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Assessment of prognostic significance of cytoplasmic survivin expression in advanced oesophageal cancer

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Abstract: Survivin is a member of the family of proteins, which inhibit apoptosis (inhibitor of apoptosis proteins - IAP). Expression of survivin was found in colorectal cancer, neuroblastoma, bladder cancer, non-small cell lung cancer, and breast cancer. There is some recent data indicating the correlation of poor prognosis and worse response to chemotherapy in patients with oesophageal squamous cell carcinoma (OSCC) expressing survivin. The aim of the present study was to assess survivin expression in cancerous tissue of patients with advanced OSCC and to test the potential correlation between survivin expression and clinicopathological data. Forty two patients (mean age 58.36±8.97 yrs), who were oesophagectomised due to squamous cell carcinoma of the thoracic oesophagus between 1998 and 2000, were retrospectively analysed. Cytoplasmic survivin expression, examined immunohistochemically, was found in 35 (83.33%) cases. No statistically significant correlation between survivin expression in the tumour and patients' gender, TNM stage, or vascular involvement was noted. The mean survival of patients with cytoplasmic survivin expression (17.81±5.51 months) was not statistically different to those with negative survivin staining (16±6.28 months) as assessed by Mantel-Cox test ($p=0.49$). Univariate regression analysis revealed UICC staging as the only predictor of survival in the analysed group ($p<0.05$). These results indicate that the cytoplasmic survivin expression does not seem to be the prognostic factor in advanced cases of OSCC.

Key words: Oesophageal squamous cell carcinoma - Survivin, cytoplasmic

Introduction

Survivin is a new member of the family of proteins, which inhibit apoptosis (inhibitor of apoptosis proteins - IAP) [12, 15, 17]. It has been shown to directly inhibit terminal protease effectors of apoptosis, *i.e.* caspase-3 and caspase-7 [6, 12, 17, 19]. There is a growing body of evidence indicating that survivin plays an important role in inhibition of apoptosis in cells exposed to diverse apoptotic stimuli by associating with microtubules of mitotic spindles. Loss of control over defects in the structure of mitotic spindle and/or in chromosome configuration during cell division caused by survivin is responsible for its antiapoptotic action [11].

Expression of survivin was found in colorectal cancer, neuroblastoma, bladder cancer, non-small cell lung cancer, or breast cancer [1, 2, 10, 13, 15, 16, 18]. There is some recent data indicating the correlation of poor prognosis and worse response to chemotherapy in patients with oesophageal squamous cell carcinoma expressing survivin [3, 4, 6, 7].

The aim of the present study was to assess survivin expression in cancerous tissue of patients with advanced oesophageal squamous cell carcinoma and to test the potential correlation between survivin expression and some selected clinicopathological parameters such as depth of tumour invasion, lymph node involvement, or overall survival.

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Materials and methods

Patients. Forty two patients oesophagectomised at the 2nd Department of General Surgery, Skubiszewski Medical University, Lublin, between 1998 and 2000, were analyzed. In all these patients the diagnosis of squamous cell carcinoma of the thoracic oesophagus was confirmed histopathologically. The analysed group included 38 men and 4 women, with male to female ratio 9.5/1. The age of the patients ranged from 40 to 70 yrs (mean 58.36±8.97). Neither neoadjuvant chemo- nor radiotherapy was applied before surgery. No adjuvant chemotherapy was applied in this group. Patients who died of postoperative complications within 30 days - (7) and lost in follow-up (6) were excluded from survival analysis. The clinicopa-

