

Original Article

Association of nuclear annexin A1 with prognosis of patients with esophageal squamous cell carcinoma

Gaohua Han^{1,2}, Ye Tian¹, Bensong Duan^{3,4}, Haihui Sheng⁴, Hengjun Gao^{3,4}, Junxing Huang²

¹Department of Oncology, The Affiliated Second Hospital of Suzhou University, Suzhou, Jiangsu, China; ²Department of Oncology, Taizhou People's Hospital, Taizhou, Jiangsu, China; ³Institute of Digestive Disease, Department of Gastroenterology, Tongji Hospital, Tongji University, Shanghai, China; ⁴National Engineering Center for Biochip at Shanghai, Shanghai, China

Received December 12, 2013; Accepted December 31, 2013; Epub January 15, 2014; Published February 1, 2014

Abstract: Although recent progress has been made in the diagnosis and treatment of cancer, the prognosis of esophageal squamous cell carcinoma (ESCC) remains poor. The identification of biomarkers for ESCC prognosis is important for treatment decisions. The aim of this study was to evaluate the relationship between the expressions of Annexin A1 (ANXA1), three prime repair exonuclease 1 (TREX1) and apurinic/aprimidinic endonuclease-1 (APE1) and clinical outcome of patients with ESCC. The expressions of ANXA1, TREX1 and APE1 in 93 pairs of ESCC and paracancerous tissues were tested using immunohistochemistry. ANXA1, TREX1 and APE1 were dysregulated in ESCC. Nuclear expressions of ANXA1 and APE1 were significantly associated with pathologic type ($P = 0.004$ and 0.040 , respectively). Patients with low expression of nuclear ANXA1 had a better prognosis than those with high expression of nuclear ANXA1 (HR = 0.448, 95% CI 0.236-0.849, $P = 0.014$), especially for those with histologic grade 1 and 2 (HR = 0.303, 95% CI: 0.155-0.593, $P < 0.001$). In conclusion, nuclear ANXA1 may be potentially used as a prognostic biomarker for ESCC.

Keywords: Annexin A1, three prime repair exonuclease 1, apurinic/aprimidinic endonuclease-1, esophageal squamous cell carcinoma, prognosis

Introduction

Esophageal cancer is the fifth most common cancer and the fourth most cause of cancer-related death in China [1]. Esophageal squamous cell carcinoma (ESCC) is the most common histological type, accounting for > 90% of cases [2]. Despite the improvement achieved in the diagnosis and treatment, the prognosis of ESCC remains poor, with the five-year survival rate of ~30% [3]. Most ESCC patients who undergo curative resection will eventually relapse and die of treatment-resistant disease, with local and regional recurrence being the most prevalent pattern of failure. Treatment for ESCC remains one of the most challenging tasks for cancer clinicians. Therefore, there is an urgent need for safer and more effectively therapies to improve the prognosis of ESCC patients.

Annexins are a family of Ca^{2+} -regulated phospholipid-binding proteins, with 12 members in

mammals, which have been implicated in the regulation of several biological processes, including cell differentiation, proliferation and apoptosis [4]. There is increasing evidence that annexins play important roles in cancer incidence and progression [5-11]. Annexin A1 (ANXA1), the first characterized member of the annexin superfamily, is an intracellular protein which is aberrantly expressed in many types of cancer, such as gastric [6, 9, 12], breast [10, 13] and esophageal cancer [14, 15]. Dysregulation of ANXA1 is related to the incidence, invasion, metastasis and drug resistance of cancers [16].

DNA repair enzymes are vitally important for protecting cells against damage caused by endogenous and exogenous agents. Apurinic/aprimidinic endonuclease-1 (APE1) is the major AP endonuclease, which is important for the base excision repair pathway. In addition to DNA repair activity, APE1 has important roles in protection against oxidative stress- and hypox-

Table 1. Clinicopathological characteristics of patients with ESCC

Characteristics	No. of Patients	%
Age (years)		
median	63	
range	49-85	
Gender		
female	22	23.7
male	71	76.3
Pathologic type		
medullary	23	24.7
ulcerative	55	59.1
others	13	14.0
unknown	2	3.2
AJCC stage		
stage I	7	7.5
stage II	52	55.9
stage III	29	31.2
stage IV	1	1.1
unknown	4	4.3
LNM		
negative	55	59.1
positive	37	39.8
unknown	3	3.2
Tumor size (cm)		
median	4.0	
range	1.7-10	
Histologic grade		
well	15	16.1
moderate	63	67.7
poor	15	16.1

ia-induced apoptosis and necrosis [17]. Overexpression of APE1 in cancer cells is closely linked to poor prognosis and chemo- and radio-resistance [18]. Three prime repair exonuclease 1 (TREX1), the major 3' to 5' exonuclease in mammalian cells, excises bases from 3' end of single- and double-stranded DNA templates with a preference for mismatched nucleotides [19]. In human fibroblasts, TREX1 was predominantly localized in the cytoplasm and translocated into the nucleus upon ultraviolet light exposure [20]. However, TREX1 is overexpressed in the nucleus of cancer cells [20].

