Clinical Research

Localization of Hepatocyte Growth Factor and Its Receptor met in Endocrine Cells and Related Tumors of the Gut and Pancreas: An Immunohistochemical Study

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Abstract

Hepatocyte growth factor (HGF), a stimulator of angiogenesis and cell migration, regulates the growth of a wide variety of cells by binding to its high-affinity receptor met and is involved in the growth and aggressiveness of several tumors. In this study we investigated the expression of HGF and met in normal endocrine cells and related neoplasms of the gut and pancreas to verify their possible role in tumor pathogenesis, growth, and aggressiveness. Normal tissues and 60 different endocrine tumors were immunostained using specific antibodies directed against HGF, met, and various hormones. HGF immunoreactivity (IR) was found in antroduodenal G cells, rectal enterochromaffin (EC) cells, and pancreatic A and B cells, whereas met IR was detected in antral EC and G cells, and in pancreatic B cells; 46 of 60 tumors examined were positive for HGF, and they were mainly represented by ECL-, EC-, and L-cell neoplasms. met IR was identified in 50/60 tumors of various phenotypes. HGF and met coexpression was found in 42/60 cases, most of which were represented by EC-cell tumors. HGF/met coexpression was significantly more frequent in ileocolonic EC-cell tumors, which in the majority of cases were malignant, than in appendiceal EC-cell tumors, which were all benign. Our results demonstrated, for the first time, that HGF and met are specifically distributed in normal gut and pancreatic endocrine cells and, in addition, suggest that HGF and met may be implicated as autocrine/paracrine factors regulating the growth of gastroenteropancreatic endocrine tumors, mainly of ileocolonic EC-cell carcinoids.

Key Words: Hepatocyte growth factor; met; endocrine tumor; gut; pancreas; immunohistochemistry.

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Introduction

Growth factors are polypeptides that stimulate cell proliferation and differentiation, and, furthermore, are able to regulate cell motility and cytoskeletal structure [1]. Their biological activities depend on binding to specific high-affinity cell membrane receptors. Unlike hormones, growth factors are often produced by more than one cell type, show a wide range of overlapping biological actions, and generally act over short distances either in a paracrine or in an autocrine fashion [2].

Among the large number of growth factor peptides discovered in the last few years, hepatocyte growth factor (HGF), also known as scatter factor, has been recently reported as an important peptide that stimulates angiogenesis [3] and regulates the growth of hepatocytes and cells distributed

in several tissues, including kidney, placenta, brain, lung, and pancreas [4,5]. HGF, encoded by a gene located on chromosome 7, was originally identified in rat serum after partial hepatectomy [6]. It is a heterodimer glycoprotein consisting of a heavy α-chain and a light β-chain, held together by disulfide bonds [7]. It is secreted as an inactive single-chain precursor that needs proteolytic cleavage to become a mature biologically active heterodimer [8,9]. HGF, first recognized as a growth factor implicated in liver regeneration, exerts a wide spectrum of biological actions on many cell types, especially of epithelial and endothelial origin [3,4]. These actions comprise mitogenic activity for hepatocytes and various other epithelial cells, a powerful motogenic (scattering) effect on several epithelial cell lines in vitro [10] a morphogenic effect (tubule formation) on kidney tubular cells [11] and breast carcinoma cells [12], and an angiogenic effect, which is due to mitogenic and motogenic stimulation of vascular endothelial cells [13].

HGF exerts its biological activities through the binding to a high-specific-transmembrane type II tyrosine kinase receptor (met), encoded by the proto-oncogene c-met, located on chromosome 7 [14–17]. Met is highly expressed in several normal epithelial adult tissues, including pancreas, stomach, intestine, skin, uterus, lung, and kidney [15,18], although it does not seem to be expressed in mesenchymal cells [17]. In addition, met has been detected in fetal epithelial cells of stomach and pancreas and fetal endocrine cells of the gut [19]. This widespread distribution of HGF and met in several adult and fetal normal tissues suggests their importance in regulation of growth, morphogenesis, and cell functions of several tissues.

The presence of HGF and met, sometimes simultaneously, in different types of human neoplasms, including those of the breast [20], melanocytes [21], pancreas [22,23], prostate [24], stomach [25], peripheral nerve sheath [26], and thyroid [27] suggests that HGF and its receptor may be involved in tumor pathogenesis, and growth and development of distant metastases.

Endocrine tumors of the gut and pancreas represent a heterogeneous group of neoplasms, whose pathogenesis and mechanisms of tumor progression are unknown. They have been recently found to express several growth factors and growth factor receptors, including TGFα, EGFR, TGFβ, PDGF, insulin-like growth factor-I, VEGF, activin A, aFGF, bFGF, and FGFR4 [28–40]. The precise role of these peptides remains unclear, although they seem to be implicated, at least as regards ECL-cell and EC-cell carcinoids, in the pathogenesis of stromal lesions and of smooth muscle layer hyperplasia associated with these tumors [30,37,39].

To verify whether HGF and met are involved in the pathogenesis, growth, and metastatic dissemination of gut and pancreatic endocrine tumors, we investigated their expression in a series of 60 endocrine tumors, and, in comparison, in normal endocrine cells of the gut mucosa and pancreatic islets.

