Expression and Prognostic Roles of β-Catenin in Hepatocellular Carcinoma: Correlation with Tumor Progression and Postoperative Survival

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ABSTRACT

Purpose: Although hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the human liver, the molecular changes and mechanisms that regulate its development and progression remain unclear. In the present study, we investigated the correlation between β-catenin expression and clinical outcome in 51 patients with relatively small (maximal diameter < 30 mm), solitary HCCs.

Experimental Design: The tumors were classified according to histological tumor differentiation (grade I, 11 tumors; grade II, 28 tumors; grade III, 12 tumors). Using immunohistochemical methods to detect nuclear accumulation of β-catenin, we investigated the correlation between β-catenin expression and clinical outcome and compared the correlation with cyclin D1, Ki-67, and E-cadherin.

Results: Focal or generalized nuclear β-catenin expression was observed in 36.4% (4 of 11) of the grade I tumors, 39.3% (11 of 28) of the grade II tumors, and 25% (3 of 12) of the grade III tumors. Nuclear β-catenin-positive grade III tumors were associated with significantly poorer survival (P = 0.004), whereas none of the patients with nuclear β-catenin-negative grade I tumors died. With regard to proliferative activity, positive nuclear β-catenin staining correlated significantly with an increased Ki-67 labeling index in grade I (P < 0.0001) and grade III (P = 0.0045) tumors and with reduced epithelial cadherin expression in the cell membrane (P < 0.001). In contrast, no association with the expression of cyclin D1, one of the target factors of β-catenin, was detected.

Conclusions: Our present data suggest that β-catenin plays important roles in promoting tumor progression by stimulating tumor cell proliferation and reducing the activity of cell adhesion systems and is associated with a poor prognosis, especially in patients with poorly differentiated HCCs.

INTRODUCTION

HCC is one of the most common cancers worldwide and is a major cause of death in many countries, especially in Asia (1, 2). Although morbidity and mortality rates have decreased in recent years in patients with surgically treated HCC (3), the long-term prognosis remains unsatisfactory because of high recurrence rates (4–6). In addition, the mechanisms underlying the development of HCC remain unclear, and its prognosis is difficult to predict because HCC exhibits a wide spectrum of clinicopathological features (7).

Recently, frequent oncogenic mutations in the β-catenin gene and its correlation with nuclear expression have been identified in human and mouse liver tumors (8–15). β-Catenin protein, originally identified as a submembrane component of the cadherin-mediated cell-cell adhesion system, functions as a downstream transcriptional activator of the Wnt signaling pathway, forming a complex with the DNA-binding proteins T-cell factor and lymphoid-enhancer factor 1 (16, 17). The APC tumor suppressor gene product regulates the level of β-catenin protein by cooperating with glycogen synthase kinase-3β via phosphorylation of serine/threonine residues coded on exon 3 of the β-catenin gene (16, 18, 19). This phosphorylation is followed by degradation of β-catenin through the ubiquitin-proteasome pathway (20, 21). In some human cancers, mutation of either the APC gene or the β-catenin gene itself leads to the accumulation of β-catenin within the cancer cells (16, 18, 22, 23). Recently, cyclin D1 has been identified as a target of the β-catenin/T-cell factor/lymphoid-enhancer factor complex (24). In addition, cyclin D1 has essential functions in cellular proliferation and is known to be overexpressed in several organ cancers including HCC (25–29).

Because large HCC nodules exhibit variable characteristics, the correlation between β-catenin expression and survival of surgically treated patients is not clearly understood. To clarify any correlation between β-catenin expression and patient outcomes, the investigation of small, solitary HCCs is required to
standardize the tumor background. Although there are many reports regarding outcomes in HCC patients (30), none of these describe any correlation with β-catenin in such tumors. We therefore selected small (maximal diameter ≤ 30 mm), solitary HCCs and investigated their β-catenin expression using immunohistochemical methods. We also investigated the relationships between β-catenin expression and cyclin D1 (to determine whether β-catenin promotes cell proliferation via the Wnt pathway), Ki-67 (a maker of tumor proliferative activity), and E-cadherin (to detect any alterations in cell-cell adhesion).

