Int J Clin Exp Pathol 2018;11(12):6025-6031
www.ijcep.com /ISSN:1936-2625/IJCEP0086349

Original Article

Prognostic and clinicopathologic significance of AEG-1/MTDH and E-cadherin expression in human gallbladder carcinoma

Song-Tao Xu[1,3*], Yong-Chao Ma[2*], Cai-Hong Wang[4*], Yue Xu[5*], Guo-Jian Gu[6,7]

Departments of [1]Clinical, [2]Basic Medical Science, Luohe Medical College, Luohe, Henan, PR China; [3]Innovative Science and Technological Team of Tumor Occurrence and Prevention in Henan Province, Luohe , Henan Province, PR China; Departments of [4]Medical Imaging, [5]Neurosurgery, [6]Pathology, Taicang Affiliated Hospital of Soochow University, Taicang, Jiangsu, PR China; [7]Department of Pathology, The First People's Hospital of Taicang, Taicang, Jiangsu, PR China. [*]Equal contributors.

Received October 5, 2018; Accepted October 25, 2018; Epub December 1, 2018; Published December 15, 2018

Abstract: Astrocyte elevated gene-1 (AEG-1) and E-cadherin are associated with tumorigenesis and progression. The aim of this study is to investigate the expression of AEG-1 and E-cadherin in human gallbladder cancer (GBC) and explore their clinical and pathological significance. The expression of AEG-1 and E-cadherin protein were detected in 71 cases of human GBC and 22 cases of tumor-adjacent tissue by the immunohistochemical method. Our results demonstrate that the positive expression (high expression) rate of AEG-1 was 62.0% in human GBC which was higher than that in tumor-adjacent tissues (13.6%), P<0.001. The positive expression of AEG-1 protein was correlated with tumor TNM classification, histologic grade, and lymph node metastasis (P=0.037, P=0.033 and P=0.020, respectively). The positive expression rate of E-cadherin was 40.8% in GBC, which was lower than that in tumor-adjacent tissues (77.3%), P=0.003. Negative expression (Low expression) of E-Cadherin was significantly related with tumor TNM classification, histologic grade and lymphatic metastasis (P=0.028, P=0.003 and P=0.040, respectively). The expression of AEG-1 was negatively correlated with the expression of E-Cadherin (r=0.530, P<0.001). The log-rank test statistical analysis suggested that patients with positive expression of AEG-1 or negative expression of E-Cadherin protein had shorter overall survival time. Cox multivariate analysis showed that tumor TNM classification, histologic grade and lymphatic metastasis, AEG-1 and E-cadherin expression were independent factors for prognosis of GBC (P=0.013, P=0.019, P=0.001, P=0.011 and P=0.025 respectively). In conclusion, positive expression of AEG-1 and negative expression of E-Cadherin are markedly correlated with tumor TNM classification, histologic grade and lymphatic metastasis. The expression of AEG-1 was negatively correlated with the expression of E-Cadherin. Cox multivariate analysis showed that tumor TNM classification, histologic grade and lymphatic metastasis, positive expression of AEG-1 and negative expression of E-Cadherin were risk factors for prognosis of GBC. Detection of AEG-1 and E-Cadherin may be helpful to evaluate prognosis and infiltrative capability of gallbladder carcinoma.

Keywords: AEG-1, gallbladder carcinoma, survival, E-cadherin, prognosis

Introduction

Gallbladder carcinoma is the most common malignant tumor of the biliary system. There were 145,203 new cases of GBC in the world, accounting for 1.1% of all cases of the new malignant tumors in 2008 [1]. GBC's early symptoms are not obvious, and it presents at advanced stage. Therefore, patients will miss the opportunity of radical resection, and the 5 year survival rate is poor [2] making early diagnosis important. At present, the diagnosis of GBC mainly relies on non-invasive auxiliary imaging and traumatic examination, such as laparoscopy and biopsy. However, there is still lack of ideal tumor markers for the diagnosis and prognosis of GBC.

