Immunohistochemical Detection of Fibroblast Growth Factor Receptors in Normal Endocrine Cells and Related Tumors of the Digestive System

Stefano La Rosa, M.D., *Silvia Uccella, M.D., *Silvia Erba, B.Sc,
*Carlo Capella, M.D., and *Fausto Sessa, M.D.

Endocrine tumors (ETs) of the digestive system produce several growth factors including acidic and basic fibroblast growth factors (aFGF and bFGF, respectively), which are thought to be involved in the growth of tumor cells and in the proliferation of tumor stromal cells. Their actions depend on binding to four specific receptors—FGFR1, FGFR2, FGFR3, and FGFR4—whose distribution in normal endocrine cells and related tumors of the gastroenteropancreatic (GEP) system has previously been examined. Formalin-fixed, paraffin-embedded normal tissues and 60 well-characterized GEP endocrine tumors were immunostained using specific antibodies directed against various GEP hormones, aFGF, FGFR1, FGFR2, FGFR3, and FGFR4. Acidic FGF immunoreactivity (IR) was found in gut EC cells; FGFR1 immunoreactivity in rare duodenal endocrine cells and in pancreatic A cells; FGFR2 immunoreactivity in gastric and duodenal G cells, pancreatic B cells, and rectal EC cells; FGFR3 immunoreactivity in duodenal G cells; and FGFR4 immunoreactivity in rectal L cells and in pancreatic B, PP, and A cells. Immunoreactivity for at least one of the four FGFRs was found in all tumors, independently of FGFR expression in the putative cell of origin. EC cell tumors, which were all positive for aFGF, were found to express at least three different FGFRs. FGFRs also were localized in the stromal cells of all the tumors examined. The tumor stroma was more abundant in EC cell tumors than in other types of neoplasms. The results suggest that aFGF-FGFR interaction may be involved in the modulation of normal endocrine cell functions and in the regulation of tumor growth and stromal proliferation of EC cell carcinoids.
Key Words: Acidic fibroblast growth factor—Endocrine tumors—Fibroblast growth factor receptor—Immunohistochemistry.

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Gastroenteropancreatic (GEP) endocrine tumors (ETs) show several characteristic stromal patterns, including desmoplastic reaction around tumor cells, proliferation

of smooth muscle cells within the tumor stroma, and nerve and muscular hypertrophy of the wall infiltrated by the neoplasm. Moreover, in the case of EC cell carcinoids, fibrosis of the peritoneal and retroperitoneal tissue is observed, often in association with obliterative elastic sclerosis of mesenteric blood vessels and sometimes with fibroelastosis of the inside surfaces of the right cardiac chambers and valvular leaflets (1–3).

The tumorigenesis and growth of GEP ETs and mechanisms involved in the pathogenesis of the above described stromal lesions have not been fully clarified. In recent years, the role of various growth factors and their receptors in the pathobiology of GEP ETs has been investigated (4–17). Among various growth factors, it has been suggested that acidic and basic fibroblast growth factors (aFGF and bFGF, respectively) are possibly involved in the pathogenesis of fibromuscular proliferation within the tumor stroma (14,15,18). Moreover, de novo expression of FGFR4 in neoplastic cells seems to be implicated in the tumorigenesis of EC cell tumors (15).

Acidic FGF and bFGF are the best characterized members of the fibroblast growth factor family, which includes at least 20 structurally related proteins (19–31). Acidic FGF and bFGF show approximately 55% of structural homology and are involved in a wide array of biologic functions and pathologic mechanisms including fetal development, tissue repair, angiogenesis, proliferation of epithelial- and mesoderm-derived cells (fibroblasts and smooth muscle cells), and tumor development and growth (32).

The biologic functions of these two growth factors depend on binding to highly specific cell surface receptors. The family of fibroblast growth receptors (FGFRs) includes at least four peptides—FGFR1, FGFR2, FGFR3, and FGFR4. Their structure consists of an extracellular ligand-binding domain, an intracellular tyrosine-kinase domain, and a transmembrane domain (33). Acidic FGF and bFGF can bind to all of these receptors, but with different affinity. The expression of FGFRs in normal endocrine cells and related tumors of the gut and pancreas has been poorly investigated (8,12, 15,16), and, to the best of the authors' knowledge, systematic studies on their specific distribution in the cells

of GEP endocrine system have not been reported previously.

The current study was designed to investigate immunohistochemically the specific cell distribution of FGFRs in ETs and related normal cells of the GEP endocrine system.

MATERIALS AND METHODS

Sixty normal tissues of the gut and pancreas (8 from subjects without neoplastic lesions and 52 from histologically normal peritumoral tissues of patients with the endocrine neoplasms investigated in the current study) and 60 immunophenotypically well-characterized (Table 1) GEP ETs were collected at surgery, fixed in buffered formalin (formaldehyde 4% [wt:vol] and acetate buffer 0.005 mol/L, pH7.4) for 24 hours, and then embedded in paraffin. As reported in Table 2, the functional cell types of the gut and pancreas have been identified and characterized based on their hormonal expression (34). Sections were stained with hematoxylin and eosin and Grimelius' silver impregnation for histopathologic evaluation and Van Gieson stain for the evaluation of tumor fibrous stroma. According to previously published criteria (15), the amount of fibrous stroma was scored as scant (1+), moderate (2+), or abundant (3+), whereas the smooth muscle cell component of the tumor stroma, evaluated immunohistochemically using anti-α-smooth muscle specific actin antibody, was scored as scant (1+) or abundant (2+). The amount of both fibrous and muscular stroma was assessed for every case, and the mean values for total, fibrous, and muscular stroma were indicated for the various tumor groups.