MATERIALS AND METHODS

Patient Material. We reviewed 51 consecutive patients who had undergone surgical resection for small, solitary HCCs (maximal diameter ≤ 30 mm) at the Ibaraki Prefectural Central Hospital and Cancer Center between January 1989 and March 2000. Most of the HCCs were associated with cirrhosis or chronic hepatitis due to HCV (42 patients) or HBV (7 patients) infection. One patient had both HCV and HBV infection, whereas one patient had neither HCV nor HBV infection. To detect and evaluate the tumors, we used abdominal US, computed tomography, magnetic resonance imaging, and angiography. Intraoperative US was also performed on all patients to avoid missing other liver lesions. The ages of the patients ranged from 45–79 years, with an average age of 63.5 years. The patients had any history or evidence of current hepatitis viral infection. One patient had both HCV and HBV infection, chronic hepatitis due to HCV (42 patients) or HBV (7 patients) infection. Each paraffin block was sliced into 3-μm sections.

Table 1. Briefly, the sections were deparaffinized using a graded ethanol series, and endogenous peroxidase activity was blocked by soaking in 0.3% hydrogen peroxidase and 0.1% sodium azide in distilled water for 15 min. Thereafter, the sections were processed in 10 mmol/liter citrate buffer (pH 6.0) and heated to 121°C in an autoclave for 20 min to retrieve the antigen. After rinsing in PBS (pH 7.2), 10% bovine serum (Dako Co., Kyoto, Japan) was applied for 10 min to block any nonspecific reactions. The sections were then incubated overnight at 4°C with the primary antibodies. Immunostaining was performed using the streptavidin–biotin–peroxidase complex method (LSAB universal kit; Dako), which involved incubation with a second-stage biotin-conjugated antibody for 30 min, followed by incubation with peroxidase-conjugated streptavidin for 30 min. After rinsing in PBS, the peroxidase reaction was visualized by incubating the sections with 3,3′-diaminobenzidine tetrahydrochloride in 0.05 mol/liter Tris buffer (pH 7.6) containing 0.03% H2O2. After rinsing in water, the sections were counterstained with hematoxylin, dehydrated, and coverslipped.

Immunohistochemical Evaluation. Two observers (S.I. and M.I.) independently evaluated the immunostaining results. The concordance ratio was >90%. Differences of opinion

Table 1 Clinical profile of 51 patients with HCC

<table>
<thead>
<tr>
<th>Histological grade</th>
<th>(n = 11)</th>
<th>(n = 28)</th>
<th>(n = 12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>7/4</td>
<td>17/11</td>
<td>9/3</td>
<td>NS</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>65.4 ± 6.2</td>
<td>64.3 ± 5.3</td>
<td>60.2 ± 5.9</td>
<td>NS</td>
</tr>
<tr>
<td>Viral infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg positive alone</td>
<td>0</td>
<td>4 (14.3%)</td>
<td>3 (25.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HCV positive alone</td>
<td>11 (100%)</td>
<td>23 (82.1%)</td>
<td>8 (66.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>HBsAg/Anti-HCV positive</td>
<td>0</td>
<td>1 (3.6%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HBsAg/Anti-HCV negative</td>
<td>0</td>
<td>0</td>
<td>1 (8.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>2.05 ± 0.59</td>
<td>2.43 ± 0.55</td>
<td>2.44 ± 0.73</td>
<td>NS</td>
</tr>
<tr>
<td>Capsule infiltrationa</td>
<td>4/10 (40.0%)</td>
<td>18/23 (78.3%)</td>
<td>5/10 (50.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Portal invasion</td>
<td>0</td>
<td>6 (21.4%)</td>
<td>6 (50.0%)</td>
<td>0.017</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>11 (100%)</td>
<td>27 (96.5%)</td>
<td>10 (83.3%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Tumor marker (AFP)c</td>
<td>224.1 ± 478.3</td>
<td>618.2 ± 2381.8</td>
<td>918.8 ± 2430.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

