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High expression of ADAM8 correlates with poor prognosis in hepatocellular carcinoma

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ABSTRACT

Objectives: To evaluate the association between ADAM8 tissue expression and patient prognosis in hepatocellular carcinoma (HCC).

Methods: ADAM8 expression was analyzed using immunohistochemical staining methods on tissue samples from a consecutive series of 105 HCC patients who underwent resections between 2000 and 2006. The correlation of ADAM8 expression and patients' clinicopathological parameters was evaluated. Survival analysis was performed using the Kaplan–Meier method and Cox's proportional hazards model.

Results: ADAM8 was highly expressed in 54.3% of the HCC patients. The ADAM8 expression level was closely associated with serum AFP elevation, tumor size, histological differentiation, tumor recurrence, tumor metastasis, and tumor stage. Kaplan–Meier survival analysis showed that a high expression level of ADAM8 resulted in a significantly poor prognosis of HCC patients. Multivariate analysis revealed that ADAM8 expression level was an independent prognostic parameter for the overall survival rate of HCC patients.

Conclusions: These findings provide evidence that a high expression level of ADAM8 serves as a biomarker for poor prognosis for HCC. Thus, we speculate that ADAM8 may be a potential target of antiangiogenic therapy for HCC.

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death, with increasing incidence worldwide.[1,2] It is more prevalent in Central Africa and parts of Asia, and half of the worldwide instances are reported in China. Most patients with HCC have a very poor prognosis, and the annual number of newly diagnosed cases is almost equal to the number of deaths.[3] This tragic situation stems from a lack of early diagnosis, the deteriorated condition of the cirrhotic liver from which most HCC cases develop, and the high resistance of HCC to chemotherapy.[4] The identification of novel molecular mechanisms involved in the development and progression of HCC may provide new strategies for the diagnosis and treatment of this life-threatening disease.

ADAM family members are implicated to be involved in the proteolytic processing of membrane-bound precursors, and they modulate cell–cell and cell–matrix interactions. ADAM8

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encodes a protein of 824 amino acids with a COOH-terminal transmembrane domain and potential extracellular adhesion and protease domains.[5,6] This molecule, which is localized to the plasma membrane, is processed by autocatalysis into two forms: one is derived through the removal of a prodomain and the other is a remnant protein composed of the extracellular region with a disintegrin domain at the NH2 terminus.[7] ADAM8 behaves as an active metalloprotease in vitro and hydrolyzes myelin basic protein and a variety of peptide substrates based on the cleavage sites of membrane-bound cytokines, growth factors, and receptors.[8–11] Other studies have demonstrated the overexpression of some ADAM family proteins in a variety of human tumors,[12,13] but no report is available on the actual expression level of ADAM8 and the correlation between clinicopathological features and prognosis of HCC patients.

Hepatocellular carcinoma is a highly malignant disease with poor prognosis. In clinical practice, it would be valuable to have a predictive marker to evaluate the behavior of the tumor and its response to treatment. Here, we examined the immunoexpression of ADAM8 in hepatocellular carcinoma tissue array specimens in relation to clinicopathological parameters.

Materials and methods

Tissue specimens

HCC specimens were obtained from 105 patients who underwent surgery between January 2000 and December 2006 in Yixing People's Hospital. All patients were from Jiangsu province, China. Their diagnosis was made by a pathological examination, and none of the patients recruited in this study had chemotherapy or radiotherapy before the surgery. The histomorphology of all specimens was assessed by the Department of Pathology at Yixing People's Hospital. Histological cell types were assigned following the WHO classification criteria. The specimens were fixed in 10% formaldehyde and embedded in paraffin for histological sectioning. Clinical information was collected and stored in a database. Postoperative surveillance included routine clinical and laboratory examinations every third month, computed tomography scans of the abdomen, and radiographs of the chest every third month. For 5-year survival analysis, person-months were calculated from the date of resection to the date of death or 60 months for those who survived for >5 years.

