RESEARCH ARTICLE

Altered Distribution and Expression Pattern of E-cadherin in Hepatocellular Carcinomas: Correlations with Prognosis and Clinical Features

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Abstract

Objective: E-cadherin has been identified as a tumor suppressor in many types of carcinoma. However, some studies recently suggested that the role and expression of E-cadherin might be more complex and diverse. In the present study, we evaluated the prognostic value of E-cadherin expression with reference to levels in membranes and cytoplasm, and the membrane/cytoplasm ratio, in hepatocellular carcinomas (HCCs) after curative hepatectomy. Methods: The expression of E-cadherin was assessed by immunohistochemistry in HCC tissue microarrays from 125 patients, and its prognostic values and other clinicopathlogical data were retrospectively analyzed. Patients were followed for a median period of 43.7 months (range 1 to 126 months). Results: Univariate analysis demonstrated that a high membrane/cytoplasm (M/C) ratio of E-cadherin expression was associated with poor overall survival (OS) (P =0.001) and shorter time to recurrence (TTR) (P =0.038), as well as tumor size, intrahepatic metastasis, and TNM stage. In contrast, neither membrane nor cytoplasmic expression of E-cadherin was related with OS and TTR. Furthermore, multivariate analysis confirmed the M/C ratio to be an independent predictor of OS (P =0.031). χ^2 tests additionally showed that the M/C ratio of E-cadherin expression was related with early stage recurrence (P =0.012), rather than later stage recurrence. Conclusion: The M/C ratio of E-cadherin expression is a strong predictor of postoperative survival and is associated with early stage recurrence in patients with HCC.

Keywords: Hepatocellular carcinoma - E-cadherin - tumor suppressor

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common aggressive malignant tumor in the world. Regions of high prevalence include East and Southeast Asia with sub-Saharan Africa and China ranked 2nd worldwide in cancer-related deaths with a 5-year overall survival rate of less than 5% (Kensler et al., 2003). Surgical resection is the preferred standard treatment for patients with resectable HCC, but high postoperative recurrence, especially early recurrence, and metastasis rate remain the major obstacles that influence long-term survival (Tang et al., 2004). Thus, improving our understanding of the postoperation metastasis and recurrence mechanisms, and identifying recurrence markers of HCC is essential for improving clinical outcomes in treatment.

E-cadherin is a 120kDa calcium-dependent transmembrane glycoprotein widely expressed at adhesion junctions in most normal epithelial tissues and well-differentiated cancer cells (Bussemakers et al., 1993). Once synthesized, E-cadherin has a short half-life (5-10h) with dynamic distribution between cell membrane and cytoplasm and degradation eventually in lysosome (Jiang and Mansel, 2000). It is reported that E-cadherin, although catalytically inactive, are able to translate environmental cues into complex intracellular signals through the interaction between themselves and the process of endocytosis and recycling to membrane (Mosesson et al., 2008). E-cadherin play a pivotal roles in establishing cell polarity, maintaining epithelial integrity and cellular differentiation (Wijnhoven and Pignatelli, 1999). Reduced expression of E-cadherin may disrupt the E-cadherin-catenin complex and inactivate the E-cadherin-mediated invasion suppressor system. Loss of cell adhesion, dedifferentiation and metastasis, results in invasive and metastatic properties (Thiery, 2002; Cowin et al., 2005). Thus E-cadherin was identified as a tumor suppressor in many types of carcinoma (Ross et al., 1995; Cespedes et al., 2010; Montserrat et al., 2011).

However, along with going deep into of research

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gradually some studies suggest that the role and expression of E-cadherin might be more complex and diverse. Some puzzled observations about E-cadherin expression in breast cancer, for example, suggesting loss of E-cadherin is generally considered a harbinger of metastasis, Researchers have also found that most breast cancer that has spread retains E-cadherin expression (Kavgaci et al., 2010). Likewise, ovarian tumors have paradoxically been found to produce more and more E-cadherin as they grow (Shim et al., 2009). E-cadherin expressed in glioblastomas did not function to keep cells stuck together. Instead, they promoted tumor growth and migration and linked to aggressive cell behavior and poor prognosis. E-cadherin expressed in glioblastoma functioned like an oncogene and these similarities in function could transcend in many breast, ovarian, and other tumors types (Lewis-Tuffin et al., 2010). Expressions of E-cadherin in hepatocellular carcinoma were also diverse and seemingly paradoxical. It has been reported that liver metastases of gastric tumors are composed strongly E-cadherin-positive cells during outgrowth in the liver environment (Mayer et al., 1993). E-cadhrin can also promote lymphovascular and intraepithelial invasion in other tumor types, enforced intercellular adhesion mediated by E-cadherin might favor the intake and expansion of tumor cells in liver and the shift of E-cadherin between membrane and cytoplasm may be associated with metabolite and functional changes of E-cadherin (Wei et al., 2002).