aNS, not significant. HBsAg, hepatitis B surface antigen.
bCapsule infiltration, capsule formation cases/capsule formation cases.
cAFP, α-fetoprotein.
ion were resolved by reaching a consensus with the assistance of a third evaluator (M. H.). When evaluating the β-catenin protein immunoreaction, we regarded β-catenin overexpression in the nucleus as a positive result. Cyclin D1 was considered to display aberrant overexpression when >5% of the carcinoma cells were positive for the protein. Ki-67-positive cells were counted by monitoring at least 500 HCC cells from at least 10 randomly selected fields. We then calculated the percentage of antigen-positive nuclei among the total number of nuclei counted to obtain the nuclear LI. To allow univariate and multivariate analyses, we divided the Ki-67 LIs into two groups (LI < 10% and LI ≥ 10%, respectively). E-cadherin expression was regarded as decreased when there was a definite reduction in E-cadherin immunoreactivity in the tumor cell membrane compared with the adjacent noncancerous liver tissue.

Survival Data. Postoperative follow-up was performed using US and/or computed tomography as well as by measuring serum levels of a tumor marker, α-fetoprotein. Survival data were analyzed for all patients during follow-up periods of 1–99.5 months (mean, 28.3 months). Survival curves were constructed using the Kaplan-Meier method.

Statistical Analyses. All values are expressed as mean ± SD. Statistically significant differences in survival rates were determined using the log-rank test. Differences in the male:female ratio, the mean age, tumor marker serum levels, clinicopathological parameters, and the proportion of each tumor grade group showing immunoreactivity for the antigens of interest were analyzed using the χ² test, ANOVA, Fisher’s exact test, the Scheffe method, and the Kruskal-Wallis test, respectively. For multivariate analyses, we used the Cox proportional hazards model.

P < 0.05 was considered statistically significant, and a P between 0.05 and 0.1 was regarded as borderline significance.

RESULTS

Clinical Profile of the Patients

The clinical features of the three groups, including sex, age, viral markers, tumor sizes and various histopathological parameters, are shown in Table 1. There were no significant differences in sex, age, types of viral infection, tumor size, capsular infiltration, or tumor marker levels between the three groups. However, whereas none of the patients with grade I tumors had portal invasion, 6 of the 28 (21.4%) patients with grade II tumors and 6 of the 12 (50%) patients with grade III tumors showed this feature, and these differences in frequency were significant (P = 0.017). In contrast, cirrhosis was observed more frequently in patients with grade I disease (P = 0.038). Although there were no significant differences in tumor marker frequency between the three groups, there was a slight tendency toward an increase as tumor differentiation became more marked.

Immunohistochemistry Results

β-Catenin. Weak β-catenin immunoreactivity was observed in almost all normal bile duct epithelial cells and their adjacent liver cells. About two-thirds of the tumors (60.7%) exhibited increased membranous and/or cytoplasmic expression of β-catenin compared with the adjacent noncancerous liver tissue. Focal or generalized nuclear accumulation of β-catenin was observed in 18 of 51 (35.3%) tumor specimens (Fig. 1A), but not in cirrhotic nodules or dysplastic liver cells in the adjacent liver. When analyzed according to the histological tumor grading classification, overexpression was observed in 4 of 11 (36.4%) grade I, 11 of 28 (39.3%) grade II, and 3 of 12 (25.0%) grade III tumors. Although grade I and II tumors exhibited a slightly higher percentage of β-catenin than grade III tumors, there were no statistically significant differences in β-catenin expression between the three groups (Table 3). Moreover, there was no significant association of nuclear β-catenin expression with any of the clinicopathological parameters or tumor marker levels.

Cyclin D1. No specific nuclear cyclin D1 immunoreactivity was observed in normal bile ducts. Nuclear cyclin D1 overexpression was observed in 17 of 51 (33.3%) tumor specimens (Fig. 1B). When analyzed according to histological tumor grade, overexpression was observed in 2 of 11 (18.2%) grade I, 8 of 28 (28.6%) grade II, and 7 of 12 (58.3%) grade III tumors, and these differences showed a trend toward significance (Table 3). There were no significant associations between cyclin D1 overexpression and any of the clinicopathological parameters.

