

## Original Article

# Astrocyte elevated gene-1 expression in urothelial bladder carcinoma and its clinical significance

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**Abstract:** Objectives: To determine the expression of astrocyte elevated gene-1 (AEG-1) in urothelial bladder carcinoma and its clinical significance. Methods: AEG-1 and CD34 expressions in 95 cases of urothelial bladder carcinoma tissues and 10 cases of normal bladder peritumoral tissues were detected by immunohistochemical method. The relation between AEG-1 expression and clinical pathology features in patients with urothelial bladder carcinoma, as well as micro-angiogenesis and its prognosis significance were analyzed. Results: AEG-1 is not expressed in normal bladder mucous membrane tissues. Among 95 cases of urothelial bladder carcinoma tissues, 42 cases (44.2%) had positive expression of AEG-1. AEG-1 expression was positively related to tumor classification and periodization. AEG-1 expression was closely related to tumor micro-angiogenesis and the micro-vessel density of the positive group was evidently higher than that of the negative group. AEG-1 had no evident influence on tumor-free survival of patients with urothelial bladder carcinoma, but it is not an independent prognosis factor. Conclusions: In the urothelial bladder carcinoma tissues, AEG-1 expression increases and it is related to biological behavior of malignant tumor and tumor micro-angiogenesis. AEG-1 may be a novel molecular marker and potential therapeutic target for diagnosis and prognosis prediction of urothelial bladder carcinoma.

**Keywords:** Bladder tumor, cancer, astrocyte elevated gene-1, micro-vessel density

## Introduction

Bladder transitional cell carcinoma is the most common malignant tumor in the urothelium. Study on the molecular mechanism underlying its occurrence and development will be helpful for clinical physicians to make relevant treatment plans according to its recurrence and development risk, so it is of great clinical significance.

Recent studies have found that AEG-1 can promote the proliferation, differentiation, invasion and metastasis of tumor cells. On the contrary, knockdown of AEG-1 gene can effectively inhibit the proliferation, migration and invasion of tumor cells, promote the cell apoptosis and decrease the pulmonary metastasis of breast cancer cells in the body [1-4]. AEG-1 can induce the formation of aggressive tumors in nude mice [5]. These results suggest that AEG-1 is closely related to the tumor malignant biological behavior and promotes the occurrence and development of tumors. Studies on solid tumors such as breast cancer, non-small cell

lung cancer, and esophageal cancer have shown that increased expression of AEG-1 is closely related to tumor differentiation and development, and influences the prognosis of patients [6-8]. But there are no relevant reports about AEG-1 expression in urothelial bladder carcinoma and its clinical significance.

Currently, many studies indicated that the expression level of astrocyte elevated gene-1 (AEG-1) increased in various tumors and it played an important role in the development and metastasis of those tumors, while the expression and its significance of AEG-1 in human urothelial bladder carcinoma have not been studied yet. This study is to investigate AEG-1 expression and its clinical significance in urothelial bladder carcinoma.

## Materials and methods

### Case selection

Ninety-five patients with urothelial bladder carcinoma that had received surgical treatment

from 2000~1 to 2006~1 were collected. The tissue samples were taken from paraffin-embedded tissues saved by the department of pathology in hospital. There were 83 male cases, 12 female cases; with the 25~77 years old age range and the 63 years old median age. Tumor grading and staging were conducted according to the pathology grading standard of World Health Organization (WHO) and the clinical pathological staging standard of International Union against Cancer (UICC): 63 cases of Ta/T1, 23 cases of T2, 9 cases of T3/T4, 23 cases of G1, 57 cases of G2, 15 cases of G3.

There were 58 cases that received transurethral resection of bladder tumor by electrotome and 37 cases of radical bladder resection. Ten cases of normal bladder tissues 5 cm to tumors were taken as controls. All patients were followed up and the survival time was defined as the period between the first surgery to death or to the end of follow-up visit. The longest survival time was 93 months, the shortest 18 months and the average survival time was  $69.2 \pm 17.53$  months. During the follow-up visits, recurrence occurred to 31 cases and 13 cases died. All patients did not receive any adjuvant therapy before surgery and patients who preserved bladder were all given standard infusion chemotherapy with mitomycin.