To gain further insight into E-cadherin involvement of in HCC, we use immunohistochemistry to examine the levels of E-cadherin expression in 125 primary HCC samples. E-cadherin expression on the cell membrane (M), cytoplasm(C) accumulation and membrane/cytoplasm ratio (M/C) were assessed separately. Univariate and multivariate survival analyses were used to determine the potential predictive and prognostic value of E-cadherin expression in these patients.

Materials and Methods

Patient's selection and evaluation

We used anonymized primary tumor tissue samples from total of 125 consecutive patients diagnosed with HCC pathologic stage I to III a (according to the 2002 international Union Against Cancer TNM classification system (Sobin, 2002)) at the University of Fudan and Zhongshan Hospital liver cancer institute. All 125 patients, receiving curative hepatectomy, had complete medical records, had been followed by the tumor registries for survival time and outcome, and had adequate paraffin embedded fixed tissue blocks. Tumor differentiation was graded by the Edmondson grading system (Brunt, 2000). Liver function was assigned by Child-Pugh scoring system. The study was approved by the ZhongShan Hospital Research Ethics Committee.

Patients' demographics, tumor, and operative characteristics were evaluated. The following variables were analyzed: age, gender, extent of cirrhosis, AFP, hepatitis B, and preoperative ALT. Pathologic specimens were reviewed for tumor characteristics: number and size of tumors, tumor grade, vascular invasion, intrahepatic metastasis, and microscopic margins.

Patients were followed until March 31 2010 with a median follow-up of 43.7 months. None of the patients received radiotherapy or chemotherapy before surgery. Patients were monitored by abdominal ultrasonography, serum α-fetoprotein and chest radiography with an interval of 2–6 months according to the postoperative time. If recurrence was suspected, computed tomography (CT) scanning or magnetic resonance imaging (MRI) was performed immediately. Overall survival (OS), time to recurrence (TTR) was defined as the interval between surgery and death or recurrence respectively. If recurrence was not diagnosed, patients were censored on the date of death or the last follow-up. Early recurrence (ER) was defined as intrahepatic, regional or systemic recurrence within 1 year of surgery, which is regarded as one of the most important factors impacting the prognosis and outcomes of HCC (Regimbeau et al., 2004) were also analyzed.

Tissue microarrays construction and immunohistochemistry protocols and evaluation

The most representative tumor areas to be sampled for the tissue microarray (TMAs) were carefully selected and marked on the hematoxylin and eosin slide. Tissue microarrays were constructed as described previously (Xu et al., 2011). Briefly, two cores were taken from each formalin-fixed paraffin-embedded tumor tissue within a distance of 10 mm to construct TMAs slides (in collaboration with Shanghai Biochip Company, Shanghai, China). Taking tumor heterogeneity into account, duplicate cylinders from two different areas from each patient were obtained and representative areas were away from necrotic and hemorrhagic materials.

Immunohistochemistry was carried out according to appropriate protocols described (Qian et al., 2006). Briefly, TMA blocks were baked at 60°C 2 hours, deparaffinized in xylene, hydrated in graded alcohol. Antigen retrieval were achieved by microwave-treated with citrate buffer (low pH, Dako, carpinteria, CA, USA) at middle power for 5min. Endogenous peroxides activity was blocked with incubation of the slides in 0.3% H_2O_2 in room temperature for 15 min. Sections were then incubated with 5% bovine serum albumin (BSA, Sigma-Aldrich, Inc.) at room temperature for 30min and primary antibody at 4°C overnight. Sections were placed in the detection system of the Envision Detection Kit (Dako, carpinteria, CA, USA). The negative control was Dako Cytomation mouse IgG serum diluted at the same concentration as the primary antibody. At last, TMA slides was counterstained with haematoxylin, dehydrated with ethanol, and permanently cover slipped. Slides were washed in PBS (pH7.4) after every step but not after incubation with 5%BSA. The primary antibodies used were mouse monoclonal antihuman E-cadherin (1:200, Dako, carpinteria, CA, USA), and the mouse polyclonal antihuman E-cadherin (1:200, Santa Cruz, California, USA). Two pathologists double-blinded independently reviewed the slides Categorization of E-cadherin expression followed as cells were considered positive for the protein if their membranes or cytoplasm had yellow or brown staining. Membrane