Materials and Methods

Sixty endocrine tumors of the gut and pancreas and samples of normal adult pancreatic, gastric, and intestinal tissues were collected at surgery, fixed in buffered formalin (formaldehyde 4% w/v and acetate buffer 0.05 *M*, pH 7.4) for 24 h and embedded in paraffin. Sections were stained with hematoxylin–eosin (H&E) and Grimelius' silver impregnation for the routinely histopathologic evaluation. For

Table 1. Antisera and Antibodies Employed

Antibodies/antisera	P/M (clone)[a]	Dilution	Source
Insulin (Ins)	M (AE9D6)	1:200	BioGenex Laboratories, San Ramon, CA
Glucagon (Gluc)	P	1:1250	Milab, Malmo, Sweden
Glucagon/glicentin (Glic)	P	1:2500	Milab
Pancreatic polypeptide (PP)	P	1:4000	Cambridge Research Biochemicals, Cambridge, UK
Somatostatin (Som)	P	1:500	Dako, Copenhagen, Denmark
Serotonin (5HT)	M (YC5)	1:50	Biogenesis, Bournemouth, UK
C-terminus gastrin-CCK-cerulein (Gastr)	M (B4)	1:10000	Farmitalia, Milan, Italy
Gastrin 34 (G34)	P	1:500	Cambridge Research Biochemicals
Secretin (Secr)	P	1:500	Milab
Peptide YY (PYY)	P	1:1000	Biogenesis
Hepatocyte growth factor (HGF)	P	1:1000	Serotec, Oxford, UK
Hepatocyte growth factor (HGF)	P	1:100	Sigma, St. Louis, MO
Hepatocyte growth factor (HGF)	M (DV-14)	1:50	Dr. M. Prat, Novara, Italy
Met receptor (met)	P	1:200	Santa Cruz Biotechnology, Inc, Santa Cruz, CA
Met receptor (met)	M (8F11)	1:100	Novocastra, Newcastle, UK

[a]P = polyclonal; M = monoclonal.

immunohistochemical stains, 3-μm-thick sections were mounted on poly-L-lysine–coated slides and then deparaffinized and hydrated through graded alcohols to water. Endogenous peroxidase activity was removed by dipping sections into 3% hydrogen peroxide for 10 min at room temperature. Thereafter, sections were incubated with the antibodies listed in Table 1 for 20 h at 4°C and then the avidin–biotin–peroxidase complex (ABC) procedure was performed. The peroxidase activity was finally revealed by 0.03% 3,3'-diaminobenzidine, and the nuclei were counterstained with Harris' hematoxylin. Colocalization studies were performed on serial paraffin sections, semithin-plastic sections, and using double label immunostains as previously described [30].

The localization of HGF was obtained using three different specific antibodies, as reported in Table 1. In detail, the anti-HGF antibody purchased from Sigma (St. Louis, MO) was a goat polyclonal antibody directed against recombinant human HGF. The anti-HGF antibody, provided by Serotec (Oxford, UK), was a sheep polyclonal antibody whose immunogen was a highly purified human HGF. The monoclonal anti-HGF antibody (clone DV-14) was raised using recombinant HGF secreted by the *Spodoptera frugiperda* insect cells (Sf9), which were infected with the baculovirus vector containing the full size human HGF cDNA, as immunogen [41]. For met protein detection, we employed a mouse monoclonal antibody recognizing the external domain of the beta chain of the c-met molecule, purchased from Novocastra (Newcastle, UK), and a rabbit polyclonal antibody raised against a 2.5 kDa peptide mapping at the carboxy terminus of human met, made by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Sections incubated with the antibodies directed against N-terminal glucagon-glicentin and somatostatin were pretreated with 0.003% subtilisin (Sigma, P4789; protease type XXVII or Nagarse protease) in 0.05 *M* Tris-buffered saline pH 7.4. Sections immunostained using both anti-met antisera and the anti-HGF goat antibody,

were pretreated with 0.01 *M* citrate buffer pH 6 for 10 min in a microwave oven at 650 W.

Specificity controls consisted of absorption of the antibody with 10–20 nmol of the related antigen, substitution of the primary antibody with nonimmune serum of the same species at the same dilution, and use of control tissues with or without the pertinent antigen. In addition, the specificity of the Sigma anti-HGF antibody and of the Santa Cruz anti-met antibody has been tested according to the criteria previously reported by Kermorgant et al. [19]. The specificity of the monoclonal anti-HGF (clone DV-14) antibody has been previously confirmed by molecular biology techniques [41].

The evaluation of HGF and met immunoreactivities in normal endocrine cells was performed either on tissues from patients without endocrine tumors or on tissues obtained from peritumoral regions of the 60 endocrine tumors investigated.

Statistical evaluation of results was performed using the Fisher exact test. Two values were considered statistically different when $p < 0.05$.

Table 2. Immunohistochemical Localization of HGF and met in Normal Endocrine Cells of the Gut and Pancreas Using Different Antibodies[a]

	HGF		met	
	Serotec	Sigma	Novocastra	Santa Cruz
Fundus	–	–	–	–
Antrum	–	G	G, EC	G, EC
Duodenum	–	G	Rare	Rare
Jejunum	–	–	–	–
Ileum	–	–	–	–
Appendix	–	–	–	–
Right colon	–	–	–	–
Rectum	EC	EC	–	–
Pancreas	–	A, B	B	B

[a]G = gastrin-producing G-cells; EC = serotonin-producing enterochromaffin cells; A = glucagon-producing A cells; B = insulin-producing B cells.

Results

Normal Tissues

In normal tissues we have found some differences regarding HGF expression in relation to the different antibodies employed (Table 2). Using the sheep polyclonal antibody directed against purified human HGF, HGF-positive cells were not identified in pancreatic islets or in gastric, duodenal, ileal, appendiceal, and right colon mucosae. On the contrary, HGF-immunoreactive (IR) cells were found in the rectal mucosa (Fig. 1), where they were mostly located in the lower part of crypts. Immunopositivity was cytoplasmic, dark brown, and granular. Double immunostains demonstrated that the rectal HGF-IR endocrine cells represented a subgroup (about 90%) of enterochromaffin (EC) cells, coexpressing serotonin, whereas HGF was not expressed in rectal endocrine cells containing other hormones such as glicentin, PYY, and somatostatin. With the goat polyclonal antibody directed against recombinant human HGF, in addition to rectal EC cells, some gastric and duodenal G cells resulted in HGF-IR (Fig. 2A). Moreover, several islet cells resulted that were more or less intensely stained by this antibody (Fig. 2B). The immunoreactivity was intense in a subgroup of glucagon-producing A cells and weak in insulin-producing B cells. The pattern of immunoreactivity was similar to that observed using the antibody directed against purified human HGF, showing a dark brown and granular staining in the cytoplasm of cells. In addition to endocrine cells, other types of epithelial cells of the gut and pancreas, including foveolar and chief cells of gastric mucosa, columnar cells of small intestinal villi, epithelial cells of the colonic mucosa and duct, and acinar cells of the pancreas, were HGF-IR with both anti-HGF antibodies employed.