### *Immunohistochemical staining*

Representative paraffin embedded tumor tissues and 10 cases of normal bladder peritumor tissue were made into 4  $\mu$ m serial sections. Dako Envision™ detection method was adopted to conduct immunohistochemical staining of AEG-1 and CD34. AEG-1 antibody was purchased from Invitrogen biotechnology Co., Ltd and CD34 antibody was purchased from Beijing Zhongshan Jinqiao biotechnology Co., Ltd. After dewaxing and hydration, tissue sections were boiled in 10 mmol/L citric acid buffer solution (pH 6.0) microwave oven for 15 min for antigen retrieval. A 3%  $H_2O_2$  was used to block the activity of endogenous peroxidase. Then, AEG-1 (1:100) and CD34 (1:200) antibodies were incubated at room temperature for 30 min. Then, the sections were treated with Envision™ + Dual Link System-HRP reagent for 30 min. DAB was used as the chromogen and the tissue sections were counterstained with Mayer's hematoxylin.

### *Results evaluation*

The quantitative and qualitative analysis of immunohistochemical staining adopted Nikon Labophot optical microscope (Tokyo, Japan) blind method. They were graded according to positive cell rate and staining intensity of tumor cell. Tumor cells were graded according to positive staining ratio: count 1000 tumor cells under high power lens: no staining as 0 point, positive staining cell rate lower than 10% as 1 point, 10-50% as 2 points, >50% as 3 points; scoring according to staining intensity: no staining as 0 point, faint yellow staining in cytoplasm or cellular nuclei as 1 point, middle intensity yellow staining as 2 points and brown deposits as 3 points. Finally, take the product of staining intensity and positive cell ratio as the value,  $\geq 3$  points as positive. Microvessel density evaluation: three regions with high microvessel density were selected and five views were counted under high power lens and their average value was taken as the value of microvessel density.

### *Statistical analyses*

All statistical analyses were conducted by SPSS 13.0 software (SPSS Inc, Chicago, IL). Correlation analysis on qualitative data adopted chi-square test or Fisher exact probability. Quantitative data adopted independent-samples *t* test. Survival analysis adopted Kaplan Meier method and its difference comparison adopted LogRank test. Relevant prognosis factor *multi-factor analysis adopted Cox regression model*.  $P < 0.05$  is considered that the difference is statistically significant.

## **Results**

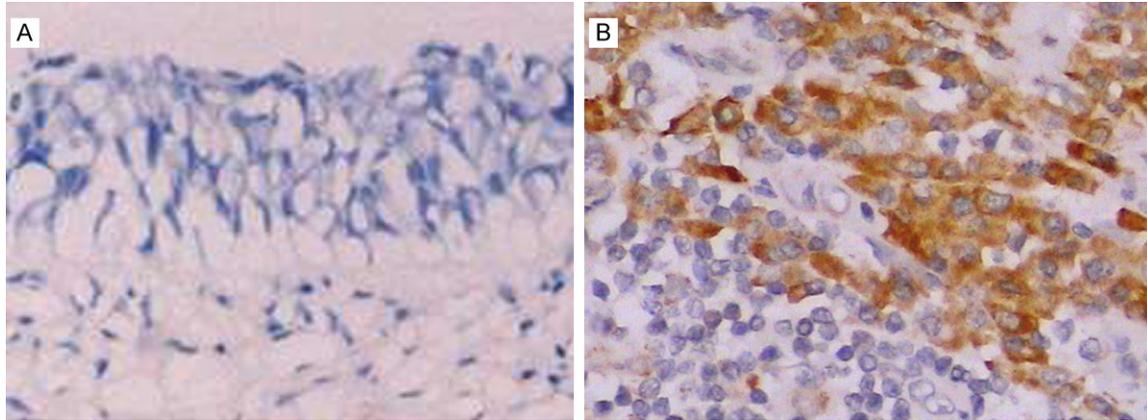
### *AEG-1 expression in urothelial bladder carcinoma*

AEG-1 is not expressed in normal bladder mucous membrane tissues. AEG-1 is mainly expressed in cytoplasm of tumor cells, for some few cases, it may be expressed in cell nucleus and the positive expression rate is 44.2% (**Figure 1**).

