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EXPRESSION OF GROWTH FACTOR PEPTIDES AND THEIR RECEPTORS IN NEUROENDOCRINE TUMORS OF THE DIGESTIVE SYSTEM

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Neuroendocrine tumors of the digestive system are slow growing neoplasms which often present with pronounced fibrosis around tumor cells and in the peritoneal cavity. In this report 30 midgut carcinoids and endocrine pancreatic tumors were examined for the expression of peptide growth factors and their receptors, both by immunohistochemistry and in situ hybridization. Our data indicate that mulitple peptide growth factors, PDGF, TGF-β, and bFGF are expressed by these tumors. PDGF was expressed on tumor cells and stroma in 70% of tissues examined. PDGF α-receptor was seen on clusters of tumor cells and occassionally on adjacent stroma, whereas PDGF β-receptor was seen only in the stroma. Our data suggest that PDGF may be involved in the autocrine stimulation of tumor cells and stimulation of stromal cell growth through paracrine and possibly autocrine mechanism. In addition, tumor tissues express all three isoforms of TGF-β in more than half of the tissues examined. Tumor cells produce small latent complexes causing an escape from potent inhibitory effect of TGF-β and stimulation of stromal cell growth and matrix deposition through paracrine mechanism. bFGF, a potent stimulant of endothelial cell growth, was expressed by all tumor tissues examined. Our data suggest that multiple peptide growth factors may have an important role in tumor progression and desmoplastic reaction accompanying these tumors.

Neuroendocrine tumors of the digestive system comprise mainly gastrointestinal carcinoids and the islet cell tumors of the pancreas. Despite their rarity they are important as they secrete multiple hormones (1), resulting in distinct clinical syndromes. Among the outstanding features of carcinoid tumors is the frequent occurrence of significant desmoplastic reaction around tumor, blood vessels and heart, and in the peritoneal cavity. Intestinal carcinoids often present with luminal obstruction due to extensive fibrosis in the peritoneal cavity. Most patients with midgut carcinoids develop pronounced endocardial fibrosis involving the valves of the right heart which results in tricuspid insufficiency and pulmonary regurgitation.

Growth factors are defined as polypeptides that stimulate cell proliferation by binding to specific high-affinity cell membrane receptors. They differ from hormones by not usually acting in an endocrine manner; they presumably diffuse short range through intracellular spaces and act locally. The multiplicity of growth factors in various tissues, and the requirement of different cell types for multiple specific growth factors provide a delicate balance for coordinated growth of cells. Growth factors are being found to play an increasingly significant role in development, wound healing, and carcinogenesis. Proliferation and differentiation of cells in normal tissues is a well coordinated process mainly under the control of multiple growth factors. An uncoupling of this process results in uncontrolled growth, giving rise to tumors. The purpose of this study was to see whether neuroendocrine tumors, besides producing hormones, also produce growth factors and what role they play in tumor progression and the resultant clinical syndrome.

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Material and Methods

Tissue material. Neuroendocrine tumors were obtained from patients by abdominal operation or by ultrasonically guided needle biopsies (diameter 1.2–2.0 mm) of liver metastases. The material comprised primary tumors and metastases. The patients presented with gastrointestinal carcinoids and endocrine pancreatic tumors as shown in Tables 1 and 2. The tumor tissue was kept frozen, cryosectioned, and fixed in cold acetone for 10 min for immunohistochemical staining. For in situ hybridization frozen tissues were cryosectioned, fixed in 4% paraformaldehyde and stored in 70% ethanol prior to hybridization. Formalin-fixed paraffin-embedded blocks of biopsies were sectioned, deparaffinized, and digested with 0.1 mg/ml proteinase K (Sigma Chemical Co., St. Louis, MO) prior to hybridization.