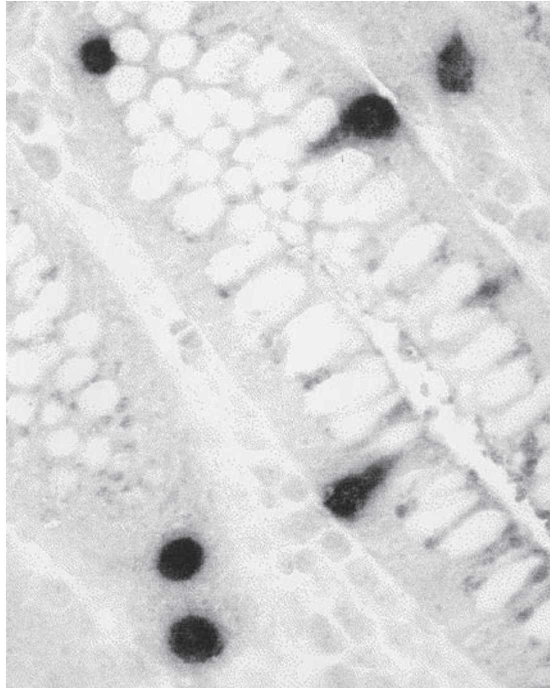

Fig. 1. Immunolocalization of HGF in scattered endocrine cells of the rectal mucosa. Original magnification ×400.

The two anti-met antibodies gave similar patterns of immunoreactivity, although the monoclonal antibody generally showed a more intense positivity in epithelial cells than the polyclonal one. On the contrary, stromal and endothelial cells were more intensely stained by the polyclonal than the monoclonal anti-met antibody. A discrete number of met-IR endocrine cells was found in antral mucosa, and colocalization studies demonstrated that they corresponded to a subpopulation of gastrin-producing (G) and serotonin-producing (EC) cells (Figs. 3 and 4). The immunoreactivity was cytoplasmic, although, in some cells, a membrane enhancement was evident. In addition to the gastric antrum, very few met-IR endocrine cells were present in the duodenal mucosa, although they were lacking in all the remaining tracts of the gut investigated. In the pancreas, an intense immunostaining for met was found

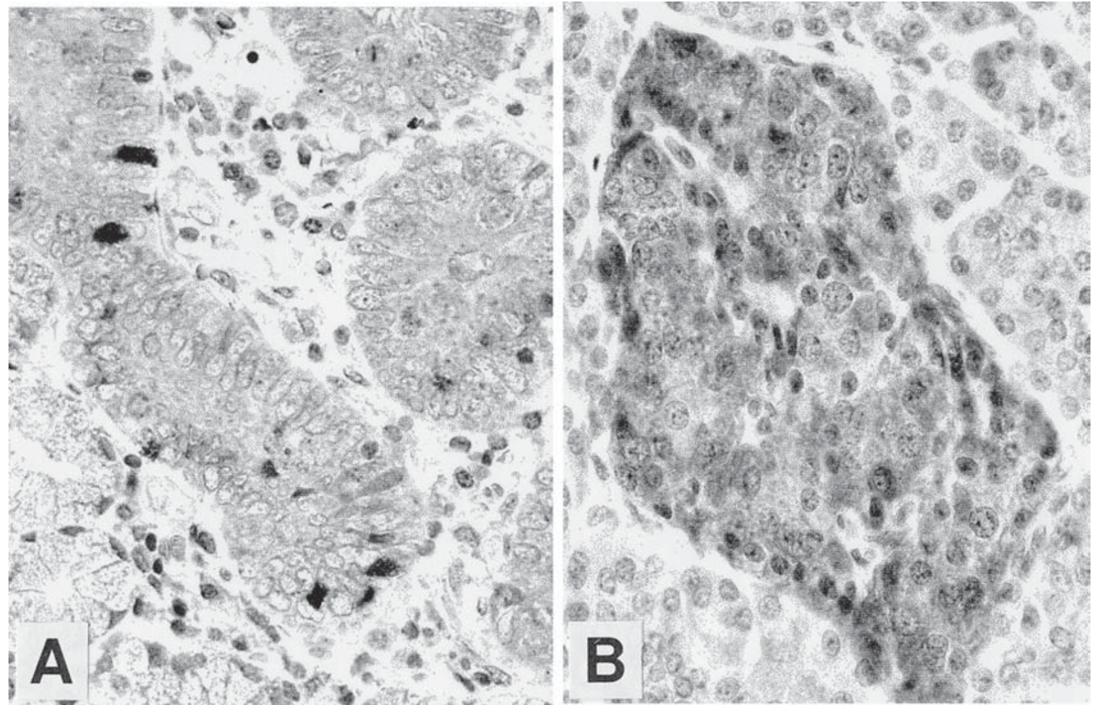

Fig. 2. HGF immunoreactivity in endocrine cells of the duodenal mucosa **(A)** and of a pancreatic islet **(B)**. Original magnification ×400.