For 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 inhibited treating sections with 3% hydrogen peroxide for 10 minutes. Primary antibody incubations (Table 3) were performed at 4°C for 18 to 20 hours, followed by the avidin-biotin-peroxidase complex (ABC) method according to Hsu et al. (35). Immunoreactions were developed with 0.03% 3,3'-diaminobenzidine tetrahydrochloride and then sections were counterstained with Harris' hematoxylin. Colocalization studies were performed using serial reverse face sections, semithin-plastic sections, and double-label immunohistochemical techniques according to Mason and Sammons (36) and to Lan et al. (37). Sections incubated with antibodies directed against N-terminal glucagon-glicentin, glucagon, and somatostatin were pretreated with 0.003% subtilisin (Sigma, P478; Sigma, St. Louis, MO; protease type XXVII or Nagarse protease) in 0.005 mol/L Tris-buffered saline, pH 7.4 for 10 minutes at room temperature. Sections immunostained for FGFR1 and FGFR3 were pretreated with 0.05% trypsin (Sigma) in 0.05 mol/L Tris-buffered saline, pH 7.4, for 20 minutes at 37°C. Finally, sections stained for aFGF, FGFR2, and FGFR4 were pretreated with 0.01 mol/L citrate buffer, pH 6, for 10 minutes in a microwave oven at 650 W. Specificity controls consisted of absorption of antibodies and antisera with 10 to 20 nM/mL of their related antigens, omission of the first layer, and use of control tissues with or without the pertinent antigen.

Results have been statistically evaluated using the Fisher exact test and the Wilcoxon test. Two variables were considered significantly different when $P < 0.05$.

RESULTS

Normal Tissues

Acidic FGF immunoreactivity (IR) was observed in the cytoplasm of several endocrine cells dispersed along the entire gastrointestinal tract (Table 4). Acidic FGF immunoreactive cells were rare in the gastric mucosa, whereas they were numerous in the small bowel, appendix, colon, and rectum. Colocalization studies showed that aFGF-positive endocrine cells were a subgroup (approximately 30%) of serotonin-producing enterochromaffin (EC)-cells. Acidic FGF immunoreactivity was absent in the other functional types of endocrine cells, including those of the pancreatic islets.

FGFR1-positive endocrine cells were rare in the ampullary region of the duodenum, whereas they were not identified in the stomach and along the intestinal tract, including the jejunum, ileum, appendix, colon, and rectum. However, several endocrine cells immunoreactive for FGFR1 were observed in the pancreas and double-label immunostains demonstrated that FGFR1 immunoreactive cells were represented by a subgroup of glucagon-producing A cells.

FGFR2 was localized in several gastrin-producing G cells of the pyloric and duodenal mucosa and in a subpopulation of serotonin-producing EC cells of the rectal mucosa. However, FGFR2-IR was not detected in any endocrine cell of the ileal, appendiceal, and right colon mucosa. In the pancreatic islets, double label immunostains demonstrated that insulin-producing B cells were strongly FGFR2-immunoreactive, whereas A, D, and PP cells were FGFR2-negative (Fig. 1).

FGFR3 IR was found in some G cells of the pyloric and duodenal mucosa (Fig. 2) and in rare pancreatic endocrine cells, whereas it was lacking in endocrine cells of the other tracts of the small and large bowel.

The expression of FGFR4 in the gut was restricted to some rectal L cells producing glicentin/PYY. In islet cells, FGFR4 was localized in A, B, and PP cells, whereas D cells did not express FGFR4.

In general, the pattern of immunoreactivity of various FGFRs was intense, finely granular, and cytoplasmic,

TABLE 1. *Summary of clinicopathologic data and immunohistochemical stainings of the 60 endocrine tumors investigated*

