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ORIGINAL ARTICLE
pISSN 2288-6575 • eISSN 2288-6796
http://dx.doi.org/10.4174/astr.2015.88.2.77
Annals of Surgical Treatment and Research

Astrocyte elevated gene-1 overexpression in hepatocellular carcinoma: an independent prognostic factor

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Purpose: Astrocyte elevated gene-1 (AEG-1) plays important roles in tumorigenesis such as proliferation, invasion, metastasis, angiogenesis, and chemoresistance. We examined the expression of AEG-1 in patients with hepatocellular carcinoma (HCC).
Methods: Eighty-five samples were collected from patients with HCC who underwent surgery and were histopathologically confirmed to have HCC. Two independent pathologists, experienced in evaluating immunohistochemistry and blinded to the clinical outcomes of the patients, reviewed all samples. They determined AEG-1 expression semiquantitatively by assessing the percentage of positively stained immunoreactive cells and staining intensity. Clinicopathological data were analyzed in association with prognosis.
Results: The association was estimated by univariate and multivariate analyses with Cox regression. Tumor size (hazard ratio [HR], 2.285; 95% confidence interval [CI], 1.175–4.447; P = 0.015), microvascular invasion (HR, 6.754; 95% CI, 1.631–27.965; P = 0.008), and AEG-1 expression (HR, 4.756; 95% CI, 1.697–13.329; P = 0.003) were independent prognostic factors for overall survival. Those for disease-free survival rate were tumor size (HR, 2.245; 95% CI, 1.282–3.933; P = 0.005) and AEG-1 expression (HR, 1.916; 95% CI, 1.035–3.545; P = 0.038). The cumulative 5-year survival and recurrence rates were 89.2% and 50.0% in the low-expressing group and 24.5% and 82.4% in the high-expressing group, respectively.
Conclusion: The results suggest that AEG-1 overexpression could serve as a valuable prognostic marker in patients with HCC.
[Ann Surg Treat Res 2015;88(2):77-85]

Key Words: Human MTDH protein, Hepatocellular carcinoma, Neoplasm metastasis

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy. It is one of the five most common cancers and the third most common cause of cancer-related deaths worldwide. HCC has a high prevalence in central and Southeast Asia because the etiology is predominantly viral hepatitis, which is a hyperendemic disease in Asia [1]. HCC is rare in Western countries, but incidence is increasing due to HCV infection and chronic alcoholism over the past 20 years [2]. HCC is also hyperendemic in Korea. According to the Korean Central Cancer Registry 2010, the age-adjusted incidence is 24.5 per 100,000 population, and HCC ranks as the second most frequent cause of cancer death in males and the fourth highest among females [3].

Received August 11, 2014, **Revised** September 5, 2014, **Accepted** September 11, 2014

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HCC is a highly aggressive and rapidly growing tumor with early vascular invasion [4]. It is difficult to operate on in many cases because of these characteristics and because it frequently occurs with liver cirrhosis. It is also highly resistant to standard chemotherapy [5]. Transarterial chemoembolization (TACE) and systemic chemotherapy have not demonstrated significant survival benefits. Target therapy including sorafenib has been introduced as a treatment for unresectable advanced HCC. Hence, there is still an urgent need for further identification of novel molecular markers to provide clinicians with useful information for patient prognosis and therapeutic options.

Astrocyte elevated gene-1 (*AEG-1*) is an oncogene, which was cloned in 2002 as an HIV and TNF-α-inducible gene in primary human fetal astrocytes. It is also called metadherin or LYsine-Rich CEACAM1 coisolated [6,7]. The *AEG-1* gene is located on chromosome 8q22, and genomic amplification occurs in multiple cancers [8]. To date, various expression analyses have demonstrated that AEG-1 is overexpressed in many tumors, including cancers of the liver, colorectum, breast, stomach, bladder, prostate, esophagus, gallbladder, lung, ovary, endometrium, lymphoma, and glioma comprared with that in respective normal tissues [9]. The AEG-1 positive rate in HCC specimens is 93.6% [10]. Several studies have shown the mechanisms of overexpression. AEG-1 is a downstream gene of the Ha-ras and PI3K pathways and is activated by c-Myc or nuclear factor-κB and the mitogen activated protein kinase and Wnt/b-catenin pathways [11]. Tumor suppressor miRNA, such as miR-375, miR-136, and miR-137 downregulates AEG-1 expression in several cancers [12-14]. Furthermore, experimental analyses of AEG-1 gene knock-in models have shown that the AGE-1 protein plays important roles in tumorigenesis such as proliferation, invasion, metastasis, angiogenesis, and chemoresistance [15]. AEG-1 expression level clearly correlates with poor survival rate and prognosis in several cancers [9,16-20].