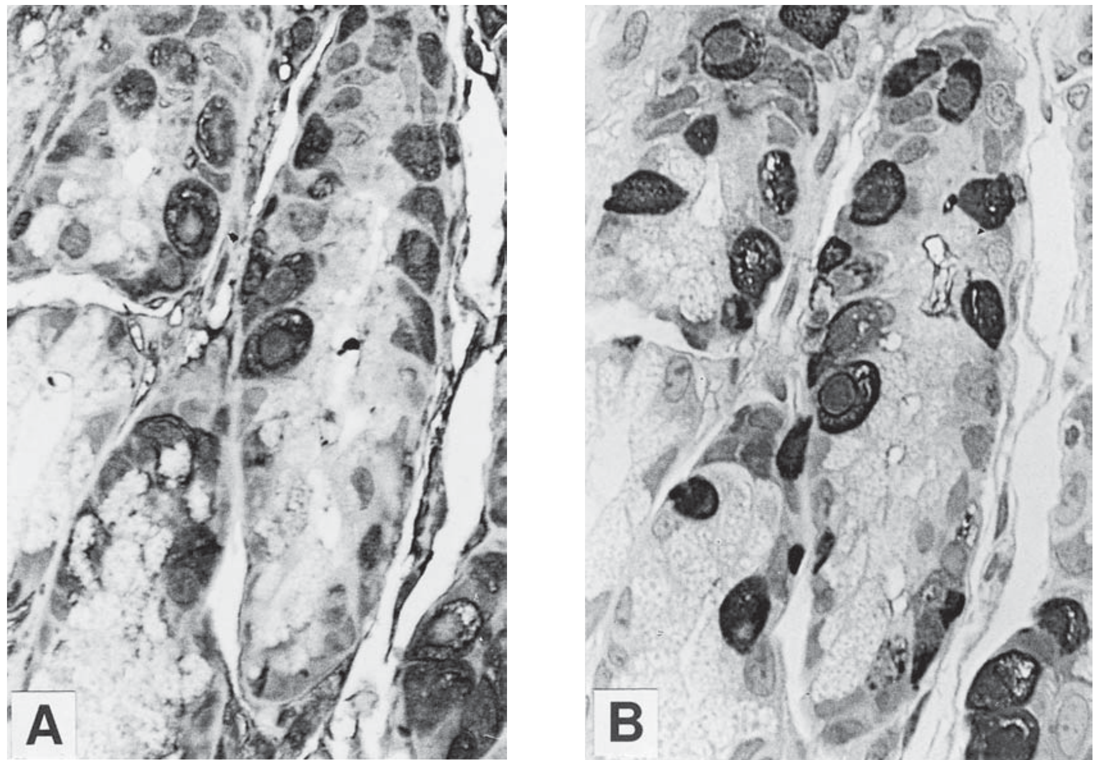

Fig. 3. Semithin serial sections of antral mucosa showing met immunoreactivity **(A)** in a subgroup of gastrin-positive cells **(B)**. Original magnification ×630.

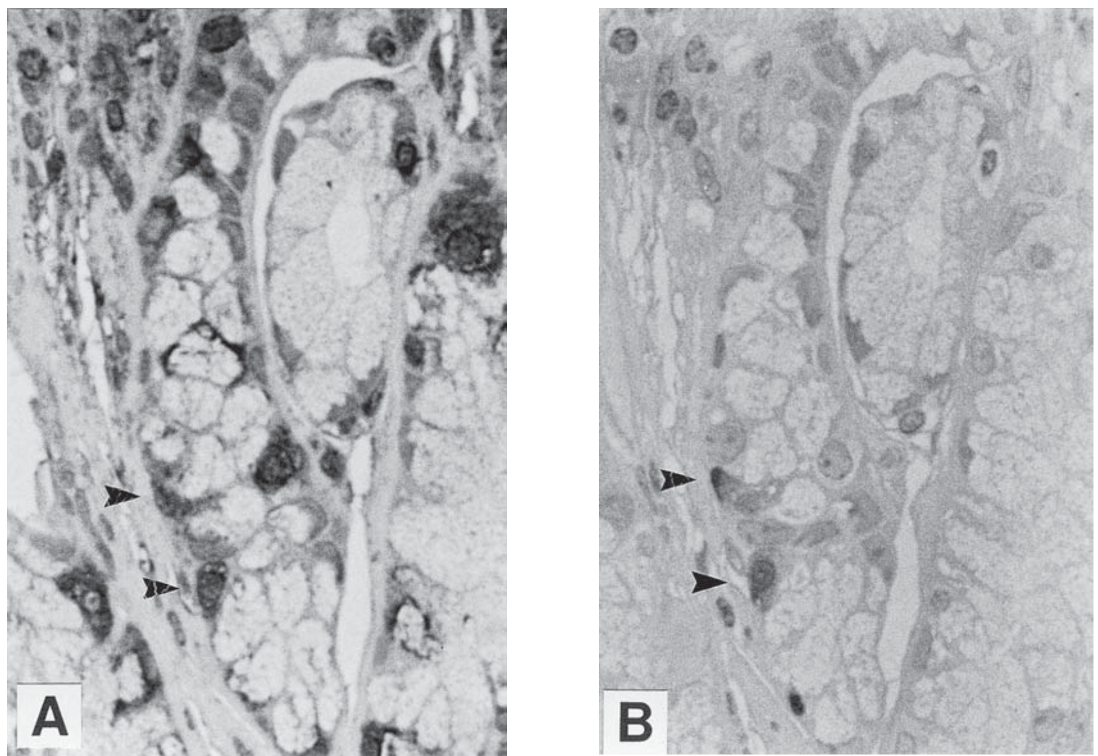

Fig. 4. Semithin serial sections of antral mucosa showing met IR **(A)** in some serotonin-positive cells **(B)** (arrows). Original magnification ×630.