In the present study, we investigated the expression levels of ANXA1, TREX1 and APE1 in 93 patients with ESCC and examined their associations with clinicopathologic factors and overall survival.

Materials and methods

Patients and tissue samples

A total of 93 patients were recruited who were underwent surgery between August 2008 and February 2010. All patients had histologically confirmed primary ESCC and had no history of other malignancy. ESCC and matched adjacent normal tissues were collected before patients were treated with any anti-cancer therapy, including surgery, chemotherapy and radiotherapy. This study was approved by the ethical committees of Taizhou People's Hospital and National Engineering Center for Biochip at Shanghai. Written informed consent was obtained from each patient before enrolling in the study. The clinicopathologic features of ESCC patients were summarized in **Table 1**.

Tumor tissue microarray (TMA) and immunohistochemistry (IHC)

ESCC TMA was constructed as previously described [21]. Hematoxylin- and eosin-stained slides were reviewed by a pathologist, and representative areas that contained invasive tumor cells were marked on both the slides and corresponding paraffin block for TMA construction. TMA was constructed using an automated tissue arrayer (Beecher Instruments, Sun Prairie, WI). A single 600- μ m tissue core was taken from each donor block and assembled into a recipient block. Subsequently, sections (4 μ m thick) were cut from the array blocks and prepared for IHC analysis.

Sections were deparaffinized with xylene and rehydrated through graded alcohol. Antigen retrieval was performed using microwave treatment for 10 min in 10 mM sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched by incubating the sections in methanol with 6% hydrogen peroxide. Slides were then incubated with antibodies for 30 min (anti-ANXA1 antibody, 1:3000; anti-TREX1 antibody, 1:250; anti-APE1 antibody, 1:200). Staining was completed after a 10-min incubation with a freshly prepared substrate-chromogen solution (20 μ L DAB chromogen per 1 mL of PBS), which results in a brown-colored precipitate at the antigen site. Slides were subsequently counterstained with hematoxylin, dehydrated, and mounted.