Immunohistochemistry. The ABC immunoperoxidase method was performed as described before (2). Endogenous peroxidase was blocked by 0.3% hydrogen peroxide in methanol and endogenous avidin-binding protein was blocked by incubating the sections sequentially with avidin and biotin in Blocking kit (Vector Laboratories, Burlingame, Ca., USA). Normal horse or goat serum diluted 1:5 was employed to block unspecific binding of the secondary antibody to tissue. Primary antibodies used in the study are as shown in Table 3. The primary antibodies were applied separately on the tissue sections for 1.5 h at room temperature. Goat anti-rabbit or anti-mouse Ig was used as secondary antibody. The immunoreaction was visualized with an Elite complex (Vector Laboratories), 0.02% hydrogen peroxide used as a substrate and 3-amino 9-ethylcarbazol in dimethylsulphoxide as chromogen. The sections were counterstained with methyl green. In order to determine the cell types in the tissue, several antibodies were applied in the sequential sections. Rabbit polyclonal antibody against von Willebrand factor (Dakopatts) was

Table 1

Summary of immunohistochemical staining of tissue sections from neuroendocrine tumors of the digestive system. The results of immunostaining were evaluated as follows: + weak staining, + + moderately strong staining, + + + strong staining; −negative staining; ND not determined

Pat. No.	Age years	Diagn.	Interferon treatm.[a]	Tissue[b]	PDGF α-receptor		PDGF β-receptor		PDGF	
					stroma	tumor	stroma	tumor	stroma	tumor
1	18	FC	yes	P	−	−	+ +	−	ND	ND
2	46	MC	yes	M	+	+	+	−	+	+
3	87	MC	yes	P	−	ND	+ +	−	ND	ND
4	67	MC	yes	M	−	−	−	−	−	−
5	72	MC	yes	M	+	+	+	−	−	+
6	59	MC	yes	P	+ + +	+ + +	+	−	+ +	+ +
				M	+ +	+ +	+ + +	−	−	+ +
7	59	MC	yes	P	−	−	+ + +	−	+	+
8	66	MC	yes	M	+ +	+ +	−	−	+ + +	+ +
9	58	MC	No	M	+	+ +	+ + +	−	+ +	+ +
10	40	MC	yes	M	ND	+ +	+ +	−	−	−
11	57	MC	yes	M	+ +	+ +	+ +	−	+	+
12	50	MC	No	M	+ + +	+	+	−	+ +	+
13	67	MC	yes	M	−	+	+ + +	−	+	+ +
14	54	MC	No	M	+	+	+	−	+	+
15	79	MC	No	M	+	+	+ +	−	−	−
16	67	MC	No	M	+ +	+	−	−	−	−
17	61	MC	No	M	+ +	+ +	+ +	−	−	−
18	50	MC	yes	M	+ +	+ + +	+	−	ND	+ +
19	48	MC	No	M	+	+	+ +	−	+ +	+ +
20	63	MC	No	P	ND	ND	+ +	−	−	−
				M	+	+	+ +	−	+	+
21	70	MC	No	M	−	−	+ +	−	+ +	+ +
22	66	HC	yes	M	ND	+	−	−	ND	+
23	49	EPT	No	P	+	+ +	+ + +	−	+	+
24	59	EPT	yes	M	+	−	−	−	ND	+
25	37	EPT	No	P	+	+	+	−	−	−
26	59	EPT	No	P	+	+ +	+ +	−	−	−
				M	+	+	+ +	−	+ +	+ +
27	22	EPT	No	M	+	+ +	+	−	−	−

[a] Certain tumors were derived from patients treated with interferon-α, whereas others were from untreated patients.
[b] Original area from where tissue was derived. P = primary; M = metastases; FC = foregut carcinoid; MC = midgut carcinoid; HC = hindgut carcinoid; EPT = endocrine pancreatic tumor.

Table 2

Summary of immunohistochemical staining of tissue sections from neuroendocrine tumors of the digestive system for TGF-β