Astrocyte elevated gene-1 (AEG-1) also is named Metadherin (MTDH) or LYRIC, and is located on human chromosome 8 (8 q22) [3, 4]. Many studies have found that AEG-1 is highly

Table 1. Expression of AEG-1[a] and E-cadherin in gallbladder carcinoma and tumor-adjacent tissue

Related Factor	Gallbladder carcinoma tissue		Tumor-adjacent tissue		*P* Value
	Negative (-)	Positive (+)	Negative (-)	Positive (+)	
AEG-1	27 (38.0)	44 (62.0)	19 (86.4)	3 (13.6)	<0.001
E-cadherin	42 (59.2)	29 (40.8)	5 (22.7)	17 (77.3)	0.003

[a]Astrocyte elevated gene-1.

Table 2. Analysis of AEG-1[a] and E-cadherin positive expression and related factors

Related Factor	n	AEG-1 expression		*P* Value	E-cadherin expression		*P* Value
		Negative (-)	Positive (+)		Negative (-)	Positive (+)	
Sex							
Male	29	13 (44.8)	16 (55.2)	0.327	20 (69.0)	9 (31.0)	0.322
Female	42	14 (33.3)	28 (66.7)		22 (52.4)	20 (47.6)	
Age (year)							
≥65	43	16 (37.2)	27 (62.8)	0.634	25 (36.1)	18 (63.9)	0.829
<65	28	12 (42.9)	16 (57.1)		17 (60.7)	11 (39.3)	
Tumor size (cm)							
<2.5	40	19 (47.5)	21 (52.5)	0.062	22 (55.0)	18 (45.0)	0.419
≥2.5	31	8 (25.8)	23 (74.2)		20 (16.0)	11 (84.0)	
TNM classification							
I-II	26	14 (53.8)	12 (46.2)	0.037	11 (42.3)	15 (57.7)	0.028
III-IV	45	13 (28.9)	32 (71.1)		31 (68.9)	14 (31.1)	
Histologic grade							
Well + moderate	50	23 (46.0)	27 (54.0)	0.033	24 (48.0)	26 (52.0)	0.003
Poor	21	4 (19.0)	17 (81.0)		18 (85.7)	3 (14.3)	
Lymphatic metastasis							
No	52	24 (46.2)	28 (53.8)	0.020	27 (51.9)	25 (48.1)	0.040
Yes	19	3 (15.8)	16 (84.2)		15 (78.9)	4 (21.1)	

[a]Astrocyte elevated gene-1.

expressed in cancer tissues such as bladder cancer, lung cancer and human Retinoblastoma, which can inhibit cell apoptosis, and promote tumor angiogenesis, and cell proliferation, invasion [5-7].

E-cadherin is located on 16q22.1 chromosome, and is a calcium-mediated cell adhesion molecule that mediates cell-cell contacts and maintains normal epithelial cell integrity [8, 9]. It is a well-known tumor cell proliferation and metastasis suppressor protein, and the down regulation or loss of its expression is treated as a molecular marker of epithelial-mesenchymal transition (EMT) [10-12].

EMT is associated with cancer invasion and tumor progression. It can cause cells to lose polarity and cell-cell contact and some tumor cells to detach and metastasize [13, 14]. However, the role of AEG-1 and E-cadherin in gallbladder carcinoma has rarely been reported. In this study, we explored the expression of AEG-1 and E-cadherin in 71 cases of gallbladder carcinoma by SP immunohistochemistry. The aim is to find a convenient tool for the diagnosis and prognosis of the GBC.