Annexin A1 and ESCC

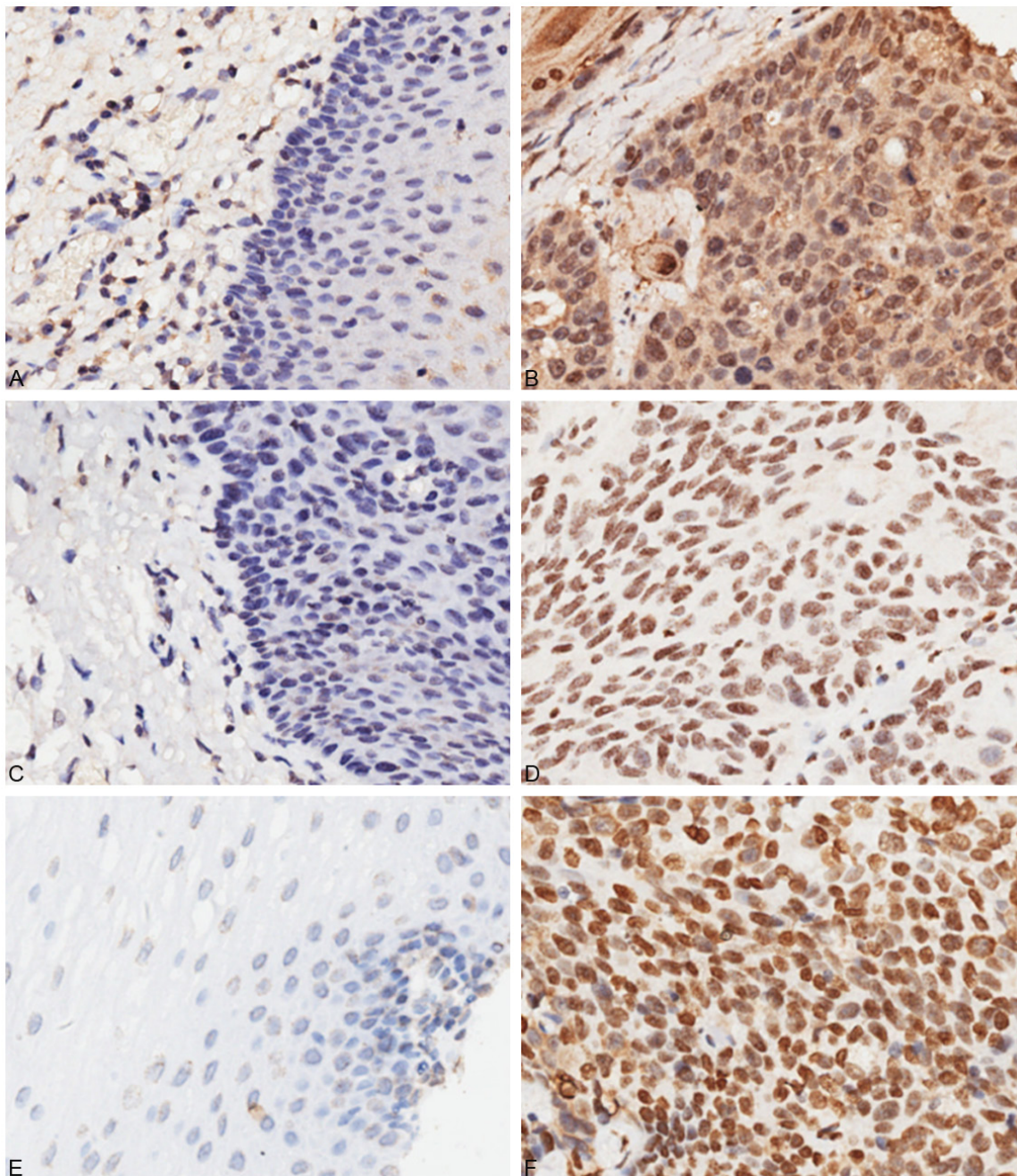


Figure 1. Immunohistochemical analysis of ANXA1, TREX1 and APE1 in ESCC and paracancerous tissues. A: Negative ANXA1 expression in adjacent non-cancerous tissue. B: Positive ANXA1 expression in ESCC tissue (cytoplasmic and nuclear staining). C: Negative TREX1 expression in adjacent non-cancerous tissue. D: Positive nuclear expression of TREX1 in ESCC tissue. E: Negative APE1 expression in adjacent non-cancerous tissue. F: Positive nuclear expression of APE1 in ESCC tissue.

The slides stained by IHC were assessed by two pathologists who were blinded to clinical information. The staining intensity of cancer cells was scored as 0, negative; 1, weak; 2, moderate; 3, strong staining. For statistical evaluation, tumors were scored as 0, non-staining; 1,

1-10%; 2, 11-50%; 3, 51-80%; 4, 81-100% positive cells. The total histological score, which was the result of multiplication of intensity and percentage scores, was utilized to determine the result. The total histological score < 4 indicated as a low level of expression, whereas a

Annexin A1 and ESCC

Table 2. Associations between the expressions of ANXA1, TREX1 and APE1 and the clinicopathological features

Variables	Nuclear ANXA1			Cytoplasmic ANXA1			Nuclear TREX1			Nuclear APE1		
	Low	High	P value	Low	High	P value	Low	High	P value	Low	High	P value
Age (years)			0.271			0.334			0.809			0.469
≤ 60	18	15		22	11		9	24		10	23	
> 61	40	20		46	14		15	45		14	46	
Gender			0.803			1			0.578			0.578
male	45	26		52	19		17	54		17	54	
female	13	9		16	6		7	15		7	15	
Size (cm)			0.83			0.636			0.472			0.811
> 4	25	14		30	9		12	27		11	28	
≤ 4	33	21		38	16		12	42		13	41	
Pathologic type			0.004			0.376			0.765			0.040
medullary	19	4		19	4		5	18		8	15	
ulcerative	27	28		40	15		16	39		9	46	
others	11	2		8	5		3	10		6	7	
Histologic grade			0.923			0.626			0.349			0.677
well	9	6		10	5		3	12		3	12	
moderate	39	24		48	15		19	44		18	45	
poor	10	5		10	5		2	13		3	12	
LNM			0.263			0.458			0.627			0.617
positive	25	10		28	7		10	25		7	28	
negative	32	23		39	16		13	42		14	41	
AJCC stage			0.764			0.606						0.937
I	4	3		4	3		3	4	0.293	2	5	
II	32	20		38	14		10	42		12	40	
III	20	9		23	6		10	19		7	22	
IV	1	0		1	0		0	1		0	1	

total histological score ≥ 4 denotes a high level of expression [21].

Statistical analysis

The expression levels of ANXA1, TREX1 and APE1 between ESCC and paracancerous tissues were compared using the non-parametric Mann-Whitney U-test. The relationship between ANXA1, TREX1 and APE1, and clinicopathologic characteristics were tested by Chi-square test or Fisher's exact test. The Kaplan-Meier method and the long-rank test were performed to compare the survival rates. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were calculated using Cox regression models for overall survival in uni- and multivariate analysis. A *P*-value < 0.05 was deemed statistically significant. All analyses were performed using the SPSS 19.0 software package (Chicago, IL, USA).

