# Expression of HSP27 in Hepatocellular Carcinoma

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**Abstract.** *Background/Aim: Heat-shock protein 27 (HSP27), a low molecular weight stress protein, is recognized as a molecular chaperone. The expression of HSP27 has been detected in some human tumors and while HSP27 is phosphorylated as a reresponse to stress, the function of phosphorylated HSP27 (p-HSP27) is not known. The aim of this study was to investigate what kind of effect expression of HSP27 and p-HSP27 in HCC has on clinicopathological characteristics and prognosis. Materials and Methods: An immunohistochemical study for HSP27 and p-HSP27 was performed on 194 resected HCC cases. We analyzed the correlation of HSP27 expression with various parameters statistically. Results: There was no correlation between expression of HSP27 and the clinicopathological characteristics and prognosis from the analysis of 194 cases. From the analysis of the hepatitis C virus (HCV)-positive group of 142 cases, those that were p-HSP27-positive had a larger tumor diameter and the portal vein invasion rate was high. Conclusion: The expression of total HSP27 may serve as a new, clinically useful marker of HCC. In addition, the present study suggests that the expression of phosphorylated HSP27 is useful in the screening and grading of HCC occurring in the setting of HCV.*

To date, eight isoforms of heat-shock protein (HSP), HSP10, 27, 40, 60, 70, 90, and 110, that are synthesized in cells in response to heat stress, have been identified (1). They maintain normal cell and tissue homeostasis mainly through: assistance in the formation of the 3-D structure of proteins (protein folding) in cells, detection and repair or degradation of abnormal proteins, involvement in the intracellular transport of proteins, and involvement in the regulation of cell death, *i.e.* apoptosis and necrosis (2). These functions provide organ protection and stress tolerance at the biological level. Recent studies have reported HSP expression in several types of cancer (2). High HSP expression in gastric (3), colorectal (4), pancreatic (5), breast (6) and ovarian (7) cancer, and leukemia (8) has been reported. It has also been reported that a higher expression level of HSP in ovarian (7), colorectal (9), prostate (10), and breast (11) cancer is associated with a poorer prognosis of patients. In addition, some studies reported the involvement of HSP in cancer cell proliferation, and in invasion and angiogenesis, and its functions in cancer are becoming increasingly clear (2).

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver (12). Although many patients with HCC have concomitant chronic hepatitis or liver cirrhosis occurring in the setting of hepatitis B or C (HBV, HCV) virus infection (13-15), HCC has also been reported in patients with non-viral hepatitis, suggesting that various factors influence HCC carcinogenesis. The 5-year recurrence rate of HCC following radical hepatectomy is as high as 70%, and it is associated with a very poor prognosis (16). Therefore, in order to control HCC, it is necessary to elucidate the mechanism of HCC, including the background liver status.

Although the main function of HSP27, a low-molecular-weight HSP, has been reported to be cytoprotective (2), an association with tumor progression and prognosis has also been indicated (17). Recently, the high expression of phosphorylated HSP7 has been reported in advanced and refractory cancer (18-21), and the expression and functions of HSP27 in cancer are becoming clearer. However, only a few studies have evaluated HSP27 expression in HCC. Therefore, we performed a clinicopathological study on patients who had undergone hepatectomy for histologically proven HCC.

## Materials and Methods

*Patients.* A total of 149 patients who had undergone hepatectomy at the Department of Surgery, Kurume University Hospital Japan between 1993 and 1997 were included in this study. They consisted of 150 men and 44 women, with a mean age of 64 years. Basic clinical data for these patients are given in Table I.



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*Key Words:* Heat-shock protein, phosphorylation, Hepatocellular carcinoma, hepatectomy, hepatitis C virus.

Table I. *Clinicopathological parameters of patients who underwent hepatectomy for hepatocellular carcinoma.*

| Factor | | Total patients (N=194) |
|---|---|---|
| Age, years | Median (range) | 64 (32-86) |
| Gender, n | Male | 150 |
| | Female | 44 |
| Histological grade, n | Well | 20 |
| | Moderate | 144 |
| | Poor | 30 |
| Tumor diameter, mm | Median (range) | 38 (10-230) |
| HBs antigen, n | Positive | 31 |
| | Negative | 163 |
| HCV, n | Positive | 142 |
| | Negative | 52 |
| Stage (UICC), n | I | 8 |
| | II | 68 |
| | III | 106 |
| | IV | 12 |
| Portal invasion, n | Positive | 112 |
| | Negative | 82 |
| Intrahepatic metastasis, n | Positive | 79 |
| | Negative | 114 |

UICC: Union for International Cancer Control; HBs: hepatitis B virus surface antigen; HCV: hepatitis C virus.

