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Analysis of Gene Expression Profiles in Fatal Hepatic Failure After Hepatectomy in Mice

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Background. **We developed 90%-hepatectomized mice that were the fatal model, and analyzed the gene expression profiles using a complementary DNA (cDNA) microarray to clarify the mechanisms of hepatic failure after excessive hepatectomy.**

Materials and Methods. **Ribonucleic acid (RNA)s from the remnant hepatic tissue of 70%- and 90%-hepatectomized mice were labeled with fluorescent dyes, and hybridized to the Riken set of 39,168 full-length enriched mouse cDNA arrays. The gene expression profiles in 90%- and 70%-hepatectomized mice were analyzed by scanning date for fluorescent dye signals.**

Results. **The down-regulated genes in 90%-hepatectomized mice were genes activating extracellular matrix (ECM) remodeling (matrix metalloproteinases, laminins, and integrins), genes related to cytokines (tumor necrosis factorα converting enzyme, and Janus kinase 3) that were related to the priming, genes related to growth factor (heparin-binding epidermal growth factor-like growth factor and others), and genes promoting cell cycle progression (cyclin D1, D2, and E2) that were related to the progression of hepatocytes. The up-regulated genes were genes inhibiting ECM remodeling [plasminogen activator inhibitors (PAIs)].**

Conclusions. **Hepatic failure after hepatectomy was characterized by the inhibition of hepatic cell cycle priming and progression both induced by ECM remodeling in liver regeneration. Particularly, the overexpression of PAIs was thought to play the major role in the first step of inhibition of ECM remodeling.**

Key Words: **plasminogen activator inhibitor; extracellular matrix; mitogen activation protein kinase; cyclin; cDNA microarray; hepatic failure; hepatectomy.**

INTRODUCTION

Many rat models have been established to clarify the mechanism of fatal hepatic failure after excessive hepatectomy [1–3]. We developed a new technique of hepatectomy in rats, a procedure that enabled all 90%-hepatectomized rats to survive, while all 95%-hepatectomized rats died of hepatic failure within 96 h [4]. It was reported that apoptosis of hepatocytes was one of the main causes of fatal hepatic failure after excessive hepatectomy [5]. However hepatic damage was reduced by transfection of the human bcl-2 gene to rat livers in order to inhibit apoptosis after hepatectomy, but the rats finally died of hepatic failure [6].

By contrast, research into liver regeneration has used many mice that have undergone 70% hepatectomy using the methods introduced by Higgins and Anderson [7]. But no fatal model of hepatic failure after excessive hepatectomy has been developed and reported in mice. Therefore, we developed a fatal model of hepatic failure after excessive hepatectomy in mice in our previous

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study [8]. In the present study, gene expression profiles in 70%- and 90%-hepatectomized mice were analyzed using a complementary DNA (cDNA) microarray to clarify the mechanisms of fatal hepatic failure after excessive hepatectomy.

MATERIALS AND METHODS

Animals

Eight-wk-old male C57BL/6 J mice weighing 20 to 25 g (mean weight ± standard deviation = 23.7 ± 0.77 g) (purchased from CLEA Japan Inc., Tokyo, Japan) were used. All mice were kept in a temperature-controlled room with alternating 12-h dark and light cycles, and fed a standard diet. They were anesthetized with diethyl ether and fasted for 6 h before hepatectomy. Mouse hepatectomy was performed using the methods introduced by Higgins and Anderson [7], and Gaub and Iversaon [3] with minor modifications. We employed two models of hepatectomy: 70% hepatectomy, consisting of the removal of the left lateral and median hepatic lobes, and 90% hepatectomy, in which all hepatic lobes except the caudate lobe were resected. Midventral laparotomy was performed as a sham operation. Mice that underwent the sham operation were used as controls. Animals were sacrificed by cervical dislocation after operation. Mice were given 1 mL of 10% glucose solution *via* a gastric tube and had free access to 10% glucose solution after hepatectomy. The animal experiments were conducted in accordance with the guidelines of the Yokohama City University Graduate School of Medicine and National Institutes of Health.

Animal Experimental Protocol

Mice were sacrificed at 0.5, 1, 3, 5, and 10 h after hepatectomy. At each time point, five mice were used for the collection of remnant hepatic tissue.

