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PRODUCTION OF HEPATOCYTE GROWTH FACTOR IN VARIOUS RAT ORGANS

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The production and content of hepatocyte growth factor (HGF) in various organs has not been fully investigated. In this study, mRNA and immunoreactive material (IRM) for HGF were investigated in order to speculate its role in various organs. Nineteen organs from adult Sprague-Dawley rats were excised and the contents of their mRNA and IRM for HGF were measured. They varied among various organs, found to be highest in the liver, lung, and kidney. Although the gastrointestinal tract showed a modest expression of HGF, they were undetectable in the testis, skin, or skeletal muscle.

Key words : pro HGF, Northern blot analysis, enzyme immunoassay, gastrointestinal tract, liver.

INTRODUCTION

Hepatocyte growth factor (HGF), which is produced by mesenchymal cells, is one of the most potent growth factors of various epithelial cells (1,2). After being produced and secreted from the mesenchymal cells in a single chain inactive form (pro HGF), pro HGF is stored on the cell surface or in the nearby extracellular matrix bound to various molecules including collagen, fibronectin, thrombospondin, and heparan sulfate proteoglycans (3-6). Once activated by HGF activating serine protease, which is circulating in the plasma, stored pro HGF is cleaved to the active heterodimeric form of HGF that can stimulate the proliferation of epithelial cells in the local tissue (7,8). Therefore, a direct way to speculate the importance of HGF as a growth factor in various organs may be assessment of the production and content of HGF in each organ. Although the production of HGF in the liver, lung, and kidney has ever been reported (9,10), it has not been fully investigated in other organs. This study was, therefore, designed to investigate the production and content of HGF in various rat organs.

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MATERIALS AND METHODS

Animals

Twenty seven-week-old male Sprague-Dawley rats were used for the experiment. They were first anesthetized with pentobarbital and then the blood was removed by perfusing the rats with sterilized saline from the left ventricle. Each organ was removed and snap-frozen, and then stored in liquid nitrogen until extraction of total RNA. For the extraction of HGF protein, the organs were removed and quickly homogenized in ice-cold 4 vol of 50mM Tris-HCl (pH 8.5) containing 0.15M NaCl, 10mM EDTA, 100 μM nafamostat mesylate, and 1mM phenylmethylsulfonyl fluoride (4,11). Soon thereafter the homogenized tissue was processed for extraction of HGF protein.

Extraction of RNA and Northern Blot Analysis

Total RNA was obtained by extraction with the RNA extraction solution Isogen® (Nippon gene, Tokyo, Japan). The total RNA was separated by 0.66M formaldehyde-1% agarose gel in 0.4M 3-(N-morpholino) propane sulfate, 0.1M sodium acetate, and 0.02M EDTA. After the transfer to nitrocellulose membranes, the nucleic acid was fixed to the membrane by UV cross-linking. The probe used for

Northern blot analysis was 0.58Kb cDNA of rat HGF as previously reported (2). DNA probes for Northern blot analysis were radiolabeled by a random prime labeling kit (Boehringer Mannheim, Germany). Hybridization was carried out at 42℃, and the filters were washed twice for 20min at 55℃ in 0.1×ssc/0.1% SDS. Radiolabeled DNA probes were determined by a bioimaging analyzer BAS 2000 (Fujix, Tokyo, Japan). As internal control, rat β-actin expression was detected.

Extraction of Protein and Enzyme Immunoassay

Protein from the various organs was extracted as described previously (4,11). After homogenized in the homogenization buffer, the homogenate was centrifuged at 25,000g for 20min, and the supernatant was again centrifuged at 100,000g for 60min. An aliquot of the supernatant containing 100mg of protein was applied to a SP-Sepharose column (bed volume 0.5ml) pre-equilibrated with 20mM Tris-HCl (pH 8.5), and containing 0.15M NaCl, 0.1% 3-(3-cholamidopropyl)-dimethylammonio-1-propanesulfonate, and 100 μM nafamostat mesylate. The column was washed with 6ml of 0.4M NaCl buffer. HGF was eluted with 3ml of 0.1M NaCl buffer. The elute was then concentrated by ultrafiltration (Centricon 30, Amicon, Beverly, Mass., USA).

The HGF concentrations in extracted protein were measured by a rat HGF·EIA kit (Institute of Immunology, Tokyo, Japan). All values are expressed as means±S.E.

RESULTS

HGF mRNA expressions in various rat organs determined by Northern blot analysis are shown in Figure 1. The liver and lung were two major organs which had a strong HGF gene expression, followed by the spleen, brain, and kidney. The gastrointestinal tract showed a moderate expression of HGF, relatively higher in the esophagus, glandular stomach, and colon. However, there was no detectable HGF mRNA in the pancreas, testis, skin, or muscle.

Although the liver and lung showed the strongest HGF gene expression, the kidney is the organ that had the highest level of immunoreactive HGF protein, followed by the lung (Figure 2). The HGF content in the liver was not found high instead of its very strong gene expression, suggesting the early clearance or catabolism of HGF in the liver. There was only a negligible amount of immunoreactive HGF found in the pancreas, testis, skin, and muscle.