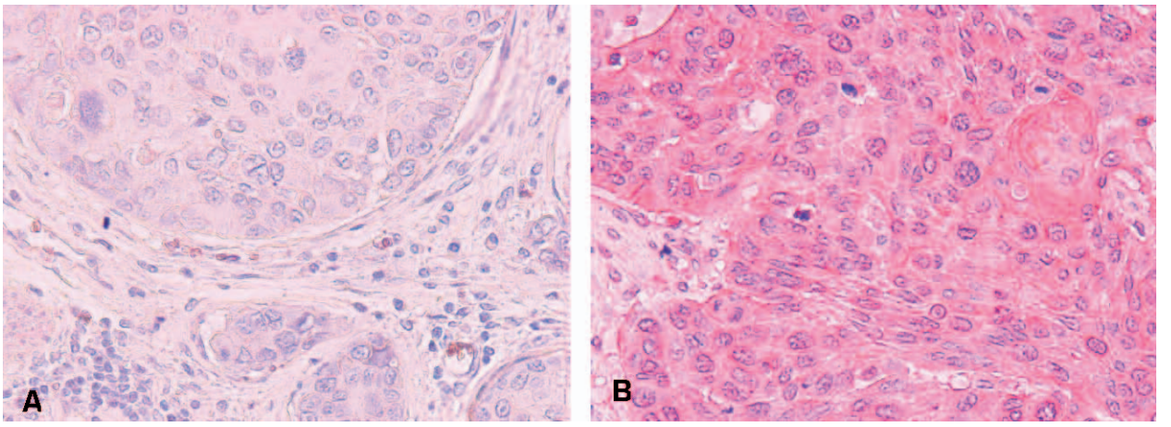

Fig. 1. Immunohistochemical detection of survivin in oesophageal cancer. Survivin-negative (**A**) and survivin-positive (**B**) cancer cells showing cytoplasmic immunostaining.

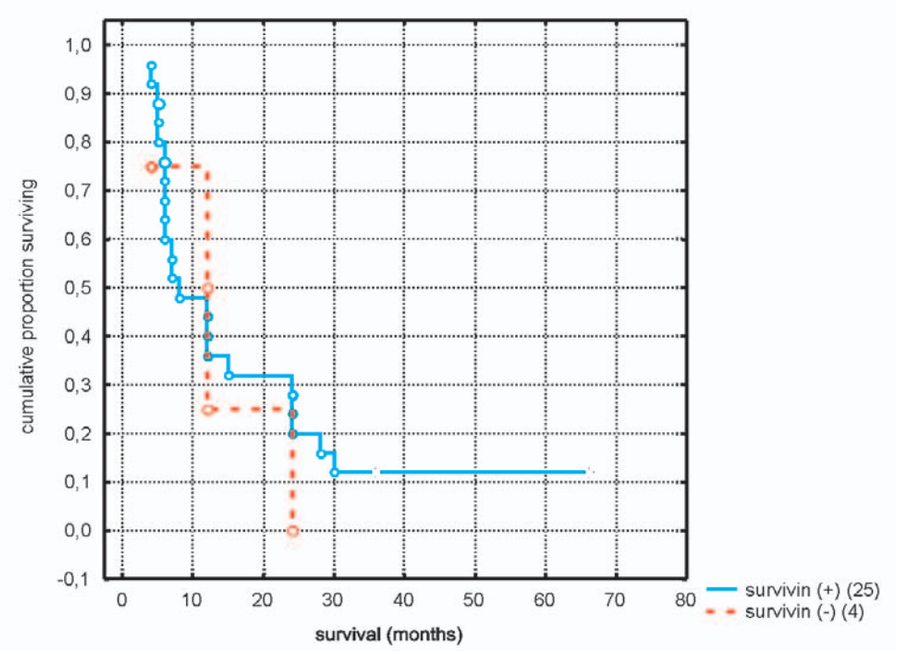

Fig. 2. Cumulative proportion surviving of the patients with positive and negative cytoplasmic survivin expression.

thological data of all patients who underwent surgery are shown in Table 1. Patients who smoked at least 20 cigarettes a day (smokers) and drank at least 20 g of ethanol daily for 5 or more years were assessed separately.

