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EXPRESSION OF HEPATOCYTE GROWTH FACTOR GENE IN ENDOTHELIAL AND KUPFFER CELLS OF DAMAGED RAT LIVERS, AS REVEALED BY IN SITU HYBRIDIZATION

Sumihare Noji[1], Kosuke Tashiro[2], Eiki Koyama[1], Tsutomu Nohno[3], Kazuhiko Ohyama[1], Shigehiko Taniguchi[1], and Toshikazu Nakamura[2]

[1]Department of Biochemistry, Okayama University Dental School, 2-5-1 Shikata-cho, Okayama City 700, Japan

[2]Department of Biology, Faculty of Science, Kyushu University, Fukuoka City 812, Japan

[3]Department of Pharmacology, Kawasaki Medical School, Kurashiki City, 701-01, Japan

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SUMMARY: Hepatocyte growth factor (HGF) has been demonstrated to be synthesized and secreted by non-parenchymal liver cells for liver regeneration after hepatic injury. We performed in situ hybridization to identify HGF-producing cell types in rat liver hepatitis induced by administrating carbon tetrachloride as a hepatotoxin. We found that transcripts of the HGF gene are localized in the Kupffer and endothelial cells in normal livers and increased remarkably in the Kupffer cells of the damaged livers. Thus, HGF is concluded to be synthesized in the Kupffer and endothelial cells to repair the liver tissue in paracrine fashion. No significant increase in the transcripts of the HGF gene was observed in livers after partial hepatectomy, indicating that a mechanism on liver regeneration after the hepatectomy differs from that on liver repairs. Since the HGF gene expression was also found in lung and kidney, HGF may be a ubiquitous factor for tissue repairs.

Hepatocyte growth factor (HGF) is a heterodimer consisting of a large 69-kDa α-subunit and a small 34-KDa β-subunit (1-3). HGF is known to stimulate remarkably the DNA synthesis of adult rat hepatocytes in primary culture. Since HGF was found in the sera of partially hepatectomized rats (4), rat platelets (5-7), the plasma of a patient with fulminant hepatitis (8) and that of a patient after partial hepatectomy (9), HGF may be a hepatotrophic factor triggering liver regeneration. Recently, Nakamura et al. (10) and Tashiro et al. (11) cloned cDNAs of human and rat HGF, respectively, and deduced their entire

Abbreviations: HGF, hepatocyte growth factor; UTP, uridine triphosphate.

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amino acid sequences. With the HGF cDNA as a probe, Kinoshita et al. (12) demonstrated that HGF mRNA increased extensively in the livers of rats when experimental hepatitis was induced by administrating carbon tetrachloride or D-galactosamine as a hepatotoxins. Furthermore, they found that HGF mRNA increased in the non-parenchymal liver cells, but not in the parenchymal hepatocytes (12).

In the present study, we performed in situ hybridization to identify the HGF-producing cells in the damaged liver. In addition, we examined the presence of the HGF transcripts in lung and kidney.

MATERIALS AND METHODS

Animals: Adult male Wistar rats, weighing 150-180 g, were used for in situ hybridization. Rats were fixed by perfusion with 4% paraformaldehyde at 12 h after administration of oil containing 1.0 ml/kg b.w. carbon tetrachloride.

Probe preparation: A 1.4-kb EcoRI fragment of a rat HGF cDNA (RBC1 clone (11)) was subcloned into a pGEM7 vector to synthesize both antisense and sense run-off transcripts labeled with [α-35S]UTP (400 Ci/mmol, Amersham Co.). The labeled transcripts were alkali hydrolyzed to 50-150 nucleotides for riboprobes (13).

In situ hybridization: In situ hybridization was performed as described previously (14). Briefly, the fixed tissues were embedded in paraffin (Paraplast) and cut serially at 5 μm thickness. The sections were collected on slide glasses coated with poly-L-lysine. The sections were deparaffinized conventionally and treated with glycine and acetic anhydride for reduction of non-specific binding. The sections were hybridized with the riboprobes for 15 h and then washed with 0.1xSSC at 50oC for 1 h after RNase A treatment. The slide glasses were dipped into emulsion (1:1 dilution of Kodak NTB2) and exposed in dark for two weeks. The sections were developed with Kodak D-19, fixed, rinsed, and then stained with hematoxiline and eosin.

