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RESEARCH ARTICLE

Astrocyte elevated gene-1 is a novel biomarker of epithelial–mesenchymal transition and progression of hepatocellular carcinoma in two China regions

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Abstract Astrocyte elevated gene-1 (AEG-1) is involved in important biological processes including cell invasion, metastasis, and carcinogenesis. However, its clinical significance has remained largely unknown in hepatocellular carcinoma. Here, specimens from 144 patients with hepatocellular carcinomas in Beijing and Heilongjiang regions were investigated by immunohistochemical staining for AEG-1, vimentin, and E-cadherin expressions. A clinicopathological study revealed that AEG-1 expression level in tumor cells was significantly correlated with TNM stage ($P=0.001$) and Edmonson grade ($P<0.0001$). In addition, AEG-1, vimentin, and E-cadherin (epithelial–mesenchymal transition (EMT) biomarker) expressions were correlated with each other. These findings suggest that AEG-1 may be an epithelial–mesenchymal transition-associated biomarker in human hepatocellular carcinoma and play important roles in the progression of hepatocellular carcinoma. In addition, the AEG-1 gene is a potential target for elimination of hepatocellular carcinoma in the future.

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Introduction

Hepatocellular carcinoma is a common cause of cancer death worldwide [1]. Due to the increases in environmental pollution and aging populations worldwide, the upward trend in hepatocellular carcinoma incidence will continue [2]. Reports from China and the USA indicate that hepatocellular carcinoma has a high mortality rate; this mortality rate is often ascribed to metastasis and late diagnosis [1–3]. However, the mechanisms of hepatocellular carcinoma are not well understood.

Epithelial–mesenchymal transition (EMT) is a central event in hepatocellular carcinoma progression [4, 5]. A cell undergoing EMT has the ability to intravasate and pass the circulatory system of hepatocellular carcinoma [4, 5]. It is also implicated in the conversion of early-stage tumors into an invasive cancer [4, 5]. The hallmarks of the EMT are the loss of E-cadherin and the increase of vimentin expressions [4, 5].

Astrocyte elevated gene-1 (AEG-1), also known as metadherin and lysine-rich co-isolated protein, was first cloned and identified as a novel human immunodeficiency virus (HIV)-1 or tumor necrosis factor alpha-inducible gene in primary human fetal astrocytes in 2002 [6, 7]. The human AEG-1 gene is located at chromosome 8q22, and its genomic strengthening has also been found in various tumors in contrast with its common counterparts [7–25]. It regulates the transformed state of various cancers by activation of different signaling molecules, such as phosphoinositide 3-kinase (PI3K)–Akt, AP-1, and extracellular signal-regulated kinase

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[7–25]. In HeLa cells, it also enhances DNA binding and transcriptional activities of nuclear factor κB [9–11, 24]. Nuclear factor κB regulates the expression of various molecules, such as metalloproteinases, chemokines, and cell adhesion proteins, all of which initiate cancer cell invasion and metastasis [9–11, 24]. Silencing AEG-1 in malignant cells significantly blocks their proliferation, migration, and invasion, whereas upregulation of AEG-1 inhibits apoptosis and increases the invasive ability of these cells [7–26]. Using a similar method, researchers have also found that microRNA-375 targets AEG-1 in hepatocellular carcinoma and suppresses liver cancer cell growth both in vivo and in vitro [27].

Although AEG-1 is an oncogene that has been implicated in pathways critical to hepatocarcinogenesis [22], AEG-1 was also found to control the expression of vimentin and E-cadherin [26–30]. These findings suggest that AEG-1 may participate in the EMT of hepatocellular carcinoma. In this study, we clinically investigated whether AEG-1 overexpression strengthens the epithelial–mesenchymal transition of hepatocellular carcinoma and further affects its progression.

Materials and methods

Samples

The study was performed in accordance with the institutional ethical guidelines and has been approved by the Committee on Research and Ethics and the Scientific Committee of the authors' university. Paraffin sections (4 μm) from 158 liver samples were collected as follows: hepatocellular carcinoma tissues and matched normal tissues. Detailed clinicopathological information was obtained from patient records, and all patients were treated in the authors' hospital. Patient ages ranged from 47 to 78 years, with an average age of 55 years. Thirty-three of the patients were women, and 111 were men. The judgment of clinicopathologic features was mainly referred to the findings of Zhu et al. [28].