Immunohistochemistry. Paraffin-embedded tumour specimens were examined. Four-µm sections were mounted on slides precoated with poly-L-lysine (SIGMA). Deparaffinized sections were immersed in citrate buffer (0.01 M, pH 6.0), heated in a microwave oven to 100˚C twice for 5 min in order to retrieve the antigens, and then allowed to cool down for 45 min in the same buffer. Next, the slides were rinsed thoroughly with deionised water and PBS, incubated with serum-free blocking solution (DAKO) and primary antibody was applied for 60 min at room temperature. Rabbit anti-human survivin antibody (200 µg/mL, Santa Cruz Biotechnology) was used pre-diluted 1:20 in blocking buffer. The negative controls, obtained by substitution of the primary antibody with blocking buffer, were also included in the study. Incubation with secondary antibody and product visualization was performed employing LSAB2 Kit (DAKO) with AEC Substrate-Chromogen, according to the manufacturer's protocol. Specimens were counterstained with Mayer's hematoxylin for 2 min, washed with deionised water and mounted in Glycergel (DAKO). The slides were examined under Olympus BH2 light microscope, fitted with S Plan Apo × 100 immersion objective. The cases were scored as survivin-positive when more than 5% of the cells reacted with the anti-survivin antibody, as proposed by Kawasaki *et al.* [8] (Fig. 1).

Statistical analysis. The obtained results were mostly analysed as dichotomised variables: tumour stage T3 vs. T4; lymph node invol-

vement N0 (-) vs. N1-N2 (+); remote metastases M0 vs. M1; blood vessels involvement (+) vs. (-). The Spearman nonparametric correlation test or U-Mann-Whitney test for two independent probes were used for statistical comparison of the results. Overall survival was assessed by the Kaplan-Meier method. The significance of differences in overall survival was calculated by the Mantel-Cox test. The differences were considered to be significant for $p<0.05$. All statistical analyses were performed using SPSS software (Statistica).

Results

Cytoplasmic survivin expression in tumour cells was detected in 35 (83,33%) cases of oesophageal squamous cell carcinoma. Nuclear survivin expression was not observed in the examined tumour specimens. No statistically significant correlation between cytoplasmic survivin expression in the tumour and patient's gender, TNM stage, or vascular involvement was noted. No correlation was found, either, between cytoplasmic survivin expression and tobacco smoking. Lower cytoplasmic survivin expression was noted in patients with history of chronic alcohol intake but this trend did not reach statistical significance ($p=0.08$). The results are shown in Table 1.

Kaplan-Meier survival curves for patients with oesophageal squamous cell carcinoma categorised according to survivin expression are shown in Figure 2. The mean survival of patients with cytoplasmic survivin expression (17.8 months±15.51) was not statistically different to those with negative survivin staining (16 months±6.28) according to Mantel-Cox test ($p=0.49$). Univariate regression analysis revealed UICC staging as the only predictor of survival ($p<0.05$). The other parameters did not reach statistical significance.

Table 1. Clinicopathological data of patients and tumours according to cytoplasmic survivin expression

	Patients		Cytoplasmic survivin		
	N	(%)	expression (+)	(%)	P value
Total	42	100	35	83.33	
Male	38	90.47	31	81.57	0.57 (n.s.)
Female	4	9.53	4	100	
T-stage					
T3	26	61.90	21	80.76	0.86 (n.s.)
T4	16	38.10	13	81.25	
N-stage					
N(-)	10	23.80	9	90	0.528 (n.s.)
N(+)	32	76.19	26	81.25	
M-stage					
M0	34	80.95	29	85.29	0.49 (n.s)
M1	8	19.04	6	75	
Vascular involvement					
(+)	32	76.19	26	81.25	0.52 (n.s.)
(-)	10	23.81	9	90	
Differentiation					
G1-G2	28	66.6	24	85.7	0.58 (n.s)
G3	14	33.3	11	78.57	
UICC-stage					
II	3	7.14	3	100	0.63 (n.s.) vs. IV 0.68 (n.s.) vs. II 0.72 (n.s.) vs. III
III	31	73.80	27	87.09	
IV	8	19.04	6	75	
Tobacco					
(+)	35	83.33	28	80	0.20 (n.s.)
(-)	7	16.66	7	100	
Alcohol					
(+)	31	73.80	24	77.41	0.08 (n.s.)
(-)	11	26.20	11	100	