Few studies have reported the clinical correlation between AEG-1 expression and prognosis in patients with HCC. In this study, we examined AEG-1 expression in different HCC tissues by immunohistochemistry (IHC) and determined the association among AEG-1 expression, clinicopathological characteristics, and survival of patients with HCC.

METHODS

Patients and samples

Eighty-five samples were collected from patients with HCC who underwent surgery and were histopathologically confirmed to have HCC at Soonchunhyang University, Cheonan and Bucheon Hospitals between January 2001 and January 2009. All patients with HCC were investigated, including 69 males and 16 females (mean age, 53 years; range, 27–74 years). None of the patients had undergone chemotherapy or radiation therapy before surgery, and no patient died within 30 days after surgery. All 85 patients were followed up. Cases lost during follow-up were not included within the sample. All clinicopathological data were collected by reviewing medical charts and pathological records, including liver cirrhosis, tumor size, Child-Pugh score, viral markers, histological differentiation, preoperative serum α-FP, prothrombin induced by vitamin K absence II (PIVKA-II), microvascular invasion, portal vein invasion, TACE, distant metastasis, and tumor stage (Table 1). The clinicopathological parameters followed the general rules for

Table 1. Demographic and clinicopathological parameters of HCC patients (n = 85)

Variable	Value
Gender	
Male/female	69 (82.2)/16 (18.8)
Age (yr), median (range)	53 (27–74)
Tumor size (cm), median (range)	4.63 (1.2–16.5)
Liver cirrhosis	
(–)/(+)	60 (70.5)/25 (29.5)
Child-pugh stage	
A/B/C	72 (84.7)/11 (12.9)/2 (2.4)
Viral marker	
HBV/HCV/none	71 (83.5)/3 (3.5)/11 (13)
Histological grade	
GI/GII/GIII/GIV	3 (3.5)/39 (45.8)/37 (43.5)/6 (7.2)
AJCC stage	
I/II/III/IV	48 (56.5)/18 (21.2)/17 (20.0)/2 (2.3)
Okuda stage	
I/II	74 (87.5)/11 (12.5)
BCLC stage	
A/B/C/D	15 (17.6)/39 (45.9)/27 (31.8)/4 (4.8)
Modified UICC stage	
I/II/III/IV	5 (5.9)/47 (55.3)/29 (34.1)/4 (4.7)
Portal vein invasion	
(–)/(+)	71 (83.5)/14 (16.5)
Microvascular invasion	
(–)/(+)	56 (65.9)/29 (34.1)
Recurrence	
(–)/(+)	29 (34.1)/56 (65.9)
Metastasis	
(–)/(+)	59 (69.5)/26 (30.5)
a-FP (<15 ng/mL)	
(–)/(+)	34 (40.0)/51 (60.0)
PIVKA-II (<40 mAU/mL)	
(–)/(+)	26 (30.6)/59 (69.4)
TACE	
(–)/(+)	51 (60)/34 (40)

Values are presented as number (%) unless otherwise indicated.
HCC, hepatocellular carcinoma; AJCC, American Joint Committee on Cancer; BCLC, Barcelona Clinic Liver Cancer; UICC, Union for International Cancer Control; PIVKA-II, prothrombin induced by vitamin K absence II; TACE, transarterial chemoembolization.

the study of primary HCC, 3rd ed. June 2007, Korea. Tumor stage was defined according to TNM classification of the American Joint Committee on International Union against Cancer, Okuda staging, Barcelona Clinic Liver Cancer (BCLC) staging of the American Association of for the Study of the Liver Disease and Modified Union for International Cancer Control (UICC) staging. Tumor differentiation (grade) was assessed according to the Edmondson and Steiner nuclear grading system (ES grade). This study was approved by the Institutional Review Board of the Soonchunhyang University, Cheonan Hospital.