Materials and methods

Patients and tissue samples

The study protocol was approved by the ethics committee of the Taicang Affiliated Hospital of Soochow University. 71 cases of GBC and 22 cases of tumor-adjacent tissues samples were collected and patients with appropriate informed consent from March 2011 to March 2017. The average age of the 71 patients who underwent surgery therapy was 64.8 ranging

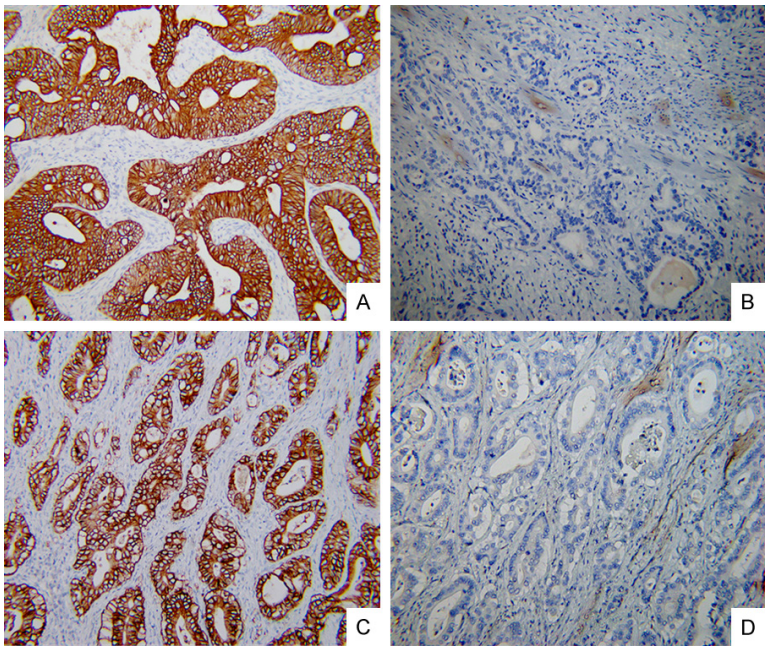

Figure 1. AEG-1 were positive expression in GBC tissues and its cytoplasm was stained brown (200×) (A). AEG-1 had negative expression in GBC tissues and its cells were not stained (200×) (B). E-cadherin were positive expression in GBC tissues and its cell membrane or cytoplasm was stained yellow (200×) (C). E-cadherin were not expressed in GBC (200×) (D).

from 45 to 82 years old. TNM classification system was proposed by American Joint Committee on Cancer (AJCC) in 2010 [15]. GBC patients in the experimental group were shown in **Tables 1** and **2**. All sections were confirmed as GBC by pathologists. They were followed up for 3 to 72 months via telephone. None of these patients received pre-operative chemotherapy or radiotherapy.

Immunohistochemistry

Immunohistochemical analysis was performed as previously [5, 16]. In brief, all specimens were cut into 4 μm sections and baked, followed by deparaffinized and rehydrated. The specimens were incubated 10 min by 3% H_2O_2, then they were incubated in citric acid buffer solution and boiled. The sections were incubated with rabbit anti-human AEG-1 antibody (1:100; Wuhan Boster Biological Technology, Ltd; Wuhan, China) or rabbit anti-human E-Cadherin antibody (1:100 Wuhan Boster Biological Technology, Ltd; Wuhan, China), overnight at 4°C. After washing with phosphate buffer saline (PBS), the specimens received secondary antibody (Wuhan Boster Biological Technology, Ltd; Wuhan, China). The specimens were incubated with streptavidin-horseradish peroxidase, and DAB (Wuhan Boster Biological Technology, Ltd; Wuhan, China) was droped for visualization and followed by hematoxylin counter-staining. The normal non-immune serum was replaced by AEG-1 or E-cadherin antibody as negative controls.

Evaluation of AEG-1 and E-cadherin staining

The staining intensity score was 0 (negative), 1 (light yellow), 2 (yellow brown), and 3 (brown). The rate of positive cells is 0 (0%), 1 (0-50%), 2 (51-100%). The proportional score and the intensity score were added to give the total score. The expression was defined as positive (+, high expression) when score was more than or equal to 4, and negative (-, low expression) when score was less than 4 [5].

