Reduced expression of E-cadherin/catenin complex in hepatocellular carcinomas

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AIM: To examine the immunoreactivity of E-cadherin and four subtypes of catenin family in human hepatocellular carcinomas (HCCs) and to investigate the correlation between expression of E-cadherin/catenin complex and clinicopathologic parameters of HCC patients.

METHODS: An immunohistochemical study for E-cadherin and catenins was performed on 97 formalin-fixed, paraffin-embedded specimens of HCC. The adhesion mediated by E-cadherin to the extracellular part of E-cadherin mediates cell-cell adhesion depending on calcium. Homophilic interaction of the extracellular part of E-cadherin mediates cell-cell adhesion. The adhesion mediated by E-cadherin is significantly implicated in the establishment of cell polarity, the transformation of epithelial/interstitial cells

RESULTS: Reduced expression of E-cadherin, α-, β-, γ-catenin and p120 was observed in 69%, 76%, 63%, 71% and 73%, respectively. Both expressions of E-cadherin and catenin components were significantly correlated with tumor grade (P = 0.000). It showed significant difference between expression of catenin members and tumor stage (P = 0.003, P = 0.017, P = 0.007 and P = 0.000, respectively). The reduced expression of E-cadherin in HCCs was significantly correlated with intrahepatic metastasis (IM) and capsular invasion (P = 0.008, P = 0.03, respectively). A close correlation was also observed between the expression of catenins and the tumor size (P = 0.002, P = 0.034, P = 0.016 and P = 0.000, respectively). In addition, the expression of each catenin was found correlated with IM (P = 0.012, P = 0.049, P = 0.026 and P = 0.014, respectively). No statistically significant difference was observed between the expression level of E-cadherin/catenin complex and lymph node permission, vascular invasion and satellite nodules. Interestingly, only expression of p120 showed correlation with AFP value (P = 0.035). The expression of E-cadherin was consistent with α-, β-, γ-catenin and p120 expression (P = 0.000). Finally, the abnormal expression of E-cadherin/catenin complex was significantly associated with patients' survival (P = 0.0253, P = 0.0052, P = 0.003, P = 0.0105 and P = 0.0016, respectively). Nevertheless, no component of E-cadherin/catenin complex was the independent prognostic factor of HCC patients.

CONCLUSION: Down-regulated expressions of E-cadherin, catenins and p120 occur frequently in HCCs and contribute to the progression and development of the tumor. It may be more exact and valuable to detect the co-expression of E-cadherin/catenin complex than to explore one of them in predicting tumor invasion, metastasis and patient's survival.
and the classification of cell types in the development. The cytoplasmic part of E-cadherin is linked to the actin cytoskeleton via the catenins, including α-catenin, β-catenin, γ-catenin and p120. E-cadherin/catenin complex is widely acknowledged as both tumor and metastasis suppressor, and the search for strategies to repress metastasis has led to intense studies of the mechanisms and molecules regulating E-cadherin function. In addition, several recent trials have displayed the bright future of E-cadherin/catenin complex as a targeted therapy for human cancers.

The expression of E-cadherin/catenin complex has been shown to associate with tumor histological features (tumor size, grade, stage, invasion, metastasis, prognosis, etc.) in several cancers. For liver cancer, some studies have reported that E-cadherin gene methylation or β-catenin exon 3 mutation occurred frequently and there was significant correlations between these abnormal biologic behavior and tumor development and progression. In addition, some studies have explored the relationship between the clinicopathologic parameters of hepatocellular carcinoma (HCC) and the expression of E-cadherin/catenins complex, but the conclusions are still controversial. Endo et al revealed over-expression of α-, β-, and γ-catenins in most HCCs, which was inversely correlated with histological grade of HCC, whereas E-cadherin expression was down-regulated and displayed a significant positive correlation with HCC grade. However, Ihara et al showed over-expression of E-cadherin in their 66 HCCs, which was inversely correlated with tumor histological grade.