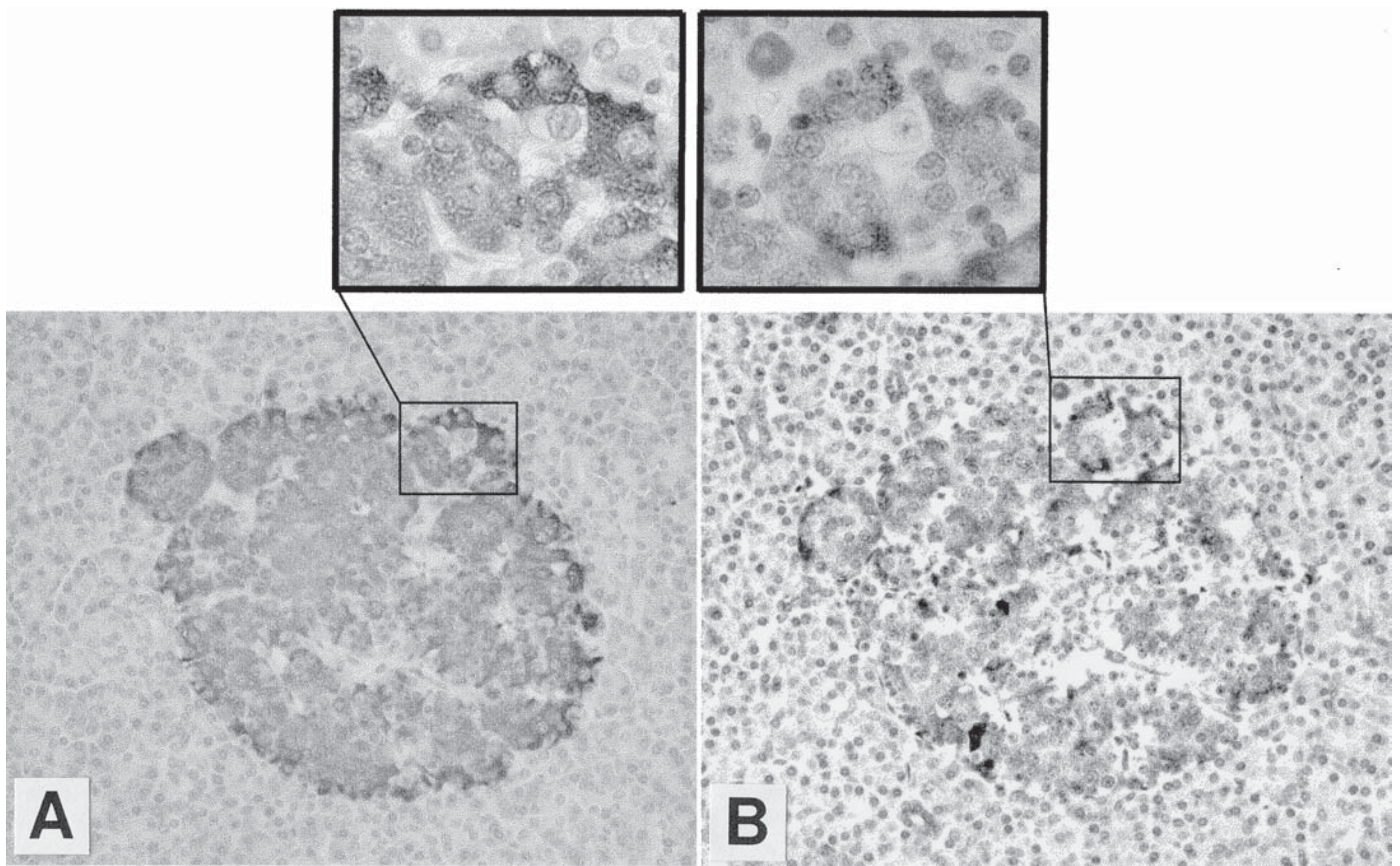

Fig. 5. Serial section showing that the majority of insulin-immunoreactive B cells **(A)** are also positive for met **(B)** (×200). The insets show a detail demonstrating the colocalization.

in islet cells (Fig. 5), which, by the colocalization studies, appeared to be insulin producing B cells. Met immunoreactivity was also detected in epithelial nonendocrine cells of the gut and pancreas, including gastric foveolar and chief cells, columnar cells of the intestine, Brunner glands, colonic crypt cells, and duct and acinar cells of the pancreas. In addition to epithelial cells, endothelial and smooth muscle cells of the gut and pancreas were also met-IR.

Endocrine Tumors

The 60 endocrine tumors studied included 14 pancreatic and 46 gut (8 gastric, 6 duodenal, 1 jejunal, 10 ileal, 12 appendiceal, 3 right colon, 6 rectal) neoplasms. The clinico-pathologic features of these tumors are summarized in Table 3. Lymph node and distant metastases and local invasion were found in 29 cases (23 gut and 6 pancreatic tumors).

HGF-immunoreactive cells were identified in gastroenteropancreatic (GEP) endocrine tumors using the three different anti-HGF antibodies (Table 1). The results regarding the HGF expression, obtained using the two polyclonal anti-HGF antibodies, were concordant in 34/49 tumors tested; in cases positive for both antibodies, the number of positive cells was sometimes different in relation to the antibody employed. With the antiserum directed against purified human HGF, immunoreactive cells were found in 32/60

(53%) tumors, whereas with the antibody directed against recombinant human HGF, they were detected in 38/49 (77%) tumors. In general, the second of the two antibodies mentioned above appeared to be more sensitive than the first one in detecting HGF-IR cells. Owing to the small amount of the antibody available, eight selected tumors (cases 8, 11, 14, 18, 28, 40, 45, 58) were tested with the monoclonal (clone DV-14) anti-HGF antibody. The results confirmed well those obtained with the polyclonal antibody directed against the recombinant HGF. Although HGF-IR tumors were located in all sites of the GEP system, the majority of them were in the stomach, ileum, and rectum, and corresponded to gastric ECL-cell, ileocolonic EC-cell, and rectal and appendiceal L-cell tumors. Immunoreactivity was generally diffuse and intense in the cytoplasm, although, with the polyclonal antibody directed against human purified HGF and with the monoclonal anti-HGF antibody, some cells showed paranuclear dot-like positivity. Among EC-cell tumors, a HGF-IR was found in 7/11 (63%) appendiceal cases and in 13/15 (86.6%) ileocolonic neoplasms. Ten of 14 (71%) pancreatic neoplasms were HGF-IR for at least one of the two antibodies and the immunoreactivity did not appear to be restricted to a unique tumor type, being found in one insulinoma, one gastrinoma, two VIPomas, and four nonfunctioning tumors (three well and one poorly differentiated).