*Method of HSP analysis*. The paraffin sections of hepactectomy tumor samples from these patients were deparaffinized in xylene, rehydrated in graded alchohol, and transferred to phosphate-buffered saline. Endogenous peroxidase was inactivated by incubating the sections with 0.3% hydrogen peroxide for 30 minutes at room temperature. Immunohistochemical staining was performed using VECTASTAIN ABC KIT (PK-4001; Vector Laboratories, Inc., Burlingame, CA, USA). The primary antibodies used were rabbit polyclonal antibody against human HSP27 (dilution, 1:2,000; Stressgen, Chicago, IL, USA), and rabbit polyclonal antibody against human phosho-HSP27 pSer15 (dilution, 1: 2,000; Affinity BioReagents, Rockford, IL, USA). Color was developed using the diaminobenzidine peroxidase substrate kit (Vector Laboratories). The staining was evaluated by three independent observers and in comparison with the non-neoplastic liver parenchyma. When the staining intensity of the neoplastic liver parenchyma was at least twice that of the non-neoplastic liver parenchyma, the staining of sample was considered positive.

*Statistical analysis*. The association between HSP27 expression and clinicopathological variables was analyzed using the Chi-square test. The effect of each variable on overall survival (OS) was evaluated by univariate analysis using the Cox proportional hazards model. The variables that were found to be statistically significant by univariate analysis were further tested by multivariate analysis. OS curves for two groups were generated by the Kaplan–Meier method, and tested with the log-rank test. Statistical analysis was performed using JMP 11 (SAS Institute Japan Inc., Osaka, Japan).

Table II. *Association between the expression of total heat-shock protein (HSP27) and clinicopathological parameters of 194 patients with hepatocellular carcinoma.*

| Factor | Total HSP-27 expression | | |
|---|---|---|---|
| | + | – | *p*-Value |
| Age at surgery, mean±SD (years) | | | |
| 62.8±0.84 | 118 | - | 0.8656 |
| 63.0±1.05 | - | 76 | |
| Gender, n | | | |
| Male (n=150) | 97 | 53 | 0.0449 |
| Female (n=44) | 21 | 23 | |
| Histological grade, n | | | |
| Well (n=20) | 14 | 6 | 0.4624 |
| Moderate (n=144) | 84 | 60 | |
| Poor (n=30) | 20 | 10 | |
| Tumor diameter, mean±SD (mm) | | | |
| 50.73±3.1 | 118 | - | 0.5146 |
| 47.53±3.8 | - | 76 | |
| HBs, n | | | |
| Positive (n=31) | 20 | 11 | 0.6443 |
| Negative (n=163) | 98 | 65 | |
| HCV, n | | | |
| Positive (n=142) | 85 | 57 | 0.648 |
| Negative (n=52) | 33 | 19 | |
| Stage (UICC), n | | | |
| I & II (n=76) | 42 | 34 | 0.2037 |
| III & IV (n=118) | 76 | 42 | |
| Portal invasion, n | | | |
| Positive (n=112) | 71 | 41 | 0.3923 |
| Negative (n=82) | 47 | 35 | |
| Intrahepatic metastasis, n | | | |
| Positive (n=79) | 50 | 29 | 0.5269 |
| Negative (n=114) | 67 | 47 | |

UICC: Union for International Cancer Control; HBV: hepatitis B virus surface antigen; HCV: hepatitis C virus.

## Results

Figure 1 shows the results of immunohistochemical staining for total and phosphorylated forms of HSP27. Both forms were stained granularly in the cytoplasm and nuclei of HCC cells. The total and phosphorylated HSP27-positive rates were 60. 8 and 35. 1%, respectively. Total HSP27 expression in the resected HCC specimens was not significantly associated with the clinicopathological parameters of patients, except that the rate of total HSP27-positivity was significantly higher in male than in female patients with HCC ($p$=00449; Table II). The rate of phosphorylated HSP27-positivity was low in the hepatitis B virus surface antigen (Hbs)-positive patients but it was high in the HCV antibody-positive patients (Table III).