At present, there are little reports to demonstrate the relationship between the co-expression of above-mentioned five cell adhesion molecules and the clinicopathologic parameters (including patients’ prognosis) in HCC. Especially, there was no report about expression of p120 catenin in HCC patients. In the present study, we used immunohistochemical staining of E-cadherin/catenin complex in the primary lesion of surgically resected HCC specimens to clarify the correlations between the expression of E-cadherin/cadherin complex and the development and progression of HCC.

MATERIALS AND METHODS

Patient selection and definition of clinicopathologic parameters

In this study, we selected 97 cases of HCC that had been collected and diagnosed at the Eastern Hepatobiliary Surgery Hospital, the Second Military Medical University between October 1998 and March 2004. The patients consisted of 67 men and 30 women and ranged in age from 34 to 72 years, with an average age of 54 years. In each case, HCC tissues and nontumorous liver tissues were obtained for pathological examination. The detailed pathologic results were gained from the department of pathology of our hospital. Background liver showed cirrhosis in 72 cases (74.2%) and chronic hepatitis in 69 cases (71.1%) which included hepatitis B virus in 60 cases and hepatitis C virus (HCV) in 5 cases, and cryptogenic in 4 cases.

Clinicopathologic parameters include histological grades, stages, size, capsular and vascular invasion (portal vein cancer thrombus), satellite nodules, IM, lymph node permission, AFP value and patients’ survival. HCC was classified into grade I, II, III and IV according to Edmondson and Steiner. Histological types of HCC were adopted according to the system of World Health Organization. HCC staging was performed according to TNM staging system of the International Union against Cancer VIcc. IM was defined as recurrent tumors consisting of moderately or poorly differentiated HCC with the same or lower degree of differentiation compared with the differentiation of the primary tumors. The satellite nodules of this HCC represent either intrahepatic spread of the tumor or multicentric origin of the tumor. The patients were followed up for 8–68 mo.

Immunohistochemistry

All samples were collected from 5-μm-thick histological sections. They were cut from the formalin-fixed paraffin-embedded material. Dako EnVision Kit (Dakocytomation Company, Denmark) was used for the immunohistochemical staining of E-cadherin, α-, β- and γ-catenins and p120. Sections were dewaxed, and incubated with methanol containing 30% H2O2 for 20 min to block endogenous peroxidase activity. To enhance antigen retrieval, sections were treated at this stage in a microwave oven. Briefly, sections were immersed in 0.01 mol/L citrate buffer (pH 6.0) and heated in a microwave oven at 100°C for 20 min. Subsequently, they were washed three times with distilled water, and then blocked with 1% BSA for 30 min. Sections were incubated overnight at 4°C with rabbit polyclonal IgG of E-cadherin, α-catenin and p120 (Santa Claus Corporation, USA, dilution 1:200) and mouse monoclonal IgG of β-catenin and γ-catenin (Santa Claus Corporation, USA, dilution 1:200). A subsequent reaction was carried out using second antibodies (Dakocytomation Company, Denmark, dilution 1:200) for 30 min at 37°C. Sections were washed three times with PBS buffer and subsequently displayed color with DAB for about 5 min. Nuclei were lightly counterstained with hematoxylin. No staining was obtained when nonimmune serum or PBS was used instead of the primary antibodies, thus confirming the specificity of each primary antibody.

Evaluation of immunostaining

We used a scoring system to evaluate semiquantitatively the immunoexpression. The expression of non-tumorous tissue served as an internal control. Briefly, immunoreactivities were assessed by the extent (broadness) and intensity (color strength). Depending on the percentage of positive cells, the extent score was classified as follows: 0, no positive cell or less than 5%; +1, 5%-25%; +2, 26%-50%; +3, 51%-75% and +4, 76%-100% positive cells. The intensity score was also
categorized into four groups: 0, no immunoreaction; +1, mild immunoreaction; +2, moderate immunoreaction; and +3, marked immunoreaction. Preserved E-cadherin or catenin expression was defined when the composite score was 6 or 7. In contrast, the expression of E-cadherin or catenin was also defined as “absent or loss” when the total score was 0.