Results

The expression levels of ANXA1, TREX1 and APE1 in ESCC and paracancerous tissues

The expression levels of ANXA1, TREX1 and APE1 in 93 ESCC tissues were evaluated by IHC. The ANXA1-positive site was located in the cytoplasm and nucleus (**Figure 1**). The majority of ESCC showed negative or low expression of cytoplasmic and nuclear ANXA1 (68/93, 73.1%; 58/93, 62.4%, respectively), whereas a subset (25/93, 26.9%; 35/93, 37.6%) of ESCC showed high expression of cytoplasmic and nuclear ANXA1. The levels of cytoplasmic ANXA1 in cancer tissues were significantly higher than those in paracancerous tissues ($P < 0.001$), but nuclear ANXA1 showed a lower expression in ESCC tissues ($P < 0.001$). TREX1 was mainly localized in nucleus of both ESCC and adjacent non-cancerous cells. A weak positive cytoplas-

Annexin A1 and ESCC

Table 3. Univariate and multivariable Cox regression analysis of overall survival ($n = 93$)

Features	Univariate analysis		Multivariable analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (years), > 60 vs ≤ 60	1.016 (0.540-1.914)	0.961		
Gender, female vs male	0.782 (0.374-1.635)	0.513		
Pathologic type, medullary vs others	0.932 (0.564-1.540)	0.784		
Histologic grade, 1+2 vs 3	0.648 (0.255-1.649)	0.362		
Tumor size (cm), > 4 vs ≤ 4	1.313 (0.703-2.449)	0.393		
LNM, positive vs negative	2.181 (1.092-4.357)	0.027	1.425 (0.443-4.591)	0.552
TNM, III+IV vs I+II	2.677 (1.235-5.802)	0.013	3.515 (0.973-12.700)	0.055
Nuclear ANXA1 (low vs high)	0.464 (0.252-0.855)	0.014	0.448 (0.236-0.849)	0.014
Cytoplasm ANXA1 (low vs high)	1.129 (0.554-2.301)	0.738		
Nuclear TREX1 (low vs high)	1.138 (0.560-2.316)	0.721		
Nuclear APE1 (low vs high)	1.328 (0.635-2.777)	0.451		

mic staining for TREX1 was observed in 26% (29/93) of cancer samples, whereas positive nuclear staining was found in 93.5% (87/93) of cancer samples. There were significant difference in cytoplasmic and nuclear expression of TREX1 between cancer and paracancerous tissues ($P = 0.038$ and < 0.001 , respectively). APE1 was mainly localized in nucleus, whereas cytoplasmic APE1 expression was relatively rare. Nuclear expression of APE1 was observed in 100% (93/93) of cancer samples. The levels of nuclear APE1 in cancer tissues were also significantly higher than those in paracancerous tissues ($P < 0.001$).

The association of ANXA1, TREX1 and APE1 with clinicopathologic characteristics of ESCC patients

Since the expressions of cytoplasmic TREX1 and APE1 were low and rare in ESCC, respectively, cytoplasmic expressions of TREX1 and APE1 were excluded from further analysis. Nuclear expressions of ANXA1 and APE1 were significantly associated with pathologic type ($P = 0.004$ and 0.040 , respectively) (Table 2). No other difference between ANXA1, TREX1 and APE1 expression and clinicopathologic characteristics was found.

Survival analyses

Overall survival was calculated as the time from the date of ESCC diagnosis to death. Follow-up period ranged from 0.13 month to 52 months (median, 35 months). Among 93 patients with ESCC, 42 (45.2%) patients died as a result of disease progression during the follow-up. The

overall survival rates at 1 and 3 years were 65.6% and 52.0%, respectively. Twenty patients of the 35 who had high expression of nuclear ANXA1 died from ESCC (20/35, 57.1%). In the group of patients with low expression of nuclear ANXA1, 22 died from the disease (22/58, 37.9%). ESCC patients with low expression of nuclear ANXA1 had a significantly longer survival time compared with those with high expression of nuclear ANXA1 (HR = 0.464, 95% CI 0.252-0.855, $P = 0.014$) (Table 3, Figure 2). Furthermore, TNM and LNM were significantly associated with shorter survival time in univariate analyses (Table 3). Upon multivariate analysis, only low expression of nuclear ANXA1 was significantly associated with better survival rate (HR = 0.448, 95% CI 0.236-0.849, $P = 0.014$). Stratified analysis revealed that low expression of nuclear ANXA1 was significantly associated with better prognosis in ESCC patients with TNM stages III and IV (HR = 0.212, 95% CI: 0.050-0.899, $P = 0.035$) or histologic grade 1 and 2 (HR = 0.371, 95% CI: 0.193-0.713, $P = 0.003$). After adjustment for LNM and TNM, the association between nuclear ANXA1 and overall survival in patients with histologic grade 1 and 2 remained significant (HR = 0.303, 95% CI: 0.155-0.593, $P < 0.001$).