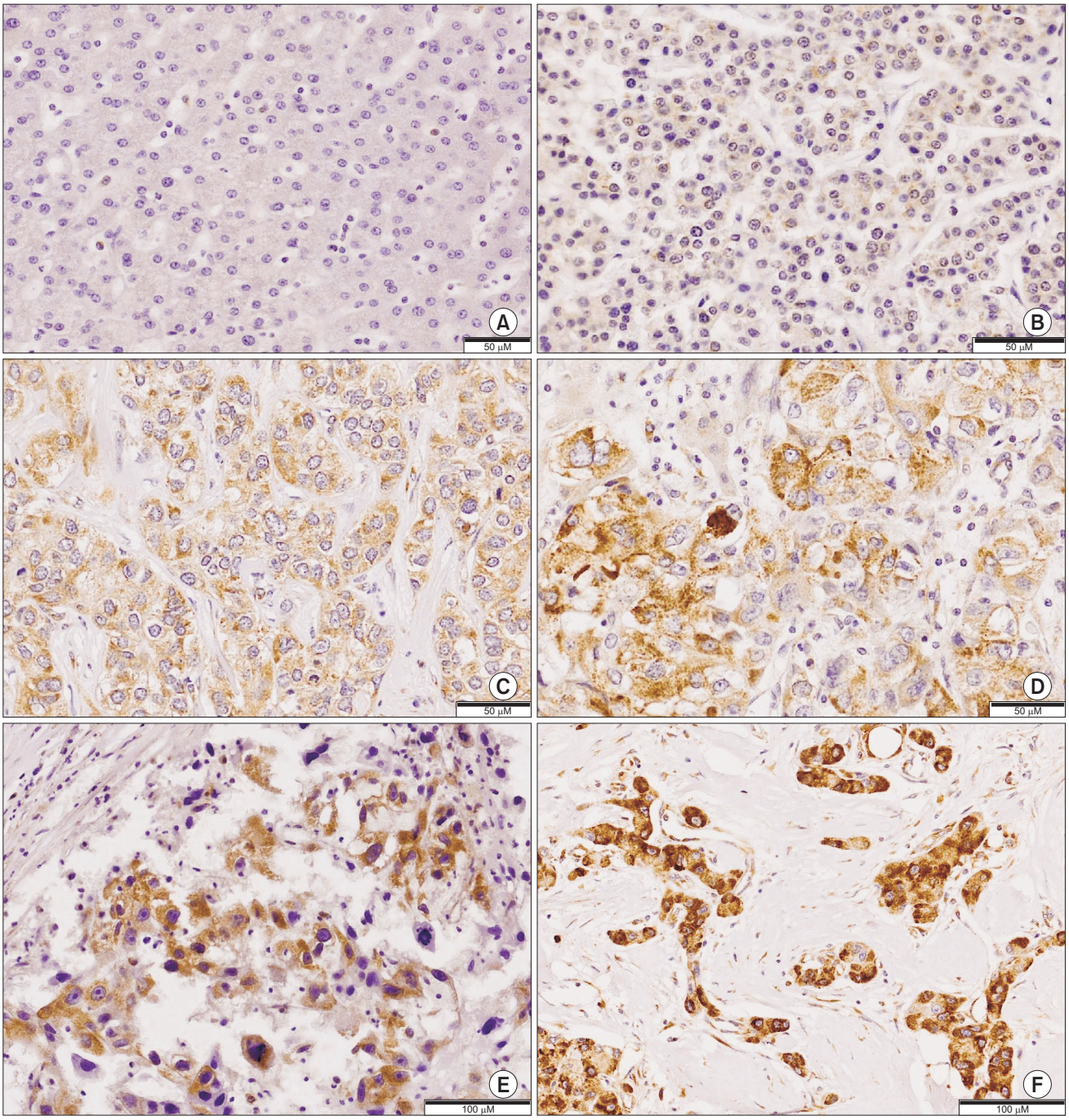

Fig. 1. (A) Astrocyte elevated gene-1 (AEG-1) was not expressed in the cytoplasm of normal hepatocytes. (B) AEG-1 was mildly expressed in tumor cells of Edmondson and Steiner (ES) grade I hepatocellular carcinoma (HCC) (score, 1). (C) AEG-1 was moderately expressed in the cytoplasm of ES grade II HCC (score, 2). (D) AEG-1 was strongly expressed in the cytoplasm of ES grade III HCC (score, 3). (E) AEG-1 was strongly expressed in the cytoplasm of ES grade IV HCC (score, 3). (F) AEG-1 was strongly expressed in the cytoplasm of highly infiltrative HCC (score, 3). Scale: A-D, 50 μm; E-F, 100 μm.