Met immunoreactivity for at least one of the two anti-met antibodies employed was found in 50/60 (83.3%) tumors. In only seven cases a met-IR was obtained with only one of the two antibodies employed, while in the remaining neoplasms tested with both antibodies, results were similar in terms of positive or negative staining. However, in some cases we observed a different percentage of met-IR cells depending on the antibody employed. Met-IR was intense and cytoplasmic, with a membrane enhancement in some cells (Fig. 6), especially with the polyclonal antibody. Met-positive endocrine tumors for at least one of the two antibodies were located in all sites of the GEP system and included 6/6 ECL-cell and 2/2 PDEC of the stomach, 15/15 ileocolonic EC-cell carcinoids, 3/4 D-cell and 2/2 G-cell tumors of the duodenum, and 5/5 rectal and appendiceal L-cell carcinoids. However, in the appendix and pancreas met-IR tumors were less numerous. In particular, in the appendix they were 7/10 (70%) EC-cell carcinoids and in the pancreas 9/14 (64%) endocrine tumors including three insulinomas, one VIPoma, one gastrinoma, and four nonfunctioning neoplasms. Metastases and/or local invasion were present in 22/48 (46%) met-IR and 5/9 (55%) met-negative tumors, without any significant difference in rela-

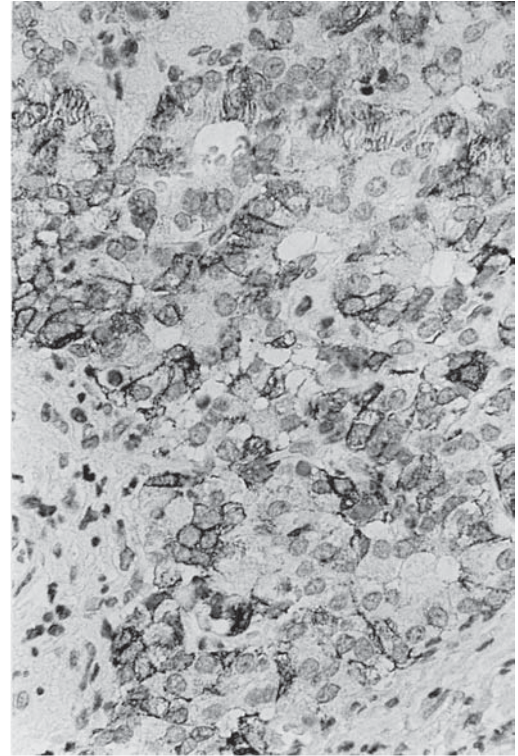

Fig. 6. Met-IR in a pancreatic insulinoma localized at membrane level in some cells.

Table 3. Clinicopathologic Profile of Endocrine Tumors[a]

N	Sex	Age	Site	Type	Size (cm)	Metastases or invasion	*Ab anti-HGF purified	Ab anti-HGF recombinant	Met MAb	Met PAb
1	F	65	Stomach	Type 1^	NE	No	0	100	90	90
2	F	90	Stomach	Type 1	NE	No	0	NE	90	NE
3	M	76	Stomach	Type 1	2	No	5	5	40	20
4	M	43	Stomach	Type 3^	2	Node	10	3	100	0
5	M	26	Stomach	Type 3	2	Node/liver	10	NE	90	NE
6	F	56	Stomach	Type 3	8.3	Node	0	90	100	70
7	M	68	Stomach	PDEC	3	Node/liver	5	0	60	30
8	M	52	Stomach	PDEC	5	Node	60	100	60	30
9	F	52	Duodenum	D cell	1.5	Node	0	0	0	0
10	F	38	Duodenum	D cell	1	Node	10	10	100	60
11	F	46	Duodenum	D cell	2.5	Node/liver	0	90	60	70
12	M	52	Duodenum	D cell	4	Node	0	0	40	40
13	F	42	Duodenum	G cell	1	No	0	3	90	90
14	F	55	Duodenum	G cell	0.5	No	3	100	80	80
15	F	38	Jejunum	Undefined cell	1.2	Node/liver	3	5	100	60
16	F	67	Ileum	EC cell	2	Node/liver	0	0	100	5
17	M	52	Ileum	EC cell	2	Node/liver	20	20	100	90
18	M	NE	Ileum	EC cell	2.5	No	30	80	100	50
19	F	46	Ileum	EC cell	1.5	Node	60	20	80	80
20	M	59	Ileum	EC cell	2.5	Liver	20	20	100	100
21	M	58	Ileum	EC cell	2.5	Node/liver	50	NE	70	NE
22	M	39	Ileum	EC cell	3	Node	70	90	80	50
23	F	75	Ileum	EC cell	1.5	Liver	0	0	80	80
24	F	69	Ileum	EC cell	2	Node	20	20	70	50
25	M	72	Ileum	EC cell	1	Omentum	20	2	20	80
26	M	53	Appendix	EC cell	2.5	No	0	50	0	90
27	M	25	Appendix	EC cell	0.5	No	0	NE	0	0
28	F	95	Appendix	EC cell	2	No	0	60	30	60
29	F	14	Appendix	EC cell	0.6	No	0	0	0	0
30	F	17	Appendix	EC cell	0.5	No	0	30	0	30
31	F	24	Appendix	EC cell	0.2	No	0	40	0	80
32	M	27	Appendix	EC cell	0.5	No	0	NE	60	NE
33	F	21	Appendix	EC cell	0.3	No	40	NE	100	NE
34	M	27	Appendix	EC cell	1	No	0	20	0	0
35	F	15	Appendix	EC cell	0.2	No	0	NE	0	0
36	F	42	Appendix	EC cell	0.9	No	0	0	80	80
37	F	24	Appendix	L cell	1.5	No	60	60	100	100
38	NE	NE	Right colon	EC cell	NE	Liver	20	NE	90	50
39	F	50	Right colon	EC cell	NE	Omentum	0	90	100	80
40	M	46	Right colon	EC cell	8	Liver	40	90	100	80
41	F	51	Rectum	L cell	0.7	No	60	NE	100	NE
42	F	66	Rectum	L cell	NE	No	70	NE	40	NE
43	M	39	Rectum	L cell	0.3	No	40	80	80	30
44	M	54	Rectum	EC cell	2	No	10	10	100	100
45	M	66	Rectum	L cell	1	No	70	60	100	90
46	M	70	Rectum	EC/L cell	2	Liver	90	90	90	30
47	F	52	Pancreas	Somatostatinoma	5	Duodenum	0	0	0	0
48	F	53	Pancreas	Insulinoma	2.5	No	0	0	10	100
49	F	54	Pancreas	Insulinoma	1	No	10	0	80	100
50	M	85	Pancreas	Insulinoma	NE	No	0	0	80	10
51	F	40	Pancreas	Gastrinoma	2	Liver/node	40	100	0	50
52	M	72	Pancreas	VIPoma	10	No	5	100	70	80
53	F	57	Pancreas	VIPoma	5	Liv/node/brain	0	70	0	0
54	M	62	Pancreas	NF	3	Duodenum	5	60	0	0
55	F	66	Pancreas	NF (gluc)	1.2	No	0	100	40	0
56	F	62	Pancreas	NF (gluc)	1.7	No	0	NE	30	NE
57	F	50	Pancreas	NF (5HT)	2	No	0	60	70	5
58	F	75	Pancreas	NF (5HT)	3	No	0	100	0	0
59	F	59	Pancreas	NF (PP)	8	Liver/node	60	60	0	10
60	M	58	Pancreas	PDEC	3.5	Liver/node	5	1	0	0

