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Original Article

Correlation between aldolase c (ALDOC) expression and the prognosis of esophageal cancer

Xiangxiang Tao[1], Bing Chen[1], Yifeng Yan[2]

Departments of [1]Pathology, [2]Forensic Pathology, Wannan Medical College, No. 22, Wenchang West Road, Wuhu 241001, Anhui, China

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Abstract: Objective: To study the correlation between ALDOC expression and the prognosis of esophageal carcinoma (EC). Methods: Tissue was taken from all EC patients (n = 100) included in this study and ALDOC expression was determined by immunohistochemistry and the correlation between ALDOC expression and the prognosis of EC was evaluated. Results: High ALDOC expression in cancer tissue significantly correlated with EC (Z = -7.179, P = 0.000), tumor grade (r^2 = -0.253, P = 0.011) and T stage (r^2 = -0.244, P = 0.016). Moreover, survival analysis indicated that ALDOC expression in cancer tissues served as an independent correlation factor. Conclusion: High ALDOC expression correlates with poor prognosis in EC patients.

Keywords: ALDOC, esophageal cancer, immunohistochemistry, prognosis, tissue chip

Introduction

Esophageal cancer (EC) is one of the most aggressive malignancies worldwide and ranks in the top five of the deadliest cancers in China [1]. Estimated numbers of new EC cases and deaths were 291,238 and 218,957 in China, 2011, respectively [2]. One of the key characteristics of EC is its heterogenic appearance; EC has a higher incidence in Asian countries compared to European and American countries [3]. Despite recent advances in EC prognosis and treatment, the pathogenesis of EC is still not well understood. Therefore, it is of utmost importance to uncover the underlying mechanism of tumor cell infiltration and metastasis, as well as understanding the molecular mechanism of action of EC for the improvement of EC therapy. Consequently, identifying molecular targets for treatment may lead to novel effective therapies for the treatment of EC.

Aldolases (ALDOs) are a class of essential enzymes that play a key role in processing glycolysis. ALDOs also has non-glycolytic roles, for example, it interactions with vacuolar-H^+-adenosine triphosphate synthase and other molecules [4]. The ALDO family includes 3 members: ALDOA, which is present in muscle [4], ALDOB, which is predominantly expressed in the liver [5], and aldolase C (ALDOC), which is observed in the brain [6]. Due to its specific expression in distinct subpopulations of cerebellar Purkinje cells (PCs), ALDOC (= zebrin II) has long been used as a marker for studying cerebellar compartmentalization [7]. To date, no reports are available that focus on studying ALDOC expression in EC. Various *in vitro* and *in vivo* experiments as well as more clinically-oriented studies have demonstrated high ALDOC gene expression in tissue derived from various squamous cell carcinoma tumors [8]. One finding identified Aldolase positively regulated the canonical Wnt signaling pathway. Numerous studies have indicated the association between cell signaling pathways and tumorigenesis [9]. In this respect, the Wnt signaling pathway plays an important role during development and self-renewal of EC [10] and indicates ALDOC may be involved in the process of tumor initiation and development. The current study focuses on the correlation between ALDOC expression and EC prognosis, which is crucial for exploring the underlying molecular mechanism of ALDOC in participating in EC invasion and metastasis. The above results suggest that ALDOC is a

Table 1. Expression of ALDOC in relation to pathologic and clinical parameters

Parameter	Case No. (%)	ALDOC expression		r^2	P
		Low	High		
Sex					
Male	74 (74.0%)	73	1	-0.186	0.064
Female	26 (26.0)	26	0		
Age (year-old)					
≤ 65	51 (51.0%)	50	1	-0.048	0.636
> 65	49 (49.0%)	49	0		
Tumor size					
≤ 5 cm	58 (58.0%)	57	1	0.021	0.848
> 5 cm	27 (27.0%)	27	0		
Grade					
I	6 (6.0%)	6	0	-0.253	0.011
II	66 (66.0%)	65	1		
III	28 (28.0%)	28	0		
T Stage					
T1	4 (4.1%)	4	0	-0.244	0.016
T2	11 (11.3%)	11	0		
T3	79 (81.4%)	78	1		
T4	3 (3.1%)	3	0		
Lymph node metastasis (N region)					
N0	45 (45.9%)	45	0	-0.118	0.247
N1	31 (31.6%)	31	0		
N2	17 (17.3%)	16	1		
N3	5 (5.1%)	5	0		
Clinical stage					
1	4 (4.2%)	4	0	-0.195	0.057
2	42 (43.8)	42	0		
3	50 (52.0%)	49	1		

r^2: correlation coefficient. Positive r^2 relates to positive correlation, whereas a negative r^2 is indicative of a negative correlation. The closer the absolute value of r^2 to 1, the greater the relativity.

promising marker for EC prognosis and a potential novel target for EC therapy.