Pat No.	Age years	Diagn.	Interferon treat.[a]	Tissue[b]	Binding protein		TGF-β1		TGF-β2		TGF-β3	
					tumor	stroma	tumor	stroma	tumor	stroma	tumor	stroma
1	18	FC	yes	P	−	+	−	−		++	−	−
2	46	MC	No	M	+	++	+	+	++	ND	−	−
3	87	MC	yes	P	−	++	−	−	+	−	−	−
4	67	MC	yes	M	−	++	−	−	+	ND	−	ND
5	72	MC	yes	M	+	+++	+	−	++	−	+	−
6	59	MC	yes	P	−	++	+	−	+	+++	+	+
				M	−	++	+	+	−	−	+	−
7	59	MC	yes	M	−	+++	−	−	−	++	+	−
8	66	MC	yes	M	−	++	+	−	+	++	−	−
9	58	MC	No	M	−	+++	ND	ND	++	++	ND	ND
10	40	MC	yes	M	+	++	+	−	+	−	−	−
11	57	MC	yes	M	−	++	−	−	−	−	+	−
12	50	MC	No	M	+	+	−	−	+	ND	−	−
13	67	MC	yes	M	−	+	−	ND	++	−	−	−
14	54	MC	No	M	+	++	−	−	+	ND	−	−
15	79	MC	No	M	+	++	+	ND	−	−	+	ND
16	67	MC	No	M	−	++	−	++	−	+	+	++
17	61	MC	No	M	+	+++	−	−	+	ND	ND	+
18	50	MC	yes	M	−	+++	−	−	−	−	−	−
19	48	MC	No	M	−	+++	+	−	++	−	+	−
20	63	MC	No	P	−	++	ND	ND	ND	ND	ND	ND
				M	−	+++	+	−	++	+++	+	+
21	70	MC	No	M	−	+++	+	−	−	++	+	+
22	66	MC	yes	M	−	++	+	−	++	++	−	−
23	49	EPT	No	M	−	+++	−	−	−	+	−	++
24	59	EPT	yes	M	+	++	ND	ND	ND	ND	ND	ND
25	37	EPT	No	P	−	ND	−	ND	−	ND	−	ND
26	59	EPT	No	P	−	++	−	−	−	+	−	+
27	59	EPT	No	M	−	++	−	−	+	+	+	+
28	22	EPT	No	M	−	+	−	−	+	+	−	−

[a] Certain tumors were derived from patients treated with interferon-α, whereas others were from untreated patients

[b] Original area from where tissue was derived. P = primary; M = metastases; FC = foregut carcinoid; MC = midgut carcinoid; EPT = endocrine pancreatic tumor.

used as a marker for endothelial cells, muscle cells were identified with mouse monoclonal antibody against muscle actin HHF 35 (3), a kind gift from Dr. Ross, and monocytes were identified by Leu M5 monoclonal antibody (Bengston-Dickinson). Peripheral nerves were identified by staining with an antibody against neurofilament 200 Kd (Sigma), Schwann cells with a monoclonal antibody against glial acidic fibrillary protein (GFAP).

In situ hybridization. Frozen tissues were sectioned (6 μm), fixed in 4% paraformaldehyde and stored in 70% ethanol. In situ hybridization was performed as described elsewhere (4). ^{35}S-labelled antisense RNA probes were transcribed in vitro from plasmids as shown in Table 4. The in vitro transcription was done as previously described (4). Labelled probe (1×10^6 cpm per section) was mixed with hybridization buffer and added to the tissue sections on slides. To check the integrity of the RNA, the slides were hybridized with β-actin RNA probe transcribed from a 1761 bp Bam HI cDNA fragment cloned in pSP65 (5). As negative control TGF-β2 sense RNA probe was used. Hybridized preparations were autoradiographed with NTB 2 nuclear track emulsion (Eastman Kodak) diluted with distilled water. After exposure for 5 days at 4°C, slides were developed in Dektol (Kodak) developer at 15°C for 4 min, soaked in distilled water for 5 min and air dried. Slides were counterstained with Mayer's haematoxylin and eosin.

Results

Platelet-derived growth factor:

The results from immunohistochemical staining for PDGF and PDGF receptors in 22 carcinoid tumors and 5 endocrine pancreatic tumors are summarized in Table 1.

The PGF 007 antibody (Mochida Co., Tokyo, Japan), raised against a synthetic peptide corresponding to amino acids 73–97 of PDGF B-chain, stained tumor cells in most of the tissues examined (Table 1). Weak stromal staining

Table 3

Primary antibodies used in immunohistochemical study of neuroendocrine tumors of the digestive system

No.	Antibody	Type	Method	Dilution	Tissue
1	PDGF BB	Mouse monoclonal	ABC elite	1:1000	Frozen
2	PDGF β-Receptor	Mouse monoclonal	ABC elite	1:150	Frozen
3	PDGF-α-receptor	Rabbit polyclonal*	ABC elite	1:2	Frozen
4	TGF-β1-LAP	Rabbit polyclonal*	ABC elite	1:2	Frozen
5	TGF-β2-LAP	Rabbit polyclonal*	ABC elite	1:2	Frozen
6	TGF-β3-LAP	Rabbit polyclonal*	ABC elite	1:2	Frozen
7	Binding protein	Rabbit polyclonal	ABC elite	1:6000	Frozen
8	bfibroblast growth factor	Rabbit polyclonal	ABC elite	1:20	Frozen
9	bfibroblast growth factor receptor	Mouse monoclonal	ABC elite	1:40	Frozen