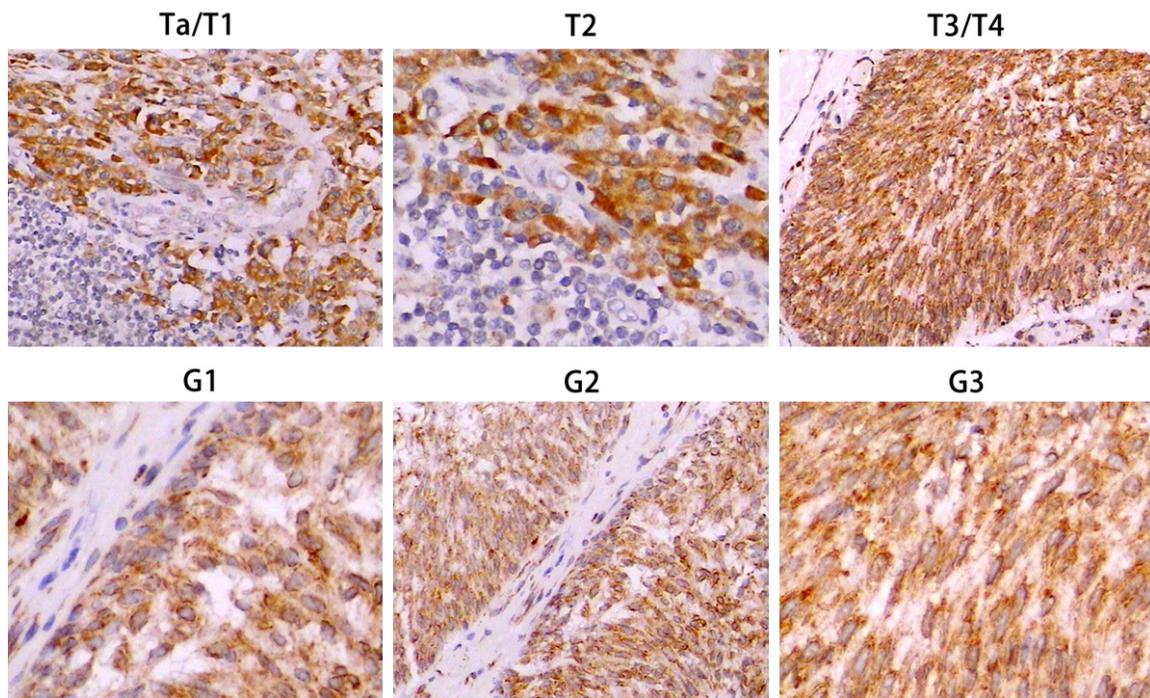
### *Relation between AEG-1 expression and clinical pathologic features and microvascular density*

AEG-1 expression is closely related to tumor classification and periodization: with the incre-

## AEG-1 in urothelial bladder carcinoma



**Figure 1.** The expression of AEG-1 in normal bladder tissue (A) and urothelial cell carcinoma (B).



**Figure 2.** Expression of AEG-1 in urothelial bladder carcinoma tissues with different tumor grading and stage.

ase of tumor grade and stage, the expression positive rate is higher (**Figure 2** and **Table 1**). In this group, they all have CD34 expression in urothelial bladder carcinoma, the microvessel density was about 4.7~60.2, averagely  $22.3 \pm 10.82$ . AEG-1 positive group was  $25.48 \pm 12.08$ , negative group is  $19.71 \pm 9.03$  and the difference between two groups had statistical significance ( $t = -2.663$ ,  $P = 0.009$ ) (**Table 1**), indicating that AEG-1 may promote urothelial bladder carcinoma micro-angiogenesis.

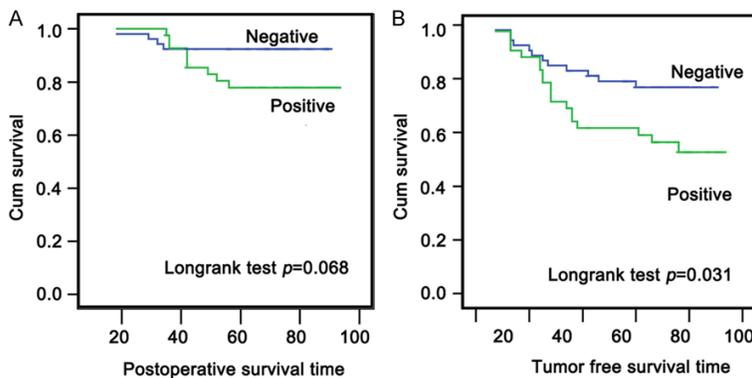
### *Relation between AEG-1 expression and prognosis of patients with urothelial bladder carcinoma*

Kaplan Meier analysis indicated that AEG-1 expression influenced tumor-free survival rate of patients with urothelial bladder carcinoma. Five-year tumor-free survival rate of the positive rate was 56% and that of the negative group was 76%; the difference between two groups had statistical significance, but had no

## AEG-1 in urothelial bladder carcinoma

**Table 1.** The correlations between AEG-1 and clinicopathological features and MVD in patients with bladder urothelial cell carcinoma

	AEG-1 expression		X <sup>2</sup> (t)	P
	Positive	Negative		
Tumor grading				
I	7	16	9.739	0.007
II	23	34		
III	12	3		
Tumor stage				
Ta/T1	22	41	6.529	0.035
T2	14	9		
T3/T4	6	3		
CD34	25.48±12.01	19.71±9.03	-2.663	0.009



**Figure 3.** The survival analysis of patients with bladder urothelial cell carcinoma by Kaplan-Meier method according to AEG-1. A. Effect of AEG-1 on the disease specific survival of patients with bladder transitional cell carcinoma. B. The effect of AEG-1 on survival of tumor-free patients.