Immunohistochemical analysis

All 105 tissue specimens were subjected to immunohistochemical analysis using the avidin–biotin–peroxidase method. The sections were deparaffinized in xylene and dehydrated using a graded alcohol series before the endogenous peroxidase activity was blocked with 0.5% H_2O_2 in methanol for 10 min. Nonspecific binding was blocked by incubating sections with 10% normal goat serum in phosphate-buffered saline (PBS) for 1 h at room temperature. Without washing, the sections were incubated with anti-ADAM8 antibody (1:100; Abnova, Taipei, Taiwan) in PBS at 4 °C overnight in a moist box. Biotinylated goat anti-rabbit immunoglobulin G (IgG) (1:400; Sigma, St. Louis, MO, USA) was incubated with the sections for 1 h at room temperature and detected with a streptavidin–peroxidase complex. The brown color indicative of peroxidase activity was developed by incubating sections with 0.1% 3,3-diaminobenzidine (Sigma) in PBS with 0.05% H_2O_2 for 5 min at room temperature. The tissue specimens were viewed double blind. The expression of ADAM8 in the HCC specimens was evaluated by scanning the entire tissue specimen under low magnification (40×) and then confirmed under high magnification (200× and 400×). An immunoreactivity score (IRS) system was applied as described elsewhere.[14] The percent of positive cells was scored as '0' (<5%, negative), '1' (5%–25%, sporadic), '2' (25%–50%, focal), and '3' (>50%, diffuse). The staining intensity was scored as '0' (no staining), '1' (weakly stained), '2' (moderately stained) and '3' (strongly stained). Both the percent of positive cells and cell staining intensity were decided in a double-blinded manner. The final ADAM8 immunostaining score was calculated using the percent of positive cell score × staining intensity score ranging 0–9. A high ADAM8 expression level was defined as a total score ≥4, and a low ADAM8 expression level was defined as a total score <4.

Statistical analyses

All statistical analyses were performed using the SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA). The correlation of ADAM8 expression with immunohistochemistry and clinicopathological parameters was evaluated by chi-square test or Fisher's exact probability test. Survival curves were plotted using the Kaplan–Meier product-limit method, and differences between survival curves were tested using the log-rank test. Cox's proportional hazards model was used to identify the factors that had a significant influence on survival. Statistical significance was set at $P < 0.05$.

Results

Expression of ADAM8 in HCC tissues

The expression level of ADAM8 protein in 105 HCC tissue samples was measured by immunohistochemical staining. ADAM8 localized at the plasma membrane and in the cytoplasm of tumor cells. Overall, ADAM8 was positively and negatively expressed in 82 (78.1%) and 23 (21.9%) of the 105 HCC patients, respectively. ADAM8 was highly and lowly expressed in 57 (54.3%) and 48 (45.7%) of the 105 HCC patients, respectively (Fig. 1). The ADAM8 expression level in HCC was associated with serum alpha fetoprotein (AFP) elevation ($P = 0.045$) but not with age, gender, chronic HBV infection, or liver cirrhosis (Table 1). Histologically, high ADAM8 expression occurred more often in larger tumors ($P = 0.036$) and in late-stage (stage III–IV) HCC ($P = 0.023$). In addition, ADAM8 expression level was associated closely with histological differentiation ($P = 0.037$), tumor recurrence ($P = 0.003$), and tumor metastasis ($P = 0.033$) (Table 1).

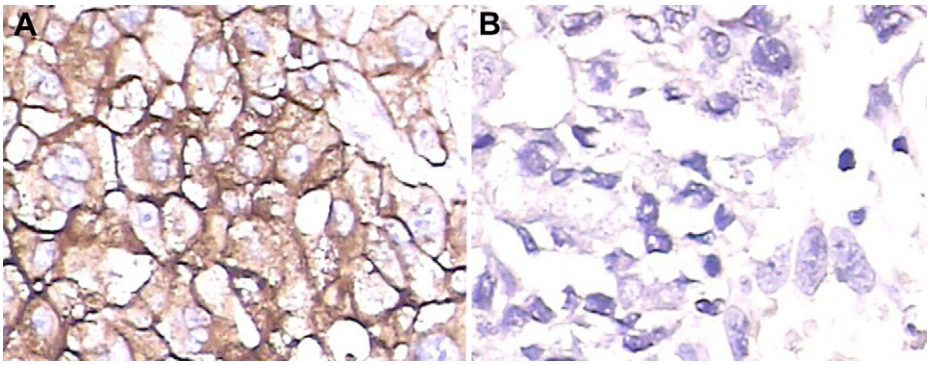

Fig. 1 – Immunohistochemical analysis of ADAM8 in hepatocellular carcinoma patients. A: high expression level of ADAM8. B: low expression level of ADAM8; A, B: original magnification 200×.