Immunohistochemistry

Specimens from all cases were fixed in a 10 % formaldehyde solution and embedded in paraffin. Briefly, tissue sections (4–5 μm) from representative paraffin blocks were deparaffinized in xylene and then rehydrated through graded alcohol solutions. Endogenous peroxidases were blocked using 3 % hydrogen peroxide. Antigen retrieval of vimentin and E-cadherin was enhanced by microwaving the slides in 0.01 M citrate buffer (pH=6) for 20 min. Furthermore, AEG-1 antigen retrieval was enhanced by microwaving the slides in EDTA (pH=8.0, ZLI-9066 and ZLI-9067, Zhong Shan Golden Bridge, China). The primary antibodies used were rabbit polyclonal anti-AEG-1 (dilution 1:200; Abcam, UK), anti-E-cadherin monoclonal antibody (dilution 1:2,500; BD Biosciences, San Diego, CA, USA), and mouse monoclonal anti-

Table 1 The relationship between the gene expression level and the main clinical characteristics of the hepatocellular carcinoma

	No.	AEG-1			Vimentin			E-cadherin		
		H	L	*P* value	H	L	*P* value	L	H	*P* value
Age				0.2			0.8			0.9
≤52	22	22	0		18	4		20	2	
>52	67	62	5		55	12		63	4	
Sex				0.7			0.8			0.9
Male	67	64	3		56	11		62	5	
Female	22	20	2		17	5		21	1	
HBsAg				0.2			0.4			0.5
Positive	80	75	5		65	15		74	6	
Negative	8	8	0		7	1		8	0	
BCLC stage				0.2			0.6			0.4
B/C	80	75	5		65	15		74	6	
0/A	7	7	0		6	1		7	0	
Edmonson grade				<0.0001			0.0003			<0.0001
I/II	47	46	1		44	3		41	6	
III/IV	42	38	4		29	13		42	0	
TNM stage				0.001			0.004			<0.0001
II/III	75	72	3		65	10		69	6	
I	14	12	2		8	6		14	0	

H high expression, *L* low expression

Table 2 The relationship between vimentin and AEG-1 expressions in hepatocellular carcinoma

		AEG-1		*P* value
		H	L	
Vimentin	H	72	1	<0.0001
	L	12	4	

H high expression, *L* low expression

vimentin (dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were incubated with the primary antibodies overnight at 4 °C, and bound primary antibodies were detected with the PV-9000 Polymer Detection System for Immuno-Histological Staining kit (Zhong Shan Golden Bridge). The reaction product was counterstained with hematoxylin.

Evaluation of immunoreactivity

The immunoreactivity in the tissues was evaluated independently by two pathologists who were blinded to the clinical data and other immunohistochemical results. Membrane and nuclear stainings for each protein were evaluated separately [10–13]. AEG-1, vimentin, and E-cadherin are mainly expressed in membranes. AEG-1, vimentin, and E-cadherin were evaluated on the basis of staining intensity and distribution using the immunoreactive score [26–30].

Statistical analysis

Statistical analysis was performed using the SPSS software package for Windows, release 15.0 (SPSS Inc., Chicago, IL, USA). Correlations of these protein expression levels with the clinicopathological parameters were analyzed using the Spearman correlation and the chi-square test for these data. The significance level was defined as $P<0.05$.

Table 3 The relationship between vimentin and E-cadherin expressions in hepatocellular carcinoma

		E-cadherin		*P* value
		L	H	
Vimentin	H	67	6	<0.0001
	L	16	0	

H high expression, *L* low expression

Table 4 The relationship between E-cadherin and AEG-1 expressions in hepatocellular carcinoma

		E-cadherin		*P* value
		L	H	
AEG-1	H	78	6	<0.0001
	L	5	0	

H high expression, *L* low expression

Results

Clinicopathologic features of hepatocellular carcinoma

One hundred forty-four patients (selected from Beijing and Heilongjiang regions) with hepatocellular carcinomas included in this study ranged from 31 to 78 years, and the mean age at the time of surgery was 52.0 years. One hundred eleven were men (77 %), and 33 were women (23 %). At the time of diagnosis, 89 hepatocellular carcinomas were included in this study.

