**Original Article**

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

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**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...
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...
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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% H2O2, then they were incubated in citric acid buffer solution and boiled. The sections were incubated with streptavidin-horseradish peroxidase, and DAB (Wuhan Boster Biological Technology, Ltd; Wuhan, China) was dropped 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-
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=0.033 and P=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 expression 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 para-cancerous 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, 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-
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Table 3. COX analyses of different clinicopathologic variables and AEG-1 and E-cadherin expression status as predictors for overall survival in gallbladder carcinoma tissues

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

*Astrocyte elevated gene-1.

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

<table>
<thead>
<tr>
<th>E-cadherin</th>
<th>AEG-1</th>
<th>Contingency coefficient (r)</th>
<th>χ²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>-0.530</td>
<td>19.910</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>0.530</td>
<td>19.910</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Astrocyte elevated gene-1.

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 to previous 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.
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Disclosure of conflict of interest

None.

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