Statistical analysis

The GraphPad Prism version 5.0 and SAS 9.3 software were used for statistical analysis. Chi-square test was carried out with categorical variables. The overall survival rates were conducted with the Kaplan-Meier method. Multivariate analysis was used for the Cox proportional hazards model. *P*-values <0.05 was considered significant.

Results

AEG-1 and E-cadherin expression in GBC and tumor-adjacent tissue

As shown in **Figure 1**, AEG-1 staining was predominantly observed in the cytoplasm of tumor cells (**Figure 1A**). E-cadherin staining was predominantly observed on the cell membrane or cytoplasm of tumor cells (**Figure 1C**). The expression level of AEG-1 in the tumor was significantly increased compared with tumor-adjacent tissue (P<0.001; **Table 1**). The expression level of E-cadherin in the tumor was significant-

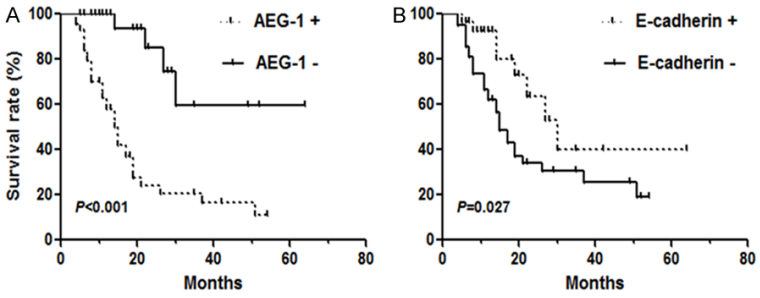

Figure 2. Kaplan-Meier survival curves of GBC patients based on AEG-1 expression (A) or E-cadherin expression (B).

ly decreased compared with tumor-adjacent tissue (P=0.003; **Table 1**).

AEG-1 and E-cadherin expression were associated with clinicopathologic characteristics of GBC

AEG-1 and E-cadherin protein expression and clinicopathologic features of GBC were examined, as shown in **Table 2**. Positive expression (high expression) rate of AEG-1 was 62.0% in GBC, which was higher than in tumor-adjacent tissues (13.6%), P<0.001. Positive of AEG-1 protein was correlated with tumor TNM classification, histologic grade, and lymph node metastasis(P=0.037,P=0.033andP=0.020,respectively). However, AEG-1 protein expression was not associated with sex, age and tumor size. Negative expression (low expression) of E-Cadherin was significantly related with tumor TNM classification, histologic grade, and lymph node metastasis (P=0.028, P=0.003 and P=0.040, respectively). However, E-cadherin protein expression was not associated with sex, age, and tumor size.

AEG-1 and E-cadherin expression correlate with survival in patients with GBC

Correlations are shown in **Figure 2**. Kaplan-Meier survival curves of GBC patients are based on AEG-1 and E-cadherin expression. Patients with positive expression of AEG-1 protein showed significantly worse survival compared with those with negative expression (P<0.001, log-rank test) (**Figure 2A**). Patients with negative E-cadherin protein showed significantly worse survival compared with those patients who were positive (P=0.027, log-rank test) (**Figure 2B**).

The prognostic values of variables were further tested by Cox multivariate analysis. This showed that tumor TNM classification, histologic grade, lymph node metastasis, AEG-1, and E-cadherin expression were independent factors for prognosis of GBC (P=0.013, P=0.019, P=0.019, P=0.001, P=0.011 and P=0.025 respectively), **Table 3**. TNM classification, histologic grade and lymphatic metastasis, positive expression of AEG-1, negative expression of E-cadherin were risk factors for prognosis.

Correlations between AEG-1 and E-cadherin expression in GBC tissue

In GBC tissues, the expression of AEG-1 protein was negatively correlated with the expression of E-cadherin protein (r=-0.530, P<0.001), **Table 4**.