IHC staining

Sections (4 μm) of the formalin-fixed, paraffin-embedded tumor samples were deparaffinized in xylene and hydrated in a graded ethanol-distilled water series. The deparaffinized sections were immersed in 0.01M citrate buffer (pH 6.0) and heated (95°C) for 15 minutes in a microwave oven. Endogenous

Table 2. Relationship between AEG-1 expression and clinicopathological data in HCC

Characteristic	No.	AEG-1 expression		P-value[a]	r (P-value)[b]
		Low	High		
Gender				0.307	0.111 (0.313)
Male	69	21	48		
Female	16	7	9		
Age (yr)				0.925	0.010 (0.926)
≤53	51	17	34		
>53	34	11	23		
Liver cirrhosis				0.258	0.123 (0.263)
(–)	25	6	19		
(+)	60	22	38		
Tumor size (cm)				0.002	0.404 (<0.001)
≤5	52	25	27		
>5	33	3	30		
Histological differentiation				0.009	0.397 (<0.001)
G1	3	2	1		
G2	39	20	19		
G3	37	5	32		
G4	6	1	5		
BCLC stage				0.001	0.390 (<0.001)
A	15	11	4		
B	39	12	27		
C	27	5	22		
D	4	0	4		
Modified UICC stage				0.009	0.351 (0.001)
I	4	1	5		
II	19	28	47		
III	5	24	29		
IV	0	4	4		
Portal vein invasion				0.371	0.109 (0.322)
(–)	71	25	46		
(+)	14	3	11		
Microvascular invasion				0.007	0.293 (0.006)
(–)	56	24	32		
(+)	29	4	25		
Recurrence				0.030	0.235 (0.031)
(–)	29	14	15		
(+)	56	14	42		
Distant metastasis				<0.001	0.411 (0.001)
(–)	59	27	32		
(+)	26	1	25		
α-FP				0.073	0.194 (0.075)
<15	34	15	19		
≥15	51	13	38		
PIVKA-II				<0.001	0.404 (<0.001)
<40	26	16	10		
≥40	59	12	47		

HCC, hepatocellular carcinoma; BCLC, Barcelona Clinic Liver Cancer; UICC, Union for International Cancer Control; PIVKA-II, prothrombin induced by vitamin K absence II.
[a]Chi-square test. [b]Spearman rank correlation.

peroxidase activity and nonspecific immunoglobulin binding were consecutively blocked by sequential incubation with 3% hydrogen peroxide in methanol for 15 minutes and 10% normal goat serum for 10 minutes. The sections were incubated for 1 hour with primary rabbit anti-AEG-1 antibody (dilution, 1:100) (Abcam, Cambridge, MA, USA) at room temperature.

After washing in phosphate buffered saline (PBS, pH 7.4) three times for 5 minutes each at room temperature, the sections were incubated with Dako EnVision+System-HRP labeled perymer antirabbit (Dako, Carpentaria, CA, USA) for 40 minutes at room temperature. After washing in PBS, the sections were visualized with the Dako Liquid DAB+Substrate Chromogen System. The immunostained slides were observed by two independent pathologists after counterstaining with hematoxylin.

Semiquantitative analysis of AEG-1 staining

Two independent pathologists, experienced in evaluating IHC and blinded to the clinical outcomes of the patients, reviewed all samples. They determined AEG-1 expression semiquantitatively by assessing the percentage of positively stained immunoreactive cells and staining intensity. The percentage of immunoreactive cells was scored as follows: 0 points, <10%; 1 point, 10%–50%; and 2 points, >50%. The staining intensity rating was as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. As a result, the overall score for AEG-1 expression was the sum of the scores. The samples were divided into two groups for statistical analysis according to the overall scores: low expression scores from 0–2 and high-expression scores from 3–5. After the initial independent evaluation, both pathologists combined their scores and discussed the results to resolve any disparities in their independent scores.