No.	Sex	Age	Site	Cell type	Class	Fibrous stroma*	Muscular stroma*	Metastases or invasion	aFGF	FGFR1	FGFR2	FGFR3	FGFR4
1	F	65	stomach	ECL cell, type 1	WDET-B	NE	NE	no	0	100	80	90	NE
2	F	90	stomach	ECL cell, type 1	WDET-B	NE	NE	no	0	100	90	60	0
3	M	76	stomach	ECL cell, type 1	WDET-U	2+	2+	no	0	0	30	60	0
4	M	43	stomach	ECL cell, type 3	WDEC	1+	2+	node	0	0	10	5	0
5	M	26	stomach	ECL cell, type 3	WDEC	1+	2+	node/liver	0	40	0	10	0
6	F	56	stomach	ECL cell, type 3	WDEC	1+	1+	node	0	0	0	90	40
7	M	68	stomach	undefined	PDEC	1+	2+	node/liver	0	0	0	0	0
8	M	52	stomach	undefined	PDEC	1+	1+	node	0	0	0	90	0
9	F	52	duodenum	D cell	WDEC	1+	1+	node	0	60	0	70	0
10	F	38	duodenum	D cell	WDEC	1+	1+	node	0	20	2	20	50
11	F	46	duodenum	D cell	WDEC	1+	1+	node/liver	0	100	0	90	0
12	M	52	duodenum	D cell	WDEC	1+	1+	node	0	0	0	0	0
13	F	42	duodenum	G cell	WDET-B	2+	1+	no	0	100	0	100	0
14	F	55	duodenum	G cell	WDET-B	1+	1+	no	0	20	0	80	0
15	M	49	duodenum	undefined	PDEC	1+	1+	node	0	60	0	0	10
16	F	38	jejunum	undefined	WDEC	1+	1+	node/liver	0	80	5	70	90
17	F	67	ileum	EC cell	WDEC	2+	2+	node/liver	5	90	90	0	100
18	M	52	ileum	EC cell	WDEC	3+	2+	node/liver	80	NE	NE	90	100
19	M	NE	ileum	EC cell	WDEC	2+	2+	no	60	60	30	60	70
20	F	46	ileum	EC cell	WDEC	2+	2+	node	70	90	0	100	40
21	M	59	ileum	EC cell	WDEC	2+	1+	NE	70	20	NE	100	70
22	M	58	ileum	EC cell	WDEC	2+	2+	node/liver	70	80	90	5	30
23	M	39	ileum	EC cell	WDEC	3+	2+	node	5	40	40	70	80
24	F	75	ileum	EC cell	WDEC	3+	1+	liver	80	40	70	70	70
25	F	69	ileum	EC cell	WDEC	2+	1+	node	40	80	NE	80	60
26	M	72	ileum	EC cell	WDEC	3+	2+	omentum	40	0	60	70	5
27	M	53	appendix	EC cell	WDET-B	3+	1+	no	30	90	90	100	20
28	M	25	appendix	EC cell	WDET-B	2+	1+	no	10	60	2	80	60
29	F	95	appendix	EC cell	WDET-B	2+	1+	no	40	80	20	90	30
30	F	14	appendix	EC cell	WDET-B	3+	1+	no	70	90	5	100	20
31	F	17	appendix	EC cell	WDET-B	2+	1+	no	60	20	90	70	0
32	F	24	appendix	EC cell	WDET-B	3+	1+	no	60	90	90	90	0
33	M	27	appendix	EC cell	WDET-B	3+	1+	no	80	80	NE	100	60
34	M	27	appendix	EC cell	WDET-B	3+	2+	no	60	40	90	90	0
35	F	42	appendix	EC cell	WDET-B	2+	2+	no	30	90	90	100	30
36	F	24	appendix	L cell	WDET-B	3+	1+	no	0	100	80	90	90
37	NE	NE	right colon	EC cell	WDEC	3+	2+	ne	5	30	90	70	80
38	F	50	right colon	EC cell	WDEC	2+	2+	omentum	5	40	40	100	70
39	M	46	right colon	EC cell	WDEC	2+	1+	liver	5	90	60	10	90
40	F	63	right colon	undefined	PDEC	1+	1+	node	0	0	0	60	10
41	F	51	rectum	L cell	WDET-B	1+	1+	no	0	NE	5	100	40
42	F	66	rectum	L cell	WDET-B	ne	1+	no	0	80	NE	80	70
43	M	39	rectum	L cell	WDET-B	3+	1+	no	0	30	80	10	30
44	M	54	rectum	EC cell	WDET-B	2+	1+	no	30	90	80	90	5
45	M	66	rectum	L cell	WDET-B	1+	1+	no	0	50	5	100	80
46	M	70	rectum	EC cell	WDEC	3+	2+	liver	10	80	50	70	10
47	F	33	pancreas	A cell	WDET-B	1+	1+	no	0	80	20	80	80
48	F	63	pancreas	A cell	WDET-B	1+	1+	no	0	100	0	60	10
49	M	66	pancreas	A cell	WDET-B	1+	2+	no	0	60	60	30	90
50	M	62	pancreas	undefined	WDET-U	1+	2+	no	0	90	0	60	90
51	F	60	pancreas	A cell	WDET-B	1+	1+	no	0	90	70	70	30
52	M	62	pancreas	EC cell	WDET-U	2+	2+	no	5	0	0	40	2
53	F	75	pancreas	EC cell	WDET-U	2+	2+	no	0	2	NE	0	40
54	M	70	pancreas	undefined	WDET-U	1+	1+	no	0	0	0	0	0
55	M	67	pancreas	Somatostatinoma	WDEC	1+	1+	no	0	80	NE	0	20
56	F	54	pancreas	Insulinoma	WDET-B	1+	1+	no	0	0	80	60	90
57	F	50	pancreas	Insulinoma	WDET-B	1+	2+	no	0	80	3	90	80
58	F	40	pancreas	Gastrinoma	WDEC	2+	2+	liver/node	10	20	90	30	80
59	M	72	pancreas	VIPoma	WDET-U	1+	1+	no	0	30	0	20	30
60	F	55	pancreas	VIPoma	WDEC	1+	1+	liver	0	100	100	70	80

Class, tumor classification according to the World Health Organization classification (49,50); WDET-B, well-differentiated endocrine tumor with benign behavior; WDET-U, well-differentiated endocrine tumor with uncertain behavior; WDEC, well-differentiated endocrine carcinoma; PDEC, poorly differentiated endocrine carcinoma; NE, not evaluated; type 1, ECL cell carcinoid associated with chronic atrophic gastritis; type 3, sporadic ECL cell carcinoid.

* See Materials and Methods for explanation of scoring.

TABLE 2. *Functional endocrine cell types of adult human gut and pancreas*

| Cell type | Hormone or amine | Pancreas | Stomach | | Small bowel | | | Appendix | Large bowel | |
			Fundus	Antrum	Duodenum	Jejunum	Ileum		Colon	Rectum
ECL	Histamine		+							
G	Gastrin			+	+					
D	Somatostatin	+	+	+	+	rare	rare	+	rare	+
A	Glucagon	+								
B	Insulin	+								
PP	PP	+								
S	Secretin				+	+				
M	Motilin				+	+				
CCK	CCK				+	+				
GIP	GIP				+	+				
EC	Serotonin	rare	+	+	+	+	+	+	+	+
N	Neurotensin						+			
L	GLI and PYY						+	+	+	+

PP, pancreatic polypeptide; CCK, cholecystochinin; GIP, gastric inhibitory peptide; GLI, glucagon-like peptides; PYY, peptide tyrosine-tyrosine.

with reinforcement at the cell membrane in some cells. In some cells, FGFR3 positivity also was present in the nucleus.