Discussion

Studies have shown that AEG-1 activates different pathways that promote the development of tumors. First, it activates the PI3K/AKT signal pathway to promote cell proliferation. Kikuno found that the proliferation of prostate cancer cells increased significantly after silencing of AEG-1, AKT was upregulated, and FOXO3a and p27 were upregulated [17]. AEG-1 can activate the NF-κB (nuclear factor kappa B) signal pathway, further inducing cell proliferation, angiogenesis, invasion, and metastasis [18, 19]. In addition, AEG-1 overexpression promotes EMT in lung cancer by activating Wnt/β-catenin signaling, causing tumor cell detachment and metastasis to distant tissues [20]. In the study, the positive rate of AEG-1 protein in GBC cells was significantly higher than that in the paracancerous tissue (P<0.05). The positive rate of AEG-1 was relatively higher in patients with lymph node metastasis and it increased with the increase of TNM classification, and histologic grade. This indicates that AEG-1 plays a role in the occurrence and development of gallbladder cancer, and can be used for an auxiliary marker. The study found that patients with AEG-1 positive expression had shorter overall survival time compared with those with nega-

Table 3. COX analyses of different clinicopathologic variables and AEG-1[a] and E-cadherin expression status as predictors for overall survival in in gallbladder carcinoma tissues

Variable	Hazard Ratio	95% Hazard Ratio Confidence Limits		*p* value
Sex (Male vs. Female)	1.104	0.543	2.243	0.785
Age (≥65 vs. <65)	0.940	0.405	2.182	0.886
Tumor size (<2.5 vs. ≥2.5)	2.377	0.943	5.991	0.067
TNM classification (I-II vs. III-IV)	4.609	1.390	15.278	0.013
Histologic grade (well moderate vs. poor)	2.492	1.164	5.335	0.019
Lymphatic metastasis (no vs. yes)	4.242	1.871	9.619	0.001
AEG-1 (positive vs. negative)	5.655	1.487	21.509	0.011
E-cadherin (positive vs. negative)	0.238	0.068	0.836	0.025

[a]Astrocyte elevated gene-1.

Table 4. Correlations between AEG-1[a] and E-cadherin expression in gallbladder carcinoma tissues

E-cadherin	AEG-1		Contingency coefficien (r)	χ^2	P
	+	-			
+	9	20	-0.530	19.910	<0.001
-	35	7			

[a]Astrocyte elevated gene-1.

tive expression of AEG-1 [21, 22]. Our follow-up results also showed that patients with positive expression of AEG-1 had a poor prognosis.

Cox multivariate analysis also suggested that positive expression of AEG-1 was an independent risk factor for prognosis of GBC.

This suggests that the AEG-1 protein is a good prognostic marker. With the development of molecular biology technology, many scholars found that knockdown of AEG-1 could inhibit the tumor cell migration and invasion [4, 23, 24]. These suggested that it might be a potential gene therapeutic target for tumor.

Many studies have found that E-cadherin is low expression (negative expression) in cancer tissues, and patients with low expression of E-cadherin had shorter overall survival time compared with those patients with overexpression (positive expression) of E-cadherin [25-27]. In the study, the positive rate of E-cadherin protein in gallbladder cancer cells was significantly lower than that in the paracancerous tissue ($P<0.05$). The positive rate of E-cadherin was relatively lower in the patients with lymph node metastasis, and it decreased with the increase of TNM classification, and histologic differentiation grade. It indicates that the loss of E-cadherin plays a role in gallbladder tumor development, and can be used for the auxiliary diagnosis of gallbladder carcinoma. Our follow-up results also showed that patients with negative expression of E-cadherin had a poor prognosis. Cox multivariate analysis also suggested that negative expression of E-cadherin was a risk factor for prognosis of GBC.

