European Review for Medical and Pharmacological Sciences 2013; 17: 752-757

Clinical significance of phospholipase A2 group IIA (PLA2G2A) expression in primary resected esophageal squamous cell carcinoma

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Abstract. – AIM: The aim of this study was to clarify the clinico-pathological outcome and prognostic significance of phospholipase A2 group IIA (PLA2G2A) in esophageal squamous cell carcinoma (ESCC).

PATIENTS AND METHODS: Immunohistochemical staining for PLA2G2A was performed on surgical specimens obtained from 132 patients with ESCC, and 43 from matched adjacent non-malignant sites. Differences in PLA2G2A expression and clinical characteristics were compared by χ^2 test. Correlations between prognostic outcomes and with PLA2G2A expression were investigated using Kaplan-Meier analysis and the Cox proportional hazards model.

RESULTS: Immunoreactivity of PLA2G2A was observed in 32% (42 of 132) of ESCC tissues compared with negative staining in matched adjacent non-malignant sites. In addition, PLA2G2A expression inversely correlated with pathological classification ($p < 0.05$ for T, N, and M classifications) and clinical staging ($p = 0.03$). Furthermore, patients with positive PLA2G2A had prolonged overall survival ($p < 0.01$).

CONCLUSIONS: Reduced PLA2G2A expression may be a risk factor for advanced clinicopathological classification and poor patient survival. These findings suggest that PLA2G2A may serve as a useful marker for the prognostic evaluation of ESCC patients.

Key Words:
Esophageal squamous cell carcinoma, Phospholipase A2 group IIA, Prognosis.

Introduction

Esophageal carcinoma, one of the most aggressive carcinomas of the gastrointestinal tract, is the sixth most common cause of cancer related death in the world[1]. Two main types of esophageal carcinoma, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma, with distinct etiological and pathological characteristics occur worldwide with variable geographic distribution. ESCC is more prevalent in China and other Asian countries[2-3]. Although effective surgical and radiation treatment exist for clinically localized esophageal cancer, refractory metastatic esophageal cancer remains incurable. Distinct sets of genes and proteins dictate the progression from precursor lesions to localized disease and finally to metastatic disease[4]. In this respect, an assessment of metastatic potential is important to establish appropriate therapeutic modalities for ESCC. It is of great clinical value to find sensitive and specific early biomarkers for the diagnosis and prognosis of this malignancy, as well as novel therapeutic strategies.

Phospholipase A2 (PLA2) catalyzes hydrolysis of the sn-2 fatty acyl ester bond of phosphoglycerides, releasing free fatty acids and lysophospholipids. One of the fatty acids that can be released from membrane stores by the activity of PLA2 is arachidonic acid, the critical precursor for biosynthesis of diverse eicosanoids, including prostaglandins, thromboxanes, and leukotrienes[5]. At least 15 human genes encode different PLA2 isoenzymes, including both secreted and cytosolic forms6. PLA2 group IIA (PLA2G2A) is a secreted PLA2.

PLA2G2A has been reported to be expressed in human colorectal carcinoma[7-9], gastric cancer[10], pancreatic cancer[11], and prostate cancer[13], and lung cancer[14]. PLA2G2A seems to play diverse roles in human diseases. In gastric cancer, PLA2G2A expression correlated negatively with depth of mural invasion, lymph node metastasis and tumour-node-metastasis (TNM) stage. Patients with positive PLA2G2A expression showed higher 5 year overall survival[12]. In pancreatic cancer[11], the presence of PLA2G2A was associated with a higher degree of fibrosis.

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Furthermore, there was a significant correlation between the enhanced expression of PLA2G2A and longer survival after surgery. However, in malignant esophageal adenocarcinoma cell lines[15], treatment with specific PLA2G2A inhibitor resulted in dose-dependent reductions in growth and cell number in both cell lines. Overexpression of PLA2G2A resulted in enhanced cancer cell growth, whereas gene knockdown attenuated growth. Therefore, PLA2G2A may predict survival and might be a potential biomarker for early detection and individualized therapy.