**Table 2.** Multivariate analysis of disease free survival for patients with bladder urothelial cell carcinoma

	B	SE	Wald	df	Sig.	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
Step 1 Grading	-1.489	.533	7.810	1	.005	.226	.079	.641
Step 2 Grading	-1.219	.392	9.655	1	.002	.296	.137	.638
Stage	-1.217	.412	8.698	1	.003	.296	.132	.665

evident influence on disease specific survival (Figure 3). Multiple factor analysis indicated that AEG-1 was not an independent prognosis factor of tumor-free survival for patients with urothelial bladder carcinoma (Table 2) and it may be related to the classification and periodization of bladder cancer.

### Discussion

Our materials first provided evidence about the relation between increased AEG-1 expression

in urothelial bladder carcinoma and poor prognosis of patients with urothelial bladder carcinoma. In this study, we found that in 44.2% urothelial bladder carcinoma tissues, AEG-1 presented moderate intensity expression, and some can be seen in cell nucleus, but there was no AEG-1 expression in normal bladder tissues. Further analysis indicated that AEG-1 expression was related to tumor grading and staging; with the increase of tumor grading and staging, AEG-1 expression positive rate increased and tumor-free survival rate of AEG-1 high expression group was evident lower than that of low expression group. It indicated that AEG-1 may play a role in the occurrence and development of urothelial bladder carcinoma and it may be a new molecular marker for recurrence and development of urothelial bladder carcinoma.

Currently, the mechanism of AEG-1 in tumor development is still unclear. Studies indicated that AEG-1 was closely related to carcinogenic effect of Ha-ras and it played an important role in its medicated tumor occurrence [9]. AEG-1 abnormal expression significantly increased activity of NF-κB transcription factor while inhibiting NF-κB can reverse the tumor cell growth, invasion and metastasis ability

induced by AEG-1. Activating NF-κB pathway results in increased expression of adhesion molecule and this may be related to AEG-1 promoting tumor metastasis [10]. Besides, activating AKT, AEG-1 can down-regulate apoptosis factor Bad and P21 expression, in contrary, AEG-1 can upregulate MDM2 expression and resist P53 effect, so as to play the role of anti-apoptosis [11]. Therefore, AEG-1 takes parts in the occurrence and development of malignant tumor in various ways.

Tumor cell metastasis spread is an important factor that results in poor prognosis of most tumor patients [12, 13] while neovascularization plays an important role in tumor growth and systemic spread. This study has discussed the relevance between AEG-1 expression and micro-angiogenesis in urothelial bladder carcinoma tissues and we found that micro-angiogenesis density of urothelial bladder carcinoma tissue in AEG-1 high expression group was evidently higher than that in the low expression group, indicating that AEG-1 may be related to urothelial bladder carcinoma angiogenesis. To promote tumor micro-angiogenesis is one of the roles that AEG-1 plays in the occurrence and development of urothelial bladder carcinoma.

Similar to our results, recent studies indicated that AEG-1 was related to tumor micro-angiogenesis. AEG-1 can activate various signal transmission pathways in development of liver cancer and adjust expression of angiogenesis- and chemotherapy resistance-relevant genes [14]. Studies on esophagus cancer confirmed that in tumor cells with positive AEG-1, the microvessel density increased evidently while excessive expression of AEG-1 results in expression of pro-angiogenic factors like pro-angiogenic factor-1, metal matrix protease-2 and hypoxia induction factor 1-a. In vitro studies also confirmed that AEG-1 can promote tubing ability and invasion of umbilical veins endothelial cells [5]. These research results supported the conclusion that abnormal expression of AEG-1 plays an important positive promoting effect in regulating cancerous change and angiogenesis.

All in all, we first evaluate AEG-1 expression and its clinical significance in urothelial bladder carcinoma tissues. Results showed that there was a higher expression in bladder cancer tissue and AEG-1 may promote micro-angiogenesis of the urothelial bladder carcinoma and affect the prognosis of patients. AEG-1 may be a new molecular marker and potential therapeutic target for diagnosis and prognosis prediction of urothelial bladder carcinoma, deserving further research.

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### Disclosure of conflict of interest

None.

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