In addition to endocrine cells, all FGFRs were widely expressed in epithelial, endothelial, mesothelial, and muscular cells and fibroblasts of the tissues investigated.

Endocrine Tumors

The 60 endocrine tumors investigated included 6 gastric ECL cell carcinoids, 1 jejunal ET of undefined cell type, 4 D cell and 2 G cell tumors of the duodenum, 24 EC cell carcinoids of the small and large bowel, 5 L cell tumors of the rectum and appendix, 4 poorly differentiated endocrine carcinomas (PDECs), and 14 pancreatic ETs (2 insulinomas, 1 gastrinoma, 2 VIPomas, 9 nonfunctioning tumors of different cell type). The average age of patients was 52.8 years, ranging from 14 years to 95 years. Thirty-one patients were female and 28 were male; for 1 patient, gender information was not available.

Cytoplasmic immunoreactivity for aFGF was found in 26/60 (43%) tumors (Table 5); 25 were EC cell carcinoids (Fig. 3) and 1 was a pancreatic gastrinoma with an EC cell component (case 58). The other types of ETs including ECL cell, D cell, G cell, and L cell neoplasms, PDECs, insulinomas, and VIPomas were aFGF-negative.

FGFRs were widely expressed among various types of ETs (Table 5), independent of FGFR expression in the putative cell of origin. FGFR1 was found in 47/58 (81%) cases, FGFR2 in 36/53 (68%), FGFR3 in 53/60 (88%), and FGFR4 in 44/59 (74%). All EC cell (Fig. 4) and all L cell ETs expressed at least three FGFRs, whereas gastric ECL cell tumors expressed at least two FGFRs (mainly FGFR2 and FGFR3), with FGFR4 being expressed in only one of the three type 3 carcinoids. Poorly differentiated endocrine carcinomas showed a low level of FGFR immunoreactivity, both in terms of the number of positive tumors and in terms of the number of immunoreactive cells per tumor. FGFR immunoreactivity was

TABLE 3. *Antibodies and antisera used*

Antibodies/antisera	P/M (clone)	Dilution	Source
Glicentin-glucagon (Glic)	P	1:2500	Milab, Malmo, Sweden
Glucagon (Gluc)	P	1:1250	Milab
Pancreatic polypeptide (PP)	P	1:4000	Cambridge Research Biochemicals, Cambridge, U.K.
Somatostatin (Som)	P	1:500	Dako, Copenhagen, Denmark
Somatostatin (Som)	M(YC7)	1:10	Immunochimica Labometrics, Milan, Italy
Secretin (Secr)	P	1:500	Milab
Serotonin (5HT)	M(YC5)	1:50	Biogenesis, Bournemouth, U.K.
Peptide YY (PYY)	P	1:1000	Biogenesis
Substance P (Sub P)	P	1:640	Milab
C-terminus gastrin-CCK-cerulein (Cer)	M(B4)	1:10000	Farmitalia, Milan, Italy
Gastrin 34 (Gastr)	P	1:500	Cambridge
α-smooth muscle specific actin	M(1A4)	1:10000	Sigma Immunochemicals, St. Louis, MO
Acidic fibroblast growth factor (aFGF)	P	1:100	UBI, Lake Placid, NY
Fibroblast growth factor receptor 1 (FGFR1)	P	1:100	Santa Cruz Biotechnology, Santa Cruz, CA
Fibroblast growth factor receptor 2 (FGFR2)	P	1:500	Santa Cruz Biotechnology
Fibroblast growth factor receptor 3 (FGFR3)	P	1:100	Santa Cruz Biotechnology
Fibroblast growth factor receptor 4 (FGFR4)	P	1:100	Santa Cruz Biotechnology

P/M, polyclonal/monoclonal.

TABLE 4. *Distribution of aFGF and FGFR immunoreactivity in normal endocrine cells of the gut and pancreas*

| | Stomach | | Small bowel | | | | Large bowel | | |
	Fundus	Antrum	Duodenum	Jejunum	Ileum	Appendix	Right colon	Rectum	Pancreas
aFGF	rare	rare	EC	EC	EC	EC	EC	EC	—
FGFR1	—	—	rare	—	—	—	—	—	A
FGFR2	—	G	G	—	—	—	—	EC	B
FGFR3	—	G	G	—	—	—	—	—	rare
FGFR4	—	—	—	—	—	—	—	L	A, B, PP

aFGF, acidic fibroblast growth factor; FGFR, fibroblast growth factor receptor.

cytoplasmic and diffuse, with membrane reinforcement in some cells, with the exception of nuclear FGFR3 immunoreactivity in a few cases. In addition to tumor cells, endothelial, mesothelial, and tumor stroma cells, including fibroblasts and smooth muscle cells, also expressed FGFRs.