The objective of this study was to investigate the expression of PLA2G2A in esophageal squamous cell cancer (ESCC) and further explore its clinical significance. All of the procedures in the present study were approved by the Ethics Committee, Tianjin Medical University Cancer Institute and Hospital and Key Laboratory of Cancer Prevention and Therapy.

Patients and Methods

Patients and Specimens

There were 132 patients diagnosed with ESCC who underwent R0 esophagectomy with lymph node dissection between 2000 and 2002 at the Department of Esophageal Cancer, Tianjin Medical University Cancer Institute and Hospital and Key Laboratory of Cancer Prevention and Therapy. The median age of the patients was 64.6 (range, 37-86) years. None of these patients underwent endoscopic mucosal resection, preoperative chemotherapy or radiotherapy, or had metachronous multiple cancer in other organs. Clinico pathological tumor-node-metastasis staging was determined by the extent of tumor invasion in the esophageal wall and lymphatic and venous invasion status according to the criteria proposed by International Union Against Cancer criteria[14]. All patients were followed-up after discharge, with x-ray examination performed every 1 to 3 months, computed tomography every 3 to 6 months, and ultrasonography every 6 months. Bronchoscopy and endoscopy were performed if necessary. Postoperative follow-up data were obtained from all patients, with a median follow-up period of 37 (range, 1-134) months. According to TNM classification, 16 of the 132 patients had T1 tumors, 35 patients had T2 tumors, 49 patients had T3 tumors, and 32 patients had T4 tumors.

Immunohistochemical Staining and Evaluation of PLA2G2A in ESCC

The paraffin-embedded 132 cases of ESCC samples, and 30 cases of non-cancerous adjacent tissues were collected. 4 μm thickness were prepared from the formalin-fixed and paraffin-embedded tissue blocks. After deparaffinization and blocking of endogenous peroxidase by 0.3% hydrogen peroxide in methanol for 30 min, the sections were immersed in 10 mM citrate buffer (pH 6.0), treated for 25 min in a microwave oven, and washed in 50 mM Tris-buffered saline (TBS; pH 7.6). After preincubation with normal goat serum (DAKO), the slides were incubated with the mouse monoclonal anti-PLA2G2A (1:200, Cayman Chemical, Ann Arbor, MI, USA), for 1h at room temperature. The sections were washed with TBS, incubated with the secondary Ab (diluted 1: 50) for 30 min, and washed again with TBS. The peroxidase reaction was visualized by using 0.05% diaminobenzidine tetrahydrochloride (DAB) containing 0.01% hydrogen peroxide. Counterstaining was carried out with hematoxylin.To ensure specificity of the immunostaining reactions, consecutive tissue sections were incubated either in the absence of the primary antibody or with an irrelevant IgG antibody. In both cases, no immunostaining was detected.

IHC (Immunohistochemistry) results were assessed according to a scoring system based on the criteria of Xing et al[10]. The analysis was assessed according to both the percentage of positive cells and the intensity of cytoplasmic reactivity. Each histological section was examined at ×40 to identify areas of maximum tumour staining. At ×200 or ×400, cells were analysed from five areas of maximum tumour staining from each case and the average percentage of positive cells was recorded. These averaged values were stratified into four scoring groups: –, not detected; +/-, < 10% positive cells; +, 10-20% positive cells; ++, 20-50% positive cells; +++, > 50% positive cells. In the statistical analysis, – and +/- were considered negative; + and above were considered positive.

Statistical Analysis

A standard Chi-square test was performed to assess the association between PLA2G2A expression and clinicopathological parameters, except for age, which was assessed by Student's *t*-test. Survival curves were plotted with method of Kaplan-Meier. The statistical difference in survival between different groups was compared by

the log-rank test. Survival correlation with the prognostic factors was further investigated by multivariate analysis using Cox proportional hazards model with backward stepwise likelihood ratio. Statistical analysis was performed using SPSS statistical software (SPSS Inc., Chicago, IL, USA). Statistical significance was assumed for $p < 0.05$.