Statistical analysis

All data were analyzed by PASW Statistics ver. 18.0 (SPSS Inc., Chicago, IL, USA). The chi-square and Fisher exact tests were used to compare the levels of AEG-1 expression and the various clinicopathological characteristics between the groups. Bivariate correlations between two independent variables were analyzed by calculating Spearman correlation coefficients. Survival curves for overall survival (OS) and disease-free survival (DFS) were calculated with the Kaplan-Meier method and were compared by the log-rank test. Multivariate analysis of prognostic relevance was evaluated by multivariate Cox regression analysis. A P-value of <0.05 was considered significant.

Table 3. Univariate and multivariate analysis of overall survival rate in HCC patients with Cox proportional hazards model

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Gender				
Male *vs.* female	0.510 (0.229–1.139)	0.101		
Age (yr)				
≤53 *vs.* >53	1.340 (0.763–2.353)	0.309		
Tumor size (cm)				
≤5 *vs.* >5	3.523 (1.987–6.247)	<0.001	2.285 (1.175–4.447)	0.015
Histological differentiation				
G1 *vs.* G2 *vs.* G3 *vs.* G4	1.877 (1.213–2.907)	0.005		
BCLC stage				
A *vs.* B *vs.* C *vs.* D	1.650 (1.140–2.387)	0.008		
Modified UICC stage				
I *vs.* II *vs.* III *vs.* IV	1.893 (1.215–2.948)	0.005		
Microvascular invasion				
(–) *vs.* (+)	1.769 (1.002–3.123)	0.049	6.754 (1.631–27.965)	0.008
Portal vein invasion				
(–) *vs.* (+)	1.765 (0.878–3.550)	0.111		
α-FP				
<15 *vs.* ≥15	1.690 (0.940–3.039)	0.080		
PIVKA-II				
<40 *vs.* ≥40	2.683 (1.337–5.384)	0.005		
AEG-1 expression				
Low *vs.* High	6.527 (2.764–15.415)	<0.001	4.756 (1.697–13.329)	0.003

HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; BCLC, Barcelona Clinic Liver Cancer; UICC, Union for International Cancer Control; PIVKA-II, prothrombin induced by vitamin K absence II; AEG-1, astrocyte elevated gene-1.

RESULTS

AEG-1 expression in different HCC tissues

We examined IHC staining of paraffin-embedded, archived HCC tissues from normal hepatocytes to highly infiltrated HCC to determine whether AEG-1 overexpression was related to the ES grading system. Only negative AEG-1 expression was observed in normal hepatocytes (Fig. 1A). The frequency and intensity of AEG-1 expression increased gradually from ES grades I to IV and were highest in highly infiltrative HCC (Fig. 1B–F).

Association between AEG-1 expression and HCC clinicopathological data

We analyzed the relationships between AEG-1 expression and the HCC clinicopathological data. The clinicopathological characteristics of the two groups are shown in Table 2. As a result, AEG-1 expression was strongly correlated with tumor size ($P = 0.002$), histological differentiation ($P = 0.009$), BCLC stage ($P = 0.001$), modified UICC stage ($P = 0.009$), microvascular invasion ($P = 0.007$), metastasis ($P < 0.001$), and PIVKA-II ($P < 0.001$) in patients with HCC.

However, no significant correlations were observed between AEG-1 expression and clinicopathological variable such as age, sex, liver cirrhosis, portal vein invasion, or α-FP.

Spearman rank correlation analysis was applied to further confirm the correlation between AEG-1 expression and the clinicopathological characteristics. As shown in Table 3, the correlations between AEG-1 expression level and tumor size, histological differentiation, BCLC stage, modified UICC stage, microvascular invasion, metastasis, and PIVKA-II were significant at 0.4042 ($P < 0.001$), 0.3969 ($P < 0.001$), 0.3903 ($P < 0.001$), 0.3510 ($P = 0.001$), 0.2931 ($P = 0.006$), 0.4109 ($P = 0.001$), and 0.4039 ($P < 0.001$), respectively.