The relation between the amount of tumor stroma, either fibrous or muscular, and the aFGF expression is

summarized in Table 6. Tumors expressing aFGF (practically all EC cell carcinoids) presented an abundant tumor stroma with a significant fibrous and muscular component. The fibrous stroma of EC cell carcinoids (mean score 2.5+) was significantly ($P = 0.01$) more abundant than that of non–EC cell tumors (mean score 1.2+). The total stroma, which includes both the fibrous and muscular component, also was significantly ($P =$
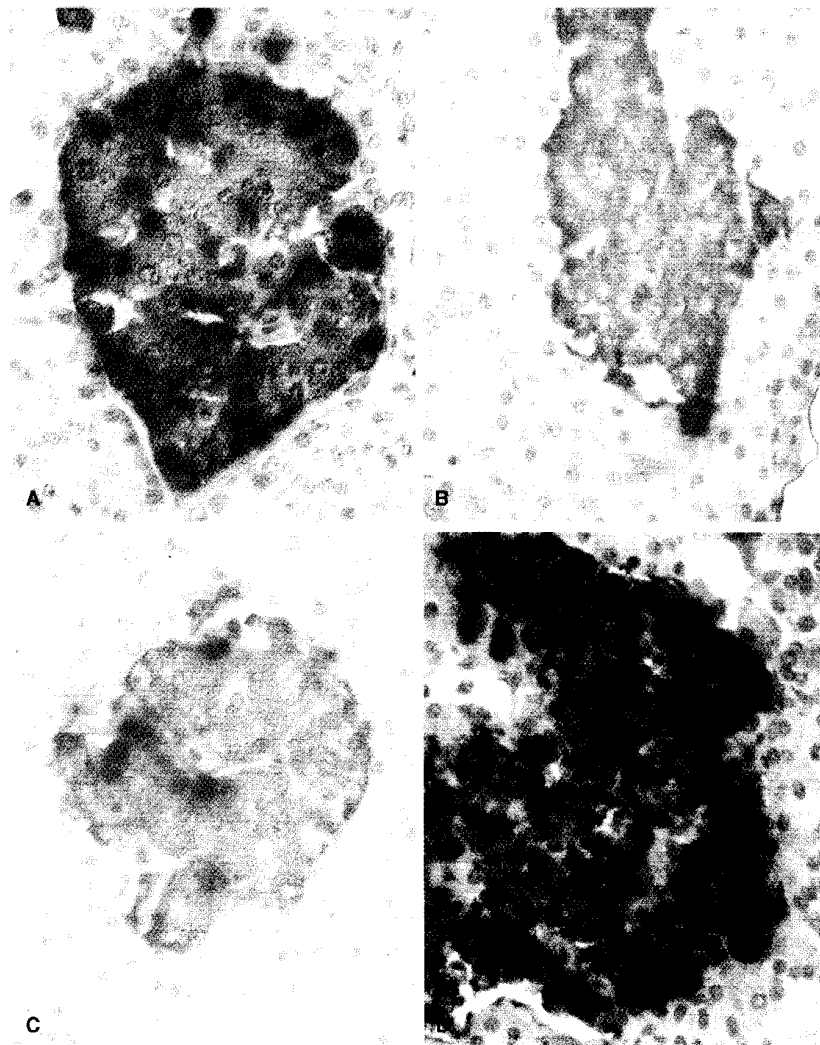

FIG. 1. Double-label immunostainings showing that FGFR2 (brown) is not expressed by glucagon immunoreactive (IR) A cells **(A)**, pancreatic polypeptide IR PP cells **(B)**, and somatostatin IR D cells **(C)**, whereas it is colocalized with insulin in B cells **(D)**. All hormones are stained in red. Avidin-biotin-peroxidase method for brown stain and alkaline phosphatase method for red stain.
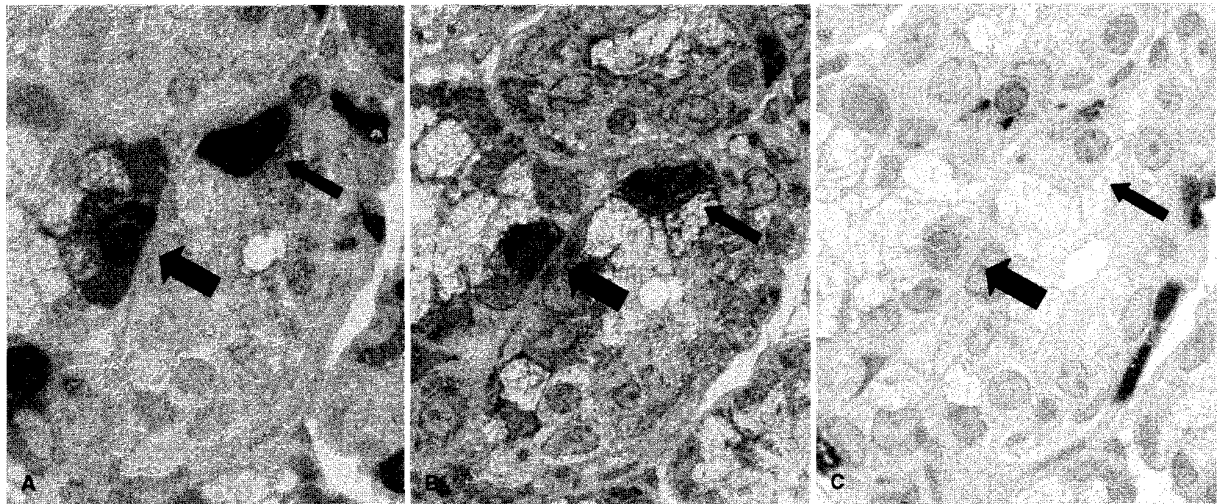
FIG. 2. Semithin serial sections of antral mucosa showing that gastrin immunoreactive (IR) cells **(A)** also are immunoreactive for FGFR3 **(B)**, which is not expressed by serotonin IR cells **(C)** (arrows). Avidin-biotin-peroxidase method.

0.01) more abundant in EC cell carcinoids than in non–EC cell tumors.