DISCUSSION

Our data clarified that the production and content of HGF varied remarkably in various organs. Liver, lung, and kidney showed a strong gene expression and high HGF content, which suggest the important role of HGF in these organs as speculated by other investigators (12-14). The HGF content in kidney was almost twice of that in lung, while its mRNA expression was less than half of that in the lung. These data suggest

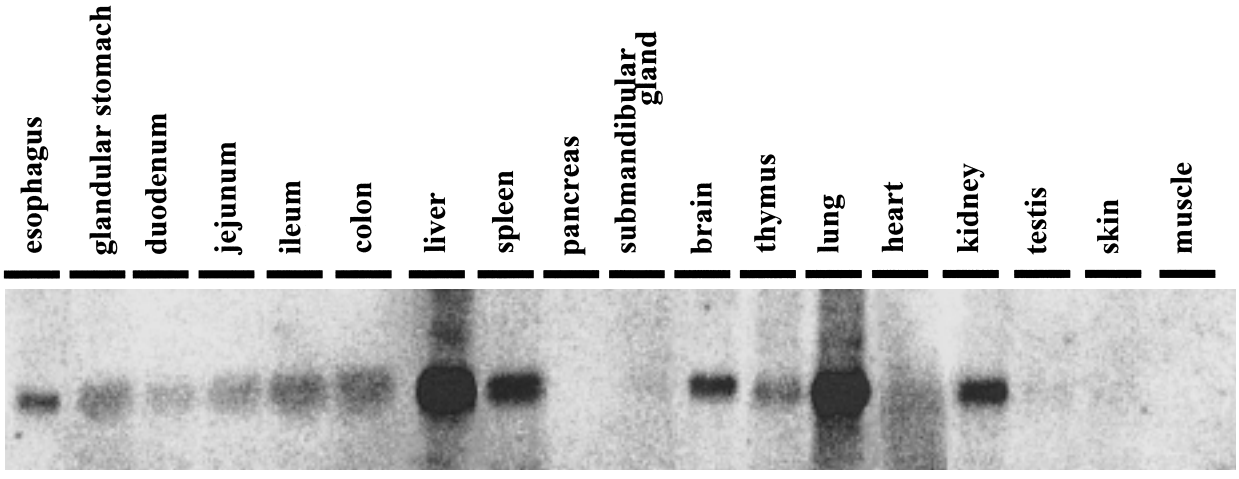

Fig. 1. Northern blot analysis of HGF mRNA in various rat organs. A strong HGF gene expression signal was detected in the liver and lung, followed by the spleen and kidney. All of the gastrointestinal tract investigated showed a significant gene expression of HGF.

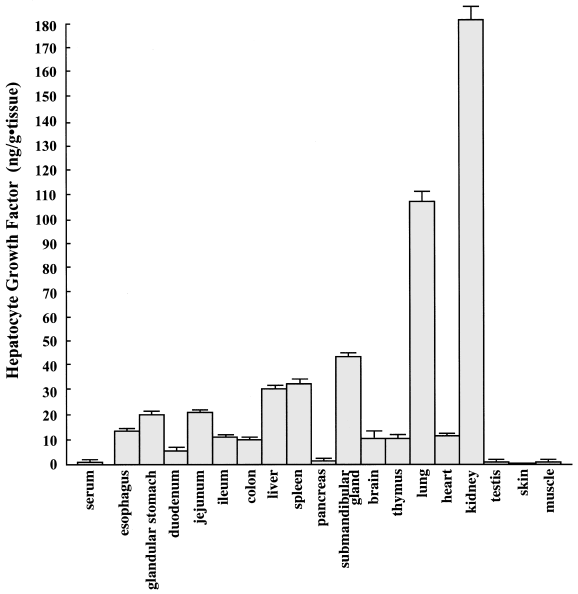

Fig. 2. Tissue content of immunoreactive HGF in various organs. The lung and kidney had a large amount of HGF. Vertical lines represent means±S.E of five rats.

that a higher amount of the secreted inactive single chain form of HGF is stored in kidney tissue by binding with the extracellular matrix, since our HGF enzyme immunoassay detected the inactive single chain form of HGF as well as the active heterodimeric HGF on an equimolar basis. The abundant extracellular matrix present in the glomerular basement membrane and intertubular connective tissue of kidney may provide the higher binding of pro-HGF.

Although the HGF gene expression was strong in the liver and lung, the immunoreactive HGF content in liver was only one third of that in the lung. This might be due to the secreted inactive of HGF cleared by the blood flow before binding to the scanty connective tissue in the liver. This observation suggests that the possible source of circulating plasma HGF may be the liver. HGF concentration in the circulating blood is, indeed, reported to be strongly influenced pathological states of liver (15-17). Since there is no active heterodimeric HGF detected in a normal uninjured liver, the low content of HGF may not be caused by active consumption by the hepatocyte after binding to the HGF receptor (18).

Skin is one of the most actively proliferating tissues with its stem cell in the basal layer of the epidermis. Several growth factors, including keratinocyte growth factor, have been reported to stimulate the proliferation of keratinocyte and to have important roles for the steady replacement of the epidermis (19,20). HGF is also one of the growth factors which can stimulate the proliferation of keratinocyte (21). However, the role of HGF as a growth factor in normal skin may not be important since its gene expression and content in normal uninjured skin were undetectable.

Our previous studies have emphasized the importance of HGF as a growth factor for gastrointestinal epithelial cells during the healing of mucosal lesions (2,4,11). Our present study has shown that the gastrointestinal tract has a modest gene expression and modest HGF content, and indicating a potential role of HGF in the gastrointestinal tract. Although both the esophagus and skin are lined by stratified squamous epithelium, the production and content of HGF were found to be quite different. Therefore, the growth control of esophageal mucosa and skin may be different, at least in HGF-related control.

In summary, we have clarified that HGF production and content varies in various organs. Although the liver, lung, and kidney are organs which produce HGF abundantly, all of the gastrointestinal tract investigated had only a modest amount of HGF production and content.

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