Statistical analysis

Results from immunohistochemistry were analyzed by Yates’ correction or Fisher’s exact tests and statistical significance was accepted when \( P < 0.05 \). For survival analysis, log-rank test was used with a significant level of \( P < 0.05 \). Survival curves were computed according to the method of Kaplan-Meier. The prognosis value of these five molecules was evaluated with univariate and multivariate analysis. The SPSS 10.1 software package for Windows (SPSS, Inc., Chicago, IL) was used.

### RESULTS

**Microscopic observations**

In nontumorous liver tissues, both E-cadherin and catenins were expressed strongly at cell membrane, but the staining strength gradually weakened from nontumoral tissue to the tumor region. In addition, bile ducts, proliferated ductiles and intrahepatic vessels strongly expressed these two molecules at the cell membrane. There was no expression in other cell types in the liver.

In 97 specimens of HCCs, the abnormal expression of E-cadherin, \( \alpha \)-, \( \beta \)-, \( \gamma \)-catenin and \( \text{p120} \) was found in 57, 65, 55, 60 and 62 cases (completely absent in 16, 19, 13, 10 and 15 cases), respectively (Table 1). The immunostaining distribution of E-cadherin was only presented at cell membrane, whereas catenins were found at the membrane in the cytoplasm and/or nucleus (Table 2, Figures 1 and 2).

### Table 1  Expression of five cell adhesion molecules in HCCs \( n (\%) \)

| E-cadherin | \( \alpha \)-catenin | \( \beta \)-catenin | \( \gamma \)-catenin | \( \text{p120} \) |
|------------|----------------------|-------------------|-------------------|----------------|
| Normal     | 40 (41.2)            | 32 (35.0)         | 42 (35.0)         | 37 (37.5)      | 35 (42.5)     |
| Reduced    | 41 (42.3)            | 46 (65.0)         | 42 (65.0)         | 50 (62.5)      | 47 (57.5)     |
| Absent     | 16 (16.5)            | 19                | 13                | 10             | 15            |

**Relationship between expression of E-cadherin/catenin complex and histological features in HCCs**

As shown in Table 3, expression of both E-cadherin and catenin components was significantly related with tumor grade \( (P = 0.000) \). There was a greater tendency for the expression of E-cadherin/catenin complex to reduce in
poorly differentiated tumors than in well and moderately differentiated tumors. Except for E-cadherin, significant difference was found between expression of catenin members and tumor stage ($P = 0.003$, $P = 0.007$ and $P = 0.000$, respectively).

Relationship between expression of E-cadherin/catenin complex and clinical parameters in HCCs
In Table 3, the reduced expression of E-cadherin in HCCs was significantly correlated with IM and capsular invasion ($P = 0.008$ and $P = 0.03$, respectively). A close correlation was also observed between expression of $\alpha$, $\beta$, $\gamma$-catenin and p120 and the tumor size ($P = 0.002$, $P = 0.034$, $P = 0.016$ and $P = 0.000$, respectively). In addition, the expression of each catenin was found correlated with IM ($P = 0.012$, $P = 0.049$, $P = 0.026$ and $P = 0.014$, respectively). No statistically significant difference was observed between the expression level of E-cadherin/catenin complex and lymph node permission, vascular invasion and satellite nodules. Interestingly, only p120 expression was correlated with AFP value ($P = 0.035$).

Relationship between expression of E-cadherin and catenins in HCCs
As shown in Table 4, the expression of E-cadherin was significantly correlated to the expression of all kinds of catenins ($P = 0.000$). There was a significant concordance between the expression of E-cadherin and catenins.