DISCUSSION

The expression of various FGFRs in normal endocrine cells of the GEP system has been poorly investigated. FGFR4 was found to be expressed by normal rectal glicentin/PYY-producing L cells (15). To the best of the authors' knowledge, no studies of the distribution of FGFR1, FGFR2, and FGFR3 have been reported. In the current study, for the first time it is demonstrated that there is a specific distribution of various FGFRs among

gut and pancreatic endocrine cell types. Interestingly, although normal gastrointestinal EC cells express aFGF (current study and 14) and bFGF (13), they do not express FGFRs, with the exception of a subpopulation of rectal EC cells expressing FGFR2. This finding suggests that, in normal mucosa, autocrine interaction between

TABLE 5. *Distribution of aFGF and FGFR immunoreactivity in endocrine tumors of the gut and pancreas*

Tumor type	aFGF	FGFR1	FGFR2	FGFR3	FGFR4
ECL cell	0/6	3/6	4/6	6/6	1/5
D dell	0/4	3/4	1/4	3/4	1/4
G cell	0/2	2/2	0/2	2/2	0/2
Undefined cell	0/1	1/1	1/1	1/1	1/1
EC cell (ileal)	10/10	8/9	6/7	9/10	10/10
EC cell (appendiceal)	9/9	9/9	8/8	9/9	6/9
EC cell (colorectal)	5/5	5/5	5/5	5/5	5/5
L cell	0/5	4/4	4/4	5/5	5/5
PDEC	0/4	1/4	0/4	2/4	2/4
Pancreas	2/14*	11/14	7/12	11/14	13/14
Total	26/60 (43%)	47/58 (81%)	36/53 (68%)	53/60 (88%)	44/59 (74%)

aFGF, acidic fibroblast growth factor; FGFR, fibroblast growth factor receptor, PDEC, poorly differentiated endocrine carcinomas.
* Of two aFGF-positive tumors, one was an EC cell tumor (case 52), and one was a gastrinoma with an EC cell component (case 58).
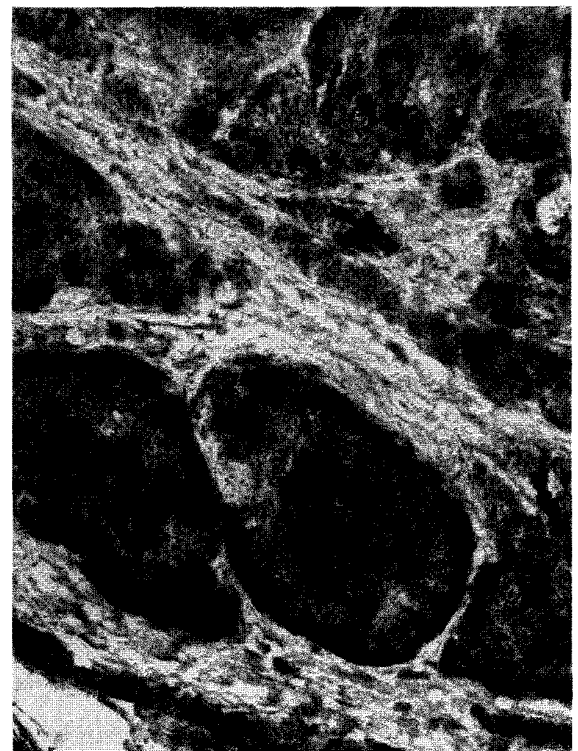

FIG. 3. Acidic fibroblast growth factor immunoreactivity in an EC cell tumor of the ileum. Avidin-biotin-peroxidase method.
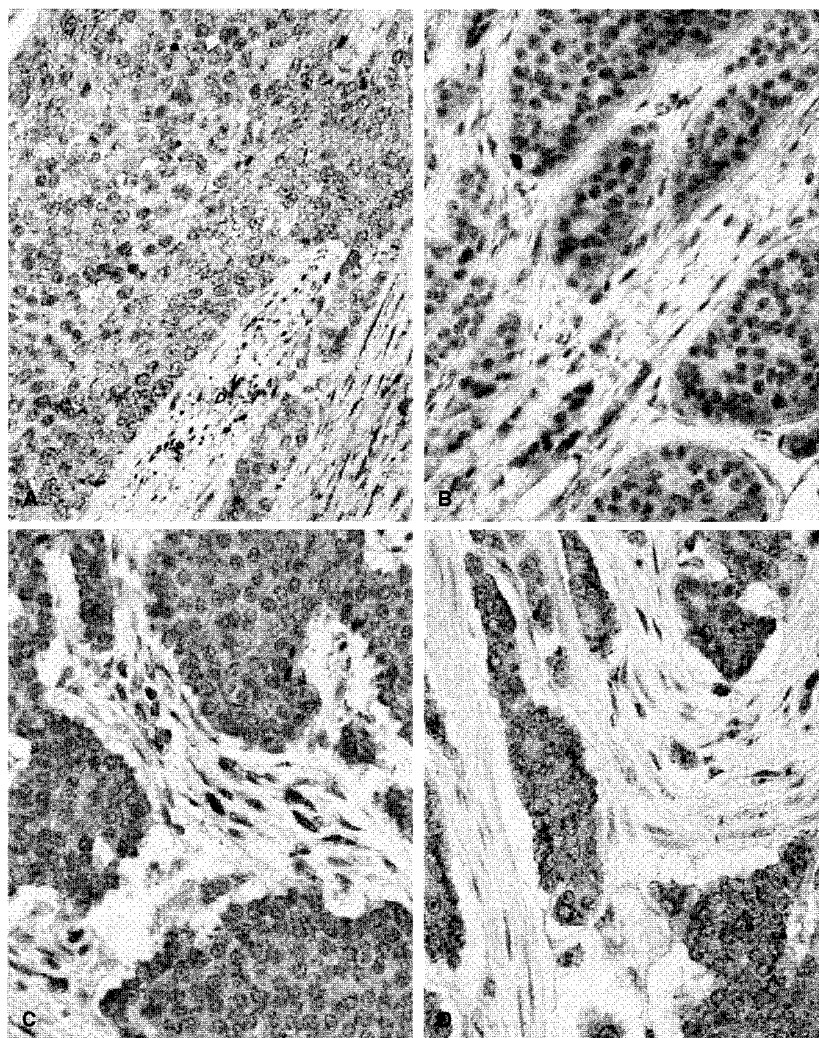
FIG. 4. Ileal EC cell tumors showing immunoreactivity for FGFR1 **(A)**, FGFR2 **(B)**, FGFR3 **(C)**, and FGFR4 **(D)**. In addition to neoplastic epithelial cells, stromal cells also are FGFRs immunoreactive. Avidin-biotin-peroxidase method.