* Affinity purified

Table 4

Summary of RNA probes used for in-situ hybridization of tissue sections from neuroendocrine tumors of the digestive system

No.	Type	cDNA	Labelling	Tissue	cmp
1	RNA	1.3 kb EcoRI fragment of PDGF A-chain	^{35}S	Paraffin	10^6/section
2	RNA	2.0 kb BamHI fragment of PDGF B-chain	^{35}S	Paraffin	10^6/section
3	RNA	0.7 kb PstI fragment of PDGF β-receptor	^{35}S	Paraffin	10^6/section
4	RNA	1.5 kb PstI/EcoRI fragment of PDGF α-receptor	^{35}S	Paraffin	10^6/section
5	RNA	0.4 kb BamHi-HindIII fragment of LTBP	^{35}S	Frozen	10^6/section
6	RNA	1.0 kb EcoRI fragment of TGF-β1	^{35}S	Frozen	10^6/section
7	RNA	2.3 kb EcoRI fragment of TGF-β2	^{35}S	Frozen	10^6/section
8	RNA	0.4 kb EcoRI-KpnI fragment of TGF-β3	^{35}S	Frozen	10^6/section

was observed around the very positive tumor cells (Fig. 1B). Endothelium of both capillary and medium-sized blood vessels stained frequently positive. Smooth muscle cells in the vessel were positive for PDGF. Macrophages identified by the Leu M5 antibody were positive for PDGF (not shown). However, macrophages were not frequently observed in most of the tissues.

The PDGF α-receptor antibody, raised against a synthetic peptide corresponding to amino acids 1066–1084 of the PDGF α-receptor peptide (4), reacted positively with most of the tumor cells in the tissues examined (Table 1). However, not all tumor cells in the same tissue section were stained (Fig. 1D) Focal staining of the stroma was observed mostly around the positive tumor cells. Endothelial cells of the blood vessels were not stained but smooth muscle layer of the vessel walls showed weak immunostaining.

The PDGF β-receptor antibody frequently showed distinct staining of the stromal tissue as observed previously (Fig. 1C). The majority of the positive cells showed fibroblastic morphology that frequently expressed muscle actin antigens.

In situ hybridization showed positive hybridization signals for mRNA of PDGF β-receptor in stromal cells in most of the examined tumor tissue sections. No significant signals were seen on tumor cells (Fig. 2C). The positive signals were present also on vascular cells. Infiltrating lymphocytes did not show any positive signals for PDGF