Table 1. Clinicopathological Characteristics of the Patients

Features	Values/Counts (n=125)
Age(years, median(range))	10/115
Gender(male/female)	51.43(18-75)
Hepatitis B history(yes/no)	105/20
Liver cirrhosis(yes/no)	97/28
Hepatitis B e antigen, positive/negative	95/30
Preoperative ALT(U/I, median(range)	46.72(2-178)
a-Fetoprotein(ng/ml, median (range)	258(0-60,000)
Portal vein thrombosis(Absent/Present)	74/51
Tumor number(Solitary/Multiple)	31/94
Tumor size(<5cm /≥5cm)	54/71
Tumor size(<3cm /≥3cm)	31/94
Microvascular invasion(yes/no)	53/72
Encapsular invasion(yes/no)	57/68
Intrahepatic metastasis	31/94
TNM stage(I/II/IIIA)	78/28/19
BCLC stage, A/B/C	36/75/14

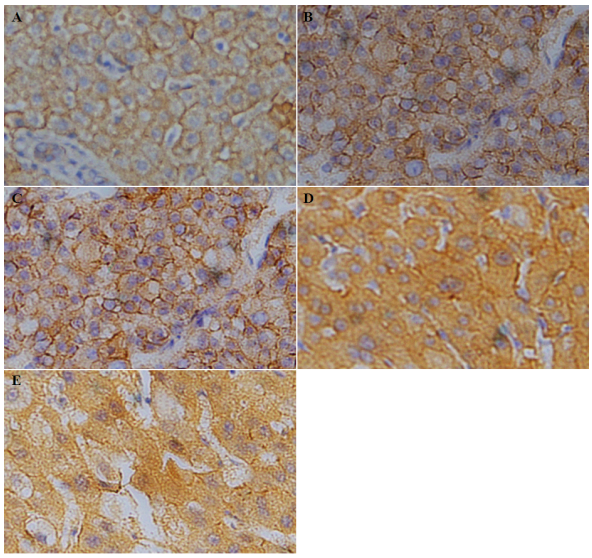

Figure 1. Photographs of E-cadherin Immunostaining in HCC Tissues were Taken for Further Analysis. The expression of E-cadherin was mainly in the membrane and cytoplasm and identify with monoclonal (A) and polyclonal (B). Photographs of high density staining in membrane(C), equal density staining between membrane and cytoplasm (D), and high density staining in cytoplasm (E)

and cytoplasm expression of E-cadherin was scored as 0 (negative), ++ (<5% positive cells), 2+ (5% to 50% positive cells) and +++ (>50% positive cells) (Han et al., 1997), scored the intensity of membrane/cytoplasm at the same time: 1 (membrane≥ cytoplasm) and 2 (membrane < cytoplasm).

Data analysis

Analysis was performed with SPSS 14.0 for windows (SPSS, Chicago, IL). The Pearson χ^2 test or Fisher exact test as applied to compare qualitative variables; the Student t test or Pearson correlation test was used to compare quantitative variables. Univariate analysis was calculated by the Kaplan-Meier method (the log-rank test). Multivariate analysis was done using the Cox multivariate proportional hazard regression model. Patients' survival was determined by Kaplan-Meier analysis, and the log-rank test was used t compare survival between subgroups. P<0.05 was considered statistically significant.