aFGF and FGRFs or bFGF and FGFRs is probably not involved in modulating EC cell functions. However, aFGF-producing EC cells and bFGF-producing gastric EC and ECL cells may regulate, in a paracrine fashion, antroduodenal G cells, which according to the current results are FGFR2- and FGFR3-immunoreactive. Although this hypothesis is intriguing, further studies are needed to better clarify the functional role of FGFRs and their ligands in the modulation of the functional activity of normal gut endocrine cells. In addition, the current results prove that FGFRs are localized in superficial and glandular exocrine epithelial cells of gut mucosa, suggesting that, in normal conditions, endocrine cells, or at least EC and ECL cells, may influence exocrine epithelia through a paracrine mechanism involving aFGF, bFGF, and FGFRs. In this context, it is interesting to recall that, in the gut, aFGF and bFGF are known to be involved in mechanisms regulating mucosal repair and cell differentiation (38–40).

Acidic FGF immunoreactivity was not found in pancreatic islet cells, agreeing with the study of Friess et al. (41), which shows that aFGF and bFGF are present in acinar and ductal cells of the pancreas, whereas they are absent in islet endocrine cells. In another report, bFGF was not detected in islet cells, confirming that this growth factor, like aFGF, is not expressed by the endocrine component of the pancreas (42). However, the authors clearly demonstrated strong immunoreactivities for FGFRs in islet cells. With the exception of FGFR3, FGFRs were well expressed and, in particular, FGFR1 was localized in A cells, FGFR2 in B cells, and FGFR4 in A, B, and PP cells. The meaning of FGFR expression in pancreatic islet cells is unknown and it does not seem to be related to aFGF and bFGF production by the same cells. However, it is important to underline that these two growth factors belong to a large family comprising at least 20 structurally related proteins and it may be hypothesized that, in pancreatic islets, other members of
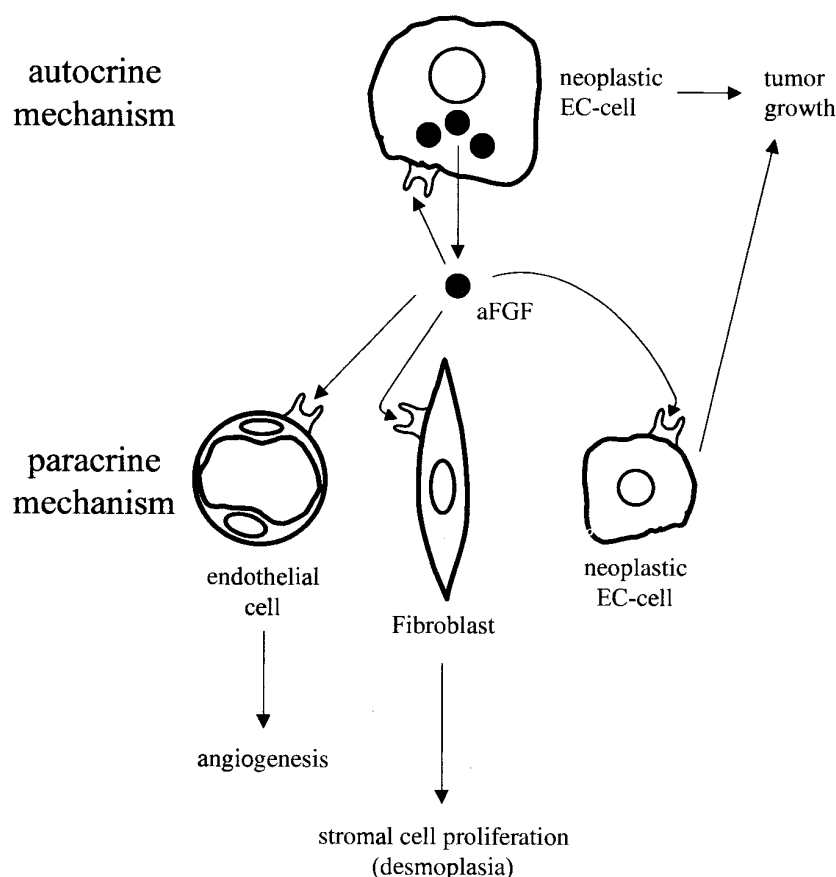
FIG. 5. Schematic representation of possible acidic fibroblast growth factor (aFGF) actions on EC cell tumors. EC cells produce aFGF, which is released with an unknown mechanism. aFGF may stimulate through paracrine mechanisms stromal and endothelial cells, which express FGFRs. The interaction between aFGF and FGFRs promotes tumor growth, stromal desmoplasia, and angiogenesis.

this family interact with FGFRs. Ishiwata et al. (43) recently have demonstrated that a few islet cells express keratinocyte growth factor, also known as FGF7, and its specific receptor (FGFR2). These results suggest that interaction between FGF7 and FGFR2 may be involved in the modulation of some functions of islet cells. In addition, because aFGF and bFGF are known to be expressed by exocrine cells of the pancreas (41,42), it may be hypothesized that exocrine pancreatic cells regulate endocrine cell function through the interaction between aFGF/bFGF and the FGFRs expressed by islet cells.