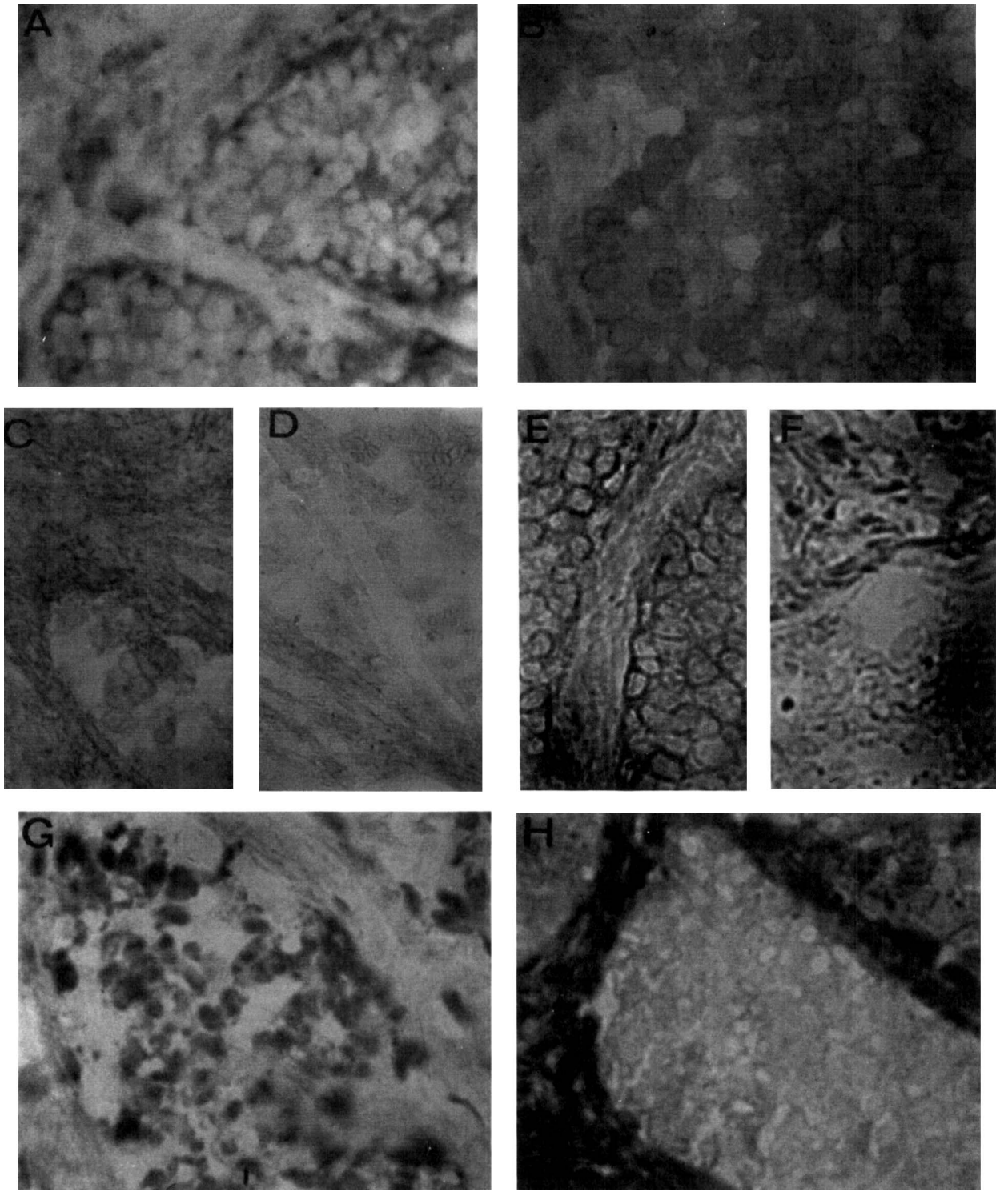

Fig. 1. A) Immunohistochemical staining of carcinoid tumor with a polyclonal antibody against bFGF. Tumor cells stained strongly perinuclearly and the surrounding stroma stained focally. B) Immunohistochemical staining with a monoclonal antibody against PDGF B-chain (PGF 007). Tumor cells stained strongly perinuclearly and stromal cells stained focally in their cytoplasms C) Double immunohistochemical stainings with PDGF antibody (PGF 007) (red brown), and the PDGF β-receptor antibody PDGFR-B2 (black). D) Immunohistochemical staining of carcinoid tumor with polyclonal antibody against PDGF α-receptor. Distinct perinucleart staining of carcinoid tumor cells was seen and stromal staining was mostly observed around positive tumor cells. E) Immunohistochemical staining of an endocrine pancreatic tumor with polyclonal antibody against TGF-β1-LAP. F) Immunoreaction with antibody against TGF-β2-LAP showed strong stromal staining as well as perinuclear staining of tumor cells. G) Carcinoid tumor cells stained strongly with antibody against TGF-β3-LAP and H) strong stromal staining was observed with antibody raised against binding protein.