Results

Expression of PLA2G2A in Esophageal Squamous Cell Carcinoma (ESCC)

PLA2G2A expression was identified in cytoplasm of ESCC and was detected rarely in noncancerous adjacent tissues. As shown in Figure 1, the intensity of PLA2G2A staining was remarkably higher in ESCC than in matched non-cancerous adjacent tissues, which had absent staining. Positive PLA2G2A expression were found in 42 of 132 neoplasms and no cases of non-cancerous adjacent tissues (32% versus 0%, $p = 0.002$).

Relationship Between PLA2G2A Expression and Clinicopathological Features

Positive PLA2G2A expression was detected less at deeper areas in ESCC. The correlations between PLA2G2A expression and clinicopathological characteristics are shown in Table I. The PLA2G2A-negative group had significantly deeper tumor invasion ($p < 0.05$), more advanced stages ($p < 0.05$), more lymphatic invasion ($p < 0.05$), poor differentiation ($p < 0.05$) and more locoregional recurrence ($p < 0.05$) than the PLA2G2A positive group. Statistical analysis showed inverse relationships between PLA2G2A expression and the tumor invasion, stages, locoregional recurrence, no significant correlation between PLA2G2A expression and age, gender of the patients, tumour size, primary location, distant metastasis, gross apperance and vascular invasion was found in this cohort (Table I).

PLA2G2A Expression and Patients' Survival

The 5-year overall survival rate was significantly higher in patients with positive PLA2G2A expression than in those with negative expression ($p = 0.0018$; Figure 2). ESCC patients with positive PLA2G2A expression had significantly longer overall survival rate compared to patients with negative PLA2G2A expression. PLA2G2A expression showed a significant correlation with disease recurrence. A total of 82.9% (68/82) of patients developing tumor recurrence had PLA2-positive original tumor, as compared to only 17.1% (14/82) patients with positive expression and had no subsequent recurrence (Table I).

Univariate and Multivariate Analysis of Survival

Table II shows the results of univariate and multivariate analyses of the factors related to patient prognosis. Univariate regression analyses revealed that depth of tumor invasion, lymph node metastasis, distant metastasis, venous invasion, locoregional recurrence, and PLA2G2A expression significantly affected postoperative outcome. Multivariate analysis indicated that PLA2G2A expression was one of the independent prognostic factors ($p = 0.005$), along with the depth of invasion ($p = 0.02$), and distant lymph node metastasis ($p = 0.002$).

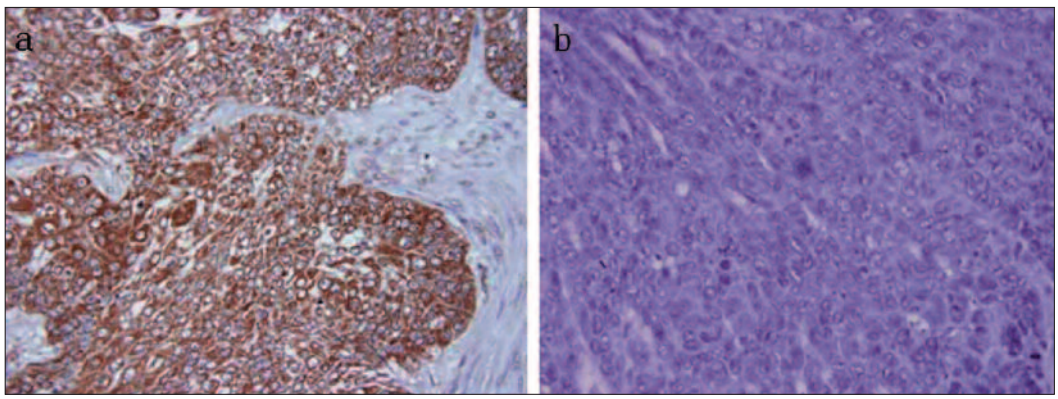

Figure 1. Immunohistochemistry of phospholipase A2 group IIA (PLA2G2A) in ESCC. ***A,*** Strong staining in ESCC tissues; ***B,*** No staining in noncancerous adjacent tissues (× 200). Positive expression of PLA2G2A was detectable in cytoplasm. Scale bar 100 mm.