Ki-67. The Ki-67 LI was calculated for all specimens. A representative example of immunohistochemical staining for Ki-67 in one of the HCCs is shown in Fig. 1C. The average LI for Ki-67-positive cancer cells in all 51 tumors was 11.1 ± 10.9%. When analyzed according to histological grade, the average Ki-67 LI was 5.72 ± 3.30% in grade I tumors and 9.39 ± 7.49% in grade II tumors, in contrast to 20.65 ± 16.38% in grade III tumors (Table 3). These differences in the LI values were significant (P = 0.008). Furthermore, the Ki-67 LI was significantly increased in a tumor that showed portal invasion (P = 0.03; data not shown).

E-Cadherin. Moderately positive E-cadherin expression was observed frequently in the membranes of noncancerous liver cells, and strong positive expression was recognized in the intrahepatic bile ducts. On the other hand, decreased E-cadherin expression was observed in 16 of 51 (31.4%) tumors (Table 3).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Class</th>
<th>m/p</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Catenin</td>
<td>14</td>
<td>IgG1</td>
<td>m</td>
<td>1:200</td>
<td>Transduction Laboratories (Lexington, KY)</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>HECD-1</td>
<td>IgG1</td>
<td>m</td>
<td>1:200</td>
<td>Takara (Tokyo, Japan)</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>DCS-6</td>
<td>IgG2a</td>
<td>m</td>
<td>1:100</td>
<td>Dako Co. (Kyoto, Japan)</td>
</tr>
<tr>
<td>Ki-67</td>
<td>p</td>
<td></td>
<td></td>
<td>1:100</td>
<td>Dako Co.</td>
</tr>
</tbody>
</table>

a m/p, monoclonal/polyclonal.
There were no significant differences in the degree of decrease in E-cadherin expression among the three tumor grade groups. In addition, no association between decreased E-cadherin expression and any of the clinicopathological parameters was detected.

**Relationships between Immunohistochemical Results, Clinicopathological Parameters, and Survival**

First, we investigated correlations between survival and the various parameters by univariate analysis using the log-rank test (Table 4). Ki-67 overexpression and histological tumor grade showed prognostic significance \((P = 0.031\) and \(0.024\), respectively). Grade III tumors had an extremely poor prognosis compared with grade I and II tumors (Fig. 2). Investigations within the respective histological tumor grades revealed that only \(\beta\)-catenin expression in grade III tumors showed prognostic significance \((P = 0.0049\); Table 4). Intrahepatic recurrence and HCC-associated death rates were also higher in patients with \(\beta\)-catenin-positive tumors than in the \(\beta\)-catenin-negative group (Table 5).

**Multivariate Analysis of Survival.** We also carried out a multivariate analysis of survival using the Cox proportional hazards regression model, including each of the immunohistochemical and clinicopathological parameters (Table 6). Only positive nuclear \(\beta\)-catenin expression showed prognostic significance \((P = 0.0098\).

**Correlation of \(\beta\)-Catenin Expression with Cyclin D1, Ki-67, and E-Cadherin Expression**

No significant correlation between positive nuclear \(\beta\)-catenin expression and the expression of cyclin D1, a target factor of \(\beta\)-catenin, was detected. However, among the grade I and III tumors, those positive for nuclear \(\beta\)-catenin expression exhibited significantly higher Ki-67 LIs than those with no nuclear \(\beta\)-catenin expression \((P < 0.0001\) and \(0.0045\), respectively, Fig. 3). Furthermore, nuclear \(\beta\)-catenin-positive tumors showed significantly decreased E-cadherin expression in their cell membranes \((P = 0.001)\).

**DISCUSSION**

Although the number of patients undergoing surgical treatment for HCC has decreased recently (4), HCC occurs widely throughout the world, especially in Asian countries (1, 2). The variable clinical features of HCC can lead to confusion over selection of the most appropriate treatment. Although possible indicators for the successful treatment of relatively small HCCs have been reported (30), it is also important to determine the most useful markers of recurrence and survival from the pathological point of view. Recently, it has been reported that \(\beta\)-catenin is an important factor in tumorigenesis and tumor progression in HCC tumors (8–15), and immunohistochemical nuclear \(\beta\)-catenin accumulation correlated with the presence of gene mutations (10–15). Therefore, in the present study, we focused on investigating the correlation between nuclear \(\beta\)-catenin expression and tumor progression using immunohistochemical methods. Because a single tumor can exhibit several different histological features, especially in large HCCs, and because the histological grade of a HCC correlates with its size (32, 33), we selected relatively small, solitary HCCs to standardize the tumor background.

