RAPID COMMUNICATION Jpn. J. Cancer Res. 83, 1262–1266, December 1992

Immunohistochemical Localization of Hepatocyte Growth Factor Protein in Pancreas Islet A-Cells of Man and Rats

Hiroyuki Tsuda,[1] Teruhiko Iwase,[1] Kazuyuki Matsumoto,[1] Mitsuya Ito,[1] Iwao Hirono,[1] Yoshihisa Nishida,[2] Masami Yamamoto,[3] Masae Tatematsu,[3] Kunio Matsumoto[4] and Toshikazu Nakamura[4]

[1]*Second Department of Pathology and* [2]*Department of Pediatric Surgery, Fujita Health University School of Medicine, Kutsukake-cho, Toyoake, Aichi 470-11,* [3]*Laboratory of Ultrastructure Research, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464 and* [4]*Department of Biology, Faculty of Science, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812*

Hepatocyte growth factor (HGF), a potent mitogen for adult rat hepatocytes in primary culture, has previously been shown to be primarily expressed in the nonparenchymal cells of the liver. Using polyclonal antisera against human and rat HGFs we studied the tissue distribution of HGF immunohistochemically and found the most intense staining in the pancreas islet cells in both man (autopsy cases) and the rat. Differential localization of 4 pancreas islet hormones, glucagon, insulin, somatostatin and pancreatic polypeptide, revealed HGF to be preferentially expressed within the glucagon-positive cells. The results indicate that HGF is primarily produced or stored in A-cells and may act as a growth factor in a paracrine and an endocrine fashion, like various other hormones.

Key words: Hepatocyte growth factor — Pancreas islet A-cell — Immunohistochemistry

The hepatocyte growth factor (HGF), first purified and characterized by Nakamura *et al.*[1,2] is a potent mitogen for mature hepatocytes in primary culture,[3] and its serum level is raised after partial hepatectomy or in liver disease in man and rats.[4–11] Molecular cloning of HGF demonstrated that it is a novel growth factor having kringle domains and a serine protease-like domain.[12–14] *In situ* hybridization has revealed high levels of HGF mRNA in non-epithelial cells of the liver including the Kupffer cells,[15] synthesized protein being taken up by hepatocytes via a receptor coded for by the proto-oncogene c-*met*.[14,16]

It has been shown that HGF mRNA or protein is expressed in a variety of tissues other than the liver,[17–19] such as kidney tubule cells, skin keratinocytes, melanocytes, mammary duct cells and bronchial epithelial cells.[6,19,20–24] Thus, it seems likely that HGF may be a growth factor existing in non-epithelial cells, which release HGF in response to the loss of epithelial cells, possibly in a paracrine manner. This raises the possibility of endocrine organ involvement. Although a number of studies have concentrated on HGF mRNA expression, no information is yet available concerning the pancreas islet, which releases several hormones, such as insulin, glucagon, somatostatin and pancreatic polypeptide. In the present report we describe the distribution of HGF in pancreas islet cells of man and rats, using an immunohistochemical aproach and specific HGF antisera.

Pancreas tissue: Human pancreas tissue was obtained at autopsy within 2 h after death from 3 males and 2 females aged 63.2±2.2 years old. Autopsy diagnoses of cause of death were lung adenocarcinoma, thyroid adenocarcinoma, intestinal hemorrhage and pulmonary infection (2 cases). The pancreas tissues, all of which were free of tumor metastasis or focal infection, were excised, cut into 10×10×3 mm blocks and immediately immersed in ice-cold acetone. After fixing for 2 weeks in dehydrated acetone at 4°C, tissue blocks were processed for paraffin embedding through an acetone-xylol-paraffin series. Rat pancreas tissues were taken from untreated F344 rats aged 7 weeks old (5 males and 5 females) and 75 weeks old (2 males), purchased from Charles River Japan Inc., Atsugi. The animals, which were completely free from neoplastic lesions, were fed on Oriental MF basal diet (Oriental Yeast Co. Ltd., Tokyo) *ad libitum*. Tissues were processed for paraffin embedding as in the human material case.

Preparation of HGF antibody: Human and rat recombinant HGF were purified from culture media of Chinese hamster ovary (CHO) cells transfected with expression vector containing cDNAs of human or rat HGF.[14,25] Polyclonal antibodies against human and rat recombinant HGF were raised in rabbits as described previously.[26] These antisera reacted with either human or rat HGF, recognizing both α- and β-chains of HGF, with no

Abbreviations: HGF, hepatocyte growth factor.

cross species reaction being evident (deta not shown). In addition, they did not recognize other proteins, including plasmin, insulin, epidermal growth factor, transforming growth factor (TGF)-α, TGF-β, tissue plasminogen activator, acidic fibroblast growth factor (FGF) and basic FGF.