Mouse cDNA Microarray

The mouse 40 K cDNA microarray was purchased from Riken Genomic Science Center (GSC). The mouse 40 K Riken set of 39,168 full-length enriched mouse cDNA arrays was used in the experiments. The total number of clones was 42,336, and 21,168 clones, including a number of clones of β-actin cDNA and G3-PDH cDNA as positive controls, and plant cDNA, 3xSSC, Cot-1 cDNA, and ssDNA as negative controls, were spotted on a glass slide. After exclusion of these control spots, 39,168 clones were used following data analysis.

RNA Extraction and Preparation of Probe cDNA

Total ribonucleic acid (RNA) was extracted from the frozen hepatic tissues by the improved AGPC method described by Carninci *et al.* [9]. The RNAs from 5 animals at each time point were pooled to reduce the effect of biological variation and to improve efficiency for microarray experiments according to previous investigations [10, 11].

Sixty μg of prepared total RNA was used to make probe cDNA. Reverse transcription of total RNA from the remnant tissues taken at each time point from each hepatectomized and each sham-operated mouse was performed with 2.5 mM amino-allyl UTP, oligo-dT primer, and SuperScript II (GIBCO/BRL, Burlington, Canada) for 1 h at 42 °C. After reverse transcription, probe cDNA was cleaned up using GFX PCR DNA and a Band Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ). After that, the probes from 70%- and 90%-hepatectomized mice were labeled with Cy3 monofunctional reactive dye (Amersham Pharmacia Biotech), and the probes from sham-operated mice were labeled with Cy5 dye for 1 h at room temperature in the dark.

Hybridization and Data Scanning

Probe cDNA from hepatectomized mice and sham-operated mice at the same time point were mixed and hybridized to a cDNA microarray. After filtration, separate images from each fluorescent signals were scanned by a ScanArray 5000 confocal laser scanner (PerkinElmer, Inc., Waltham, MA). The scanning images were analyzed by using a Digital Genome (MolecularWare, Inc., Boston, MA). The color images of the hybridization were made by representing the Cy3 fluorescent image as red and the Cy5 fluorescent image as green, and then merging the two color images. Each signal was evaluated by using the Digital Genome for data analysis.

Data Analysis

To increase the accuracy of the data, we carried out the same experiment twice and calculated the means of each pair of values. The data were normalized by the global normalization method using the filtering program PRIM [12]. This program (1) deletes the results with "flags" added manually to corrupted spots, (2) eliminates spots with signal intensities less than the mean + 3× standard deviation of the background signal intensity in either Cy3 or Cy5, and (3) eliminates spots located outside the least-mean squares line ± 2× standard deviation. After the filtering was finished, we compared the results of the two experiments by calculating a Pearson's correlation coefficient. If the coefficient was equal to or greater than 0.7, we used the data in these analyses [13]. The final data were represented as log-ratio (base 2), log2 (Cy3/Cy5). The expression ratio was defined according the following formula:

$$\begin{aligned}\text{Expression ratio} &= \log_2(90\% - \text{hepatectomized mice}/70\% - \text{hepatectomized mice})\\ &= \log_2[(\text{Cy3}(90\% - \text{hepatectomized mice})/\text{Cy5}(\text{sham} - \text{operated mice}))/\\ &\quad [(\text{Cy3}(70\% - \text{hepatectomized mice})/\text{Cy5}(\text{sham} - \text{operated mice}))\\ &= \log_2\text{Cy3}(90\% - \text{hepatectomized mice})/\text{Cy5}(\text{sham} - \text{operated mice})\\ &\quad - \log_2\text{Cy3}(70\% - \text{hepatectomized mice})/\text{Cy5}(\text{sham} - \text{operated mice})\end{aligned}$$

The up-regulated genes in 90%-hepatectomized mice were defined as having an expression ratio at least 2, and the down-regulated genes –2 or less according to a previous study [14]. Hierarchical clustering to both axes was applied by the weighted pair-group method with a centroid average as implemented by the program CLUSTER (M. Eisen: http://rana.stanford.edu/software). The results were expressed by using TreeView (M. Eisen: http://rana.stanford.edu/software).