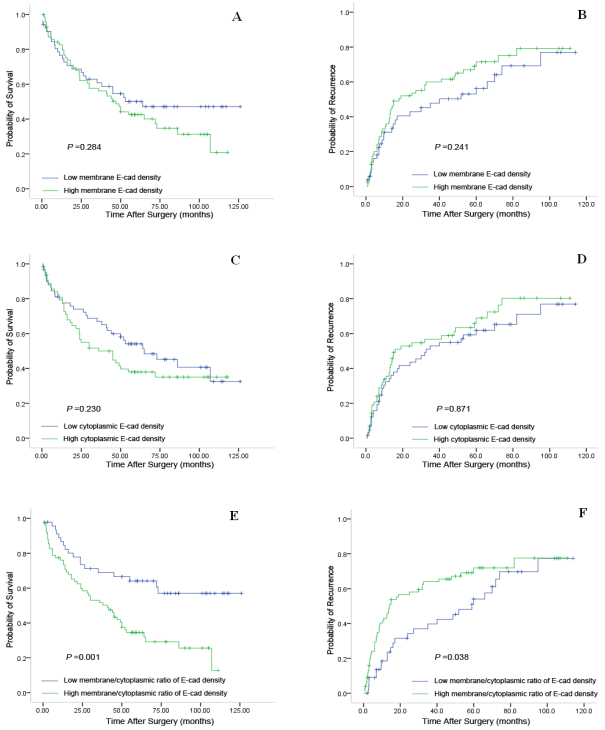

Figure 2. Kaplan-Meier Curves of Overall Survival (OS) and Time to Recurrence (TTR) Different among Membrane E-Cadherin Expression, cytoplasm E-cadherin expression and membrane/cytoplasm ratio E-Cadhrein expression. Membrane E-cadherin expression was not significantly associated either with TTR (A) and OS (B). Cytoplasm E-cadherin expression also was not significantly associated either with TTR (C) and OS (D). Membrane/cytoplasm ratio E-cadhrein expression was significantly associated with TTR (E) (P=0.001) and OS (F) (P=0.038)

Results

Clinicopathologic data

The clinicopathologic features of the patients enrolled in this study were described in Table 1. All patients underwent curative hepatectomy for HCC. The majority of the patients were male (92%) and the median age was 51.43 years (rang, 18 to 75 years). About 84% patients had HBV-infectious background, and 77.6% patients companied with liver cirrhosis. The mean preoperative ALT and AFP were 46.72 U/L and 258 ng/ml respectively. Less half patients had portal vein thrombosis (40.8%), microvascular metastasis (42.4%), and encapsular invasion (45.6%) in our study. About 24.8% patients happened intrahepatic metastasis, 75.2% patients developed more than 3cm tumor, and 56.8% larger than 5cm. Most patients were TNM stage I and BCLC stage B. At the time of the last follow-up, 70 patients had died, including 13 patients who had died of liver failure without record of tumor recurrence; 78 patients had tumor recurrence, with 41 early recurrences(ER). Resection, radiofrequency ablation, transcatheter arterial chemoembolization, radiotherapy and support treatment were administered according to a uniform guideline. The 1-, 3- and 5-year OS rates were

Table 2. Relationships Between E-cadherin Expression and Clinicopathologic Features

Variables	Cell membrane E-cadherin density[1]			Cell cytoplam E-cadherin density[1]			Membrane/cytoplasm E-cadherin density[1]		
	High (n=53) No. of patients	Low (n=71) No. of patients	P	High (n=64) No. of patients	Low (n=60) No. of patients	P	low (n=47) No. of patients	High (n=77) No. of patients	P
Age(years)[2]	51.02	51.73	0.714	49.98	52.79	0.741	50.1	52.2	0.763
Gender[3]			0.965						0.568
female	48	66		61	53	0.136	43	71	
male	5	5		3	7		4	6	
Hepatitis B history			0.421			0.257			0.442
yes	44	10		56	49		38	67	
no	9	61		8	11		9	10	
Hepatitis Be antigen			0.421			0.498			1
positive	39	55		48	46		11	19	
negative	14	16		16	14		36	58	
Liver cirrhosis			0.065			0.102			0.515
yes	45	51		53	43		38	58	
no	8	20		11	17		9	19	
AFP(ng/ml)	3335.07	3906.12	0.543	1929.64	5489.48	0	4388.94	22443.5	0.121
Preoperative ALT,U/L	48.36	45.8	0.137	44.7	49.29	0.455	47.5	46.5	0.438
Tumor size			0.473			0.281			0.853
≤5	24	37		28	33		24	37	
>5	29	34		36	27		23	40	
Tumor size, cm	7.9	5.1	0.002	6.67	6.68	0.036	5.91.	7.09	0.036
Tumor encapsulation			0.418						0.46
complete	28	40		34	34	0.415	19	37	
none	25	31		30	26		28	40	
Microvascular invasion			0.25			0.545			0.049
yes	32	42		38	36		13	43	
no	21	29		26	24		34	34	
Intrahepatic metastasis			0.233.			0.267			0.002
yes	42	51		46	47		39	44	
no	11	20		18	13		8	33	
TNM stage			0.213			0.079			0.019
I	9	5		3	11		14	3	
II	29	37		34	32		29	34	
IIIa	1	25		24	15		15	25	
IIIb	1	4		3	2		2	2	

1 core of 5 and 1patients were unexpectedly detached from TMA sectons during immunostaining; 2, 3 cells have expected count less than 5; Fisher exact test; 2 Student t test; 3 Twenty-five percent of all cells have an expected count of <5; Fisher exact test; ALT, alanine aminotransferase; AFP, α- fetoprotein

85%, 62% and 46%, respectively, and the 1-, 3- and 5-year probabilities of recurrence were 17.2%, 48% and 60%, respectively.