Table I. Relationship between PLA2G2A expression and clinicopathologic findings.

Clinicopathological factors	PLA2G2A expression		p
	Positive (n=42)	Negative (n=90)	
Age(yr) (mean ± SD)	63.9 ± 10	64.2 ± 10	0.942
Gender			0.146
Male (n=113)	36	77	
Female (n=19)	6	13	
Primary location			0.357
Upper (n=16)	4	12	
Median (n=84)	29	55	
Lower (n=32)	9	23	
Tumor size			0.086
< 3 cm (n=45)	18	27	
> 3 cm (n=87)	24	63	
Gross appearance			0.263
Ulcer type (n=96)	28	68	
Medullar type (n=10)	3	7	
Sclerotic type (n=18)	7	11	
Mushroom type (n=8)	4	4	
Tumor status			0.012
T1 (n=15)	11	4	
T2 (n=12)	9	3	
T3 (n=71)	17	54	
T4 (n=34)	5	29	
Lymph node involvement			0.03
Positive (n=84)	16	68	
Negative (n=48)	26	22	
Distant metastasis			0.104
Positive (n=32)	12	20	
Negative (n=100)	30	70	
Lymphovascular invasion			
Positive (n=40)	18	22	0.156
Negative (n=92)	24	68	
Stage (TNM)			0.03
I (n=16)	10	6	
II (n=35)	19	16	
III (n=49)	8	41	
IV (n=32)	5	27	
Cell differentiation			0.028
Well (n=20)	13	7	
Moderate (n=86)	24	62	
Poor (n=26)	5	21	
Locoregional recurrence			0.014
Negative (n=50)	28	22	
Positive (n=82)	14	68	

Discussion

This is the first report to show an association between PLA2G2A expression and clinicopathological findings in ESCC. PLA2G2A expression positivity was observed in 32% of ESCC tumors, and no staining in noncancerous adjacent tissues was found, suggested that overexpression of cytoplasmic PLA2G2A in tumor cells played an important role in ESCC development.

In pancreatic cancer, PLA2G2A immunoreactivity was present in 65% of the cancer samples[11]. There was a significant correlation between the enhanced expression of PLA2G2A and longer survival after surgery ($p < 0.03$). Spearman analysis disclosed no correlation between PLA2G2A mRNA levels and any variable of the tumour, node, metastasis (TNM) classification, the tumour stage, or the histological tumour grading. In gastric cancer, cytoplasmic immunoreactivity of PLA2G2A was observed in 27% of gastric cancer tissues compared with negative staining in normal mucosa. PLA2G2A expression correlated negatively with depth of mural invasion, lymph node metas-

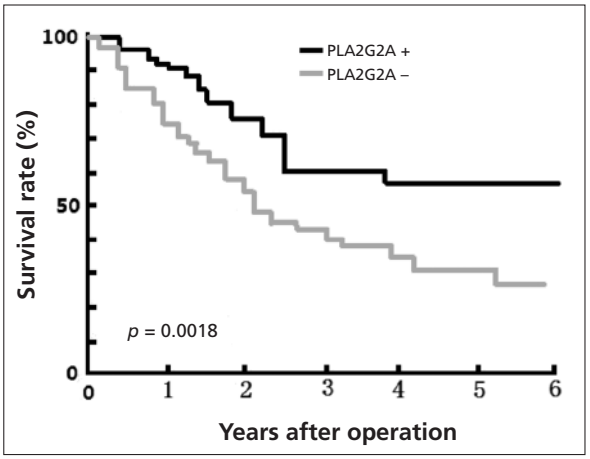

Figure 2. Overall 5 year survival curves of patients with esophageal cancer according to the expression of PLA2G2A. Patients who were positive for PLA2G2A expression exhibited significantly better prognosis than those negative for PLA2G2A expression.

tasis and tumour-node-metastasis (TNM) stage. Patients with positive PLA2G2A expression showed higher 5 year overall survival[10]. However, in colorectal cancer, althought 55% percent of all tumors were positive for PLA2G2A, patients with PLA2G2A-positive tumors living significantly shorter. Furthermore, no significant correlation between PLA2G2A expression and variable of the tumour[17] was detected.