[a]Ab = antibody; MAb = monoclonal antibody; PAb = polyclonal antibody; * = percentage of positive cells; ^ = classified according to Rindi et al. [52]; NE = not evaluated; PDEC = poorly differentiated endocrine carcinoma; NF = nonfunctioning.

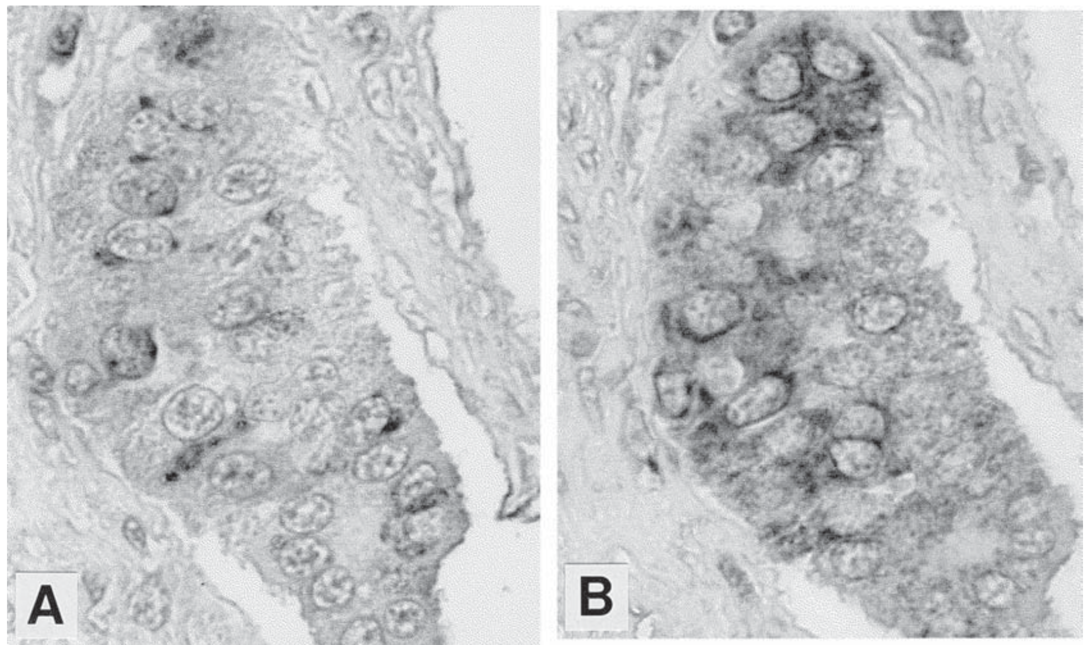

Fig. 7. Serial sections of an ileal EC-cell tumor indicating that neoplastic cells express HGF **(A)** and met **(B)** (×1000).

tion to tumor type. Among gut EC-cell carcinoids, 13/22 (59%) met-IR tumors were metastatic and/or invasive, while none of the four met-negative EC-cell tumors (all localized in the appendix) was associated with metastases ($p = 0.04$).

Coexpression of HGF and met in the same tumor was found in 42/60 (70%) cases, 21 of which (50%) were metastatic or locally invasive (Table 3). The majority of cases showing HGF and met coexpression were gastric ECL-cell (6/6 cases), ileocolonic EC-cell (13/15 cases) (Fig. 7), and rectal L-cell tumors (4/4 cases). Among them, metastases or gross invasion of adjacent organs or vessels were observed in type III gastric ECL-cell, and in EC-cell tumors. Among 18 tumors not expressing HGF and met or expressing only one of them, 8 showed metastases or local invasion (Table 3). Considering neoplasms, without differentiating them in relation to cell type and site, the biological aggressiveness of HGF/met-positive tumors was not statistically different from that of tumors expressing only one or none of these proteins. In the group of gut EC-cell neoplasms, coexpression of HGF/met statistically ($p = 0.03$) correlates with tumor site, in fact it was found in 13/15 (86.6%) ileocolonic EC-cell tumors, 11 of which (85%) were malignant, and in 5/11 (45%) appendiceal EC-cell neoplasms, which were all benign.

Peritumoral tissues were evaluated in 43 cases. They were histologically normal, with the exception of mucosae neighboring the three type 1 gastric ECL-cell carcinoids, which showed histological features of chronic autoimmune atrophic gastritis with intestinal and pseudopyloric metaplasia. The pattern and distribution of immunoreactivity for HGF and met in peritumoral examined reflected those observed in normal tissues not associated with endocrine tumors. In particular, the majority of foveolar and chief gastric cells, columnar cells of small intestinal villi, colonic crypt cells, some duct and acinar

pancreatic cells were HGF and met-IR, the latter being also expressed in Brunner gland cells.

Discussion

Growth factors are polypeptides that interact with specific receptors to modulate several cell functions, such as proliferation, differentiation, and motility [1]. In addition, growth factors are also involved in mechanisms regulating tumorigenesis, during which cells lose their normal physiologic functions and acquire an aggressive phenotype. HGF and its receptor met have been found to play physiologic roles in gastric ulcer healing [42,43] and human organogenesis [44], but they also seem to be involved in tumorigenesis and progression of several malignancies [18,20–25].