Immunohistochemistry: Serial sections cut at 2.5–3 μm were processed for the following immunostaining. Binding of specific antisera was demonstrated with dilutions of 1:500 for the human and 1:75 for the rat tissues. Antibodies to human pancreatic islet hormone peptides, including glucagon, insulin, somatostatin and pancreatic polypeptide were purchased from Nichirei Co. Ltd., Tokyo or Dako Japan Co., Ltd., Tokyo. For the single staining of islet hormones, both avidin-biotin complex (ABC)[27] (Vectastain ABC kit, Vector Laboratories Inc., Burlingame, CA or DAKO LSAB Kit, Dako Japan Co., Ltd., both peroxidase or alkaline phosphatase-labeled) and peroxidase-antiperoxidase-peroxidase(PAP)(DAKO PAP Kit, Dako Japan Co., Ltd.) methods were used. For the double staining, HGF was stained using the ABC (DAKO LSAB Kit) method followed by demonstration of binding of one of the 4 islet hormones, except rat somatostatin and pancreatic polypeptide, by the PAP procedure. Double staining of rat HGF and somatostatin or pancreatic polypeptide was done by using the PAP followed by ABC (DAKO LSAB Kit) method. Visualization dye for the PAP was antibody-labeled peroxidase-3,3′-diaminobenzidine (brown color)[27] and for the ABC (LSAB), alkaline phosphatase-conjugated streptavidin-new fuchsin (red color).[28, 29] Specificity of binding for all antibodies was examined by applying non-immune sera instead of specific antibodies. Immunostaining results were carefully evaluated by comparing cellular localization of reaction products on singly stained serial sections and on double-stained sections.

Essentially similar staining patterns with pancreatic hormones and HGF antibodies were observed in the pancreas tissues of both humans and young and old rats of both sexes. In no case was any positive staining of acinar or ductal cells evident. In the human and rat islets, scattered HGF-positive cells showed a tendency to be localized at the periphery (Fig. 1A). Positive binding was only apparent in the cytoplasm with no staining of the nuclei. Comparison with expression of the different pancreatic hormones in serial sections allowed distinction of the islet cell type positive for HGF (Table I). In many cases coexistence of HGF protein and glucagon could be unequivocally detected within the same cell in serial sections (Fig. 1B). No islet cells were found to stain only red (HGF) or brown (glucagon) on double-staining, positive binding being demonstrated by a reddish-brown deposit (Fig. 1C). Furthermore, cellular localization of HGF was completely separate from that of the 3 other

Table I. Localization of HGF in Pancreatic Islet Cells

Islet cell type	Pancreatic hormone peptide	HGF positivity	
		Man	Rat
A-cell	glucagon	+	+
B-cell	insulin	−	−
D-cell	somatostatin	−	−
PP-cell	pancreatic polypeptide	−	−

+, positive; −, negative.

peptides, insulin (Fig. 1D), somatostatin (Fig. 1E), and pancreatic polypeptide, as evidenced by the double staining. In rats, HGF was also positive in the glucagon-secreting A-cells, which were localized only at the periphery of the islets. HGF-positive cells were clearly separated from insulin-(Fig. 1F), somatostatin- or pancreatic polypeptide-positive cells.

The present results provide strong evidence that glucagon-secreting A-cells in the pancreas of both man and rats contain high levels of HGF protein. Whether this is a direct reflection of gene expression or uptake and storage remains to be elucidated. This is the first demonstration of preferential localization in the pancreatic islets. The reason for the discrepancy between our findings and those published earlier by Zarnegar *et al.*[18, 30] regarding acinar cell positivity is unclear although differences in methodology or specificity of the antibodies used presumably played a role.

In the liver, endothelial cells and Kupffer cells might be responsible for intra-hepatic production of HGF.[15] It was also reported that lung alveolar macrophages express HGF mRNA but they are apparently not responsible for the induction of HGF mRNA in the lung observed after partial hepatectomy, carbon tetrachloride-induced hepatic injury and unilateral nephrectomy.[31] Similarly, in the kidney itself, mRNA expression has also been found in endothelial cells after carbon tetrachloride administration,[24] and in mesangial cells in primary culture.[32] However, in these proliferating tissues it is unclear whether the tissues themselves are producing the growth factor or whether receptor-mediated uptake is responsible. The number of cell types which are reported to respond to HGF includes, in addition to the hepatocyte, the kidney proximal tubule cells, nonparenchymal liver epithelial cells, melanoma cells and mouse keratinocytes,[6, 20–22, 24, 32] but none of the mesenchymal cells so far investigated. This strongly suggests that HGF is an agent with a wide-spectrum organ specificity for epithelial cell growth. The fact that in all organs so far investigated production was considered to take place in mesenchymal cells indicates a paracrine mechanism of action.[11, 32] The present finding of high levels of HGF protein in the pancreatic