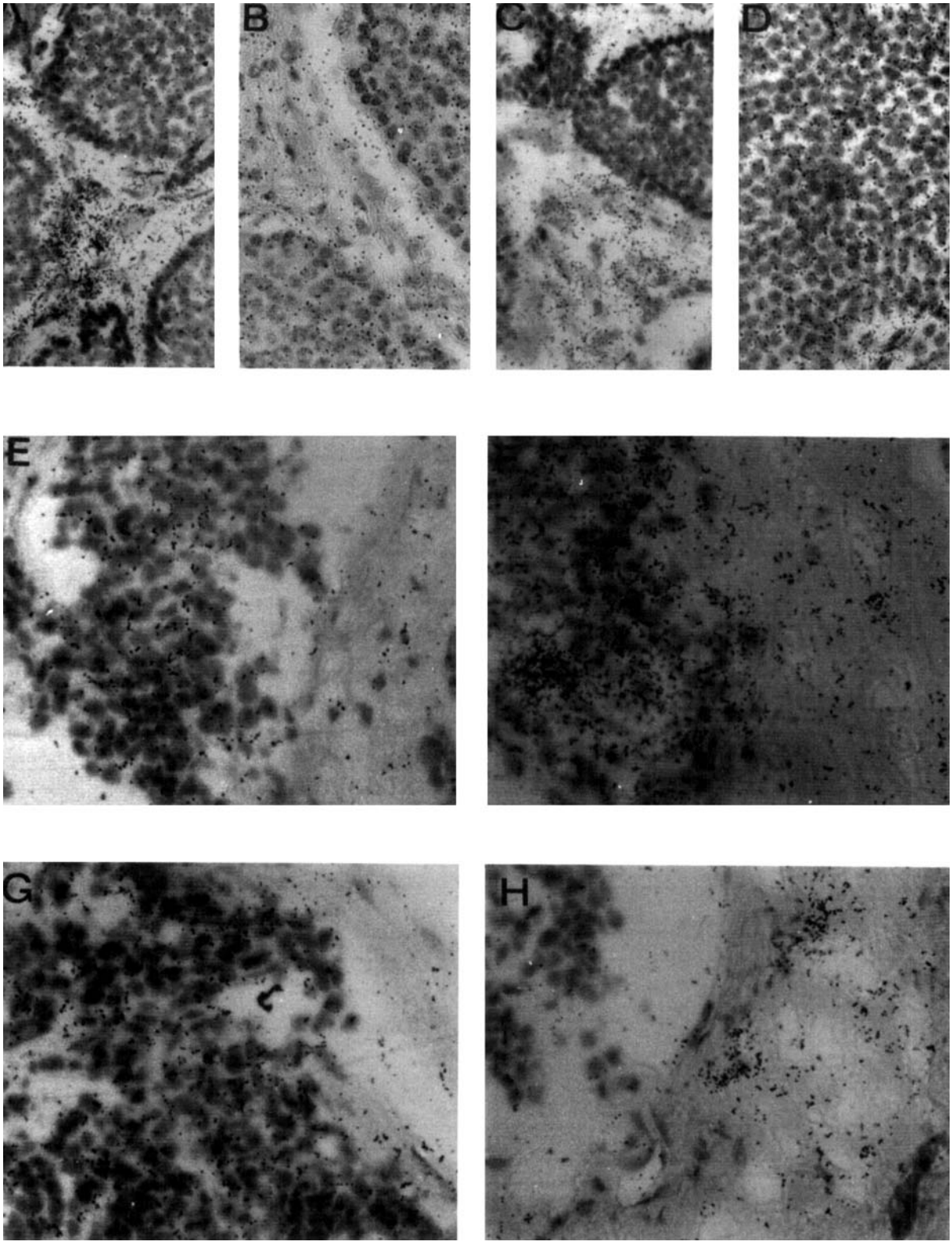

Fig. 2. A) In-situ hybridization using RNA probe for PDGF A-chain. Strong signals were observed in the stroma and weaker signals in the tumor cells. B) Weak signals in both tumor tissue and stroma were observed using RNA probe for PDGF B-chain. C) Hybridization with RNA probe for PDGF β-receptor showed signals only in stroma whereas D) in hybridization with RNA probe for PDGF α-receptor, signals were obtained both in tumor tissue and surrounding stroma. E) In-situ hybridization using RNA probes for E) TGF-β1, F) TGF-β2, G) TGF-β3 and H) binding protein.

β-receptor mRNA. A similar hybridization pattern in stromal cells was shown with PDGF A-chain probe (Fig. 2A). However, some of the positive cells contained stronger signals than those containing the PDGF β-receptor mRNA. In contrast, the frequency of the positive cells was less than that of β-receptor mRNA expressing cells. There were also several tissues containing β-receptor mRNA-expressing cells which do not contain any detectable A-chain mRNA. Hybridization with PDGF B-chain showed weak signals both on tumor cells and in the stroma (Fig. 2B). Hybridization with PDGF α-receptor showed signals both on tumor tissue and stromal cells.