Univariate and multivariate survival analysis

The Cox proportional hazard model was used to determine whether the independent factors affected the rates of OS and DFS in patients with HCC (Tables 3, 4). The univariate analysis revealed that tumor size ($P = 0.001$), histological differentiation ($P = 0.005$), BCLC stage ($P = 0.008$), modified UICC stage ($P = 0.005$), microvascular invasion ($P = 0.049$), PIVKA-II ($P = 0.005$), and AEG-1 expression ($P < 0.001$) were significant prognostic factors for OS in patients with HCC. Significant factors for DFS were tumor size ($P = 0.001$), portal vein invasion ($P = 0.028$), α-FP ($P = 0.020$), PIVKA-II ($P = 0.002$), and AEG-1 ($P = 0.002$).

Table 4. Univariate and multivariate analysis of disease-free survival rate in HCC patients with Cox proportional hazards model

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Gender				
Male *vs*. female	0.684 (0.356–1.315)	0.255		
Age (yr)				
≤53 *vs*. >53	1.435 (0.865–2.380)	0.162		
Tumor size (cm)				
≤5 *vs*. >5	2.923 (1.737–4.918)	0.001	2.245 (1.282–3.933)	0.005
Histological differentiation				
G1 and G2 *vs*. G3 and G4	1.244 (0.755–2.050)	0.391		
BCLC stage				
A and B *vs*. C and D	1.349 (0.808–2.252)	0.252		
Modified UICC stage				
I and II *vs*. III and IV	1.395 (0.840–2.315)	0.198		
Microvascular invasion				
(–) *vs*. (+)	1.487 (0.887–2.493)	0.132		
Portal vein invasion				
(–) *vs*. (+)	2.004 (1.080–3.720)	0.028		
α-FP				
<15 *vs*. ≥15	1.873 (1.103–3.180)	0.020		
PIVKA-II				
<40 *vs*. ≥40	2.637 (1.444–4.814)	0.002		
AEG-1 expression				
Low *vs*. high	2.530 (1.420–4.508)	0.002	1.916 (1.035–3.545)	0.038

HCC, hepatocellular carcinoma; HR, hazard ratio; CI, confidence interval; BCLC, Barcelona Clinic Liver Cancer; UICC, Union for International Cancer Control; PIVKA-II, prothrombin induced by vitamin K absence II; AEG-1, astrocyte elevated gene-1.

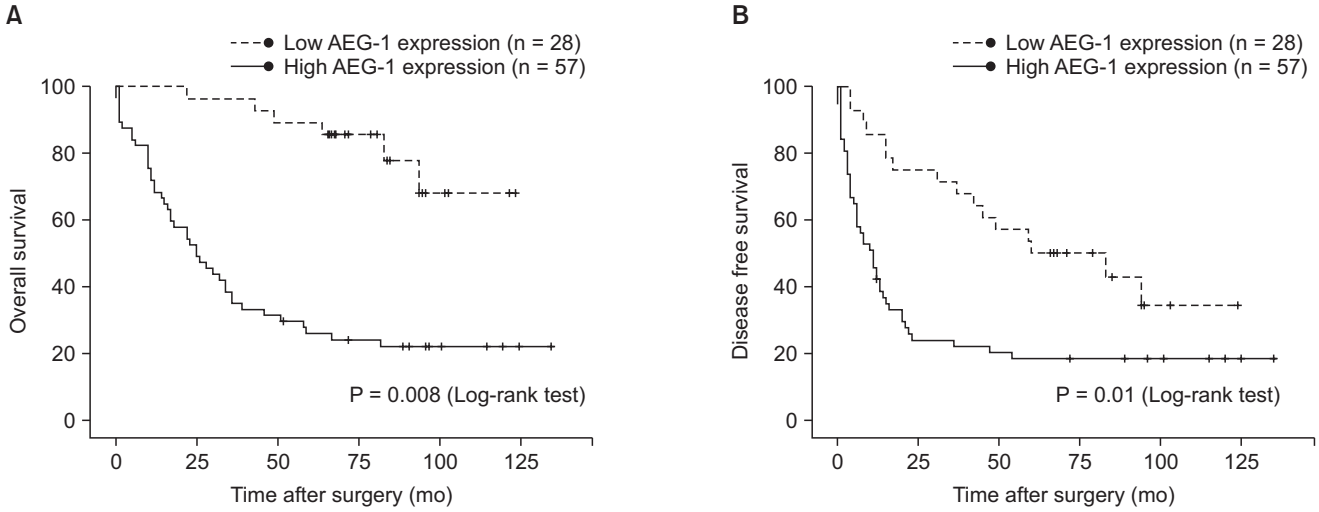

Fig. 2. Overall (A) and disease-free survival (B) of all the patients with hepatocellular carcinoma (HCC) in the two astrocyte elevated gene-1 (AEG-1) expression groups. The 5-year overall (A) and disease-free survival (B) were 24.5% and 17.6% in the AEG-1 high expressing group (thick line) and 89.2% and 50% in the low AEG-1 expressing group (dotted line).