Expressions of vimentin, E-cadherin, and AEG-1 in hepatocellular carcinoma and matched normal tissues

AEG-1 and vimentin expressions were mainly detected in the cell membrane of hepatocellular carcinoma tissues. Evaluation of immunoreactivity was gradually elevated from normal to cancer tissues. Vimentin-positive expression was observed in nine tumor cases and in one normal tissue. E-cadherin-positive expression was observed in 6 tumor cases and in 13 normal tissues. AEG-1-positive expression was observed in seven tumor cases and in one normal tissue. Overall, the expression of vimentin, E-cadherin, and AEG-1 has a significant deviation between hepatocellular carcinoma and matched normal tissues ($P<0.05$).

Expressions of vimentin, E-cadherin, and AEG-1 in the hepatocellular carcinoma

We analyzed the expression levels of vimentin, E-cadherin, and AEG-1 in 89 hepatocellular carcinomas. The relationship between the gene expression level and the main clinical characteristics of the patients analyzed in this study is detailed in Table 1.

The relationship between vimentin, E-cadherin, and AEG-1 expressions

We analyzed the relationship among vimentin, E-cadherin, and AEG-1 using the Spearman correlation. It was showed

that AEG-1, vimentin, and E-cadherin expressions were correlated with each other (Tables 2, 3, and 4).

Discussion

This study quantified the upregulation of AEG-1 and vimentin and the aberrant expression of E-cadherin in primary hepatocellular carcinoma patients by immunohistochemistry. EMT has been demonstrated to occur during the metastasis process of hepatocellular carcinoma [4, 5]. The relationship between the expression levels of these molecular markers and the clinicopathological features of the patients was analyzed in this manuscript. The expression of AEG-1 in hepatocellular carcinoma has not been reported previously in Beijing and Heilongjiang regions. Here, we investigated both its expression level in human hepatocellular carcinoma as well as the correlation between the expression level and clinicopathological features. In our study, AEG-1 expression in hepatocellular carcinoma was higher than that in matched normal liver tissues. Similarly, vimentin was also overexpressed in the membrane of hepatocellular carcinoma.

Tumor progression is critical to the outcome of hepatocellular carcinoma patients [1]. Patients with advanced stage have a higher death rate than those with early stage [2, 3]. Previous studies have showed that EMT is strongly correlated with the metastasis of hepatocellular carcinoma [4]. According to our studies and the findings of other researchers, the AEG-1 pathway may be involved in the EMT process of hepatocellular carcinoma, which causes cancer cell metastasis [4, 5, 26–30]. In some cancer cell lines, a mechanism involving the AEG-1–vimentin interaction has been suggested [26–30]. Recently, vimentin has also been reported in breast cancer cell lines, which was attributed to a mechanism involving the activation of AEG-1 [23, 29]. Taken together, these findings suggest that AEG-1 binds upstream of the vimentin gene, causing it to be overexpressed. Vimentin induces a decrease in the expression of E-cadherin, allowing tumor cells to overcome barriers against local invasion and metastasis. AEG-1 also plays a role in carcinogenesis with regard to human hepatocellular carcinomas and by other investigations [22, 27, 28].

In conclusion, our findings suggest that AEG-1 is overexpressed in hepatocellular carcinoma and indicate the importance of the AEG-1 pathway in the process of tumor progression through EMT. However, there are some limitations in our paper. Further multicenter investigation and cooperation are needed for this study (at present, research like this has been carried out only in Shanghai, Wuhan, and Xian in China). In the future, we will also test this pathway in vitro and combine other biomarkers to predict the metastatic risk of hepatocellular carcinoma patients [31, 32]. In the next step, we also use Chinese herbs (like Huaier granules) [33] suppressing hepatocellular carcinoma metastasis and EMT, likely via the anti-AEG-1 pathway.

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Conflicts of interest None

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