During the present study, although \(\beta\)-catenin accumulation in the nucleus was decreased in poorly differentiated tumors, \(\beta\)-catenin expression was not correlated with survival in grade I and II tumors. On the contrary, none of the patients with \(\beta\)-catenin-negative grade I tumors died, whereas patients with \(\beta\)-catenin-positive grade III tumors had a significantly poorer prognosis. In other words, we demonstrated that \(\beta\)-catenin expression, especially in poorly differentiated tumors, is an indicator of poor prognosis. Indeed, the mortality rate of the \(\beta\)-catenin-positive patients (8 of 18 patients, 44.4%) was higher than that among the \(\beta\)-catenin-negative...
several conflicting reports concerning the association of \( \beta \)-catenin expression with tumor differentiation, the prognosis of poorly differentiated HCC was contrary to that discussed in previous reports. This discrepancy might be due to the fact that we selected small, solitary HCCs strictly to standardize the background and that most of the HCCs were due to HCV infection. Furthermore, we investigated tumor proliferation through the signaling pathway and cell adhesion mechanism. To determine whether \( \beta \)-catenin plays a role in oncogenic activation, we investigated the expression of cyclin D1, which has been reported to be a target factor of \( \beta \)-catenin in colon cancer (24). In the present study, the number of patients with cyclin D1 overexpression increased as the tumors became more poorly differentiated. However, no correlation between cyclin D1 and survival was observed. Furthermore, we found no immunohistochemical correlation between nuclear \( \beta \)-catenin expression and cyclin D1 overexpression in any of the histological tumor grades.

We also investigated the correlation between nuclear \( \beta \)-catenin expression and Ki-67 immunoreactivity, which has been reported to be a useful marker of tumor proliferative activity (35, 36). Patients with \( \beta \)-catenin-positive grade I and III tumors showed markedly increased Ki-67 LI, in agreement with a previous report (14). This correlation between \( \beta \)-catenin expression and Ki-67 LI suggests that one possible reason for the poor prognosis of HCC is high proliferative activity. Furthermore, considering the point of distant metastasis, we hypothesize that as \( \beta \)-catenin begins to be expressed in the nucleus, cell adhesion in the tumor may be weakened, thus predisposing to distant metastasis. To clarify this point, we investigated immunoreactivity for E-cadherin, a component of cell adhesion systems. The results supported our hypothesis; namely, reduced E-cadherin expression in the cell membrane was associated with nuclear \( \beta \)-catenin expression. Similar findings have also been reported previously (37, 38). Based on our results regarding tumor proliferative activity and cell adhesion systems, we believe that \( \beta \)-catenin is not only involved in the oncogenic process but also plays an important role in tumor patients (6 of 33 patients, 18.2%). Furthermore, two of the eight (25.0%) patients in the \( \beta \)-catenin-positive group who died showed multiple distant metastases, whereas only one of the six (12.5%) \( \beta \)-catenin-negative patients who died exhibited such features. On the other hand, there are some reports that \( \beta \)-catenin mutations were not associated with the histological tumor grade and that mutant nuclear \( \beta \)-catenin was associated with a better survival rate (10–12). Although our study also defined no significant association of nuclear \( \beta \)-catenin expression with tumor differentiation, the prognosis of poorly differentiated HCC was contrary to that discussed in previous reports. This discrepancy might be due to the fact that we selected small, solitary HCCs strictly to standardize the background and that most of the HCCs were due to HCV infection, as compared with those in previous reports. Furthermore, there are several conflicting reports concerning the association of \( \beta \)-catenin gene mutations with tumorigenesis and tumor progression (13, 14, 34). We defined no correlation between \( \beta \)-catenin and survival in grade I and II tumors, which are relatively well differentiated, whereas there was a wide difference in the survival rates for grade II and III tumors. Kondo et al. (13) reported that \( \beta \)-catenin accumulation and gene mutation are not early events in hepatocarcinogenesis but may be associated with the malignant progression of HCC. These findings may help to explain the correlation between \( \beta \)-catenin and survival, especially in poorly differentiated HCCs, in our study. We suspect that even if nuclear \( \beta \)-catenin accumulation is detectable in low-grade tumors, the mean amount present in nuclei might differ according to the tumor grade. Thus, there will be pathological and biological differences between moderately and poorly differentiated HCCs.