Recently, high HSP27 expression was reported in patients with HCC occurring in the setting of HCV infection,

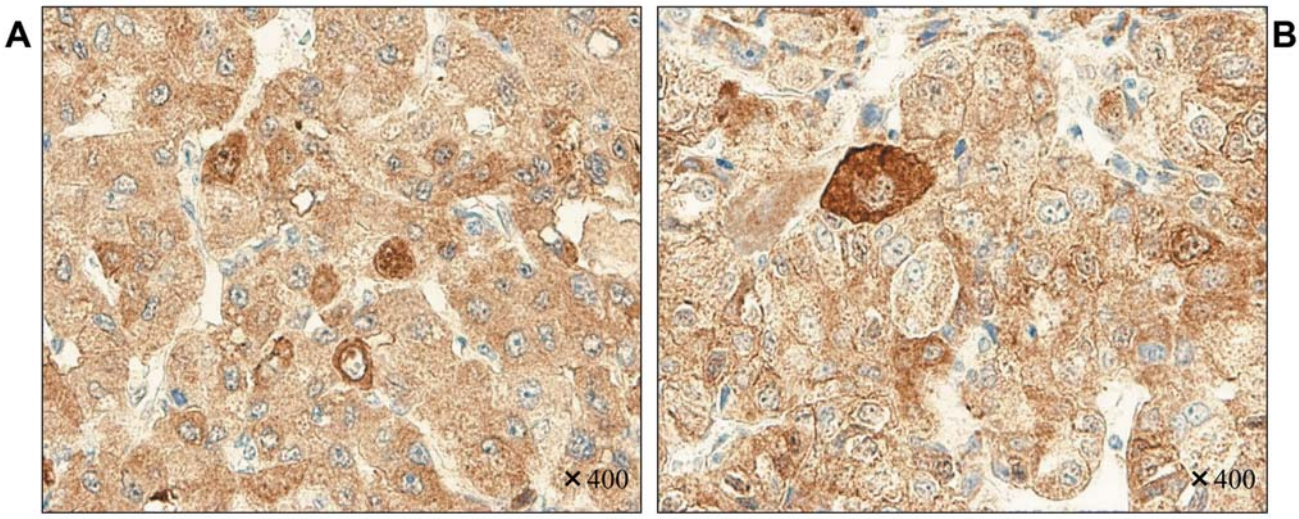


Figure 1. *Expression of total and phosphorylated heat-shock protein (HSP27) in hepatocellular carcinoma. Total HSP27 (A) and phosphorylated HSP27 (B) were stained granularly in the cytoplasm and nuclei of hepatocellular carcinoma cells (original magnification, ×400).*

suggesting the involvement of HSP27 in hepatocellular carcinogenesis (22). Thus, we investigated the association between the expression of total and phosphorylated HSP27 and the clinicopathological parameters of 142 patients with HCC occurring in the setting of chronic hepatitis C. We found that although total HSP27 expression was not significantly associated with the clinicopathological parameters of these patients, phosphorylated HSP27 expression was significantly associated with larger tumor diameter ($p$=0.0369) and portal vein invasion (Table IV). The presence or absence of total or phosphorylated HSP27 was not significantly correlated with the prognosis (Kaplan–Meier analyses, data not shown).

## Discussion

The rate of positive expression of total HSP27 in HCC was relatively high, at 60.8%, which was similar to that previously reported (23). In addition, this positivity rate did not significantly vary with the degree of HCC differentiation, making it a promising tumor marker for HCC. Luk *et al*. noted that total HSP27 expression was higher in HCC tissue than in the surrounding non-neoplastic tissue (24). HSP27 expression may be useful for the differentiation of a dysplastic nodule (precancerous lesion) from cancer. HSP27 has been shown to be released into the blood (25, 26). It was reported that serum HSP27 levels were higher in patients with HCC than in those without, and in patients with HCC derived from HCV than in those with HCC derived from alcoholic steatohepatitis or HBV (22). Our study also showed that the rate of expression of phosphorylated HSP27 was much higher in patients with HCC occurring in the setting of HCV than HBV. These observations suggest that the phosphorylated form of HSP27 is involved in the carcinogenesis of HCC derived from HCV. Thus, we investigated the correlation between the expression of phosphorylated HSP27 in patients with HCC derived from hepatitis C and their clinicopathological parameters. We found that phosphorylated HSP27 expression was highly positively associated with the tumor diameter and portal vein invasion. It has been reported that phosphorylated HSP27 is associated with apoptosis resistance (27) and epithelial–mesenchymal transition (19), and is involved in invasion and metastasis in ovarian and prostate cancer (28, 29). These findings suggest that phosphorylated HSP27 is involved in HCC proliferation, invasion, and metastasis. On the other hand, the presence or absence of total or phosphorylated HSP27 was not significantly correlated with the prognosis (Kaplan–Meier analyses, data not shown). In general, the prognosis of patients with HCC is influenced not only by the tumor stage but also by the liver function (hepatic functional reserve). Therefore, adverse effects (if any) of HSP27 on cancer cells may not have been reflected in the prognosis. Moreover, Yasuda *et al*. described a different function of HSP27: they reported that progression of HCC stage was associated with reduced expression of phosphorylated HSP27, suggesting its inhibitory role in cancer progression (30). The various functions of HST27 need to be further explored.