Relationship between expression of E-cadherin/catenin complex and patients’ survival
The overall patient survival according to the expression of E-cadherin and catenins in tumor is shown in Figure 3. Analysis of the survival for all patients showed that abnormal expression of E-cadherin and $\alpha$, $\beta$, $\gamma$- and p120 catenins were correlated with poor survival ($P = 0.0253$, $P = 0.0052$, $P = 0.003$, $P = 0.0105$ and $P = 0.0016$, respectively). However, when E-cadherin/catenins complex status and other clinicopathological parameters were analyzed by the Cox regression model, abnormal expression of the E-cadherin/catenin complex was not found to be an independent prognostic factor (data not shown).

DISCUSSION
In accordance with previous reports[22,23], the expressions of all the members of E-cadherin/catenin complex were down-regulated in HCC, among of which the reduced expression of $\alpha$-catenin was found most frequently. The expression of these five molecules was inversely correlated with tumor differentiation degree, thus all of them may be considered as good differentiation markers of HCC. Our result, however, is different from the reports of Endo and Ihara et al.[16,17], which respectively showed over-expression of E-cadherin or $\alpha$, $\beta$- and $\gamma$-catenins in most HCCs. Therefore, together with CpG methylation of E-cadherin in HCC[13], it can be concluded that E-cadherin may act as a modulator of the maintenance of HCC histological architecture.

In addition, the reduced expression of all catenins,
but E-cadherin, was significantly associated with tumor stage and tumor size in our study. The mechanism of these findings was unknown, but they were consistent with tumor clinical features, the small liver cancers have a relatively better clinical course. Interestingly, p120 expression was also correlated with AFP value. It is well known that AFP is only an indicator of malignant liver tumor, and its value is not always related to the classification of tumor cells, so it may be a coincidence and needs to be investigated in the future. However, the findings above may further demonstrate that E-cadherin/catenin complex, at least catenins, correlate with HCC’s biological behavior to some degree.

We found some distinct locations for different molecules of complex in HCC, especially for β-, γ-, and p120 catenin. In this study, hepatocytes, endothelial cells of bile ducts, proliferated ductiles and intrahepatic vessels of nontumorous liver tissue strongly expressed all of five molecules at cell membrane, but the staining strength gradually weakened from nontumorous tissue to tumor region. Besides expressing mostly at the membrane, these three members of armidillo protein family were also found in the cytoplasm or nucleus. It has been shown to be important in the development of several tumors that constitutionally activated the Wnt/Wingless signaling pathway by stabilization and accumulation of β-catenin in the nucleus and cytoplasm[24-27]. Like β-catenin, γ-catenin can also bind to APC protein and is able to translocate into the nucleus participating in the Wnt signaling pathway[28].

Several studies demonstrate that p120 can enter the nucleus and the Kaiso may be its receptor in nucleus[29]. Upon loss of E-cadherin, p120 translocates from

### Table 3 Relationship between expression of E-cadherin/catenin complex and histological features and clinical parameters in HCCs

|                                | E-cadherin | α-catenin | β-catenin | γ-catenin | p120ctn |
|--------------------------------|------------|-----------|-----------|-----------|---------|
| Histological grade             | 23         | 19/4      | 22/1      | 30/3      | 19/4    |
| Histological stage             | 47         | 22/25     | 27/20     | 25/22     | 27/20   |
| Tumor size                     | 29         | 17/12     | 18/11     | 17/12     | 19/10   |
| Capsular invasion              | 23         | 15/14     | 16/35     | 15/15     | 14/15   |
| Satellite nodules              | 10         | 15/30     | 14/24     | 12/12     | 10/12   |
| Vascular invasion              | 22         | 10/16     | 11/27     | 10/27     | 9/19    |
| Lymph node permission          | 17         | 4/13      | 3/14      | 4/14      | 3/14    |
| Intrahepatic metastasis        | 12         | 4/13      | 3/14      | 4/14      | 3/14    |
| AFP-value                      | 28         | 6/12      | 16/12     | 16/12     | 15/13   |
| Table 4 Relationship between E-cadherin and catenin expressions in HCCs