E-cadherin expressions in HCC

E-cadherin staining was mainly in the membrane and cytoplasm of HCC cells, and staining results with both antibodies (monoclonal, polyclonal) were essentially identical (Figure 1A and B). The staining was even on HCC tissue and no staining on mesenchyma stroma. Cores of tumor tissue from 1 patient were detached completely from TMA sections during immunostaining and exclude from our study. The low expression of E-cadherin in membrane and cytoplasm was found in 53, and 64 cases, respectively, whereas high expression was found 71 and 60 patients. The ratio of membrane/cytoplasm (M/C) E-cadherin expression was low in 47 cases (Figure 1C-D), and high in 77 cases (Figure 1E). Patients with high E-cadherin expression M/C ratio were prone to have large tumor size (P =0.036), presence of intrahepatic metastasis (P =0.002), microvascular invasion (P =0.049), and high TNM stage (P =0.019) (Table 2).

Prognostic significance of E-cadherin expression in HCC

In univariate analysis, as shown in table 2, high TNM stage, tumor size, and presence of intrahepatic metastasis were risk factors for both OS and TTR. Liver cirrhosis was only associated with OS. Neither membrane nor cytoplasm expression of E-cadherin was associated with OS or TTR (P=0.284, P=0.241, P =0.230, and P=0.871 respectively, Figure 2A-D). But E-cadherin M/C expression ratio was associated with poor prognosis and TTR (P=0.001 and P =0.038 respectively; Figure 2E and 2F). The median OS and TTR were 84.1 and 56.97 months, respectively, in patients with low ratio of M/C E-cadherin exression, both were significantly longer than those with high ratio patients (48.57 months and 40.07 months, respectively). All clinicopathologic factors were adopted in multivariate Cox proportional hazards analysis. The ratio of M/C E-cadherin expression was an independent risk factor for OS (hazard ratio [HR] =1.99; 95% confidence interval [CI], 1.063-3.708; P =0.031), in addition to TNM stage for OS (HR=2.071; 95%CI, 1.135-3.777; P =0.018). For TTR, E-cadherin M/C expression ratio was not an independent risk factor (Table 3).

Table 3. Univariate and Multivariate Analyses of Factors Associated with Survival and Recurrence

Features	OS				TTR			
	Univariate	Multivariate			Univariate	Multivariate		
	P value	HR	95% CI	P value	P value	HR	95% CI	P value
Age: <51 vs. ≥51 years	0.798			NA	0.695			NA
Gender: female vs. male	0.208			NA	0.207			NA
Hepatitis B history: yes vs. no	0.548			NA	0.981			NA
Hepatitis B e antigen: positive vs. negative	0.691			NA	0.241			NA
Liver cirrhosis: yes vs. no	0.022			NA	0.088			NA
AFP:<400 vs. ≥400 ng/ml	0.068			NA	0.116			NA
Preoperative ALT: <75 vs. ≥75 U/L	0.72			NA	0.281			NA
Tumor size:<3 vs.≥3 cm	0.002				0.01			
Tumor size:<5 vs.≥5 cm	0			NA	0.004			NA
Tumor encapsulation: complete vs. none	0.062			NA	0.229			NA
Microvascular invasion: yes vs. no	0.129			NA	0.743			NA
Intrahepatic metastasis: yes vs. no	0			NA	<0.008	0.372	0.153-0.904	0.029
TNM stage: IIIa vs.II vs.I	0	2071	1.135-3.777	0.018	0	3.059	1.848-5.064	0
E-cadherin membrane/cytoplasm ratio	0.001	1.99	1.063-3.708	0.031	0.038			NA