Endocrine tumors of the GEP system show different clinicopathologic features depending on the cell type from which they derive (34,44). In addition, GEP ETs, particularly gut ETs, show a wide spectrum of proliferation of host tissue including fibromuscular reaction of the gut wall, desmoplastic reaction of tumor stroma, fibrosis of peritoneal and retroperitoneal tissues, and fibroelastosis of mesenteric blood vessels (1–3). The tumorigenesis of ETs and the pathogenesis of these stromal lesions are not clear. Recent studies have provided evidence that GEP ETs produce several growth factors including insulin-like growth factor-I (IGF-I), transforming growth factor-α (TGF-α) and -β (TGF-β), activin A, platelet derived growth factor (PDGF), aFGF and bFGF, vascular

endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (4–17,45,46). Among these peptides, it has been suggested that aFGF and bFGF, which are well known as stimulators of fibroblast, smooth muscle, epithelial, and endothelial cell proliferation, are involved in the pathogenesis of fibromuscular proliferation within the tumor stroma (14,15,18). Because the action of aFGF and bFGF depends on their interaction with specific cell receptors, it is of interest to know the exact localization of FGFRs. The expression of FGFRs in GEP ETs has been poorly investigated. In 1993, Chaudhry et al. (8) and Ahlman et al. (12) described an immunoreactivity for an unspecified FGFR in stromal vascular smooth muscle cells and fibroblasts

TABLE 6. Relation between aFGF expression and tumor stroma

Tumor type	Stroma*			aFGF
	Fibrous	Muscular	Total	
EC cell	2.5+	1.5+	4+	25/26†
Non–EC cell	1.2+	1.2+	2.4+	0/34

aFGF, acidic fibroblast growth factor.
* Mean values of data reported in Table 1.
† The aFGF negative EC cell tumor was pancreatic (case 53).

of carcinoids showing positive staining for bFGF. In addition, Wulbrand et al. (16), using reverse transcription-polymerase chain reaction (RT-PCR), demonstrated the expression of FGFR mRNA in pancreatic gastrinomas, insulinomas, functioning intestinal EC-cell tumors, nonfunctioning gastric and intestinal endocrine tumors, but not in nonfunctioning pancreatic islet cell tumors. The type of FGFR investigated was not specified; however, checking the primer sequence used in the study at the National Library of Medicine with the BLAST basic local alignment search tool (http://www.ncbi.nlm.nih. gov/cgi-bin/BLAST/nph-newblast, accessed May 1998), the sequence used identified the Ig domains of FGFR1, FGFR2, and FGFR3 molecules. In the same study (16), it was not possible to discriminate whether the signal observed derived only from the stromal component or also from the epithelial component because of the methodologic procedure and technique used. In the current study, immunohistochemical techniques were used that, although known to be less sensitive than RT-PCR, provided the opportunity to identify the specific tumor cells expressing FGFRs. As shown in Table 5, various FGFRs were widely distributed among various endocrine tumors. ECL cell, EC cell, and L cell carcinoids represented the neoplasms in which FGFRs were more frequently expressed. The current results are comparable with those reported by Wulbrand et al. (16) who did not observe statistically significant differences in the FGFR mRNA expression among various types of ETs.

Comparing the FGFR distribution in normal endocrine cells and related tumors, it clearly appears that, with the exception of FGFR4 in rectal L cell tumors, all FGFRs are expressed de novo in the majority of ETs including ECL and EC cell carcinoids. In fact, normal ECL cells and the majority of EC cells, with the exception of few rectal EC cells expressing FGFR2, are not immunoreactive for FGFRs. The current results suggest that de novo expression of FGFRs may be involved in mechanisms of carcinoid tumorigenesis, at least for EC cell and ECL cell tumors. It is well known that de novo expression and overexpression are mechanisms involved in the oncogenetic potential of growth factors with tyrosine-kinase activity (47).

EC cell carcinoids, which produce aFGF and show immunoreactivity for at least three FGFRs, present a more abundant stroma than that of tumors negative for aFGF. This finding suggests that aFGF may be a factor inducing stromal proliferation through a paracrine mechanism. This hypothesis, which has been postulated in previous studies (14,15), is now convincingly supported by the results presented in the current article demonstrating that stromal cells express FGFRs (Fig. 5).

In addition, a paracrine aFGF/FGFR binding may also be involved in vascular proliferation (angiogenesis) (48) in the stroma of EC cell tumors, which are generally well vascularized neoplasms, because endothelial cells express FGFRs.

FGFR immunoreactivity did not correlate with the Ki67 proliferative index (data not shown) and malignancy of the endocrine tumors investigated; indeed, in highly aggressive PDECs, FGFR immunoreactivity appeared to be low. These findings indicate that FGFRs are probably involved in tumor development but do not participate in the regulation of tumor progression, which may be regulated and modulated by other growth factors such as hepatocyte growth factor and scatter factor (46).

In conclusion, the current results demonstrate that aFGF and FGFRs are expressed by specific normal endocrine cell types of the gut and pancreas suggesting their possible role in the modulation of cell functions. In addition, aFGF is expressed by EC cell carcinoids whereas FGFRs are more widely expressed among various endocrine tumors of the digestive system. The interaction between aFGF and FGFRs may be involved, at least in EC cell carcinoids, in the modulation of tumor growth and tumor stroma proliferation. □

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