

Survivin is expressed in early hepatocellular carcinoma and surrounding hepatitis tissue

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Abstract. It has been suggested that survivin, a member of the inhibitor of apoptosis protein (IAP) family, is an attractive novel target for anticancer therapy. The present study investigated the expression of survivin during the early stages of hepatocellular carcinoma (HCC) and associated hepatitis, and attempted to elucidate how this expression is correlated with clinicopathological factors. Twenty-two patients (15 men and 7 women) underwent a liver tissue biopsy for the diagnosis of HCC. In the HCC and surrounding hepatitis-infected tissues, the average survivin expression rate was 62.36 and 31.41% (median), respectively. The serum level of ALT was correlated with the survivin expression rate in the HCC ($r = -0.60$, $P < 0.01$) and hepatitis specimens ($r = -0.43$, $P < 0.05$). The data suggest that certain clinicopathological factors may in future serve as useful indicators for the selection of patients responsive to the inhibition of survivin.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third leading cause of cancer-related death in the world, with an estimated annual prevalence of >5,000,000 cases worldwide (1). Most patients with HCC have no indications for any specific therapy strategies, such as surgery, percutaneous ethanol injection therapy and radiofrequency ablation, due to multiple recurrences with small nodules (2). As a result, patients must undergo interventional radiology (IVR), such as transcatheter arterial chemoembolization (TACE). A more effective IVR drug is therefore required.

Survivin is a recently identified member of the inhibitor of apoptosis protein (IAP) family (3) that is strongly associ-

ated with apoptosis, cell proliferation and cell cycle control (4-7). By inhibiting apoptosis and promoting mitosis, survivin facilitates cancer cell survival and growth (6,8-11). Survivin is selectively expressed in the most common human neoplasms, and appears to be involved in tumor cell resistance to some anticancer agents and ionizing radiation (12).

A previous report suggests that survivin inhibition in early HCC is potentially useful as an effective interventional radiological treatment modality (13). This study investigated the expression of survivin in early-stage HCC tissue and in its surrounding hepatitis-infected tissue, and examined the correlation of this expression with clinicopathological factors.

Materials and methods

Patients. Between January 2003 and December 2005, 22 patients (15 men and 7 women; median age 68 years, range 56-81 years) underwent a concurrent tumor and non-tumor liver tissue biopsy for the diagnosis of HCC at the Jikei University Daisan Hospital, Tokyo, Japan (Table I). Three peritumoral liver tissue specimens of metastatic liver cancer (pancreatic cancer) were also selected as non-hepatitis liver controls, and were retrospectively examined. The study was approved by the Jikei University Ethics Community Institutional Review Board.

Pathologic specimens. Tumor specimens were obtained by tumor biopsy with a 21-G fine-needle aspiration kit. Non-tumorous liver tissue specimens were concurrently obtained using an 18- to 20-G needle. Formalin-fixed and paraffin-embedded liver tumor and non-tumor tissue specimens were processed for conventional histological assessment with hematoxylin and eosin staining. The tumors were histologically graded as either well or moderately differentiated.

Immunohistochemical analysis. Formalin-fixed paraffin-embedded specimens were used for immunohistochemical analysis after deparaffinization. A rabbit anti-human survivin polyclonal antibody (Diagnostic Biosystems, USA) was used at a dilution of 1:2000 as the primary antibody, and detected with Envision+Rabbit/HRP (Dako, Japan). The specimens were heated in a microwave oven in antigen retrieval solution (10 mmol/l citrate buffer, pH 6.4) at 121°C for 15 min for the retrieval of antigens, then cooled to room temperature. 3,3-Diaminobenzidine and hematoxylin were used for color

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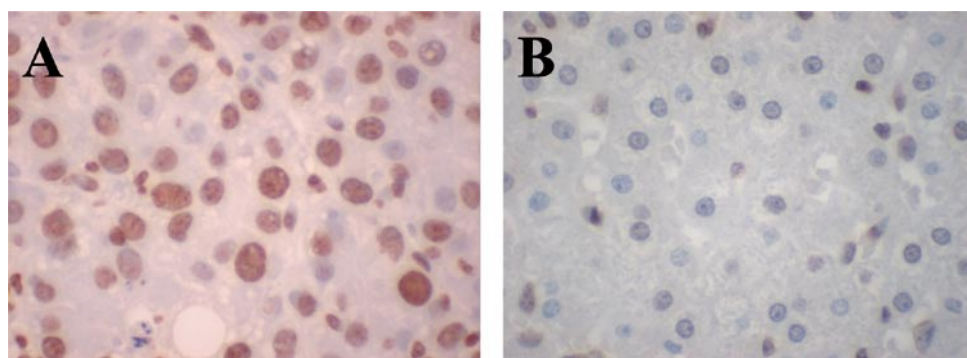