In this study, we have investigated the immunohistochemical expression of HGF and met in endocrine cells of the digestive system and in a series of GEP endocrine tumors. Immunohistochemistry, and particularly double label techniques, is useful in demonstrating the presence of two or more antigens within the same cell. The introduction of microwave-oven heating in the double-immunostaining technique has eliminated major drawbacks related to antibodies crossreactivity and has provided several advantages over traditional methods [45]. In this study we simultaneously used double label immunostainings and the ABC-immunohistochemical technique on serial paraffin and semithin-plastic sections to eliminate possible doubts of interpretation in the localization of HGF and met in specific cell types.

HGF and met appeared to be localized in specific endocrine cell types of the gut and pancreas. Although the two anti-HGF antibodies employed were specific, there were differences between them in terms of sensitivity. The antibody directed against human recombinant HGF seemed to be more sensitive than that directed against purified human HGF, in fact the former antibodies detected a greater number of HGF-positive cells in the various tumors investigated. In addition, in normal tissues the antibody directed against the recombinant peptide recognized HGF in G cells of the duodenal mucosa, and in A and B cells of pancreatic islets, which resulted negative when investigated with the antibody directed against the purified peptide. Regarding the two anti-met antibodies (monoclonal and polyclonal), we did not find significant differences in the distribution of positive cells, although the monoclonal antibody gave a more intense staining of epithelial cells, whereas the polyclonal antibody presented a stronger immunoreactivity of endothelial and stromal cells in comparison to epithelial cells.

The localization of HGF and met in normal endocrine cells of the human adult gut was restricted to G cells of the antral and duodenal mucosa, which expressed both these proteins. In addition, in the gastric antrum met-IR was also localized in EC cells. In the rectum, EC cells expressed HGF but not met. This specific distribution suggests that in human adult gut mucosa HGF/met interaction may have a regulatory function in the biology of at least gastrin-producing G-cells and serotonin-producing EC cells. However, further studies are needed to better clarify this point.

The wide expression of both HGF and met in nonendocrine epithelial cells of mucosal surface and glands suggests that a paracrine mechanism of regulation may be operative in local interactions between endocrine and exocrine cells of the normal mucosa and among the different types of exocrine cells. On the basis of our results it may be hypothesized that there may exist,

in pyloric mucosa, a paracrine interaction between exocrine epithelia and G and EC cells through a HGF/met binding system. A similar paracrine interaction may be possible between EC cells of the rectal mucosa producing HGF and the met-IR crypt cells. In the pancreas a HGF-IR was found in A and B cells, whereas a met-IR was observed only in B cells. Our results concerning the met expression in islets of normal human adult pancreas confirms previous results of Ebert et al. [23] proving that met is localized in islet cells, acinar cells, and ductal cells. Moreover, we have demonstrated, for the first time, that met expression is restricted to B cells. However, the expression of HGF in the same B cells and in part of A cells suggests that there may exist within pancreatic islets an autocrine/paracrine interaction among A and B cells through a HGF/met binding. In addition, an interaction between the exocrine and endocrine pancreas may be suggested because the exocrine component of the gland displayed an immunoreactivity for both HGF and met. However, the real functional meaning of the HGF and met expression in either exocrine or endocrine pancreas remains to be clarified, and needs to be further investigated. As regards the possible interactions between HGF and met in islet cells, it is of interest to recall that HGF has recently been demonstrated to be capable of enhancing the growth of human B cells in culture [46]. On the basis of this report and of our findings, it may be suggested that HGF produced by exocrine cells and A and B cells of the pancreas may exert a trophic action in an autocrine/paracrine fashion on met-expressing B cells.

In the normal adult tissues investigated, a HGF/met colocalization was found only in G cells of the antral mucosa. On the other hand, their coexpression was detected in 42/60 (70%) endocrine tumors. Particularly, coexpression was more frequently found in gastric ECL-cell, ileocolonic EC-cell, colorectal L-cell neoplasms, whereas in pancreatic tumors HGF and met were less frequently coexpressed. These results suggest the hypothesis that HGF and met may be implicated as autocrine/paracrine factors regulating gut endocrine tumor growth and perhaps development. In the literature, there is evidence indicating involvement of HGF and met in the development and progression of solid tumors such as sarcomas [26,47,48] and epithelial malignancies [20,22–25,49], and, in addition, the tumorigenicity of HGF and met has recently been demonstrated in some experimental studies. Cotransfection of NIH 3T3 cells with met and HGF resulted in tumorigenesis [50,51]. Our results also suggest a relationship between HGF/met expression and tumor aggressiveness in gut EC-cell neoplasms. In fact, HGF/met coexpression was different between ileocolonic EC-cell carcinoids, which are mostly malignant, and appendiceal EC-cell tumors, which are generally benign. The lower expression of HGF and met in appendiceal EC-cell tumors may be interpreted as a biological factor explaining the more favorable behavior of these neoplasms. In this context, it is interesting to note that a recent report has shown that appendiceal EC-cell neoplasms show an expression of fibroblast growth factor receptor 4 (FGFR4) lower than that of ileocolonic EC-cell tumors [30]. However, although the HGF and met involvement in gut endocrine tumor development, growth, and aggressiveness is an interesting and intriguing hypothesis, their role in endocrine tumor biology remains to be clarified, because it is unknown for certain whether HGF and met expression is a primary early event in

tumor development or a secondary event implicated in tumor progression. Further studies are needed to better clarify this point.

In conclusion, the results of the present study demonstrate, for the first time, that HGF and met are specifically expressed by different types of normal GEP endocrine cells and also by various GEP endocrine tumors, in which they may be implicated as autocrine/paracrine factors regulating their growth. The results presented in this paper can represent the beginning point for future investigations that will be able to better clarify the biological and pathobiological role of HGF and met in the physiology of normal GEP endocrine cells and in the biology of GEP endocrine tumors.

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