The multivariate analysis showed that tumor size (hazard ratio [HR], 2.285; 95% confidence interval [CI], 1.175–4.447; P = 0.015), microvascular invasion (HR, 6.754; 95% CI, 1.631–27.965; P = 0.008), and AEG-1 expression (HR, 4.756; 95% CI, 1.697–13.329; P = 0.003) were independent prognostic factors for OS (Table 3). Those for DFS were tumor size (HR, 2.245; 95% CI, 1.282–3.933; P = 0.005), and AEG-1 expression (HR, 1.916; 95% CI, 1.035–3.545; P = 0.038) (Table 4).

The Kaplan-Meier analysis and log-rank test were used to evaluate the relationship between AEG-1 expression and survival rates. The mean and median survival times in the low AEG-1 expressing group were 106 and 72 months, compared with 47 and 25 months for the high AEG-1 expressing group. Cumulative 5-year survival and recurrence rates were 89.2% and 50.0% in the low-expressing group and 50% and 82.4% in the high-expressing group, respectively. Survival time for the high expressing AEG-1 group was significantly less than that in the low AEG-1 expressing group (P = 0.001, log-rank test) (Fig. 2). Recurrence rates were markedly higher in the high AEG-1 expressing group than those in the low AEG-1 expressing group (P = 0.001, log-rank test) (Fig. 2).

DISCUSSION

Only a few reports have shown that AEG-1 is strongly expressed in HCC samples [10,16]. In our study, tumor size, stage, histological differentiation, microvascular invasion, recurrence, and distant metastasis were related to the AEG-1 expression level. AEG-1 expression was also related to short survival rates. In particular, tumor size and AEG-1 expression were independent prognostic factors for OS and recurrence. Hence, AEG-1 was significantly associated with poor prognosis, disease severity, and recurrence. Microvascular invasion was also a prognostic factor for OS.

Studies have suggested that AEG-1 is associated with HCC invasion and metastasis [10,16]. And several studies have analyzed multiple mechanisms and intermediate markers for AEG-1 expression [9]. Hepatocarcinogenesis and a decrease in IL-6 were found in a mouse model that had HepG2 human hepatoma cells by AEG-1 knockdown [21]. AEG-1 expression regulates apoptosis, invasion, HCC cell viability by LY294002, and suppressed cell growth caused by the microRNA-375 *in vitro* and *in vivo* [14,16]. Some reports have indicated a relationship between prognosis and markers, including the epithelial-mesenchymal transition, Huaier polysaccharides, and AEG-1 expression [22,23]. AEG-1 functions include steatosis, hepatocarcinogenesis, and inhibition of senescence by activating the coagulation pathway in the Alb/AEG-1 mouse model [24].

Several studies have reported that overexpression of AEG-1 in various tumors is related to clinical prognosis [9]. Other studies have shown that high AEG-1 expression occurred in 54% of 323 patients with HCC and was related to factors such as microvascular invasion (P < 0.001), pathologic satellites (P = 0.007), tumor differentiation (P = 0.002), and TNM stage (P = 0.001). However age, sex, liver cirrhosis, α-FP, tumor size, tumor encapsulation, and BCLC stage were not associated with AEG-1 overexpression. The 5-year OS and cumulative recurrence rates were 50.7% and 59.7%, respectively. The 5-year OS rate in the high AEG-1 expressing group was significantly lower than that in the low AEG-1 expressing group (37.4% *vs.* 66.9%, respectively) and the 5-year cumulative recurrence rate was also higher in the high AEG-1 expressing group (70.7% *vs.* 47.8%, respectively). Encapsulation, tumor size, microvascular invasion, TNM stage,

and AEG-1 were independent prognostic factors for both OS (HR, 1.870; P < 0.001) and recurrence (HR, 1.695; P < 0.001) [9]. Another study showed that AEG-1 expression was significantly associated with American Joint Committee on Cancer stage (P = 0.020), T classification (P = 0.007), N classification (P = 0.044), vascular invasion (P = 0.006), and histological differentiation (P = 0.020). In addition, patients with high AEG-1 levels had shorter survival times compared to those with low AEG-1 levels (P = 0.001) [5].