Univariate and multivariate analyses of prognostic variables in HCC patients

The prognostic effect of ADAM8 on the overall survival rate of HCC patients with a high or low ADAM8 protein expression level was compared using Kaplan–Meier survival curves and the log-rank test. These data showed that the high expression level of ADAM8 protein was a significant prognostic factor for the poor overall survival rate of HCC patients. The 5-year survival rate of HCC patients with a high or a low ADAM8 protein expression level was 31.6% and 64.6%, respectively. A significant difference was observed on the Kaplan–Meier survival curves for HCC patients with a high or a low expression level of ADAM8 ($P < 0.001$, log-rank test, Fig. 2). Univariate Cox regression analysis also identified that clinical variables, including serum AFP, tumor size, histological differentiation, tumor recurrence, tumor metastasis, and tumor stage, and ADAM8 expression were significantly associated with overall survival (Table 2). Furthermore, to evaluate the potential of ADAM8 expression as an independent predictor for overall survival of HCC, multivariate Cox regression analyses were performed. While the other factors failed to demonstrate independence, serum AFP, tumor size, histological differentiation, tumor recurrence, tumor metastasis, tumor stage and ADAM8 expression may play a role in predicting overall survival in HCC (Table 2).

Table 1 – Relationship between ADAM8 expression in HCC and clinicopathological features of hepatocellular carcinoma patients.

Clinicopathological variable (*n*)	Low ADAM8 (total score <4) *n* = 48	High ADAM8 (total score ≥4) *n* = 57	*P* value
Age (yr)			
<60 (59)	23	36	0.116
≥60 (46)	25	21	
Gender			
Male (55)	29	26	0.286
Female (50)	19	31	
Tumor size (cm)			
≤5 (25)	16	9	0.036
>5 (80)	32	48	
Histological differentiation			
Well/moderate (54)	30	24	0.037
Poor (51)	18	33	
Liver cirrhosis			
With (62)	30	32	0.509
Without (43)	18	25	
Recurrence			
Yes (33)	8	25	0.003
No (72)	40	32	
Metastasis			
With (23)	6	17	0.033
Without (82)	42	40	
HBsAg			
Positive (89)	39	50	0.358
Negative (16)	9	7	
AFP (ng/ml)			
≤400 (17)	4	13	0.045
>400 (88)	44	44	
Tumor stage			
I–II (32)	20	12	0.023
III–IV (73)	28	45	

Discussion

Despite many advances in the diagnostic imaging of tumors, combination chemotherapy, and radiation therapy, little improvement has been achieved within the last decade in terms of prognosis and quality of life for patients with hepatocellular carcinoma. Given the frequent failure of

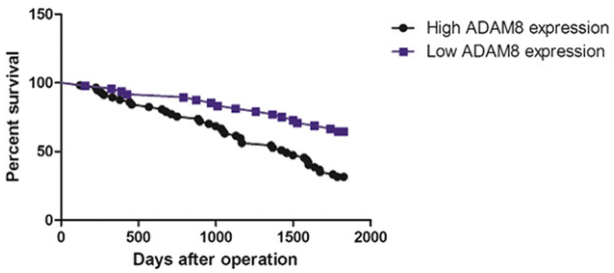

Fig. 2 – Overall survival rate of hepatocellular carcinoma patients estimated according to the ADAM8 expression level in hepatocellular carcinoma tissue samples (Kaplan–Meier method) with immunohistochemical staining ($P < 0.001$).