Figure 1. Immunohistochemical staining of survivin in hepatocellular carcinoma and peritumoral (hepatitis) biopsy tissues. (A) Tumor biopsy (x400); (B) Peritumoral biopsy (x400).

Table I. Characteristics of the patients undergoing tumor biopsy (n=22).

Features	Median value
Age	68 (56-81)
Gender (male/female)	15/7
Plt ($\times 10^3/\mu\text{l}$)	10.0 (5.1-24.5)
AST (IU/l)	68 (21-147)
ALT (IU/l)	61.5 (6-214)
T-Bil (mg/dl)	0.8 (0.4-2.3)
γ -GTP (IU/l)	48 (18-665)
AFP (ng/ml)	21.5 (3-444)
HBs Ag/HCV Ab/others	3/18/1
Tumor size (mm)	14.5 (8-23)
Cirrhosis (positive/negative)	5/17
Differentiation (well/moderate)	15/7

Data are expressed as the median (range) unless otherwise indicated. Hbs Ag, anti-hepatitis B surface antigen; HCV Ab, anti-hepatitis C antibody. Well, well-differentiated HCC; moderate, moderately differentiated HCC. Normal ranges: Plt (platelet count), $15\text{--}35 \times 10^3/\mu\text{l}$; AST (aspartate aminotransferase), 10-33 IU/l; ALT (alanine aminotransferase), 6-35 IU/l; T-Bil (total bilirubin), 0.2-1.2 mg/dl; γ -GTP (γ -glutamyl transferase), 10-50 IU/l; AFP (α -fetoprotein), >20 ng/ml.

development and counterstaining, respectively. Cells with brown-colored nuclei were scored as positive. The mean percentage of survivin-positive HCC cells was determined in three areas at $\times 100$ magnification using the nuclear labeling index (labeled nuclei/500 nuclei). The same method was applied to hepatocytes in non-tumorous biopsy specimens.

Statistical analysis. Correlations between the degree of survivin-positive cells within biopsy tissues and clinicopathological variables were analyzed using Pearson's correlation coefficient. A multiple regression analysis, in which the explanatory variables were age, gender, Plt (platelet count), ALT (alanine aminotransferase), AFP (α -fetoprotein) and size of tumor, was performed to clarify the factors associated with survivin-positive cancer cell levels. Probabilities <0.05 were

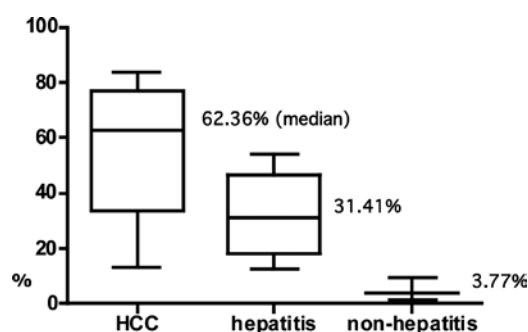


Figure 2. Survivin expression rates in hepatocellular carcinoma (HCC), hepatitis and non-hepatitis tissues.

considered to be statistically significant. The correlation analyses were performed using GraphPad Prism 4.00 (GraphPad Software, San Diego, USA), and multiple regression analysis was performed using the STATA 10.0 software program (STATA Corp., College Station, TX, USA).

Results

Survivin expression rates in early HCC and non-tumorous liver tissues. In HCC tissues, the rate of survivin expression was determined by counting survivin-positive cancer cells (Fig. 1A). The average survivin expression rate was 62.36% (median). In the peritumoral hepatitis tissues, the rate of survivin expression was determined in all hepatocytes (Fig. 1B). The average survivin expression rate was 31.41% (median). The survivin expression rate was also determined in all hepatocytes in the non-hepatitis tissues. The average survivin expression rate was 3.77% (median). Fig. 2 shows that survivin expression was higher in the tumor and peritumoral specimens than in the non-hepatitis tissues.