Discussion

In the present study, we investigated the relationship between the expressions of ANXA1, TREX1 and APE1 and clinical outcome of patients with ESCC. ANXA1, TREX1 and APE1 were dysregulated in ESCC. In addition, decreased expression of nuclear ANXA1 in ESCC was correlated with a favorable progn-

Annexin A1 and ESCC

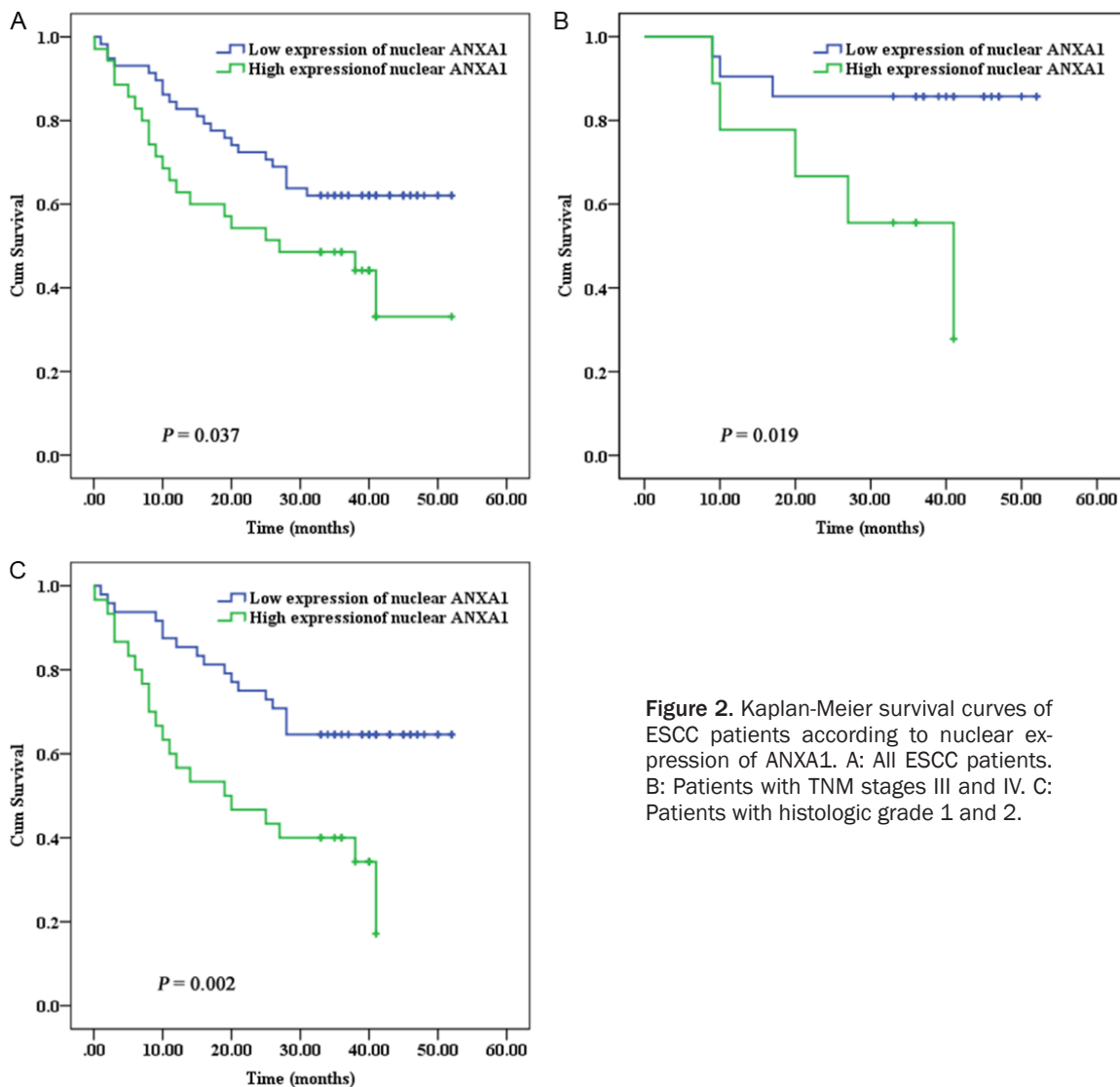


Figure 2. Kaplan-Meier survival curves of ESCC patients according to nuclear expression of ANXA1. A: All ESCC patients. B: Patients with TNM stages III and IV. C: Patients with histologic grade 1 and 2.

sis. These findings indicate that nuclear ANXA1 may have an influence on the progression of ESCC.