OS, overall survival; TTR, time to recurrence; AFP, α-fetoprotein

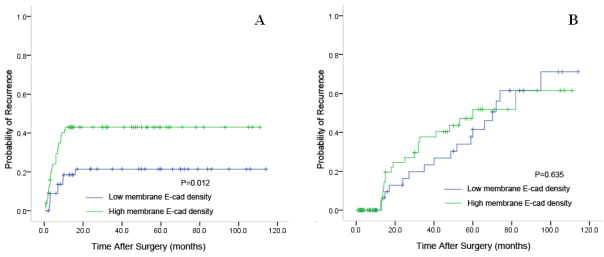

Figure 3. Using 1 Year as A Cutoff Value, Postoperative Recurrence was Discriminated into Early and Later Recurrence According to the Time to Recurrence. Early recurrence curves, but not late recurrence curves, differed between high and low E-cadherin M/C ratio expression (3A, 3B)

Significance of E-cadherin expression in early recurrence of HCC

A further study on the effects of M/C ratio of E-cadherin on recurrence was conducted. All the recurrent cases were divided into early or late recurrence groups, using 1 year as the cutoff value, as suggested by Poon's study (Poon et al., 2000). In all 78 recurrences in this study, no significant difference was found between high M/C ratio and low M/C ratio (P =0.249); but a high M/C ratio E-cadherin expression tended to have an early recurrence (P=0.012), rather than late recurrence (P =0.635) (Figure 3A, B).

Discussion

The much different prognosis of those suitable HCC patients, who received hepatic resection timely, drew attention to the importance of study heterogeneity of HCC and the necessary for seeking or reevaluating prognostic markers. In the present study of 125 diagnosed HCC with curative hepatectomy, we found that high E-cadherin membrane/cytoplasm ratio, but neither membrane nor cytoplasm E-cadherin expression, associating with large tumor size and intrahepatic metastasis, was an efficient poor prognosis predictive marker.

Although E-cadherin appears as a member of adhesion molecules correlating with better outcome in HCC (Garcia et al., 1998) and in some other tumors, expression of E-cadherin on membrane alone did not correlate with OS for HCC received curative hepatectomy according to the data presented in this study. Immunohistochemical analysis of E-cadherin expression in TMA including 125 HCCs tissues revealed obvious variations among tumor samples, ranging from complete or heterogeneous down-regulation in 42.4% of cases to striking overexpression in 34.4% of tumors, which was similar to the results of Buendia's (Wei et al., 2002), and E-cadherin level in cytoplasm was not associated with OS too. Different from the membrane and cytoplasm alone, we considered both factors in coming and found the high membrane/cytoplasm ratio was an independence prognostic factor. E-cadherin M/C ratio also serve as a predictor of HCC recurrence. Patients with low E-cadherin M/C ratio have a significantly prolonged 5-year OS (64% vs. 33%) and TTR (38% vs. 27%).

Considering the significant prognostic value of tumor size and linear relation between tumor size and E-cadherin distribution, we investigated the prognostic factors in the subgroup with small and large HCC to determine whether the distinction in prognostic seen in this study with respect to high or low membrane/cytoplasm ratio of E-cadherin reflects inherent impact of E-cadhein to prognosis or is the results of tumor-related influences. The results of stratified analyses showed that, according to tumor size, membrane/ cytoplasm ratio of E-cadherin could further discriminate the outcomes of HCC patients with large (n=71) or small (n=53) tumor size (P =0.035, P =0.009, respectively) which is a feature of poor prognosis. We therefore assumed that altered distribution of E-cadherin

is associated with prognosis involving tumor size.

In Garcia's study (Garcia et al., 1998), they found E-cadherin immunodetection was an independent prognostic factor, but the Cox multivariate analysis showed that its prognostic value was lower when compared to other prognostic factors. The reasons of different prognostic values of membrane E-cadherin may be due, on the one hand, to the different disease background and the different follow up time, on the other hand, to the multifaceted function of E-cadherin and recycle of E-cadherin.

To expand the understanding of diverse and seemingly paradoxic roles of E-cadherin in HCC, examination of the distribution and correlationship between cell membrane and cytoplasm of E-cadheirn is required. Studies showed that E-cadherin's move from membrane to cytoplasm, then recycle to the membrane is crucial for its function, derailed E-cadherin is hallmark of tumor (Mosesson et al., 2008). The distribution ratio of E-cadheirn in membrane and cytoplasm is more important than the membrane level of E-cadheirn alone. There was also evidence showed that adhesion for E-cadherin is neither necessary nor sufficient for suppressing cancer invasion, and inhibition of this invasion through the cytoplamic tail of E-cadherin but not the extracellular domain (Wong and Gumbiner BM, 2003). We speculate that it maybe related to E-cadherin's membrane– cytoplasm–membrane cycle metabolite. Furthermore, protease cleavage of the peptides could contribute. This field requires further investigation to determine the relative role of transcriptional repressors of extracellular cleavage and shedding of E-cadherin.