RESULTS AND DISCUSSION

Kinoshita et al. (12) have demonstrated by northern blot analysis that the level of HGF mRNA is negligible or undetectable in normal rat liver, whereas it increases remarkably at 5 h and reached to the maximum level (more than 20 times compared with the normal level) at 10 h after carbon tetrachloride administration. Furthermore, they found that HGF mRNA is present in normal non-parenchymal liver cells and increases markedly after administration of carbon tetrachloride, but negligible in parenchymal hepatocytes throughout (12). In order to identify cell type in non-parenchymal liver cells sythesizing and secreting HGF and to observe the expression pattern of the HGF gene in the damaged liver, we performed in situ hybridization on serial sections of rat livers treated with carbon tetrachloride for 12 h. Figure 1 shows the typical results on sections of both normal and carbon tetrachloride-treated livers. In the dark field view (right side), white particles are exposed silver

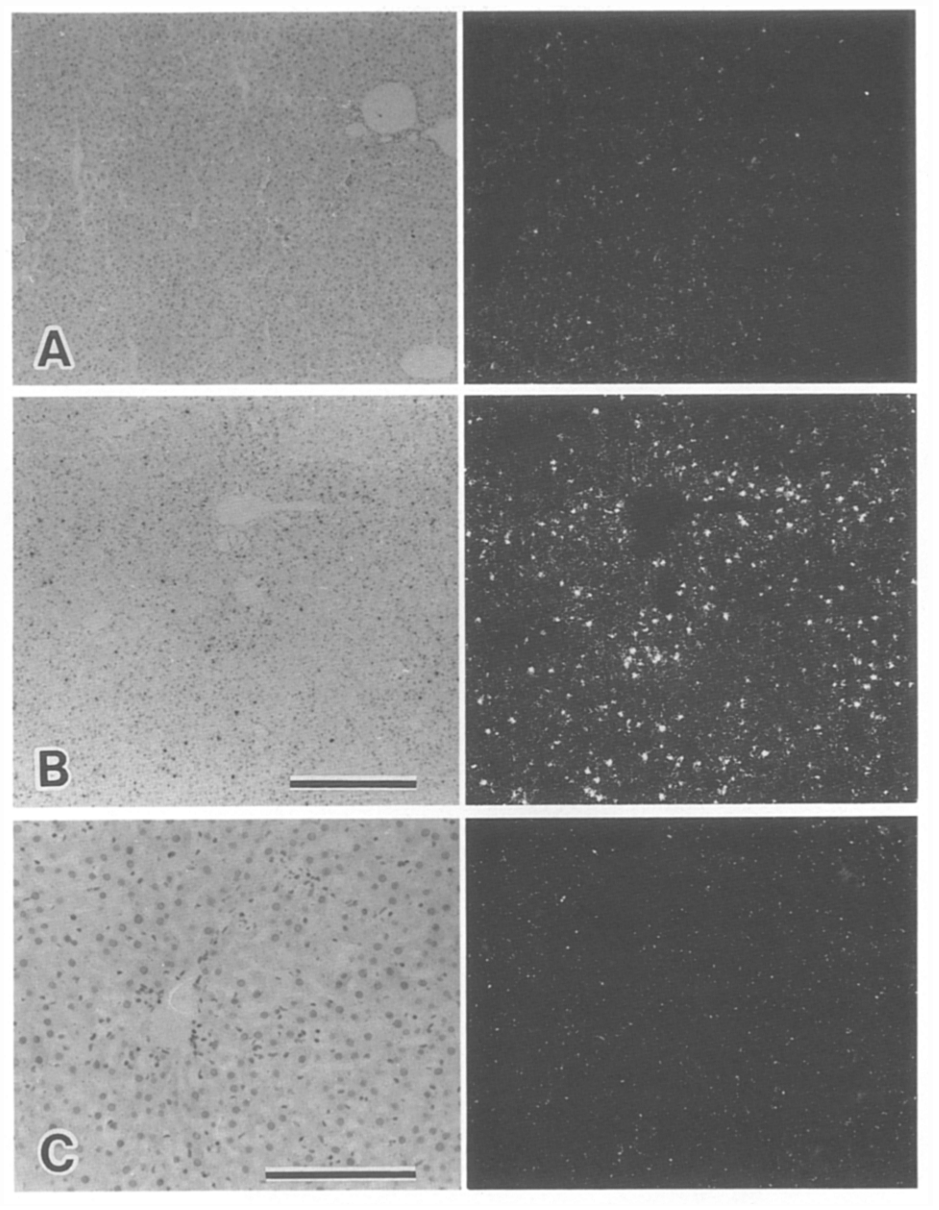

Fig. 1. Expression pattern of the HGF gene in (A) normal and (B) carbon tetrachloride-treated livers. (C) a negative control with a sense riboprobe. Bright (left) and dark (right) field views. White particles are signal silver grains in dark field view. The dense accumulation of grains is observed on endothelial and Kupffer cells in A and B (see Fig. 2). Bar= 500 μm.