In our study, interesting correlations were found between the PLA2G2A expression and some important clinicopathological parameters. First, PLA2G2A expression was correlated negatively with depth of invasion, lymph node metastasis and tumour-node-metastasis (TNM) stage. Low expression of PLA2G2A being more common among high-grade tumors, metastasis and deeper depth of invasion. Otherwise, the PLA2G2A expression rate in well-differentiated carcinoma was elevated significantly compared with that in poorly differentiated**.** This suggests the role of PLA2G2A as a biological factor that might affect the behavior of the tumor cell population.

In this study, concerning the overall survival analysis, tumor depth, lymph node metastasis, venous invasion, distant metastasis, locoregional recurrence, and PLA2G2A expression were prognostic factors on univariate analysis. We showed that PLA2G2A expression was one of independent prognostic factors, along with tumor depth, and distant metastasis on multivariate analysis. PLA2G2A expression could be used as a useful prognostic parameter predicting the survival of postoperative ESCC patients.

In this study, the 5 year overall survival rate for the patients with PLA2G2A negative expression was significantly worse than those with positive expression. This enables us to choose postoperative treatment, including chemotherapy. Moreover, we showed that low PLA2G2A expression tends to correlate with lymphatic invasion. Thus, cancerous PLA2G2A expression in a biopsy may be informative to predict lymphatic invasion. When selecting whether to perform endoscopic resection for early ESCC, we can use cancerous PLA2G2A expression as a predictor of lymphatic invasion.

Our results are controversial to the studies in colorectal cancer and pancreatic cancer[17-18] and prostate cancer[18-19]. In prostatic cancer, PLA2G2A expression increases with progression and is highest in the most poorly differentiated, highest-grade primary cancers[18-19]. In colorectal cancer and pancreatic cancer, although PLA2G2A acts as a negative prognostic determinant in stage II colorectal carcinoma and prostatic cancer, but cannot predict longer survival after

Table II. Univariate and multivariate survival analysis in patients with ESCC.

Characteristic	Univariate analysis	Multivariate analysis		
	p value	HR	95% CI	*p* value*
Age, years (< 60 vs. ≥ 60)	0.453	1.373	0.884-1.548	0.469
Gender (male vs. female)	0.286	1.564	0.638-1.892	0.732
Lymph node involvement (yes vs. no)	0.003	1.048	1.341-2.56	0.086
Distant metastasis (yes vs. no)	< 0.001	10.42	8.284-35.48	0.002
pTNM (I-II vs. III-IV)	0.0002	1.63	1.252-3.287	0.019
Tumor status (T1+T2 vs T3+T4)	0.001	1.78	1.264-3.149	0.02
Lymphovascular invasion (yes vs. no)	< 0.001	1.235	0.854-1.869	0.453
Locoregional recurrence (negative vs. positive)	0.002	1.258	0.848-2.154	0.542
PLA2G2A expression (negative vs. positive)	0.005	1.92	2.264-4.873	0.036

surgery in pancreatic cancer[17-19]. These controversial findings may be attributed to its interaction with the microenvironment and divergent genetic pathways present in different tissues (20). However, this remains only speculative at this stage, and future molecular studies are necessary to confirm this hypothesis[10].

Conclusions

PLA2G2A expression in ESCC plays a critical role in tumor invasion, and may represent a useful prognostic marker for ESCC.Low expression of PLA2G2A in ESCC may be a predictor of poor overall survival. This independent molecular marker for high-risk patients could help us in our attempts to individualize patients' therapy.

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