Table 2 – Univariate analysis and multivariate analyses showing the overall survival rate for hepatocellular carcinoma patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
ADAM8	1.987	1.023–3.674	0.015	1.876	1.082–3.543	0.014
Age	0.867	0.435–1.435	0.354			
Gender	0.679	0.367–1.231	0.442			
Tumor size	1.342	0.897–1.964	0.008	1.231	0.987–2.856	0.013
Histologic grade	1.213	0.768–2.324	0.044	1.356	0.823–1.978	0.032
Liver cirrhosis	1.432	1.121–2.465	0.453			
Recurrence	1.456	0.986–2.546	0.035	1.678	1.123–2.134	0.014
Metastasis	1.324	1.123–1.654	0.026	1.341	0.786–1.856	0.021
HBsAg	1.231	0.897–1.432	0.238			
AFP (ng/ml)	1.342	1.123–1.678	0.043	1.751	1.134–2.341	0.047
Tumor stage	1.465	0.967–1.956	0.023	1.213	0.684–1.563	0.015

conventional treatment strategies, many cancer-related molecules have been characterized toward the goal of developing novel anticancer therapies such as molecular-targeted drugs and antibodies or cancer vaccines.[15,16] Molecular-targeted therapies are expected to be highly specific to malignant cells, with minimal adverse effects due to their well-defined mechanisms of action. Equally desirable in prospect are minimally invasive, highly sensitive, and specific new diagnostic methods that would adapt readily to clinical settings. From this point of view, tumor-specific transmembrane/secretory proteins should have significant advantages because they are presented either on the cell surface, within the extracellular space and/or in serum, which makes them easily accessible as molecular markers and therapeutic targets. Some tumor-specific markers already available, such as CYFRA or Pro-GRP, which are transmembrane/secretory proteins[17,18]; rituximab (Rituxan), a humanized monoclonal antibody against CD20-positive lymphomas, provide proof that targeting specific cell-surface proteins can result in significant clinical benefits.[22] As an approach to identifying novel cancer-specific cell surface or secretory proteins, we have been exploiting the power of genome-wide expression analysis to select genes that are overexpressed in cancer cells. The analysis of candidate molecules revealed ADAM8 as a potential target for the development of novel tools for the diagnosis and treatment of hepatocellular carcinoma.

ADAM8 protein is homologous to a snake disintegrin, Reprolysin (M12B), which is a zinc metalloprotease.[6] The members of the ADAM family are cell-surface proteins with a unique structure combining potential adhesion and protease domains. A published report has suggested that the ADAM8 ectodomain is cleaved by ADAM8 itself.[7] Because various matrix metalloproteinases and ADAM family proteins had been described as being overexpressed in human cancers,[19] ADAM8 seemed likely to have a potential role in tumor development or progression. In this study, we evaluated the association between ADAM8 tissue expression and patient prognosis in hepatocellular carcinoma. In our tumor specimens, ADAM8 was positively expressed in 78.1% of HCC patients and highly expressed in 54.3% of HCC patients.

ADAM8 expression level was closely correlated with serum AFP elevation and tumor stage, confirming the association of poorly differentiated HCC with AFP elevation.[20] In addition, a high expression level of ADAM8 occurred more often in larger tumors ($P = 0.036$) and in late-stage (stage III–IV) HCC ($P = 0.023$). Late-stage HCC exhibited portal vein invasion and was correlated closely with poorer prognosis.[21] Our findings therefore support the suggestion that ADAM8 expression enhances the metastatic potential of transformed cells and human cancers, including HCC.[22,23] Our results suggest that ADAM8 expression was not related to HBV infection and liver cirrhosis, indicating that ADAM8 may be not associated with the initiation of HCC.[24]

In this study, the prognosis of HCC patients with a high expression level of ADAM8 was poor, and Cox regression analysis indicated that a high expression level of ADAM8 was a significant prognostic factor for a poor overall survival rate of HCC patients, suggesting that ADAM8 may become a novel prognostic marker for HCC.

Based on our data, there were some weaknesses in the study. Firstly, because fresh tissue was not available, western blot analyses for ADAM8 could not be performed, and these results were based on immunohistochemistry in paraffin-embedded specimens. Secondly, the exact mechanism under which the loss of ADAM8 regulates tumor development and progression is also largely unknown. Thus, more work needs to be performed.

In conclusion, our results provide a basis for the concept that a high expression level of ADAM8 in hepatocellular carcinoma may be important in tumor progression and serves as an independent biomarker for poor survival. Thus, we speculate that a high expression level of ADAM8 identifies patients at high risk and is a potential novel therapeutic target for hepatocellular carcinoma.

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