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Prognostic significance of receptor for advanced glycation end products expression in hepatocellular carcinoma after hepatectomy

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ABSTRACT

Background: The receptor for advanced glycation end products (RAGE) is recognized to be responsible for cancer progression in several human cancers. In this study, we investigated the clinical impact of RAGE expression in patients with hepatocellular carcinoma (HCC) after hepatectomy.

Materials and methods: Sixty-five consecutive patients who underwent initial hepatectomy for HCC were investigated. The relationships between immunohistochemical expression of RAGE and clinicopathologic features, clinical outcome (overall survival [OS], and disease-free survival [DFS]) were evaluated.

Results: The cytoplasmic expression of RAGE in HCC cells was observed in 46 patients (70.8%) and correlated with histologic grade (poorly differentiated *versus* moderately differentiated HCC, $P = 0.021$). Five-year OS in RAGE-positive and RAGE-negative groups were 72% and 94%, respectively, whereas 5-y DFS were 29% and 55%, respectively. There were significant differences between OS and DFS ($P = 0.018$ and 0.031, respectively). Multivariate analysis indicated that RAGE was an independent predictor for both OS and DFS ($P = 0.048$ and 0.032, respectively).

Conclusions: Our data suggest for the first time a positive correlation between RAGE expression and poor therapeutic outcome. Furthermore, RAGE downregulation may provide a novel therapeutic target for HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most commonly diagnosed malignant disease and the second most common cause of cancer death worldwide, especially in Asia and Africa [1]. Most cases of HCC are inflammation-related malignancies, which develop as a consequence of underlying liver disease, mostly viral hepatitis [2]. Hepatectomy is still considered the main curative therapy for HCC, with a 5-y OS of approximately 50%–70% after curative hepatectomy.

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However, the postoperative recurrence rate at 5 y remains as high as 70%–83.7% [3,4]. Therefore, it is necessary to identify new prognostic factors for HCC patients after hepatectomy.

The receptor for advanced glycation end products (RAGE) is a multiligand receptor classified as an immunoglobulin superfamily cell-surface molecule. RAGE was originally described as a receptor for protein adducts formed by glycoxidation (advanced glycation end products) that accumulate in diseases such as diabetes mellitus and renal failure [5,6]. The interaction of RAGE with distinct molecules is implicated in homeostasis, development, and inflammation. Several ligands binding to RAGE have been identified, such as S100/calgranulins, amyloid-β peptide [7–11], and high-mobility group box 1 (HMGB-1). HMGB-1 is considered a late mediator of endotoxin shock [12,13]. RAGE triggers activation of key cell signal pathways, such as NF-κB and cdc42/rac, which result in the production of proinflammatory cytokines [14].

Recently, RAGE has been found to be responsible for cancer progression in several human cancers, including gastric, colon, prostate, pancreatic, biliary, esophageal, lung, and oral [15–24]. *In vitro* and clinical studies suggest that RAGE activation is associated with proliferation, migration, and invasion of cancer cells [25]. Although clinical usefulness of RAGE expression for estimating inflammation-related carcinogenesis and intrahepatic metastasis has been reported [26,27], the relationship between RAGE expression and clinical outcome is unknown. This study investigates the clinical impact of RAGE expression on therapeutic outcome in patients with surgically resected HCC.

2. Materials and methods

2.1. Patient and tumor specimens

From January 2003–December 2007, 71 consecutive patients with HCC were treated by hepatectomy at the Department of Surgery, Jikei University Hospital, Japan. Of these, 65 consecutive patients who underwent initial hepatectomy were studied. The histologic grade and pTNM stage of each tumor were determined according to the general rules for the clinical and pathological study of primary liver cancer (The Liver Cancer Study Group of Japan, 2003).

2.2. Immunohistochemical staining and evaluation of RAGE expression

For the immunohistochemical study, formalin-fixed, paraffin-embedded sections were used. Tumor samples were sectioned into 3-μm thick slices. Immunohistochemical staining was performed via the streptavidin–biotin–peroxidase complex method [28,29]. The Ventana auto-immunostaining system (Ventana NexES; Roche Diagnostics, Tokyo, Japan) using polyclonal anti-RAGE antibodies (H-300; Santa Cruz, CA) was employed. Anti-RAGE antibody was used at a dilution of 1/500. The antigen retrieval procedure was performed with a microwave oven in DAKO antigen retrieval solution (DAKO Corporation, Carpinteria, CA) for 30 min at 95°C to efficiently stain the sample [30]. As a positive control, normal lung tissue was used.

To evaluate RAGE expression, four fields were selected from each cancerous area (two marginal and two internal). The RAGE-positive cells were identified at high-power field (×200) magnification, and the occupancy area of RAGE-positive cells was calculated using National Institutes of Health Image software (National Institutes of Health, Bethesda, MD). The results were then classified into two groups, that is, negative (0%–25%) and positive (over 25%), according to the average value of the occupancy rate for each of the four views.

2.3. Statistical analyses

The chi-square test was used to compare clinicopathologic characteristics of HCC and RAGE expression. The Kaplan–Meier method was used for survival analysis, and differences in survival were estimated by the log-rank test. Cox regression was performed for multivariate analyses of prognostic factors. P values of <0.05 was considered significant. All statistical analyses were performed using StatView software, version 5.0 (Abacus Concepts, Berkeley, CA).

3. Results

About the background of 65 consecutive patients in our study, their mean age was 63 y (range 31–80 y) and 58 were men. Twenty-three patients (35.4%) were positive for hepatitis B surface antigen (HBsAg) and 29 (44.6%) were positive for the antibody to hepatitis C virus. Thirteen patients (20.0%) were negative for both virus markers. Histologically, 28 patients had liver cirrhosis, 30 had chronic hepatitis, and 7 had a normal liver. The tumors were well-differentiated in 13 (20%), moderately differentiated in 42 (64.6%), and poorly differentiated in 10 (15.4%) patients. Twelve patients (18.5%) were ascertained as stage I, 36 (55.4%) as stage II, 14 (21.5%) as stage III, and 3 (4.6%) as stage IV. Median follow-up was 37.5 mo, with a range of 7–85 mo. Postoperative tumor recurrence and death occurred in 40 patients (61.5%) and 17 patients (26.2%), respectively.

RAGE immunoreactivity was observed in the cell membrane and cytoplasm of tumor cells in HCC tissues but not in the nuclei (Fig. 1). RAGE expression was observed in the sections from 46 of the 65 patients (70.8%).

First, we evaluated the relationship between RAGE and the following clinicopathologic parameters: pTNM stage, histologic grade, tumor number, tumor size, portal invasion factor, liver condition (normal liver, chronic hepatitis, or liver cirrhosis), viral HBsAg, hepatitis C virus, gender, and age. As shown in Table 1, we found that RAGE expression was significantly higher in poorly differentiated HCC than in moderately differentiated HCC ($P = 0.021$).

Five-year overall survival (OS) and disease-free survival (DFS) for the total study population were 80.4% and 36.2%, respectively. The relationship between RAGE expression and OS is shown in Figure 2A. The 5-y OS was significantly lower in the RAGE-positive group than in the RAGE-negative group ($P = 0.018$, log-rank test). Moreover, we also found significantly lower DFS with the RAGE-positive group ($P = 0.031$, log-rank test), as shown in Figure 2B.