Cytoskeleton-associated protein 2 is a potential predictive marker for risk of early and extensive recurrence of hepatocellular carcinoma after operative resection

Tatsuya Hayashi, MD, PhD,[a] Masayuki Ohtsuka, MD, PhD,[a] Daiki Okamura, MD, PhD,[a] Naohiko Seki, PhD,[b] Fumio Kimura, MD, PhD,[a] Hiroaki Shimizu, MD, PhD,[a] Hiroyuki Yoshidome, MD, PhD,[a] Atsushi Kato, MD, PhD,[a] Hideyuki Yoshitomi, MD, PhD,[a] Katsunori Furukawa, MD, PhD,[a] *and* **Masaru Miyazaki, MD, PhD,[a]** *Chiba, Japan*

Background. *De principe transplantation is an attractive strategy for the treatment of patients with hepatocellular carcinoma (HCC). The most important issue for this strategy is how to predict the risk of early and extensive recurrence. The present study aimed to identify a molecule associated with early and extensive recurrence of HCC after resection.*

Methods. *Differentially expressed genes were screened by DNA microarray analysis with the use of 12 HCC samples from patients who had different clinical courses based on the timing and extent of recurrence after operative resection. Furthermore, the obtained results were validated in 60 independent samples by quantitative real-time reverse transcription-polymerase chain reaction. Immunohistochemistry was performed to assess gene expression at the protein level.*

Results. *Microarray analysis and quantitative reverse transcription-polymerase chain reaction revealed cytoskeleton-associated protein 2 (CKAP2) as a candidate gene associated with early and extensive recurrence of HCC after resection. This observation was confirmed through examination of independent set samples, in which patients with greater-level CKAP2 mRNA expression exhibited shorter recurrence-free survival. Immunohistochemistry showed CKAP2 protein expression was associated with early (≤3 years) and extensive recurrence (beyond Milan criteria) after operative resection.*

Conclusion. *Immunohistochemical CKAP2 expression might be a potential biologic marker for identifying HCC patients at risk of early and extensive recurrence after operative resection. (Surgery 2014;155:114-23.)*

From the Department of General Surgery[a] and the Department of Functional Genomics,[b] Graduate School of Medicine, Chiba University, Chiba, Japan

Hepatocellular carcinoma (HCC) is the fifth most common cancer and is one of the leading causes of cancer deaths around the world.[1] Several treatment options are available for HCC, including operative resection, liver transplantation, percutaneous ablation therapy, and transarterial chemoembolization.[2] Optimal treatment depends on the severity of the underlying liver disease. For patients with decompensated liver cirrhosis whose HCC fulfills the Milan criteria, liver transplantation could be the optimal management approach.[3,4] In patients with adequate hepatocellular function whose HCC is anatomically resectable, operative resection is usually the first consideration, even if the HCC meets the Milan criteria.[5,6]

Although substantial improvement of survival as the result of major advances in operative procedures has been achieved in the past decade, high recurrence rates after resection remain a major obstacle to further improvements in quality of life and prognosis for patients with HCC.[7,8] Salvage transplantation may be the potential indication for such recurrent tumors and outcomes

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Reprint requests: Masayuki Ohtsuka, MD, PhD, Department of General Surgery, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuoh-ku, Chiba 260-8670, Japan. E-mail: otsuka-m@faculty.chiba-u.jp.

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of it is comparable with those of primary liver transplantation.[9] However, this strategy is not applicable to patients with early and extensive recurrent tumors. To overcome this problem, another strategy of *de principe* transplantation has been proposed.[10,11] In *de principe* transplantation, operative resection is used as an initial therapy. After resection, patients with high risk of recurrence are selected on the basis of detailed pathologic examination of the resected specimens, followed by performance of liver transplantation even in the absence of proven disease.

Currently, the most important factors used for identifying patients at high risk of recurrence are poor tumor differentiation, microvascular invasion, and multiple tumors, all of which are conventional pathologic factors. However, cases of death shortly after resection are occasionally encountered because of recurrence among patients with small solitary HCC, even without vascular invasion,[12] whereas patients with long-term survival after salvage transplantation for HCC with macroscopic portal vein thrombus also have been reported.[13] These clinical experiences suggest that HCC has heterogeneous biologic characteristics that cannot be revealed by conventional pathologic examination. Our aim, therefore, was to identify a biological molecule for the prediction of early and extensive recurrence of HCC.

The gene for cytoskeleton-associated protein 2 (CKAP2), also known as tumor-associated microtubule-associated protein, was screened as one of the up-regulated genes in our microarray analysis of HCC with early and extensive recurrence after resection. Previous reports have shown that various human malignancies, including gastric carcinoma,[14] diffuse B-cell lymphoma,[15] and cutaneous T cell lymphoma,[16] show up-regulation of the CKAP2 gene. Although the exact biologic functions of the gene product have not been fully identified, recent research has demonstrated that CKAP2 plays important functions in cell proliferation, particularly during mitosis.[17-20] The present study assessed expression of CKAP2 in HCC by the use of clinical samples and investigated the association of CKAP2 expression with early and extensive recurrence of HCC after curative operative resection.

MATERIALS AND METHODS

Microarray analysis. A total of 12 fresh operative specimens were obtained from 12 of 68 patients with HCC who underwent R0 operative resection at Chiba University Hospital between January 1996 and December 2002, with fully informed consent. Six specimens were randomly selected among 7 HCCs initially meeting the Milan criteria,[4] resulting in early (≤3 years) recurrence beyond the criteria (Group A), along with another 6 specimens from among 10 HCCs initially exceeding the criteria without recurrence ≤3 years after operative resection (Group B), to screen for genes that might be associated with early and extensive recurrence of HCC after resection. Complementary RNA was prepared from the total RNA sample of each HCC. Hybridization and signal detection using Agilent Human Genome 44 K arrays (Agilent Technologies, Palo Alto, CA) were performed in accordance with the instructions from the manufacturer.

Obtained microarray data sets were normalized using the median intensities of Group B probes. A Mann-Whitney test was performed to estimate the significance of differences in gene expression between groups. Differentially expressed genes were selected on the basis of the following criteria: $P < .01$ in the Mann-Whitney test and $\log_2$-transformed mean values of Group A greater than 1.0 or less than −1.0.

Patient selection and tissue samples for assessment of CKAP2 expression. Sixty consecutive patients with HCC who underwent initial R0 operative resection at Chiba University Hospital between January 2003 and December 2006 were enrolled for this study. These patients comprised 50 men and 10 women, with a mean age of 65.8 years (range, 29–80). HCC was diagnosed on the basis of typical clinical and radiologic findings and was confirmed histologically. Cases of combined hepatocellular-cholangiocellular carcinoma were excluded. No patients received any preoperative treatment or postoperative adjuvant therapy. At the time of surgery, 29 patients had HCC that met the Milan criteria and 31 had HCC that did not. The follow-up period for all patients was more than 3 years. Patients were followed up by measuring serum α-fetoprotein and protein induced by vitamin K absence or antagonists II (PIVKA II) levels every month, and computed tomography or ultrasonography every 3 months for the first year, then every 6 months thereafter. The presence of bone metastasis was examined by scintigraphy with ^{99m}Tc diphosphonate. Recurrence was identified when new lesions were observed on imaging, including magnetic resonance imaging, with typical appearances of HCC. Among the 60 patients, 29 showed recurrent tumors within 3 years after operative resection. Recurred tumors met the Milan criteria in 11