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Original Article

Expression of ATP7A in esophageal squamous cell carcinoma (ESCC) and its clinical significance

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Abstract: Objective: This study investigated the expression of P-type copper transporting adenosine triphosphatase ATP7A in the tumor tissues of patients with advanced esophageal squamous cell carcinoma (ESCC), and analyzed its correlation to clinicopathologic features and prognosis of advanced ESCC patients. Methods: The expression of ATP7A protein in 49 specimens of advanced ESCC patients who were treated with first line cisplatin-based chemotherapy without surgery or radiotherapy, was detected by immunohistochemistry. The correlation of ATP7A expression with clinicopathologic features and prognosis of advanced ESCC patients weas analyzed by SPSS 16.0 statistical software package. Results: Positive ATP7A staining was observed in cytoplasm of ESCC cells in 44. of tumors (22 of 49 cases), but was not detected in adjacent stroma of tumor tissue. ATP7A expression status was correlated with response to histologic grade and cisplatin-based chemotherapy (*P* values 0.02, 0.028 respectively). No significant association was found between ATP7A expression and age (P=0.085), gender (P=0.74), or PS (P=0.56). Kaplan-Meier analysis indicated that advanced ESCC patients positive for ATP7A positive had overall survival (OS) inferior to advanced ESCC patients who were ATP7A negative (*P* value was 0.037 by log-rank test). In univariate analysis, histologic grade and ATP7A expression were significantly correlated with OS (P=0.011 and 0.049 respectively); in multivariate analysis, histologic grade and ATP7A were independent factors significantly related to OS for advanced ESCC patients treated by cisplatin-based chemotherapy (*P* values 0.039 and 0.043 respectively). Conclusion: ATP7A was positively expressed in the majority of advanced ESCC tissues. The expression level of ATP7A was an important factor affecting tumor tissue's histologic grade, the response to platinum-based chemotherapy and the prognosis of advanced ESCC patients. This indicates that ATP7A might be involved in the genesis and development of ESCC, and could be a resistance marker for platinum-based chemotherapy, and a prognostic factor for survival in patients with ESCC treated by Pt-based chemotherapy.

Keywords: Esophageal squamous cell carcinoma (ESCC), platinum derivatives, copper transporter, ATP7A

Introduction

Esophageal cancer is the 6th most common cause of cancer death worldwide [1] and it is endemic in many parts of the world, particularly in developing countries, including China [2, 3]. Histologicly, esophageal cancer can be classified as adenocarcinoma or esophageal squamous cell carcinoma (ESCC) which is the most common histology in Asia [1]. For locally advanced or metastatic ESCC patients, chemotherapy can improve overall survival (OS) and progression free survival (PFS) [4, 5]. Cisplatin (DDP) is one of the most active agents with a single-agent response rate of about 20% [6]. One of the most important problems in the treatment of ESCC is intrinsic/acquired resistance to platinum derivatives (DDP, CBDCA, and L-OHP) [7]. Knowledge of the active mechanism of -resistance may lead to new treatment strategies by overcoming platinum resistance and by the selection of platinum-resistant patients for specific treatment modalities, so as to improve the survival of patients with ESCC.

As an essential trace element and catalytic factor for many enzymes, copper plays an important role in human physiology and metabolism.