Our study also found that AEG-1 was overexpressed, while the expression of E-cadherin protein in gallbladder carcinoma was low, and they were negatively correlated. This indicated that AEG-1 has a regulatory effect for E-cadherin protein. It is similar toprevious studies that AEG-1 overexpression induces EMT by activating Wnt/β-catenin signaling, leading to the down-regulation of E-cadherin [20]. However, the factors and mechanisms that affect the occurrence and development of tumors are very complex. We studied AEG-1 and E-cadherin expression by immunohistochemistry only, which is a limitation of the present study. In future studies, we can knock down the expression of AEG-1 and study its role in the occurrence and development of GBC as well as for E-cadherin.

Conclusion

Positive expression of AEG-1 or lost expression of E-cadherin were an important biomarker for progression, metastasis, and prognosis in GBC. Patients with positive expression of AEG-1 or negative expression of E-cadherin had poor survival. AEG-1 has an adjustment effect for E-cadherin protein. They were negatively correlated. Positive expression of AEG-1 or negative expression of E-cadherin correlates with lymph node metastasis, TNM classification, and histological differentiation grade. Further work is warranted on these targets in gallbladder carcinoma.

Acknowledgements

This work is supported by (a) the 2015 basic and advanced technology research project of Henan Province, P. R. C. (No. 152300410177), and (b) Fundamental Research Program (Applied foundational Research on Medical and Health) of Taicang, Jiangsu, PR China. (no. TC2018JCYL17).

Disclosure of conflict of interest

None.

Address correspondence to: Guo-Jian Gu, Department of Pathology, Taicang Affiliated Hospital of Soochow University, 58 South Changsheng Street, Taicang 215400, Jiangsu, PR China. Tel: +86-512-53610208; Fax: +86-512-53610208; E-mail: ggj1326@163.com

References

[1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.

[2] Stinton LM and Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. Gut Liver 2012; 6: 172-187.

[3] Lee SG, Kang DC, DeSalle R, Sarkar D and Fisher PB. AEG-1/MTDH/LYRIC, the beginning: initial cloning, structure, expression profile, and regulation of expression. Adv Cancer Res 2013; 120: 1-38.

[4] Wang Y, Wang T, Sun Y, Sun W and Wang X. Astrocyte elevated gene-1 promotes tumour growth and invasion by inducing EMT in oral squamous cell carcinoma. Sci Rep 2017; 7: 15447.

[5] Xu S, Gu G, Ni Q, Li N, Yu K, Li X and Liu C. The expression of AEG-1 and Cyclin D1 in human bladder urothelial carcinoma and their clinicopathological significance. Int J Clin Exp Med 2015; 8: 21222-21228.

[6] Ma Z, Chen Y, Dong S, Xu X, Liu J, Song P, Yu C and Dai L. AEG-1 mRNA expression in non-small cell lung cancer is associated with increased tumor angiogenesis. Pathol Res Pract 2017; 213: 1257-1263.

[7] Chang Y, Li B, Xu X, Shen L, Bai H, Gao F, Zhang Z and Jonas JB. Lentivirus-mediated knockdown of astrocyte elevated Gene-1 inhibits growth and induces apoptosis through mapk pathways in human retinoblastoma cells. PLoS One 2016; 11: e0148763.

[8] Kowalski PJ, Rubin MA and Kleer CG. E-cadherin expression in primary carcinomas of the breast and its distant metastases. Breast Cancer Res 2003; 5: R217-222.

[9] Deman JJ, Van Larebeke NA, Bruyneel EA, Bracke ME, Vermeulen SJ, Vennekens KM and Mareel MM. Removal of sialic acid from the surface of human MCF-7 mammary cancer cells abolishes E-cadherin-dependent cell-cell adhesion in an aggregation assay. In Vitro Cell Dev Biol Anim 1995; 31: 633-639.

[10] Zhang Z, Bu X, Chen H, Wang Q and Sha W. Bmi-1 promotes the invasion and migration of colon cancer stem cells through the downregulation of E-cadherin. Int J Mol Med 2016; 38: 1199-1207.