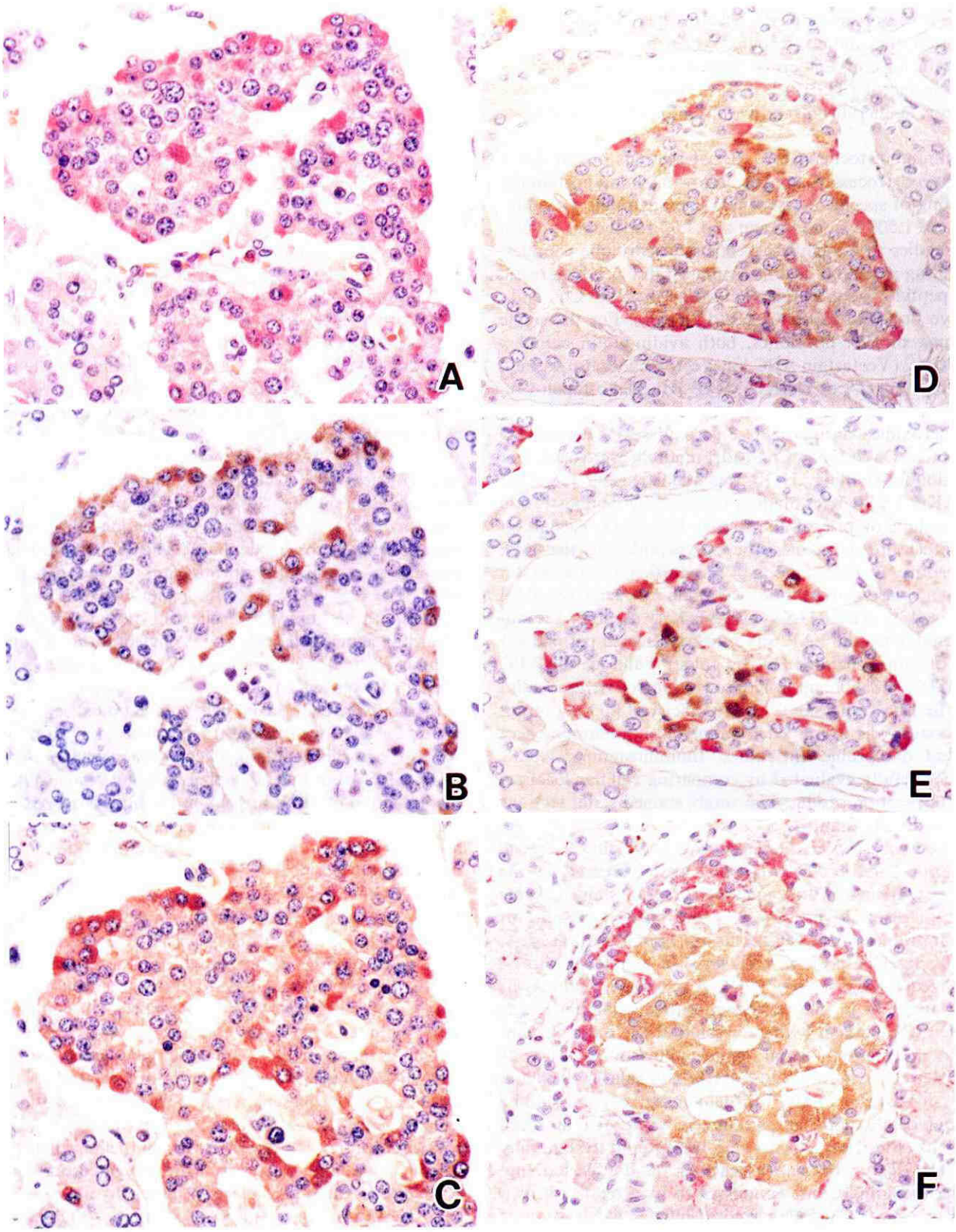
A
D
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Fig. 1A. HGF-positive cells in human pancreas islet cells visualized by alkaline phosphatase-conjugated streptavidin-new fuchsin (red color). ×100. Fig. 1A–C, serial sections.

Fig. 1B. Glucagon-positive A-cells in the same islet shown in Fig. 1A visualized by immunoperoxidase-3,3′-diaminobenzidine (brown color).

Fig. 1C. Double staining of HGF (red) and glucagon (brown) in the same islet shown in Fig. 1A and 1B. Since HGF-positive cells are also positive for glucagon-positive A-cells, the reaction product is of mixed color (reddish brown). No islet cells were found to stain only red (HGF) or brown (glucagon).

Fig. 1D. Double staining of HGF (red) and insulin (brown) in a human pancreas islet. HGF- and insulin-positive cells are localized separately. ×100.

Fig. 1E. Double staining of HGF (red) and somatostatin (brown) in a human pancreas islet. HGF- and somatostatin-positive cells are localized separately. ×100.

Fig. 1F. Double staining of HGF (red) and insulin (brown) in a rat pancreas islet. HGF- and insulin-positive cells are localized separately. ×100.

islets of both man and rats suggests that a hormonal response may in fact be involved, with release from A-cells after a stimulus for proliferation occurs. Future investigations should concentrate on localization of both mRNA and protein species under normal and stimulated conditions.

This research was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, from the Ministry of Health and Welfare, and by a Research Grant from Fujita Health University.

(Received June 29, 1992/Accepted September 14, 1992)

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