Our study also showed that high AEG-1 expression occurred in 57 of the 85 cases (67%) and the 5-year OS in the high AEG-1 expressing group was significantly lower than that in the low AEG-1 expressing group (24.5% *vs.* 89.2%). The 5-year cumulative recurrence rate in the high AEG-1 expressing group was significantly higher than that in the low AEG-1 expressing group (82.4% *vs.* 50.0%). The rate of distant metastasis and recurrence in the high AEG-1 expressing group were 96% and 75%, respectively. These results suggest that AEG-1 might be a useful biomarker to identify the progression of high-risk patients. Approximately 40% of the patients (34 of 85) were TACE cases, and these were not significantly different. The majority of patients (73 of 85) had viral hepatitis and most were HBV positive. Approximately 82% of patients diagnosed preoperatively with liver cirrhosis were included in Child-Pugh A, and AEG-1 expression was not associated with TACE, HBV positivity, or liver cirrhosis in those patients.

Treatments for HCC depend on disease stage and grade [25]. Surgical resection, radiofrequency ablation, and liver transplantation are the treatments of choice when the disease is localized [26]. Surgical resection and transplantation are not amenable in many cases because of underlying liver cirrhosis and advanced cancer stage. Alternative treatments include local ablation and TACE. TACE and systemic therapy with doxorubicin alone or a combination with cisplatin, interferon, doxorubicin, and 5-fluorouracil (PIAF) are being used for advanced disease with moderate improvements in OS of 6.8–8.6 months [4,25]. However, the recurrence rates for the remnant liver are high after surgery and the benefit to survival following chemotherapy was unclear in a randomized controlled trial [4]. In particular, AEG-1 contributes to 5-fluorouracil chemoresistance. AEG-1 augments expression of the transcription factor LSF (Late SV40 Factor), which regulates the expression of thymidylate synthase, a target of 5-fluorouracil [26]. Anticancer drugs and target therapy have been developed with advances in molecular biology. Sorafenib, an inhibitor of c-Raf and B-Raf kinases in the vascular endothelial growth factor (VEGF) receptor family, has been approved by the U.S. Food and Drug Administration. In a recent randomized controlled trial, sorafenib was associated with an increase in OS in patients with unresectable advanced HCC. Median survival for placebo-treated patients was approximately 7.9 months, whereas sorafenib-treated patients survived 10.7 months [27]. Other target therapeutic agents include those for the fibroblast growth factor receptor, the VEGF receptor, epidermal growth factor receptor, the platelet derived growth factor receptor, an mTOR inhibitor, and a VEGF antibody, which are currently being studied [28]. Hence, anticancer therapy for HCC remains a major clinical challenge.

Autoantibodies against tumor-associated antigens are present in the blood of patients with cancer [29]. Determining the anti-AEG-1 antibody titer as a serum biomarker for cancer is an encouraging approach, but comparative studies must be carried out to establish its specificity and selectivity versus other currently employed serum biomarkers. As AEG-1 overexpression is detected on the cancer cell membrane, it is hypothesized that patients with cancer may have elevated serum levels of anti-AEG-1 antibody. Sera from 483 patients with cancer were analyzed by ELISA to detect the anti-AEG-1 antibody using the lung-homing domain (amino acids, 381–443) of human AEG-1 as the antigen. In that study, the antibody titer increased with stage; thus, the anti-AEG-1 antibody might be a marker for cancer progression [18].

In conclusion, our results showed the AEG-1 was overexpressed in HCC specimens. Thus, AEG-1 expression may serve as a valuable prognostic marker. We demonstrated that AEG-1 plays a significant role in proliferation and metastasis in patients with HCC. However, some limitations of our study should be mentioned. Further multicenter investigations and functional studies are needed to verify our findings. More extensive research will be needed to use AEG-1 as a tool in clinical diagnostic laboratories, as a monitor during target therapy, cancer progression, and cancer relapse, and for defining the efficacy of cancer therapy.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This work was supported by the Soonchunhyang University Research Fund.

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