Table III. *Association between the expression of phosphorylated heat-shock protein (HSP27) and clinicopathological parameters in 194 patients with hepatocellular carcinoma.*

| Factor | Phosphorylated HSP-27 expression | | |
|---|---|---|---|
| | + | – | *p*-Value |
| Age at surgery, mean±SD (years) | | | |
| 63.5±1.11 | 68 | - | 0.4464 |
| 62.5±0.82 | - | 126 | |
| Gender, n | | | |
| Male (n=150) | 52 | 98 | 0.836 |
| Female (n=44) | 16 | 28 | |
| Histological grade, n | | | |
| Well (n=20) | 8 | 12 | 0.3108 |
| Moderate (n=144) | 53 | 91 | |
| Poor (n=30) | 7 | 23 | |
| Tumor diameter, mean±SD (mm) | | | |
| 51.5±4.04 | 68 | - | 0.5409 |
| 48.4±2.97 | - | 126 | |
| HBs, n | | | |
| Positive (n=31) | 5 | 26 | 0.0111 |
| Negative (n=163) | 63 | 100 | |
| HCV, n | | | |
| Positive (n=142) | 57 | 85 | 0.0116 |
| Negative (n=52) | 11 | 41 | |
| Stage (UICC), n | | | |
| I & II (n=76) | 22 | 54 | 0.1501 |
| III & IV (n=118) | 46 | 72 | |
| Portal invasion, n | | | |
| Positive (n=112) | 44 | 68 | 0.1466 |
| Negative (n=82) | 24 | 58 | |
| Intrahepatic metastasis, n | | | |
| Positive (n=79) | 28 | 51 | 0.9595 |
| Negative (n=114) | 40 | 74 | |

UICC: Union for International Cancer Control; HBs: hepatitis B virus surface antigen; HCV: hepatitis C virus.

Table IV. *Association between the expression of total heat-shock protein (HSP27) and clinicopathological parameters in 142 patients with hepatocellular carcinoma occurring* in the setting of hepatitis C virus.

| Factor | Phosphorylated HSP-27 expression | | |
|---|---|---|---|
| | + | – | *p*-Value |
| Age at surgery, mean±SD (years) | | | |
| 65.5±0.96 | 57 | - | 0.6116 |
| 64.9±0.79 | - | 85 | |
| Gender, n | | | |
| Male (n=150) | 43 | 67 | 0.6371 |
| Female (n=44) | 14 | 18 | |
| Histological grade, n | | | |
| Well (n=20) | 6 | 10 | 0.7764 |
| Moderate (n=144) | 45 | 63 | |
| Poor (n=30) | 6 | 12 | |
| Tumor diameter, mean±SD (mm) | | | |
| 50.5±3.42 | 57 | - | 0.0369 |
| 41.2±2.80 | - | 85 | |
| Stage (UICC), n | | | |
| I & II (n=76) | 18 | 39 | 0.0863 |
| III & IV (n=118) | 39 | 46 | |
| Portal invasion, n | | | |
| Positive (n=112) | 39 | 42 | 0.0238 |
| Negative (n=82) | 18 | 43 | |
| Intrahepatic metastasis, n | | | |
| Positive (n=79) | 25 | 32 | 0.4941 |
| Negative (n=114) | 32 | 52 | |

UICC: Union for International Cancer Control.

The expression of HSP27 in pancreatic cancer confers increased resistance to the anticancer drug gemcitabine; however, it was reported that the concomitant use of an HSP27 inhibitor and gemcitabine inhibited the proliferation of pancreatic cancer cells (31). Inhibition of the expression of HSP27 with a potent cytoprotective effect may also be applicable to patients with HCC, who often show resistance to anticancer drugs. For example, if a new drug that selectively blocks HSP27 is approved for clinical application, its concomitant use with an anticancer drug is expected to exert a synergistic effect.

In this study, we investigated the expression of HSP27 in HCC. The expression of total HSP27 may serve as a new, clinically useful marker of HCC. In addition, the present study suggests that the expression of phosphorylated HSP27 is useful in the screening and grading of HCC occurring in the setting of HCV.

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