Materials and methods

Source of samples

The EC tissue chip (HEso-Squ180Sur-04) was built by Shanghai Outdo Biotech Co., Ltd. SOBC and includes 100 samples of EC tissue and 80 samples of corresponding adjacent tissues (adjacent tissue is classified as tissue within a range of 1.5 cm from the tumor tissue). All EC patients underwent surgery between July 2006 and December 2008. The last patient interviewed was interviewed in September 2014. All cases were clinically diagnosed with EC and no presurgical treatment was given. This study incuded 74 males and 26 females with a median age of 65 years.

Immunohistochemical analysis

Immunohistochemical analysis was performed using a two-step method. Tissues underwent high-temperature, high-pressure antigen retrieval after which non-specific sites were blocked with goat serum. Next, an anti-ALDOC antibody was added (1:1000, MAB-12876, Abnova Co., Ltd) and incubated overnight at 4°C. After washing with PBS for 5 min at 4°C, tissues were incubated with a HRP-conjugated anti-rabbit antibody (DAKO, K8000) for 10 min at 4°C. Subsequently, tissues were rinsed with PBS and diaminobenzidine (DAB) solution was applied. Color development was allowed for 2 min. Slides were washed with PBS, counterstained with Hematoxylin and rinsed in running tap water. Tissues were dehydrated through 4 changes of alcohol, cleared in Xylene and mounted using Permount. Staining was evaluated by light microscopy. Positively stained cells were counted using 3 random high-power images, no less than 300 cells were counted. Positive cells were counted and staining intensity was recorded. The scoring for positive staining of cells was as follows: 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%), 4 (76%-100%). The scoring for staining intensity was: 0 (0), 0.5 (0-1+), 1 (1+), 1.5 (1+~2+), 2 (2+), 2.5 (2+~3+), 3 (3+). For each image, two scores were obtained, a "staining intensity score" and a "positive staining score". The two scores were added, resulting in a final score per image. Tissues with a score no larger than 4 were placed into a low ALDOC expression group, whereas those with a score larger than 4 are placed into high ALDOC expression group.

Statistical analysis

The expression of ALDOC in EC and adjacent cancer tissues was analyzed using a paired Wilcoxon test. The association between clinical characteristics of EC patients and ALDOC expression was analyzed using the Pearson