Transforming Growth Factor-β

The results from immunohistochemical staining for TGF-β1,-β2,-β3 precursors and LTBP in 23 carcinoid tumors and 7 endocrine pancreatic tumors are summarized in Table 2.

Anti-TGF-β1 LAP antibody stained tumor cells of about half of the tumor tissues examined (11 out of 25). Weak staining of fibroblasts and smooth muscle was observed in surrounding tumor stroma. In addition, strong positive staining was observed on peripheral nerve fibers. The staining pattern was similar to that obtained with the antibody against neurofilament on serial section. Macrophages as identified by Leu M5 stained positively for TGF-β1. Strong staining on intestinal glands was observed in normal human intestine. No staining was observed in the exocrine or islet cells of normal human pancreas but the basal portion of the ductular epithelial cells stained positive. Scattered interstitial cells also stained positive.

Rabbit antiserum to LTBP stained tumor cells only in some (8 out of 28) tumor tissues examined. However, strong staining was observed in the surrounding stroma. Endothelial cells and vessel smooth muscle stained positively. Peripheral nerve fibres also stained positive. A staining pattern similar to that of TGF β1 was observed in normal human intestine. In the normal human pancreas, ductular epithelium and interstitial cells stained positive. Fibrous partitions and blood vessel walls in the exocrine portion of the pancreas stained positive.

Anti-TGF-β2 LAP antibody strongly stained tumor cells on more than half of the tumor tissues examined (17 out of 26). Strong stromal staining was also seen with this antibody. Fibroblasts stained positive and so did vessel smooth muscle cells. Peripheral nerve fibers stained positive for TGF-β2 LAP and the staining pattern corresponds to that shown with anti-GFAP on a serial section. Smooth muscle cells in normal human intestine also stained positive. No staining was observed in intestinal glands of normal human intestine. Nor was staining seen in the exocrine or endocrine cells of normal human pancreas. Interstitial cells and ductular epithelium in the exocrine portion of the pancreas stained positive. Blood vessels and fibrous partitions of the exocrine portion of the pancreas did not show any detectable staining.

Anti-TGF-β3 LAP antibody strongly stained tumor cells in more than one-third of the tumor tissues examined (10 out of 24). No significant stromal staining was observed. No detectable staining of normal human intestine or of the exocrine or endocrine cells of the pancreas was observed. Ductular epithelium and fibrous partitions of the exocrine portion of the pancreas stained positive but no significant staining was observed in the blood vessel walls.

In situ hybridization showed positive signals on tumor cells with TGF-β1 probe. Weaker signals were observed on stromal cells. Strong positive signals with TGF-β2 were observed both on tumor cells and surrounding stroma. Smooth muscle cells and fibroblasts showed strong positive signals. Hybridization with TGF-β3 probe showed strong positive signals on tumor cells but no significant signals were observed on stromal cells. Strong positive hybridization signals with LTBP probe were observed on stromal cells but no significant signal was obtained on tumor cells. Only background levels of signals were observed when tissues were hybridized with sense probe, whereas strong signals identifying total mRNA were observed when tissues were hybridized with actin probe.

b-Fibroblast Growth Factor

All tumors stained positive with antibody against bFGF (British Bio Tech) (Fig. 1A). The stromal component stained strongly for bFGF. Macrophages stained strongly positive in all tumors where such an infiltrate could be detected. Staining with antibody against bFGF receptor (UBI, NY) was found only in the stromal component. Smooth muscle of blood vessel wall stained strongly positive for bFGF receptor.

Discussion

Neuroendocrine tumors of the gut and pancreas have certain distinct pathological characteristics, such as low malignant but high metastatic potential. These tumors also display an intense fibroblastic reaction both around the tumor and in the peritoneal cavity. Endocardial fibrosis is a common feature in patients with midgut carcinoids. Although serotonin and other vasoactive substances have been implicated as the cause, this has not been substantiated by the fact that serotonin alone is not mitogenic to Chinese hamster lung fibroblasts. Rather a host of peptide growth factors is known to stimulate growth of fibroblasts in cell cultures. The previously reported effects of TGF-β

regarding its role in certain diseases with fibroblastic response (6, 7, 8) and the association of PDGF with proliferative disorders of fibroblastic origin (9) prompted us to examine if growth factor peptides are expressed by neuroendocrine tumors themselves.

