER stress-adaptive response to the tumor microenvironment (Lu et al. 2009). Besides SEC61γ, the authors compared the expression profiles

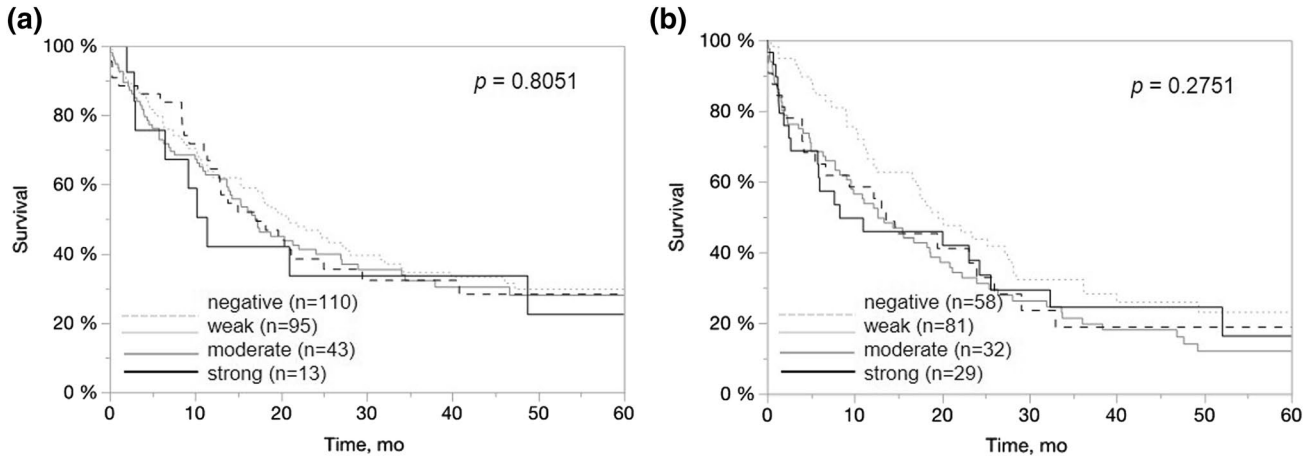

Fig. 2 Prognostic impact of Sec61α immunostaining in esophageal cancers. Relationship of Sec61α staining intensity with overall survival in EACs ($n=261$; $p=0.8051$; **a**) and ESCCs ($n=200$; $p=0.2751$; **b**)

of the other subunit genes SEC61α and SEC61β in glioblastoma multiforme samples versus control samples (Lu et al. 2009). These data showed that there was only a marginal trend of t SEC61α and SEC61β proteins towards an elevation in glioblastoma multiforme as compared to the control samples (Lu et al. 2009). Here, we demonstrated that SEC61α expression was reduced in esophageal cancer as compared to the control group. However, further studies are needed to get further impact in the functions of SEC61α in detail and its role in other cellular signaling pathways.

In the literature, other Sec proteins, such as Sec62 and Sec63, have also been linked to tumorigenesis (Linxweiler et al. 2017). For example, Sec63 frameshift mutations have been found in 37.5% of microsatellite-unstable gastric cancers (Mori et al. 2002), 48.8% of colorectal cancer (Mori et al. 2002), 56% of HNPCC-associated small-bowel cancers (Schulmann et al. 2005), and in one Lynch syndrome-associated hepatocellular carcinoma (Casper et al. 2013). Moreover, one study demonstrated a significant association between low hepatic SEC63 expression with decreased apoptosis and increased proliferation rate in the mouse model (Casper et al. 2013). Altogether, these studies indicate a potential role of Sec63 as a tumor suppressor gene in the carcinogenesis of gastric cancer, colorectal cancer and hepatocellular cancer (Linxweiler et al. 2017). Thus, it can be speculated that Sec61A1 might be also have tumor suppressive functions in esophageal cancers.

In summary, our study demonstrates that Sec61α was aberrant expressed in esophageal cancer, but was unrelated to prognosis of patients. Thus, Sec61α cannot be considered as prognostic marker in esophageal cancer.

Funding None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals The utilization of tissues and clinical data was according to the Hamburger Krankenhaus Gesetz (§12 HmbKHG) and approved by our local Ethical Committee.

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