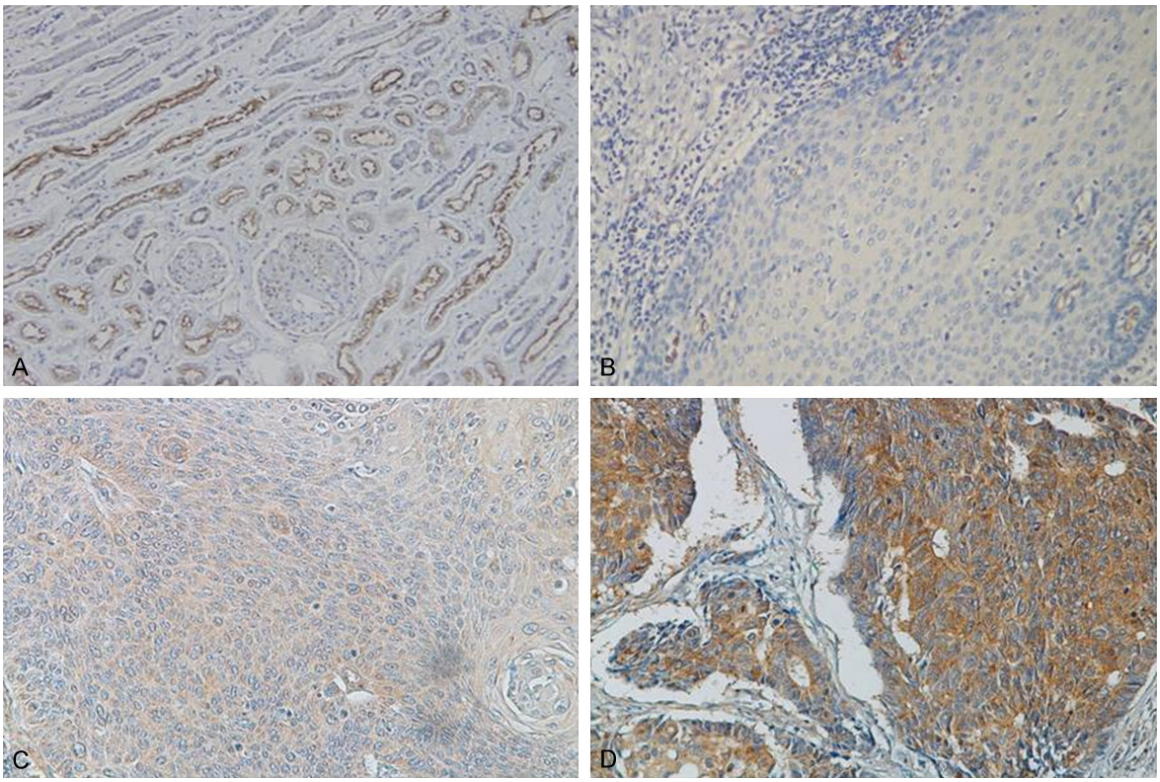

Figure 1. Immunohistochemical staining of normal and ESCC human tissues for ATP7A. A. Adult normal kidney tissue as positive control; B. Adult normal esophageal tissue as negative control; C. ATP7A negative case: ATP7A expression is not detectable in the cytoplasm; D. ATP7A positive case, almost all tumor cells show strong positive reaction in the cytoplasm.

Table 1. The clinicopathologic characteristics of 49 patients with ESCC treated with platinum-based chemotherapy

Characteristics	Number of patients			P
	Total	ATP7A(-)	ATP7A(+)	
Age				0.085
Median	52	53	51	
Range	31-81	31-81	31-77	
Gender				0.74
Male	28	16	12	
Female	21	11	10	
PS				0.56
0	24	14	10	
>0	25	13	12	
Histologic Grade				0.02
Low	19	6	13	
Moderate	20	13	7	
High	10	8	2	
Response to chemo				0.028
PR	17	13	4	
SD+PD	32	14	18	

Its homeostasis is tightly regulated, including copper uptake, intracellular distribution, and copper export [8, 9]. The three processes are mediated through the coordinated action of the copper uptake protein Ctr1, and copper export proteins ATP7A and ATP7B. Recent reports showed that import and export copper transporters are also involved in the transport of platinum. Ctr1 has been demonstrated to transport cisplatin and its analogues, such as carboplatin and oxaliplatin [10, 11]. Evidence also suggests that the two copper efflux transporters ATP7A and ATP7B regulate the efflux of DDP [12, 13]. Recently, we found that ATP7A was associated with platinum-resistance in non-small cell lung cancer (NSCLC) [14] and ESCC [15]. ATP7B, another copper efflux transporter, is also implicated in platinum-resistance [16, 17].

Whether ATP7A is a negative prognostic factor for ESCC patients treated with platinum-based chemotherapy is still in doubt. Hence,