Discussion

Recent studies indicating the role of survivin in carcinogenesis of OSCC became the inspiration to perform the present analysis [3, 4, 6, 7]. Survivin is one of the apoptosis inhibitor proteins and participates in the regulation of cell division. It has apoptotic control over G2-M checkpoint of the cell cycle [3, 6, 11, 17]. Survivin expression seems to be specific for neoplasms, but not for nonproliferating adult tissues [1, 2, 6, 10, 15, 17, 18]. Anti-survivin proteins are observed in serum of patients with malignant neoplasms more frequently than p53 protein [8].

In the present study, 42 patients treated surgically due to advanced, thoracic oesophageal squamous cell carcinoma were analysed. The vast majority of patients included in the study were classified at III or IV stage of disease according to UICC classification. Cytoplasmic expression of survivin was noted in 35 subjects (83.3%). Grabowski and co-workers [4] were the first who differentiated nuclear and cytoplasmic expression of survivin and described its translocation during carcinogenesis. They observed cytoplasmic and nuclear survivin expression in 67% and 80% of cancer patients respectively. Lack of nuclear survivin expression in tumour specimens examined in this study can be caused by the type of antibodies applied which were different from those used by Grabowski *et al.* [4]. In studies concerning other tumours, expression of survivin was noted at the level of 70.7% in breast cancer, 34.5% in gastric cancer, and 53.2% in colorectal cancer [14, 15, 18].

In the present study, no statistical correlation was found between cytoplasmic survivin expression and the analysed parameters, *i.e.* the depth of invasion, lymph node metastases and remote metastases. Statistical correlation between cytoplasmic survivin expression and overall survival revealed no significant differences between survivin-positive and -negative OSCC cases. These data are very similar to those obtained by other research groups. Ikeguchi *et al.* [6] revealed no correla-

tion between survivin mRNA expression and lymph node metastases or depth of tumour invasion in OSCC. Sarela *et al.* [15] drew the same conclusions in colorectal cancer. In the later study, Ikeguchi *et al.* [5] confirmed the previous results and showed no correlation between the level of survivin expression and the occurrence of apoptosis or tumour angiogenesis. However, survivin/GAPDH (glyceraldehyde-3-phosphate dehydrogenase) ratio positively correlated with proliferative activity and p53 nuclear accumulation in cancer cells. Grabowski *et al.* [4] noted that cytoplasimc survivin staining has no prognostic relevance in OSCC. In contrast, significant correlation was observed between nuclear survivin expression and lymph node involvement, tumour grading or overall survival. In other study, survivin mRNA expression in oesophageal cancer cells (51 cases) assessed with the use of RT-PCR was significantly higher than in the adjacent normal tissue. Furthermore, pN4 tumours showed significantly higher survivin expression comparing to pN0-3 ones [7] . Interestingly, longer median survival was noted in "survivin-negative" patients than in "survivin-postitve" group with oesophageal cancer, as well as with other malignancies [3, 4, 6, 7, 15]. Some other reports indicate association of positive nuclear survivin staining with a favourable prognosis in gastric or breast cancer [9, 14]. Kato *et al.* [7] suggest some caution in the interpretation of these results indicating very short follow-up and small number of patients included in the analyses.

In the present study, the obtained results seem to be different from those mentioned above. Certainly, the results were influenced by specificity of the analysed group of patients, which was quite similar regarding the advanced stage of the disease (mostly III or IV UICC grade). It should be emphasized that the majority of patients with OSCC in Poland is diagnosed and treated at advanced stage of the disease, so the analysed group seems to be representative for this country.

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