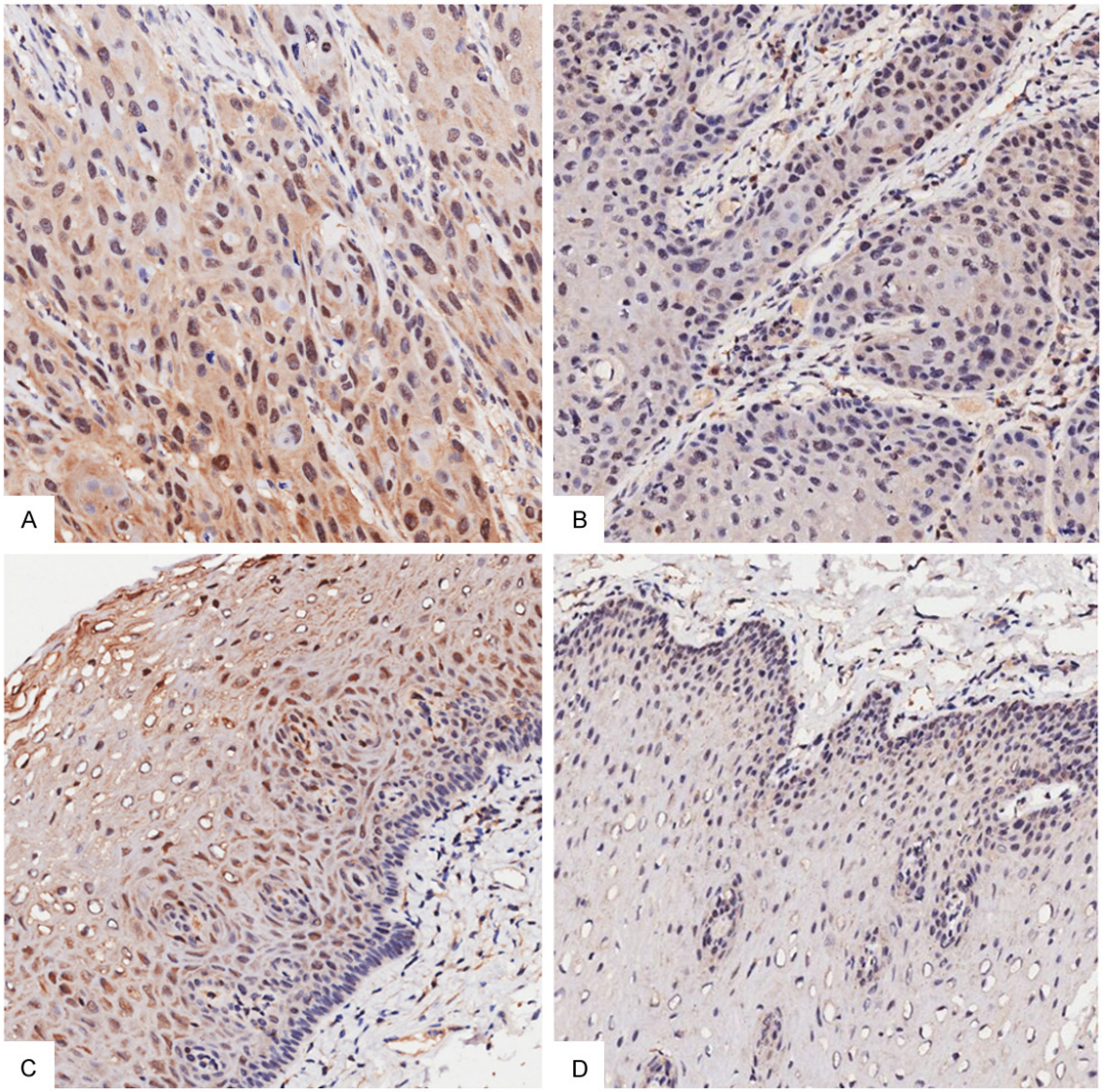

Figure 1. Immunohistochemical analysis of ALDOC protein expression. A. High ALDOC expression in cancer tissue. B. Low ALDOC expression in cancer tissue. C. High ALDOC expression in cancer adjacent tissue. D. Low ALDOC expression in cancer adjacent tissue. Magnification: ×200.

and Spearman's correlation test. The prognosis of EC and ALDOC protein expression was determined Kaplan-Meier survival analysis and Log-rank testing for univariate analysis. The significant variables from the univariate test were included in Cox multivariate regression analysis. $P<0.05$ was considered statistically significant.

Results

Study population

Clinical characteristics of 100 EC patients are shown in **Table 1**. Study subjects were matched for age and gender. Two groups were made that showed a similar ratio of patients with tumor size of ≤ 5.0 cm or > 5.0 cm. Approximately 94.0% of EC patients were categorized as grade 2 and grade 3. Based on the T stage, 4, 11, 79 and 3 patients were classified as T1, T2, T3 and T4, respectively. According to lymph node metastasis in the N region, 45 EC patients were considered N0, whereas 31 patients were classified as N1. In addition, 17 patients were classified as N2 and the remaining 5 patients were classified as N3. Clinical staging classified EC patients into stage 1 (4 people), stage 2 (42 patients) and stage 3 (50 patients).

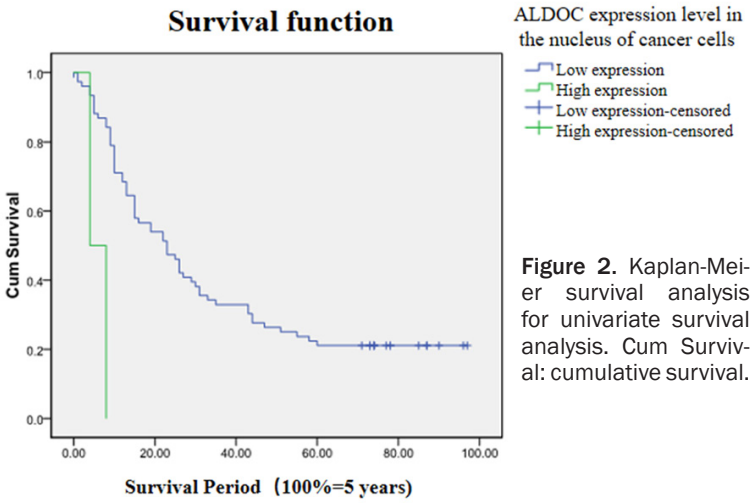

Figure 2. Kaplan-Meier survival analysis for univariate survival analysis. Cum Survival: cumulative survival.

ents with lower ALDOC expression in cancer tissue had a longer life expectancy (P = 0.002) and a significantly higher 5-year survival rate (19.2%) than those with high ALDOC expression (**Figure 2**).

Variables that showed statistical significance in the univariate analysis underwent Cox multivariate regression analysis. This indicated that ALDOC expression was an independent correlation factor of EC prognosis (P = 0.000, **Table 2**).

Expression of ALDOC in relation to EC prognosis

The immunohistochemical findings of ALDOC expression in the nucleus of EC tissue and cancer adjacent tissues (**Figure 1**) underwent Wilcoxon statistical analysis, which indicated that ALDOC expression in EC tissue was notably higher compared to that in the adjacent tissue, 5.730 ± 2.967 μg/ml versus1.474 ± 1.090 μg/ml, respectively.