grains indicating the presence of the riboprobes. In normal control liver (Fig. 1A), little signal grains are observed, while dense accumulation of the grains is observed in non-parenchymal cells in the damaged liver (Figs. 1B and 2). Since no significant signal was observed with the sense probe (Fig. 1C), the accumulation of the grains was concluded to be indicative of the presence of the HGF transcripts. We

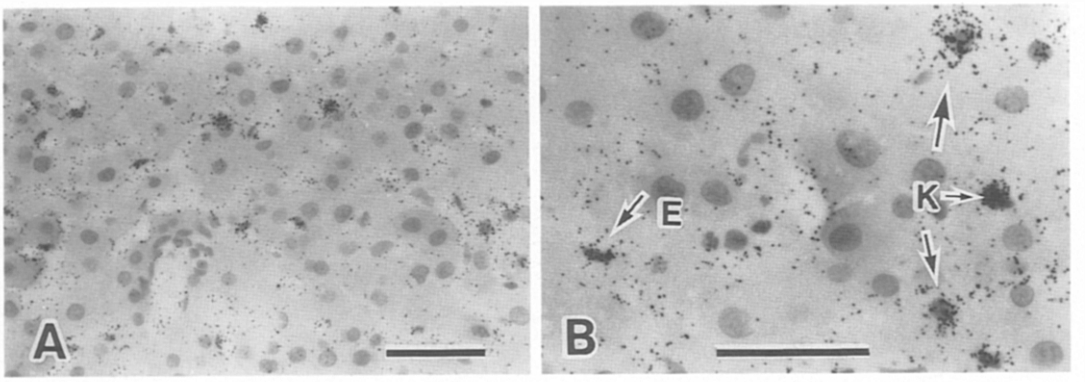

Fig. 2. Localization of the silver grains on endothelial (E) and Kupffer (K) cells in the carbon tetrachloride-treated liver. Bar= 50 μm.

observed two types of non-parenchymal cells expressing the HGF gene; one type with large nuclei (Fig. 2), the other with small flattened nuclei (Fig. 2). Since both are faced to the snusoid, the two should be two of four sinusoidal cells; Kupffer, endothelial, stellate, and pit (liver-associated NK cell) cells (15 as a review). The cell with a small flattened nucleus is an endothelial cell, judged from the cell morphology. On the other hand, the assignment of the cell with a large nucleus was not straightforward. We employed three criteria (16); (1) Kupffer cells lie upon the endothelium and are distributed uniformly in the hepatic lobule, (2) stellate cells with small nuclei are less dense in the central zone of the lobule than the peripheral area and surrounded with parenchymal cells, and (3) the non-hepatocytes account for 6.3% of parenchymal volume in the rat liver and consist of 2.8% endothelial cells, 2.1% Kupffer cells, 1.4% stellate cells, and less than 1% pit cells. Since the cells with large nuclei are distributed uniformly and about 2% of liver cells, the results can not be fully explained as far as we assumed that the positive cells are either stellate or pit cells. Thus, we concluded that the cells with large nuclei are Kupffer cells. This assignment was supported by the fact that isolated Kupffer cells and monocytes produce HGF (data not shown).

It is of particular interest that no significant induction of HGF transcripts was found in partially hepatectomized liver (data not shown). Thus, the mechanism on production of HGF in hepatectomy seems different from that in hepatitis with carbon tetrachloride.

