Expression of Fas antigen and Bcl-2 protein in hepatocellular carcinoma.

Keisuke Hamazaki* Akira Gochi† Nagahide Matsubara‡
Mazanobu Mori** Kunzo Orita††

*Okayama University,
†Okayama University,
‡Okayama University,
**Okayama University,
††Okayama University,

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Keisuke Hamazaki, Akira Gochi, Nagahide Matsubara, Mazanobu Mori, and Kunzo Orita

Abstract

Fas antigen (ag) is a cell surface protein known to trigger apoptosis in a variety of cells upon specific antibody binding. On the other hand, Bcl-2 protein, an oncogene product located at the mitochondrial inner surface, prolongs cell survival by blocking apoptosis. In this study we examined the expression of Fas ag and bcl-2 protein in 17 cases of hepatocellular carcinoma (HCC) to determine their role on HCC. By flow cytometric analysis, mean (SD) value of the expression of Fas ag on hepatocytes derived from normal liver, diseased liver (chronic hepatitis or liver cirrhosis) and HCC was 5.8 (4.7)%, 10.3 (6.9)%, and 24.0 (18.2)%, respectively. Fas ag expression on hepatoma cells was significantly greater than normal and diseased liver cells. The expression of Bcl-2 protein in normal liver, diseased liver and HCC was 4.3 (8.5)%, 0.8 (2.5)%, and 2.1 (3.4)%, respectively, and the difference was not significant. These results suggest that induction of apoptosis may be a possible therapy against HCC.

KEYWORDS: apoptosis, Fas antigen, Bcl-2, hepatocellular carcinoma

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Brief Note

Expression of Fas Antigen and Bcl-2 Protein in Hepatocellular Carcinoma

Keisuke Hamazaki*, Akira Gochi, Nagahide Matsubara, Masanobu Mori and Kunzo Orita

First Department of Surgery, Okayama University Medical School, Okayama 700, Japan

Fas antigen (ag) is a cell surface protein known to trigger apoptosis in a variety of cells upon specific antibody binding. On the other hand, Bcl-2 protein, an oncogene product located at the mitochondrial inner surface, prolongs cell survival by blocking apoptosis. In this study we examined the expression of Fas ag and bcl-2 protein in 17 cases of hepatocellular carcinoma (HCC) to determine their role on HCC. By flow cytometric analysis, mean (SD) value of the expression of Fas ag on hepatocytes derived from normal liver, diseased liver (chronic hepatitis or liver cirrhosis) and HCC was 5.8 (4.7)%, 10.3 (6.9)%, and 24.0 (18.2)%, respectively. Fas ag expression on hepatoma cells was significantly greater than normal and diseased liver cells. The expression of Bcl-2 protein in normal liver, diseased liver and HCC was 4.3 (8.5)%, 0.8 (2.5)%, and 2.1 (3.4)%, respectively, and the difference was not significant. These results suggest that induction of apoptosis may be a possible therapy against HCC.

Key words: apoptosis, Fas antigen, Bcl-2, hepatocellular carcinoma

Kerr et al. (1) reported a new form of cell death called apoptosis which is different from necrosis. The importance of apoptosis has been recognized in cancer therapy, and a greater understanding of apoptosis is required for effective treatment against cancer (2).

Yonehara et al. (3) discovered the Fas ag and reported that it was closely related to the induction of apoptosis. Since then, Fas ag-induced apoptosis has been studied extensively, particularly in thymic cells. Although Fas ag expression on malignant hematopoietic cells has been well examined, there are few reports about its expression on solid tumors and to our knowledge, there are no reports on its expression on HCC.

On the other hand, Tsujimoto et al. (4) found that B cell lymphoma leukemia-2 (bcl-2) oncogene is a suppressor of apoptosis. These findings indicate that the balance between Fas ag and Bcl-2 is very important to the occurrence of apoptosis. Accordingly, we studied both the expression of Fas ag and Bcl-2 protein in a series of HCC patients by flow cytometry.

Tissues from HCC and adjacent liver parenchyma were obtained from 17 patients undergoing surgical resection. Adjacent liver samples were obtained in 6 cases of chronic hepatitis and 11 cases of cirrhosis. Histologically normal liver tissues were obtained from 5 patients with liver metastasis of gastric cancer or colon cancer. The mean age of patients was 62 years (range 40-79) and there were 13 men and 4 women. Ten patients were positive for antibody against hepatitis C virus (HCV C100-3 antibody and second generation antibody) and 6 patients were positive for hepatitis B surface antigen (HBsAg).

Samples were frozen rapidly and stored at −80°C until examination. Frozen tissues were thawed rapidly and minced with surgical blades in 3ml of phosphate-buffered saline (PBS) containing 0.2% type I collagenase (Sigma, St. Louis, MO, USA). The suspension was incubated at room temperature for 10min and filtered through a 40-m nylon mesh. The cells in the filtrate were washed with PBS twice.