The role of ANXA1 in cancer is complicated by the fact that ANXA1 is downregulated in some cancers, including gastric [6, 12], breast [10, 13], prostate [22], cervical [23] and thyroid cancer [24], but upregulated in other types of cancer, such as pancreatic cancer [25]. A controversy exists regarding the expression of ANXA1 in gastric cancer. Cheng et al. [6] and Yu et al. [12] reported that ANXA1 was downregulated in gastric cancer, but Jorg et al. [9] showed overexpression of ANXA1 in gastric cancer. TNM stage may be the one of the main reasons for the inconsistent and contradictory findings. ANXA1 appears to play multifaceted roles in

cancer, and acts as context-dependent tumor suppressor or oncogene. Inhibition of ANXA1 facilitates the growth of prostate cancer cells [5], whereas overexpression of ANXA1 facilitates the migration and invasion of gastric cancer cells [6]. Previous studies revealed that ANXA1 was downregulated in both ESCC and esophageal adenocarcinoma [14, 15]. In the current study, the majority of ESCC showed negative or low expression of ANXA1. However, there was no association between both cytoplasmic and nuclear expression of ANXA1 and histologic grade, which was inconsistent with previous study that ANXA1 was predominantly in well-differentiated ESCC [15]. In this study, most cases were TNM stages I and II, while cases were almost TNM stage III in previous study [15, 26], which may partly explain the dis-

crepant results. Furthermore, recent studies have demonstrated that the levels of ANXA1 may influence the survival in cancer patients [6, 27-30]. Although no association between cytoplasmic expression of ANXA1 and overall survival of ESCC patients was observed, patients with low expression of nuclear ANXA1 had longer survival time, which was in agreement with previous study [27]. Further studies are required to fully understand roles of ANXA1 in ESCC.

Although TREX1 knockout mice does not show an increase in cancer incidence [31], inhibition of TREX1 promotes cell death in malignant glioma and melanoma cells treated with anti-cancer agents [20]. Furthermore, Dong et al. reported that polymorphism in TREX1 [32] was associated with survival in patients with pancreatic cancer. APE1 are frequently overexpressed in some types of cancer, such as gastric cancer [33, 34]. The levels of APE1 have previously been shown to correlate with survival in cancer patients [33]. In the present study, TREX1 and APE1 were upregulated in ESCC tissues, which were consistent with previous studies [20, 34]. However, the level of cytoplasmic TREX1 was significantly lower than those of nuclear TREX1 in paracancerous tissues, which differ from fibroblasts [20]. Tomicic et al. [20] found that nuclear TREX1 seems to be associated with replication. The details of the mechanism of nuclear TREX1 in esophageal cell and ESCC need further studies. However, nuclear expression of TREX1 and APE1 did not correlate with survival. It was amazing that nuclear expressions of both ANXA1 and APE1 correlated with pathologic type. Are there different mechanisms in carcinogenesis between pathologic types? Further studies are warranted to verify our findings and to determine molecular mechanisms underlying the pathogenesis of ESCC.

In summary, high expression of nuclear ANXA1 was found to be a strong risk factor for the overall survival of ESCC. Even though further studies are required to precisely elucidate the role and significance of nuclear ANXA1 in the setting of ESCC, it may be a valuable biomarker for the prediction of ESCC prognosis.

Acknowledgements

This work was supported by the Jiangsu Province Ministry of Health, China (grant No.

H201260), the 12th Five-Year Plan Key Project of Science and Technology, China (grant No. 2013ZX10002007), the Shanghai Committee of Science and Technology, China (grant No. 13440701500), and the Jiangsu Province Science and Technology Support Program, China (grant No. BE2012729).

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Address correspondence to: Dr. Ye Tian, Department of Oncology, The Affiliated Second Hospital of Suzhou University, 181 Sanxiang Road, Suzhou, Jiangsu, China. Tel: +86 512 67783430; Fax: +86 512 68284303; E-mail: dryetian@hotmail.com; Dr. Junxing Huang, Department of Oncology, Taizhou People's Hospital, 210 Yingchun Road, Taizhou, Jiangsu 225300, China. Tel: +86 523 86333483; Fax: +86 523 86333483; E-mail: hjxtz@sina.cn