We also found *in situ* that the HGF gene was expressed weakly in normal rat lung and kidney and that the expression was induced by administration of carbon tetrachloride in both (Fig. 3), as expected from northern blot analysis (11). From consideration of the results obtained

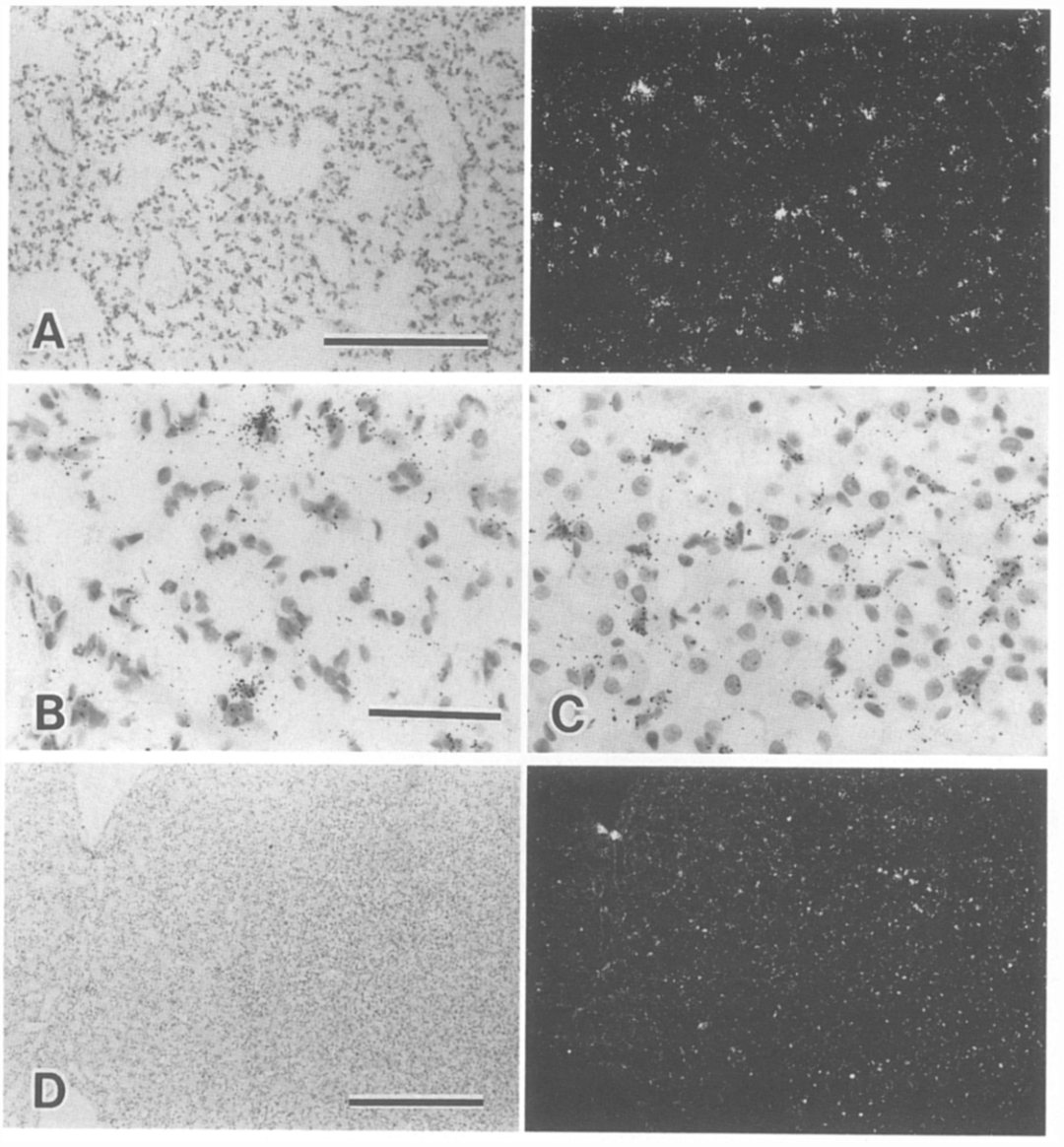

Fig. 3. Expression pattern of the HGF gene in the carbon tetrachloride-treated lung (A and B) and kidney (C and D). Bright (left) and dark (right)-field views in A and D. Bar= 500 μm in A; 50 μm in B and C; 1 mm in D.

for liver, the cells expressing the HGF gene may be alveolar macrophages and endothelial cells in lung, and fenestrated endothelial cells in kidney. These findings suggest that HGF may be a ubiquitous factor for tissue repairs.

In conclusion, HGF is synthesized and secreted by the Kupffer and endothelial cells in normal liver, and probably by macrophages and endothelial cells in normal lung and kidney. The transcription of the HGF gene is induced in those cells by some factor(s) produced in damaged

livers. The produced HGF regulates, in paracrine fashion, the growth of parenchymal hepatocytes during tissue repairs.

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