*To whom correspondence should be addressed.
To study Fas expression, cells were incubated at 4°C in a blocking solution of PBS containing 2% normal goat serum to prevent nonspecific binding of mouse immunoglobulin (Ig), then exposed to anti-Fas antibody (MBL, Nagoya, Japan) for 30 min at 4°C. Cells were washed in PBS twice, and fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgM (Tago, Burlingame, CA, USA) was added for 30 min at 4°C. After washing twice, the cells were fixed with 70% cold ethanol for 10 min at 4°C. The cells were suspended in medium containing propidium iodide (PI; Sigma) after washing.

Bel-2 staining was carried out according to the method of Aiello et al. (5). After fixation with 2% paraformaldehyde, cells were washed in PBS once, and incubated with FITC-conjugated anti-Bel-2 monoclonal antibody (DAKO, Glostrup, Denmark) at 4°C for 30 min.

As negative control, normal mouse serum was employed at 4°C for 10 min. After washing, the nuclei were suspended in medium containing PI.

Flow cytometric analysis was carried out using a FACS-CAN (Becton Dickenson, Mountain View, CA, USA) with the window set to exclude damaged cells and debris. Ten thousand cells were examined from each sample for determination of Fas ag or Bel-2 protein expression.

The results are shown as mean (SD) and statistical analyses were made with Student's t-test. A P value of less than 0.05 was considered significant.

Immunofluorescence analysis of Fas ag expression in a representative case was shown in Fig. 1.

The mean (SD) value of the expression of Fas ag in normal liver, diseased liver (chronic hepatitis and cirrhosis) and HCC was 5.8 (4.7)% 10.3 (6.9)% and 24.0 (18.2)% respectively. There was a significant difference between HCC and diseased or normal liver (Fig. 2).

The mean (SD) value of the expression of Bel-2 protein in normal liver, diseased liver and HCC was 4.3 (8.5)% 0.8 (2.5)% and 2.1 (3.4)% respectively, but lacked significance (Fig. 3).

Radiation and cancer chemotherapies have been reported to induce apoptosis, and p53 and c-myc genes are known to affect the induction of apoptosis (2). Furthermore, Fas ag has recently been shown to play a role in the induction of apoptosis. Fas ag is a 48 kDa transmembrane, cysteine-rich glycoprotein which belongs to the nerve growth factor/tumor necrosis factor receptor family.

Although extensively studied on thymocytes and hematopoietic cells, little is known about Fas ag expression in liver disease. Normal human livers express Fas...
ag, and Hiramatsu et al. (6) showed that Fas ag expression in the HCV antigen-positive group was significantly higher than that in the HCV antigen-negative group, and HCV-infected hepatocytes were found at or near the area with Fas ag expressed hepatocytes. They suggested that Fas ag may be a prerequisite for liver injury in chronic hepatitis C. However, in our study no significant difference was found in the expression of Fas ag between chronic hepatitis or liver cirrhosis and normal liver.

Further studies are needed to determine the role of Fas ag in hepatocyte injury in cases of chronic hepatitis or liver cirrhosis.

The expression of Fas ag was significantly higher in HCC compared with that of normal or diseased liver, suggesting that the induction of apoptosis with anti-Fas may be useful as a therapy for HCC. Owen-Schaub et al. (7) reported that Fas ag expression was widely distributed among solid tumors of various histological types, and the susceptibility to anti-Fas induced apoptosis was not correlated to the expression of this protein, and several solid tumor cell lines responded to anti-Fas treatment with growth stimulation rather than cell death. Whether the tumor will be stimulated to grow or will face apoptosis seems to depend on c-myc (8). Therefore, the increase of Fas expression alone does not necessarily induce apoptosis.

Cytokines, such as IFN-γ and TNF-α, are known...
to increase Fas expression (9), and therefore its expression in HCC may be related to these cytokines.

Bcl-2 was discovered by Tsujimoto et al. (4) in the course of research on human follicular lymphoma, and it was found to suppress apoptosis by influencing the susceptibility of cells to anti-cancer therapy or anti-Fas treatment. Charlotte et al. (10) examined the expression of Bcl-2 protein immunohistochemically in normal liver (12 cases), chronic hepatitis (2 cases), liver cirrhosis (10 cases), HCC (15 cases) and cholangioma (11 cases). Eight out of 11 cases in cholangioma were positive, and all cases except cholangioma were negative for Bcl-2 protein. This result is similar to our finding that expression of Bcl-2 was not increased in any liver disease studied.

Further studies are planned to elucidate the relationship between the expression of Fas ag and apoptosis in HCC and the possible control by c-myc.

References


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