References

- [1] He J and Chen W. 2012 Chinese Cancer Registry Annual Report. Military Medical Science Press 2012, pp: 27-31.
- [2] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; 61: 69-90.
- [3] Yan W, Wistuba II, Emmert-Buck MR, Erickson HS. Squamous Cell Carcinoma - Similarities and Differences among Anatomical Sites. *Am J Cancer Res* 2011; 1: 275-300.
- [4] Calmon MF, Mota MT, Babeto E, Candido NM, Girol AP, Mendiburu CF, Bonilha JL, Silvestre RV, Rosa BM, Thome JA, Medeiros GH, Soares FA, Guimaraes GC, de Arruda JG, Oliani SM, Villa LL, Vassallo J and Rahal P. Overexpression of ANXA1 in penile carcinomas positive for high-risk HPVs. *PLoS One* 2013; 8: e53260.
- [5] Mu D, Gao Z, Guo H, Zhou G and Sun B. Sodium Butyrate Induces Growth Inhibition and Apoptosis in Human Prostate Cancer DU145 Cells by Up-Regulation of the Expression of Annexin A1. *PLoS One* 2013; 8: e74922.
- [6] Cheng TY, Wu MS, Lin JT, Lin MT, Shun CT, Huang HY, Hua KT and Kuo ML. Annexin A1 is associated with gastric cancer survival and promotes gastric cancer cell invasiveness through the formyl peptide receptor/extracellular signal-regulated kinase/integrin beta-1-binding protein 1 pathway. *Cancer* 2012; 118: 5757-5767.
- [7] Kang H, Ko J and Jang SW. The role of annexin A1 in expression of matrix metalloproteinase-9 and invasion of breast cancer cells. *Biochem Biophys Res Commun* 2012; 423: 188-194.

- [8] Shimizu T, Kasamatsu A, Yamamoto A, Koike K, Ishige S, Takatori H, Sakamoto Y, Ogawara K, Shiiba M, Tanzawa H and Uzawa K. Annexin A10 in human oral cancer: biomarker for tumoral growth via G1/S transition by targeting MAPK signaling pathways. *PLoS One* 2012; 7: e45510.
- [9] Jorge YC, Mataruco MM, Araujo LP, Rossi AF, de Oliveira JG, Valsechi MC, Caetano A, Miyazaki K, Fazio CS, Thome JA, Rahal P, Oliani SM and Silva AE. Expression of annexin-A1 and galectin-1 anti-inflammatory proteins and mRNA in chronic gastritis and gastric cancer. *Mediators Inflamm* 2013; 2013: 152860.
- [10] Yom CK, Han W, Kim SW, Kim HS, Shin HC, Chang JN, Koo M, Noh DY and Moon BI. Clinical significance of annexin A1 expression in breast cancer. *J Breast Cancer* 2011; 14: 262-268.
- [11] Zhu DW, Liu Y, Yang X, Yang CZ, Ma J, Yang X, Qiao JK, Wang LZ, Li J, Zhang CP, Zhang ZY and Zhong LP. Low Annexin A1 expression predicts benefit from induction chemotherapy in oral cancer patients with moderate or poor pathologic differentiation grade. *BMC Cancer* 2013; 13: 301.
- [12] Yu G, Wang J, Chen Y, Wang X, Pan J, Li Q and Xie K. Tissue microarray analysis reveals strong clinical evidence for a close association between loss of annexin A1 expression and nodal metastasis in gastric cancer. *Clin Exp Metastasis* 2008; 25: 695-702.
- [13] Shen D, Nooraie F, Elshimali Y, Lonsberry V, He J, Bose S, Chia D, Seligson D, Chang HR and Goodglick L. Decreased expression of annexin A1 is correlated with breast cancer development and progression as determined by a tissue microarray analysis. *Hum Pathol* 2006; 37: 1583-1591.
- [14] Wang KL, Wu TT, Resetkova E, Wang H, Correa AM, Hofstetter WL, Swisher SG, Ajani JA, Rashid A, Hamilton SR and Albarracin CT. Expression of annexin A1 in esophageal and esophagogastric junction adenocarcinomas: association with poor outcome. *Clin Cancer Res* 2006; 12: 4598-4604.
- [15] Hu N, Flaig MJ, Su H, Shou JZ, Roth MJ, Li WJ, Wang C, Goldstein AM, Li G, Emmert-Buck MR and Taylor PR. Comprehensive characterization of annexin I alterations in esophageal squamous cell carcinoma. *Clin Cancer Res* 2004; 10: 6013-6022.
- [16] Guo C, Liu S and Sun MZ. Potential role of Anxa1 in cancer. *Future Oncol* 2013; 9: 1773-1793.
- [17] Xia L, Guan W, Wang D, Zhang YS, Zeng LL, Li ZP, Wang G and Yang ZZ. Killing effect of Ad5/F35-APE1 siRNA recombinant adenovirus in combination with hematoporphyrin derivative-mediated photodynamic therapy on human nonsmall cell lung cancer. *Biomed Res Int* 2013; 2013: 957913.
- [18] Dai N, Cao XJ, Li MX, Qing Y, Liao L, Lu XF, Zhang SH, Li Z, Yang YX and Wang D. Serum APE1 autoantibodies: a novel potential tumor marker and predictor of chemotherapeutic efficacy in non-small cell lung cancer. *PLoS One* 2013; 8: e58001.
- [19] Wang CJ, Lam W, Bussom S, Chang HM and Cheng YC. TREX1 acts in degrading damaged DNA from drug-treated tumor cells. *DNA Repair (Amst)* 2009; 8: 1179-1189.
- [20] Tomacic MT, Aasland D, Nikolova T, Kaina B and Christmann M. Human three prime exonuclease TREX1 is induced by genotoxic stress and involved in protection of glioma and melanoma cells to anticancer drugs. *Biochim Biophys Acta* 2013; 1833: 1832-1843.
- [21] Zhang X, He C, He C, Chen B, Liu Y, Kong M, Wang C, Lin L, Dong Y and Sheng H. Nuclear PKM2 expression predicts poor prognosis in patients with esophageal squamous cell carcinoma. *Pathol Res Pract* 2013; 209: 510-515.
- [22] Patton KT, Chen HM, Joseph L and Yang XJ. Decreased annexin I expression in prostatic adenocarcinoma and in high-grade prostatic intraepithelial neoplasia. *Histopathology* 2005; 47: 597-601.
- [23] Wang LD, Yang YH, Liu Y, Song HT, Zhang LY and Li PL. Decreased expression of annexin A1 during the progression of cervical neoplasia. *J Int Med Res* 2008; 36: 665-672.
- [24] Petrella A, Festa M, Ercolino SF, Zerilli M, Stassi G, Solito E and Parente L. Annexin-1 downregulation in thyroid cancer correlates to the degree of tumor differentiation. *Cancer Biol Ther* 2006; 5: 643-647.
- [25] Bai XF, Ni XG, Zhao P, Liu SM, Wang HX, Guo B, Zhou LP, Liu F, Zhang JS, Wang K, Xie YQ, Shao YF and Zhao XH. Overexpression of annexin 1 in pancreatic cancer and its clinical significance. *World J Gastroenterol* 2004; 10: 1466-1470.
- [26] Huang J, Hu N, Goldstein AM, Emmert-Buck MR, Tang ZZ, Roth MJ, Wang QH, Dawsey SM, Han XY, Ding T, Li G, Giffen C and Taylor PR. High frequency allelic loss on chromosome 17p13.3-p11.1 in esophageal squamous cell carcinomas from a high incidence area in northern China. *Carcinogenesis* 2000; 21: 2019-2026.
- [27] Lin CY, Jeng YM, Chou HY, Hsu HC, Yuan RH, Chiang CP and Kuo MY. Nuclear localization of annexin A1 is a prognostic factor in oral squamous cell carcinoma. *J Surg Oncol* 2008; 97: 544-550.
- [28] Wang D, Zhang H, Fang Z and Yu G. Annexin-1 downregulation is associated with clinical outcome in Chinese patients with hilar cholangiocarcinoma. *Eur Surg Res* 2010; 45: 151-157.