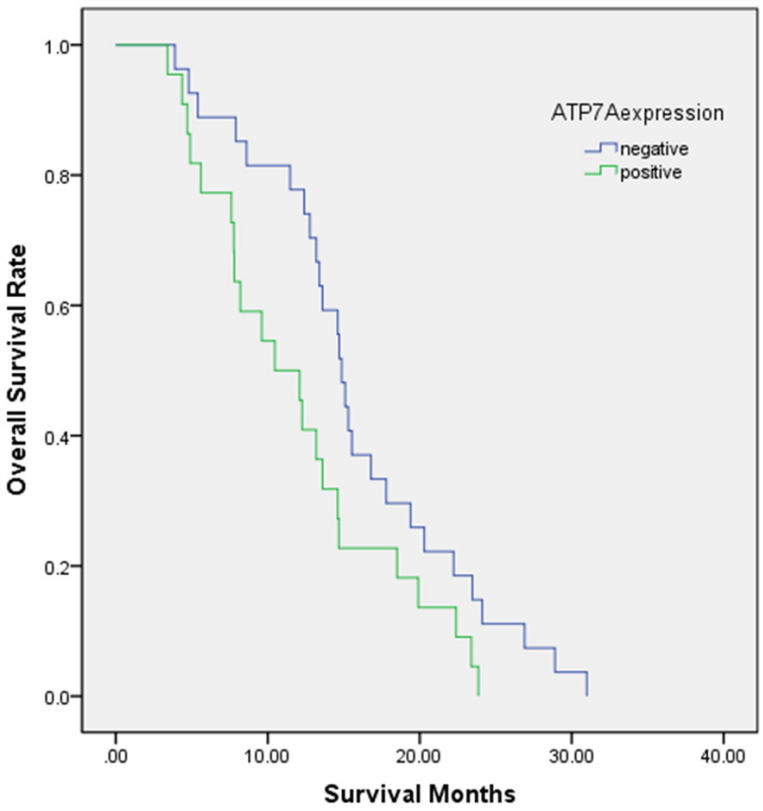

Figure 2. Overall survival curves of ESCC treated with platinum-based chemotherapy. Comparison of survival curves for patients whose tumors stained positive for ATP7A with those patients whose tumors were classified as negative for ATP7A expression. Curves present the results for all patients. *P* value was obtained by Log-rank test.

knowledge of the active mechanism of platinum resistance in ESCC may lead to new treatment strategies and allow the selection of patients for specific treatment modalities.

Methods

Immunohistochemical staining

Immunohistochemical staining was performed according to the guidelines of the Catalyzed Signal Amplification System (ZSGB-BIO, Beijin, China). The slides were incubated with a 1:200 dilution of anti-ATP7A antibody (Abcam, Cambrige, UK). Adult normal kidney and esophageal samples were taken as positive control and negative control respectively. The slides were examined and scored by two pathologists independently without knowledge of clinical information of the patients. If more than 10% of the tumor cells were stained, a sample was considered to be ATP7A-positive carcinoma [18, 19].

Statistical analysis

Data analysis was performed using SPSS 16.0 statistical software package (SPSS, IL, USA). Continuous variables were analyzed using Student's t test. As qualitative variables, the clinicopathologic characteristics of 49 patients with ESCC were analyzed using chi-square test. Survival curves were determined using Kaplan-Meier method, and differences in survival between subgroups were compared by log-rank test. Multivariate prognostic analysis was performed using a Cox proportional hazards model. All reported *P*-values were two-sided. P<0.05 was considered significant.

Results

ATP7A expression was negatively correlated with response to DDP-based chemotherapy in ESCC. To investigate the expression of ATP7A in ESCC tissue, and analyze its correlation to the clinicopathologic features and prognosis of ESCC, immunohistochemistry was performed. As shown in **Figure 1**, ATP7A was detected in cytoplasm of tumor cells in 22 of 49 (44.9%) tumors but not in adjacent stroma nor normal esophageal tissue. Associations were sought between ATP7A expression and the clinicopathologic characteristics of 49 patients including age, gender, stage, performance status (PS), histologic grade, response to chemotherapy, and overall survival (OS). The clinical of the 49 patients in this study are summarized in **Table 1**. ATP7A expression was inversely correlated with histologic grade (P=0.02) and DDP-based chemotherapy (P=0.028). No significant association was found between ATP7A expression and age (P=0.085), gender (P=0.74) and PS (P=0.56). The median overall survival (mOS) of all patients was 14.11 months. In 22 patients with ATP7A positive staining, the mOS was 11.95 months, and the mOS was 15.87 months in 27 patients with ATP7A negative staining. As shown in **Figure 2**,

Kaplan-Meier analysis indicated that ATP7A positive patients had inferior survival compared with ATP7A negative patients (P=0.037, log-rank test). By univariate analysis, histologic grade and ATP7A expression were significantly correlated with OS (P=0.011 and 0.049 respectively); Cox proportional hazards model analysis showed that 2 independent factors were significantly related to overall survival: histologic grade (P=0.039) and ATP7A (P=0.043).

Discussion

This article sheds new light on the potential function of copper transporter ATP7A in ESCC. We found that 44.9% (22/49) of ESCC patients aberrantly expressed ATP7A. However, ATP7A protein was not detected in tumor-adjacent stroma nor normal esophageal tissue. This suggested that ATP7A might be involved in the transformation of a normal differentiated cell to a malignant tumor cell. Compared with ATP7A-negative patients, ATP7A-positive patients had lower histologic grade (P=0.02), inferior response to platinum-based chemotherapy (P=0.028) and poorer OS (P=0.037). Cox proportional hazards model analysis showed that ATP7A expression was an independent prognostic factor for survival. The present study in ESCC patients also showed similar results that ATP7A-mediated resistance to platinum derivatives was increased in cancer cells, and was associated with poor survival in ESCC patients during platinum drug-based treatments [18].

Conclusions

Our results demonstrate that overexpression of copper efflux transporter ATP7A is a chemoresistance marker and a negative prognostic factor for survival in ESCC patients treated with platinum-based chemotherapy. It provides a basis for better utilization of platinum-based antitumor agents so as to improve the survival of ESCC patients.

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Disclosure of conflict of interest

None.

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