The majority of neuroendocrine tumors of the digestive system tumors expresses PDGF α-receptors, which bind PDGF A-chains as well as B-chains, suggesting that they respond to any of the three dimeric forms of PDGF. Tumor cells themselves express B-chains and lower levels of A-chains, suggesting an autocrine growth stimulation. It is also possible that surrounding stromal component, which also synthesizes A- and B-chains, may stimulate growth of tumor cells in a paracrine manner. There is increasing evidence of the importance of the stromal component as fibroblasts in the carcinoid tumors expressing both receptor types, pointing to the possibility that they are activated in vivo.

Neuroendocrine tumors express TGF-β1-β2-β3 in most of the tumor tissues which we examined. In most tissues, LTBP was found only in the stromal component. It is thus possible that tumor cells may produce small complexes of TGF-β LAP. As the association of TGF-β LAP with LTBP occurs in connection with secretion (10), it indicates that lack of LTBP might cause retention or slow secretion of TGF-β1 from tumor cells, thus escaping the potent growth inhibitory action of TGF-β. Since TGF-β1 and TGF-β2 were not found in normal intestine and pancreas, the increased expression might present an involvement of these isoforms of TGF-β in tumor fibrosis. It has also been demonstrated that fibroblasts in the cardial contain TGF-β1, -β2, -β3-LAPs which thus may be involved in matrix deposition and fibrosis (11).

The production of endothelial cell mitogens by tumor cells might aid in vascularization of solid tumors, thus helping in tumor progression. Capillaries in tumor tissue were found to express PDGF β-receptor. In larger blood vessels the smooth muscle cells of blood vessel wall were positive for PDGF β-receptor and bFGF receptor. It is possible that PDGF and bFGF may have a role in vascularization of carcinoid tumors, but other angiogenic factors may also be involved.

REFERENCES

1. Norheim I, Theodorsson-Norheim E, Brodin E, Öberg K. Tachykinins in carcinoid tumors: Their use as a tumor marker and possible role in the carcinoid flush. J Clin Endocrinol Metab 1986; 63: 605–12.
2. Funa K, Papanicolaou V, Juhlin K, et al. Expression of platalet-derived growth factor β-receptor on stromal tissue cells in human carcinoid tumors. Cancer Res 1990; 50: 748–53.
3. Tsukada T, Tippens D, Gordon D, Ross R, Gown AM. HHF35, a muscle actin-specific monoclonal antibody: I. Immunocytohistochemical and biochemical characterization. Am J Pathol 1987; 126: 51–60.
4. Funa K, Steinholtz L, Nou E, Bergh J. Increased expression of N-myc in human small cell lung cancer biopsies predicts lack of response to chemotherapy and poor prognosis. Am J Clin Pathol 1987; 88: 216–20.
5. Chaudhry A, Papanicolaou V, Öberg K, Heldin C-H, Funa K. Expression of platelet-derived growth factor and its receptors in neuroendocrine tumors of the digestive system. Cancer Res 1992; 52: 1006–12.
6. Hoyt DG, Lazo JS. Alterations in pulmonary mRNA encoding procollagens, fibronectin and TGF-β precede bleomycin induced pulmonary fibrosis in mice. J Pharmacol Exp Ther 1988; 246: 765–71.
7. Okuda S, Languino LR, Ruoslahti E, Border WA. Elevated expression of TGF-β and proteoglycan production in experimental glomerulonephritis-possible role in expansion of the mesangial extracellular matrix. J Clin Invest 1990; 86: 453–62.
8. Czaja MH, Weiner FR, Flanders KC, Giam brone MA, Wind R, Biempica L. In vitro and in vivo association transforming growth factor-beta 1 with hepatic fibrosis. J Cell Biol 1989; 108: 2477–82.
9. Smits A, Funa K, Vassbotn FS, et al. Expression of platelet-derived growth factor and its receptors in proliferative disorders of fibroblastic origin. Am J Pathol 1992; 140: 639–48.
10. Miyazono K, Olofsson A, Colosetti P, Heldin C-H. A role of the latent TGF-β1-binding protein in the assembly and secretion of TGF-β1. EMBO 1991; 10: 1091–101.
11. Waltenberger J, Lundin L, Öberg K, et al. Involvement of transforming growth factor-b in the formation of fibrotic lesions in carcinoid heart disease. Am J Pathol 1993; 142: 71–8.