A correlation was also observed between the survivin expression rate in the peritumoral cells and the expression rate in HCC specimens ($r=0.81$, $P<0.001$, Fig. 3).

Relationship between survivin expression rates and clinical parameters. Fig. 4 shows that the serum level of ALT was significantly correlated with the survivin expression rate in both the HCC specimens ($r=-0.60$, $P<0.01$) and the non-tumorous liver tissues ($r=-0.43$, $P<0.05$). No significant

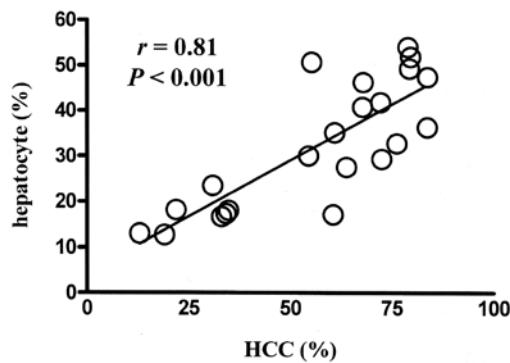


Figure 3. Correlation between the survivin expression rate in hepatocellular carcinoma (HCC) and peritumoral cells.

correlation was observed for other clinical parameters, such as the serum AFP level.

After adjustment for other covariates using multiple regression analysis, only the ALT level was thought to be a significant predictor of survivin expression ($P=0.02$, Table II).

Discussion

Survivin, a member of the recently described IAP family, is characterized by a unique structure with a single BIR and no zinc-binding domain (14). It is undetectable in terminally differentiated adult tissues, but becomes notably expressed in the most common human cancers, including esophageal, stomach, colorectal, breast and pancreatic carcinoma (15-19). Survivin has also been implicated in the control of cell cycle kinetics and the inhibition of apoptosis (20-22). These findings suggest that survivin is an attractive novel target for anticancer interventions. Several preclinical studies have demonstrated that the down-regulation of survivin expression/function by means of an antisense oligonucleotide, dominant negative mutants, ribozymes, small interfering RNAs and cyclin-dependent kinase inhibitors increases the rate of apoptosis, reduces tumor growth potential and sensitizes tumor cells to various chemotherapeutic drugs and γ -irradiation in *in vitro* and *in vivo* models of various types of human tumors (12). YM155 was the first agent designed to inhibit survivin (23). Several early phase clinical studies demonstrated this novel anticancer agent to be well-tolerated and capable of shrinking recurrent

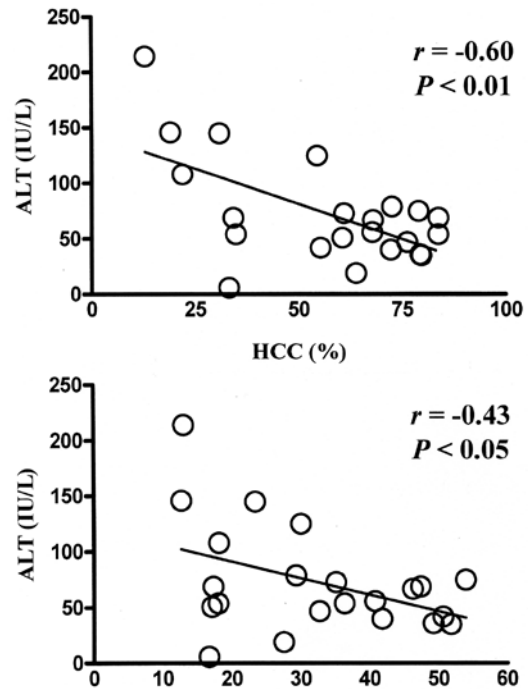


Figure 4. Correlation between serum levels of alanine aminotransferase (ALT) and survivin expression rates in hepatocellular carcinoma (HCC) and peritumoral cells.

tumors in some patients with non-Hodgkin lymphoma and hormone-refractory prostate cancer after conventional chemotherapy. In addition, interim reports indicate that it has few side effects. It is possible that survivin inhibition in early HCC could be potentially useful as an effective interventional radiological treatment modality (13). Moreover, the current study showed the expression of survivin in HCC to be correlated with peritumoral hepatocytes and serum ALT levels. This suggests that patients with HCC could benefit from survivin inhibitive therapy, even in cases where liver function has been stabilized.