Postoperative tumor recurrence is a major problem that compromises the effect of hepatectomy for HCC, which can be categorized into two types, that is, early recurrence (within 1-year) which mainly from intrahepatic metastasis and late recurrence caused by persistent cirrhosis or underlying liver diseases (Ikeda et al., 2003). Despite similar treatment, the prognosis for patients with early recurrence was worse than that of patients with late recurrence (Poon et al., 2000). So predicting early recurrence following curative resection is critical for the management of HCC. Previously reported risk factors for early recurrence including AFP, intrahepatic metastasis, tumor size, serum albumin level, and initial tumor pTNM classification in different series (Shirabe et al., 1991; Poon et al., 2000; Regimbeau et al., 2004). But the reports are conflicted and lack of consistence and much debate still exists on which, if any, factors are most important. Our statistic results showed that membrane /cytoplasm ratio of E-cadherin is a prognostic factor for TTR but not an independent factor. We further found that early recurrence is much different between high and low E-cadherin membrane/cytoplasm ratio groups and ER happened more often in the high E-cadherin membrane/cytoplasm ratio group than that in low ratio group but no different in late recurrence. Therefore, high E-cadherin M/C expression provided an alternative option for predicting early recurrence and helped to identify a high-risk subgroup of patients for whom adjuvant therapies after hepatectomy are needed.

Beyond doubt, loss of E-cadherin in HCC cells is associated with signaling pathway, inducing tumor cell growth and invasion (Fransvea et al., 2008; Du et al., 2009). Adhesion-induced ligand-independent activation of the EGF receptor could lead to Akt and MAPK activation and hence caused genetic abnormalities. In addition, E-cadherin promoted cell survival by activated Stat3 through homophilic E-cadherin interactions (Arulanandam et al., 2009), or through E-cadhrin-induced ligand-independent activation of the EGF receptor (Comoglio et al., 2003). We suspected that the shift of E-cadherin from membrane to cytoplasm may activate these different pathway and triggered cell growth and invasion. Along with the deepgoing and detailgoing of the research, E-cadherin was recognized as not "perfect" in modulate tumor growth and metastasis. One possible explanation maybe that misregulated E-cadherin expression associated with an aggressive tumor phenotype and even was regarded as a pro-tumorigenic factor (Lewis-Tuffin et al., 2010). The diversify expression and regulation of E-cadherin could lead to the multifaceted function in HCC but the underlying molecular mechanism is unknown and worthy further investigated.

To conclude, our results showed the membrane/cytoplasm ratio of E-cadherin is a strong predictor of postoperative survival and early recurrence in patients with HCC. This ratio could be incorporated to the others markers of patients with high recurrence risks, especially for the early recurrence, in order to provide them with proper and prompt interventions.

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References

Arulanandam R, Vultur A, Cao J, et al (2009). Cadherin-cadherin engagement promotes cell survival via Rac1/Cdc42 and signal transducer and activator of transcription-3. *Mol Cancer Res*, **7**, 1310-27.

Brunt EM (2000). Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. *Hepatology*, **31**, 241-6.

Bussemakers MJ, van Bokhoven A, Mees SG, Kemler R, Schalken JA (1993). Molecular cloning and characterization of the human E-cadherin cDNA. *Mol Biol Rep*, **17**, 123-8.

Cespedes MV, Larriba MJ, Pavon MA, et al (2010). Site-dependent E-cadherin cleavage and nuclear translocation in a metastatic colorectal cancer model. *Am J Pathol* **177**: 2067-2079.

Comoglio PM, Boccaccio C, Trusolino L (2003). Interactions between growth factor receptors and adhesion molecules: breaking the rules. *Curr Opin Cell Biol*, **15**, 565-71.

Cowin P, Rowlands TM, Hatsell SJ (2005). Cadherins and catenins in breast cancer. *Curr Opin Cell Biol*, **17**, 499-508.

Du GS, Wang JM, Lu JX, et al (2009). Expression of P-aPKC-iota, E-cadherin, and beta-catenin related to invasion and

metastasis in hepatocellular carcinoma. *Ann Surg Oncol*, **16**, 1578-86.

Fransvea E, Angelotti U, Antonaci S, Giannelli G (2008). Blocking transforming growth factor-beta up-regulates E-cadherin and reduces migration and invasion of hepatocellular carcinoma cells. *Hepatology*, **47**, 1557-66.