Annexin A1 and ESCC

- [29] Wang LP, Bi J, Yao C, Xu XD, Li XX, Wang SM, Li ZL, Zhang DY, Wang M and Chang GQ. Annexin A1 expression and its prognostic significance in human breast cancer. *Neoplasma* 2010; 57: 253-259.
- [30] Li CF, Shen KH, Huang LC, Huang HY, Wang YH and Wu TF. Annexin-I overexpression is associated with tumour progression and independently predicts inferior disease-specific and metastasis-free survival in urinary bladder urothelial carcinoma. *Pathology* 2010; 42: 43-49.
- [31] Morita M, Stamp G, Robins P, Dulic A, Rosewell I, Hrivnak G, Daly G, Lindahl T and Barnes DE. Gene-targeted mice lacking the Trex1 (DNase III) 3'→5' DNA exonuclease develop inflammatory myocarditis. *Mol Cell Biol* 2004; 24: 6719-6727.
- [32] Dong X, Li Y, Hess KR, Abbruzzese JL and Li D. DNA mismatch repair gene polymorphisms affect survival in pancreatic cancer. *Oncologist* 2011; 16: 61-70.
- [33] Al-Attar A, Gossage L, Fareed KR, Shehata M, Mohammed M, Zaitoun AM, Soomro I, Lobo DN, Abbotts R, Chan S and Madhusudan S. Human apurinic/apyrimidinic endonuclease (APE1) is a prognostic factor in ovarian, gastro-oesophageal and pancreatico-biliary cancers. *Br J Cancer* 2010; 102: 704-709.
- [34] Fareed KR, Al-Attar A, Soomro IN, Kaye PV, Patel J, Lobo DN, Parsons SL and Madhusudan S. Tumour regression and ERCC1 nuclear protein expression predict clinical outcome in patients with gastro-oesophageal cancer treated with neoadjuvant chemotherapy. *Br J Cancer* 2010; 102: 1600-1607.