[11] Onder TT, Gupta PB, Mani SA, Yang J, Lander ES and Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res 2008; 68: 3645-3654.

[12] Petrova YI, Schecterson L and Gumbiner BM. Roles for E-cadherin cell surface regulation in cancer. Mol Biol Cell 2016; 27: 3233-3244.

[13] Lymperatou D, Giannopoulou E, Koutras AK and Kalofonos HP. The exposure of breast cancer cells to fulvestrant and tamoxifen modulates cell migration differently. Biomed Res Int 2013; 2013: 147514.

[14] Thiery JP, Acloque H, Huang RY and Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871-890.

[15] In: Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL and Trotti A, editors. AJCC Cancer Staging Manual. 7th edition. New York, NY: Springer; 2010.

[16] Xu S, Wen W, Zhou J, Liu J, Gu G . Clinicopathological significance of expression of CIP2A and c-myc in human gallbladder carcinoma. Int J Clin Exp Pathol 2017; 10: 5868-5874.

[17] Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, Place RF, Pookot D, Majid S, Igawa M and Dahiya R. Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of FOXO3a activity. Oncogene 2007; 26: 7647-7655.

[18] Emdad L, Sarkar D, Su ZZ, Randolph A, Boukerche H, Valerie K and Fisher PB. Activation of the nuclear factor kappaB pathway by astrocyte elevated gene-1: implications for tumor progression and metastasis. Cancer Res 2006; 66: 1509-1516.

[19] Sarkar D, Park ES, Emdad L, Lee SG, Su ZZ and Fisher PB. Molecular basis of nuclear factor-kappaB activation by astrocyte elevated gene-1. Cancer Res 2008; 68: 1478-1484.

[20] He W, He S, Wang Z, Shen H, Fang W, Zhang Y, Qian W, Lin M, Yuan J, Wang J, Huang W, Wang L and Ke Z. Astrocyte elevated gene-1 (AEG-1) induces epithelial-mesenchymal transition in

lung cancer through activating Wnt/beta-catenin signaling. BMC Cancer 2015; 15: 107.
[21] Wang Y, Jin X, Song H and Meng F. AEG-1 as a predictor of sensitivity to neoadjuvant chemotherapy in advanced epithelial ovarian cancer. Onco Targets Ther 2016; 9: 2385-2392.
[22] Chen CY, Chen YY, Chen JJW, Chen KY, Ho CC, Shih JY, Chang YL, Yu CJ and Yang PC. Astrocyte-elevated gene-1 confers resistance to pemetrexed in non-small cell lung cancer by upregulating thymidylate synthase expression. Oncotarget 2017; 8: 61901-61916.
[23] Li H and Zhao J. let-7d suppresses proliferation and invasion and promotes apoptosis of meningioma by targeting AEG-1. Onco Targets Ther 2017; 10: 4895-4904.
[24] Wang J, Chen X and Tong M. Knockdown of astrocyte elevated gene-1 inhibited cell growth and induced apoptosis and suppressed invasion in ovarian cancer cells. Gene 2017; 616: 8-15.
[25] Li P, Sun T, Yuan Q, Pan G, Zhang J and Sun D. The expressions of NEDD9 and E-cadherin correlate with metastasis and poor prognosis in triple-negative breast cancer patients. Onco Targets Ther 2016; 9: 5751-5759.
[26] Schneider MR, Hiltwein F, Grill J, Blum H, Krebs S, Klanner A, Bauersachs S, Bruns C, Longerich T, Horst D, Brandl L, de Toni E, Herbst A and Kolligs FT. Evidence for a role of E-cadherin in suppressing liver carcinogenesis in mice and men. Carcinogenesis 2014; 35: 1855-1862.
[27] Yun JA, Kim SH, Hong HK, Yun SH, Kim HC, Chun HK, Cho YB and Lee WY. Loss of E-Cadherin expression is associated with a poor prognosis in stage III colorectal cancer. Oncology 2014; 86: 318-328.