Garcia S, Martini F, De Micco C, et al (1998). [Prognostic value of E-cadherin expression in hepatocellular carcinoma]. *Ann Pathol*, **18**, 98-102.

Han AC, Peralta-Soler A, Knudsen KA, et al (1997). Differential expression of N-cadherin in pleural mesotheliomas and E-cadherin in lung adenocarcinomas in formalin-fixed, paraffin-embedded tissues. *Hum Pathol*, **28**, 641-5.

Ikeda K, Arase Y, Kobayashi M, et al (2003). Significance of multicentric cancer recurrence after potentially curative ablation of hepatocellular carcinoma: a longterm cohort study of 892 patients with viral cirrhosis. *J Gastroenterol*, **38**, 865-76.

Jiang WG, Mansel RE (2000). E-cadherin complex and its abnormalities in human breast cancer. *Surg Oncol*, **9**, 151-71.

Kavgaci H, Yildiz B, Fidan E, et al (2010). The effects of E-cadherin and bcl-2 on prognosis in patients with breast cancer. *Bratisl Lek Listy*, **111**, 493-7.

Kensler TW, Qian GS, Chen JG, Groopman JD (2003). Translational strategies for cancer prevention in liver. *Nat Rev Cancer*, **3**, 321-9.

Lewis-Tuffin LJ, Rodriguez F, Giannini C, et al (2010). Misregulated E-cadherin expression associated with an aggressive brain tumor phenotype. *PLoS One*, **5**, e13665.

Mayer B, Johnson JP, Leitl F, et al (1993). E-cadherin expression in primary and metastatic gastric cancer: down-regulation correlates with cellular dedifferentiation and glandular disintegration. *Cancer Res*, **53**, 1690-5.

Montserrat N, Gallardo A, Escuin D, et al (2011). Repression of E-cadherin by SNAIL, ZEB1, and TWIST in invasive ductal carcinomas of the breast: a cooperative effort? *Hum Pathol*, **42**, 103-10.

Mosesson Y, Mills GB, Yarden Y (2008). Derailed endocytosis: an emerging feature of cancer. *Nat Rev Cancer*, **8**, 835-50.

Poon RT, Fan ST, Ng IO, et al (2000). Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. *Cancer*, **89**, 500-7.

Qian YB, Zhang JB, Wu WZ, et al (2006). P48 is a predictive marker for outcome of postoperative interferon-alpha treatment in patients with hepatitis B virus infection-related hepatocellular carcinoma. *Cancer*, **107**, 1562-9.

Regimbeau JM, Abdalla EK, Vauthey JN, et al (2004). Risk factors for early death due to recurrence after liver resection for hepatocellular carcinoma: results of a multicenter study. *J Surg Oncol*, **85**, 36-41.

Ross JS, del Rosario AD, Figge HL, et al (1995). E-cadherin expression in papillary transitional cell carcinoma of the urinary bladder. *Hum Pathol*, **26**, 940-4.

Shim HS, Yoon BS, Cho NH (2009). Prognostic significance of paired epithelial cell adhesion molecule and E-cadherin in ovarian serous carcinoma. *Hum Pathol*, **40**, 693-8.

Shirabe K, Kanematsu T, Matsumata T, et al (1991). Factors linked to early recurrence of small hepatocellular carcinoma after hepatectomy: univariate and multivariate analyses. *Hepatology*, **14**, 802-5.

Sobin LH (2002). TNM classification of malignant tumors, 6th edn. Geneva, Switzerland: International Union Against Cancer.

Tang ZY, Ye SL, Liu YK, et al (2004). A decade's studies on metastasis of hepatocellular carcinoma. *J Cancer Res Clin Oncol*, **130**, 187-96.

Thiery JP (2002). Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer*, **2**, 442-54.

Wei Y, Van Nhieu JT, Prigent S, et al (2002). Altered expression of E-cadherin in hepatocellular carcinoma: correlations with genetic alterations, beta-catenin expression, and clinical features. *Hepatology*, **36**, 692-701.

Wijnhoven BP, Pignatelli M (1999) E-cadherin-catenin: more than a "sticky" molecular complex. *Lancet*, **354**, 356-7.

Wong AS, Gumbiner BM (2003). Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. *J Cell Biol*, **161**, 1191-203.

Xu HX, Zhu XD, Zhuang PY, et al (2011). Expression and prognostic significance of placental growth factor in hepatocellular carcinoma and peritumoral liver tissue. *Int J Cancer*, **128**, 1559-69.