Furthermore, we investigated tumor proliferation through the signaling pathway and cell adhesion mechanism. To determine whether \( \beta \)-catenin plays a role in oncogenic activation, we investigated the expression of cyclin D1, which has been reported to be a target factor of \( \beta \)-catenin in colon cancer (24). In the present study, the number of patients with cyclin D1 overexpression increased as the tumors became more poorly differentiated. However, no correlation between cyclin D1 and survival was observed. Furthermore, we found no immunohistochemical correlation between nuclear \( \beta \)-catenin expression and cyclin D1 overexpression in any of the histological tumor grades.

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proliferative activity and cell adhesion systems, especially in poorly differentiated HCCs.

Our immunohistochemical study did not confirm the effect of the signaling pathway in HCC. However, it might be too early to assert that cyclin D1 expression has no significant correlation with β-catenin. As considering the reports that superexpression of an oncogenic form of β-catenin in the liver did not induce an up-regulation of cyclin D1 in transgenic mice (39) and that APC gene mutation does not occur in HCC (15, 40), there might be another pathway or target genes at work in HCC that are not present in other organ carcinomas (41, 42). Therefore, it is difficult to explain outcomes in HCC patients only in terms of oncogenic activation by β-catenin. In other words, as we defined higher cyclin D1 overexpression, which was not associated with β-catenin expression, and higher Ki-67 immunoreactivity in poorly differentiated HCC, a poor prognosis might depend on an increase in proliferative activity through mechanisms other than the signaling pathway and the reduction of cell adhesion system efficiency.

In conclusion, although we were unable to confirm its role in oncogenic activation immunohistochemically, our results suggest that nuclear accumulation of β-catenin in HCC is related to tumor progression, probably through the stimulation of tumor cell proliferation by means other than the signaling pathway and the reduction of cell adhesion system efficiency. Therefore, accumulation of β-catenin increases the risk of tumor recurrence and indicates a poor prognosis, especially in poorly differentiated HCC. Furthermore, there are histological and biological differences between moderately and poorly differentiated HCCs.

Because hepatic resection is regarded as one of the most valuable treatments for small, solitary HCCs (30), the number of the patients with recurrent HCC after resection may increase in the future. Therefore, in addition to assessing clinicopathological features during postoperative follow-up, it is important from the pathological point of view to pay attention to the possibility of distant metastasis and poor prognosis, especially in patients with poorly differentiated and β-catenin expression-positive HCCs.

Table 5 Relationship between β-catenin expression and outcome for each histological tumor grade

<table>
<thead>
<tr>
<th></th>
<th>β-Catenin positive</th>
<th>β-Catenin negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Intrahepatic recurrence</td>
<td>2 (50%)</td>
<td>3 (42.9%)</td>
</tr>
<tr>
<td>Death from HCC</td>
<td>2 (50%)</td>
<td>7 (41.2%)</td>
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</table>

Table 6 Multivariate analysis of survival of individual parameters

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>95% CI†</th>
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<tbody>
<tr>
<td>Histological grade</td>
<td>0.1744</td>
<td>0.050–1.719</td>
</tr>
<tr>
<td>Portal invasion</td>
<td>0.4177</td>
<td>0.134–2.303</td>
</tr>
<tr>
<td>β-Catenin</td>
<td>0.0098</td>
<td>0.028–0.613</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>0.3472</td>
<td>0.060–2.691</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>0.2947</td>
<td>0.538–7.707</td>
</tr>
<tr>
<td>Ki-67</td>
<td>0.1242</td>
<td>0.100–1.719</td>
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<table>
<thead>
<tr>
<th></th>
<th></th>
<th>5.000</th>
<th>0.0045</th>
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</tr>
<tr>
<td>II</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
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</tr>
</tbody>
</table>

* CI, confidence interval.

Fig. 3 Correlation between β-catenin expression and the Ki-67 LI (percentage of Ki-67-positive nuclei/all nuclei counted). □, nuclear β-catenin expression negative; ■, nuclear β-catenin expression positive. *, P < 0.0001; †, P = 0.0045.

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