Additionally, Spearman's rank correlation analysis was conducted to evaluate the relation between ALDOC expression in EC tissues and sex, age, tumor size, tumor grade, T stage, lymph node metastasis in N region and clinical characteristics of the study subjects (**Table 1**). Interestingly, we found significantly higher levels of ALDOC in patients who were classified with 2nd or 3rd grade of EC compared to those classified with 1st grade EC (r^2 = -0.253, P = 0.011). In addition, significantly higher levels of ALDOC were found in patients who suffer from 2nd or 3rd or 4th T stage of EC compared to those that were classified with 1st grade EC (r^2 = -0.244, P = 0.016).

Of the 100 EC cases included in this study, survival was analyzed between December 2008 and September 2014. Data indicated that 81 patients had died from EC with a median follow-up of 14 months (range, 1-60 months) and 19 patients had survived with a median follow-up of 76 months (range, 71-97 months). Univariate survival analysis was conducted using both Kaplan-Meier survival analysis and Log-Rank statistical testing and indicated that EC pati-

Discussion

In this study, we performed immunohistochemistry to evaluate ALDOC expression levels in cancer tissue and cancer adjacent tissue. Our results demonstrated that the level of ALDOC expression in cancer tissue was significantly higher compared to that in cancer adjacent tissue. In addition, Spearman's rank-order correlation analysis indicated a positive correlation between ALDOC expression level and EC. Moreover, the univariate survival analysis including Kaplan-Meier survival analysis and Log-rank statistical testing revealed that EC patients that showed a lower ALDOC expression in cancer tissue have a significantly higher 5-year survival rate (19.2%) compared to those with high ALDOC expression in cancer tissue. Cox multivariate regression analysis indicated that ALDOC expression was an independent correlation variable for the prognosis of EC.

Aldolase is an enzyme that is involved in one of the key steps in glycolysis, a process that takes place in all cells that consume glucose [11]. Aldoc (= zebrin II) has a long history as being a marker for the study of cerebellar compartmentalization [12]. The Wnt signaling pathway can be modified by various proteins, some are known and others have yet to be revealed [11]. Caspi et al. identified a role for ALDOC as a positive regulator of the Wnt signaling pathway and characterized its relationship with components of the Wnt cascade and proposed a mechanism of action [9]. Over-expression of aldolase induces Wnt signaling and dysregulation of Wnt/β-catenin signaling has been linked to a

Table 2. Analysis of independent correlation factors of colorectal cancer prognosis

Factor	Regression coefficient	SE	Wald	DF	*P*-value	Risk	95% CI	
							Lower	Upper
ALDOC expression	3.101	0.843	13.528	1	0.000	22.221	4.257	115.994
Sex	-0.006	0.348	0.000	1	0.986	0.994	0.502	1.967
T stage	0.637	0.398	2.555	1	0.110	1.890	0.866	4.127
Node metastasis	0.251	0.245	1.047	1	0.306	1.286	0.795	2.080
Clinical stage	0.249	0.460	0.293	1	0.588	1.283	0.521	3.161

ALDOC expression: ALDOC is primarily expression in nuclei of esophageal cancer cells; SE: standard error; DF: degree of freedom; CI: confidence interval; Lower: lower limit; Upper: upper limit.

variety of cancers [13]. The Wnt signaling pathway has been reported to be involved in many phases of vertebrate embryonic development as well as in the initiation and progression of human EC [14]. Therefore, ALDOC may play a critical role in the initiation and development of human EC.

A number of studies have reported that ALDOC is not only related to the development of lung squamous cell carcinoma but that it also contributes to the invasion of oral squamous cell carcinoma [8, 15]. However, an association between ALDOC expression levels and EC has not yet been reported. In this study, we showed a significant correlation between elevated ALDOC expression levels and EC, which strongly indicates that ALDOC plays a role in tumor development and invasion.

The mechanism of action of ALDOC in cancer cell invasion is not well understood. Therefore, our future studies will focus on understanding ALDOC+ and ALDOC- allogeneic EC cells in nude mice to increase our understanding of the underlying molecular mechanism(s) of ALDOC in the initiation and development of pancreatic cancer.

In conclusion, our results demonstrate that high levels of ALDOC expression are associated with EC grade and T stage. This suggests that ALDOC has great potential to serve as a prognostic marker by identifying patients with a relatively poor prognosis. Thus, ALDOC may become be a potential molecular target for the treatment of EC.

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Disclosure of conflict of interest

None.

Address correspondence to: Bing Chen, Department of Pathology, Wannan Medical College, No. 22, Wenchang West Road, Wuhu 241001, Anhui, China. E-mail: 281993214@qq.com

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