The expression of survivin has been detected in a variety of preneoplastic and/or benign lesions, including polyps of the colon, breast adenomas, Bowen's disease and hypertrophic actinic keratosis (24). This suggests that the expression of survivin may occur during early malignant transformation

Table II. Results of a multiple regression analysis associated with the survivin-positive rates of hepatocellular carcinoma tissues.

Clinicopathological variables	Coefficient	Standard error	95% Confidence interval of coefficient	P-value
Age	0.004790	0.006960	-0.0100-0.0196	0.501
Gender	0.076200	0.143000	-0.228-0.380	0.601
Plt	-0.004020	0.011900	-0.0294-0.0214	0.740
ALT	-0.002710	0.001040	-0.004930- -0.000484	0.020
AFP	-0.000554	0.000438	-0.00149-0.00380	0.225
Tumor size	0.000677	0.012500	-0.0260-0.0274	0.958

Plt, platelet count; ALT, alanine aminotransferase; AFP, α -fetoprotein.

or following a disturbance in the balance between cell proliferation and cell death (12). A previous study showed that the HBV X and HCV core proteins activate NF- κ B and/or STAT-3, which regulate gene expression for cell survival factors such as anti-apoptotic proteins, including survivin (25,26). In the current study, the average survivin expression in peritumoral liver tissues was 31.41% (median). Survivin expression may also occur in the presence of the hepatitis virus (13). In this study, survivin expression was correlated with the serum levels of ALT. These findings indicate that, in viral hepatitis, survivin expression induces early malignant transformation following a disturbance in the balance between cell proliferation and cell death, even if liver function has been stabilized.

In 1955, elevations in serum AST levels were reported in viral hepatitis and other hepatic diseases. Subsequently, concomitant ALT elevations were found in similar disorders. AST and ALT are abundant hepatic enzymes that catalyze the transfer of amino groups to form the hepatic metabolites pyruvate and oxaloacetate, respectively. ALT is found in the cytosol in liver cells, whereas the two AST isoenzymes are located in the cytosol and mitochondria, respectively. Both ALT and AST are released from damaged hepatocytes into the blood after hepatocellular injury or death. AST is also abundantly expressed in several non-hepatic tissues, including the heart, skeletal muscle and blood. ALT is found at low concentrations in tissues other than the liver, and is therefore frequently considered specific for hepatocellular injury (27). In this study, a negative correlation between the rate of survivin expression and the serum level of ALT was observed in HCC and hepatocytes in patients with hepatitis. This suggests that the expression of survivin occurs with the inhibition of apoptosis, or following a disturbance in the balance between cell proliferation and cell death. ALT was not significantly released into the blood by damaged HCCs or hepatocytes, suggesting that the expression of survivin inhibits cellular injury or death caused by HCC and hepatitis.

Scattered HCCs are commonly treated by the embolization of the supplying branch of the hepatic artery, or by direct hepatic artery injection of chemotherapeutic agents followed by local embolization. The main advantage of TACE is that it allows the local administration of anticancer drugs to a specific cancerous area. In this study, a correlation was observed between the rate of survivin expression in peritumoral cells and the rate of expression in HCC specimens ($r=0.81$, $P<0.001$). The average rate of survivin expression was 31.41% (median) in the peritumoral area. If side effects are taken into consideration with survivin inhibitive therapy, then interventional radiological treatment of a selective artery with a survivin inhibitor could be an effective therapy for scattered HCCs.

In conclusion, the expression of survivin was analyzed during the early stages of HCC additionally surrounded by hepatitis, and the correlation of this expression with clinicopathological factors was measured. The serum level of ALT was correlated with the rate of survivin expression in the HCC and peritumoral hepatitis specimens. Multiple regression analysis showed that only serum ALT levels were associated with survivin expression in HCC. These findings suggest that various clinicopathological factors may in future serve as useful indicators for the selection of patients responsive to the inhibition of survivin.

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