| E-cadherin | α-catenin | β-catenin | γ-catenin | p120ctn |
|------------|-----------|-----------|-----------|---------|
| +          | 28        | 12        | 33        | 7       | 29      | 11      | 31      | 9       |
| -          | 4         | 53        | 9         | 48       | 8       | 49      | 4       | 53      |
| P          | 0.000     | 0.000     | 0.000     | 0.000    | 0.000   | 0.000   | 0.000   | 0.000   | www.wjgnet.com
the membrane to the cytoplasm. Alternatively, the cytoplasmic p120 pool may have increased access to the nucleus \[30,31\]. Therefore, these three molecules may play an important role in cell signal transduction and affect the development and progress of tumor. Unfortunately, there is no further investigation in relationship between p120 nucleus expression and clinicopathologic parameters in HCC due to the small number of cases with positive nucleus expression in our study.

As a focal point, we examined the relationships between expression of E-cadherin/catenin complex and some invasion and metastasis parameters of HCC. The reduced expression of E-cadherin in our experiment was significantly correlated with IM and capsular invasion. There was also a significant correlation between expression of catenins and IM in HCC. Nevertheless, no...
statistically significant difference was observed between the expression level of E-cadherin/catenin complex and lymph node permission, vascular invasion and satellite nodules. These results were different from those of the previous studies to some degree. Endo et al. [16] pointed out that the histological features of the intrahepatic metastatic lesions are essentially the same as those of the main nodule. The proliferative activities in the intrahepatic metastatic lesions were generally higher than those in the main nodules. The fact that there are differences in the proliferative activity, despite the similarity in histology between the primary sites and the metastatic lesions, suggests that tumor cells of the metastatic lesions might acquire some characteristic advantages in forming metastasis. Preserved or recovered function of E-cadherin may be one of these advantages. Osada et al. [34] found that E-cadherin is involved in the IM of HCC. Asayama et al. [33] had a similar report in HCC-CC patients. These results indicated that E-cadherin/catenin complex might play an important role in the detachment of cancer cells.

To our knowledge, this is the first report of p120 catenin expression in human HCC. p120 was found to strangely correlate with differentiation, IM and patients’ survival, and p120 loss was associated with down-regulation of all members of the complex. Thoreson et al. [35] suggested that it is possible that morphologic and behavioral changes in some tumors are due to p120 loss and consequent destabilization of E-cadherin. The roles for p120 as either tumor suppressor or metastasis promoter during tumor progression differ with the order, in which p120 or E-cadherin was down-regulated first. If p120 is lost first, E-cadherin level will fall significantly [36], which is likely to be paralleled by reduced levels of α- and β-catenins [37]. If E-cadherin is lost first, p120 may directly and actively promote metastasis.

With regard to the relationship between the E-cadherin/catenin complex expression and patients’ survival in HCC, we found that patients with low expression of these five molecules had poorer prognosis than those with normal expression. Unlike most previous reports [38-41], which revealed only one or a few members of this complex were associated with patients’ survival, our result demonstrated that all molecules of E-cadherin/catenin complex were significantly correlated with HCC patients’ survival. However, when E-cadherin/catenin complex status and other clinicopathological parameters were analyzed by the Cox regression model, abnormal expression of the E-cadherin/catenin complex was not found to be an independent prognostic factor. Thus, it may be more exact and valuable to detect the co-expression of E-cadherin/catenin complex than to explore only one of them.

In conclusion, these data indicated that abnormal expression of E-cadherin/catenin complex is common in HCC. The complex expression is mostly located in the cytoplasm of HCCs and correlated with tumor differentiation, IM and patients’ survival. E-cadherin/catenin complex may play an important role in development and progression of human HCC.

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