Localization of Vascular Endothelial Growth Factor and Its Receptors in Digestive Endocrine Tumors: Correlation With Microvessel Density and Clinicopathologic Features

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Angiogenesis, a process related to tumor growth and malignancy, is stimulated by several growth factors. Among these is vascular endothelial growth factor (VEGF), which acts on endothelial cells by binding with 2 specific receptors, VEGFR1 and VEGFR2. Recent studies have demonstrated that VEGF expression is correlated with microvessel density (MVD) and tumor progression. Digestive endocrine tumors are heterogeneous neoplasms exhibiting variable biological aggressiveness and behavior that often are not predictable on morphologic grounds alone. The aims of this study were to evaluate the expression of VEGF, VEGFR1, and VEGFR2 in digestive endocrine tumors and to examine its correlation with MVD and malignancy. A total of 84 specimens from endocrine neoplasms and normal gut and pancreatic tissue were immunohistochemically studied using specific antibodies directed against VEGF, VEGFR1, VEGFR2, endothelial antigens, and gastroenteropancreatic hormones. Ultrastructural immunocytochemistry was performed to identify the cellular localization of VEGF and the VEGFRs. In normal tissues, VEGF immunoreactivity was detected in G cells and PP cells. Ultrastructurally, VEGF was localized within secretory granules. The VEGFRs were not significantly expressed by normal endocrine cells. VEGF-immunoreactive (IR) cells were detected in 40 of 83 tumors, mainly G cell and enterochromaffin cell neoplasms. VEGFR1-IR cells were found in 44 of 82 tumors, and VEGFR2-IR cells were found in 55 of 82 tumors, with no predilection for any specific tumor type. The expression of VEGF and its receptors did not correlate with MVD or malignancy. These results suggest that in normal tissues, endothelial functions may be regulated by VEGF produced by some endocrine cells and that a VEGF/VEGFR binding mechanism may be involved in tumorigenesis, but not in tumor progression and aggressiveness. HUM PATHOL 34:18-27. Copyright 2003, Elsevier Science (USA). All rights reserved.

Key words: **vascular endothelial growth factor; vascular endothelial growth factor receptor; angiogenesis; microvessel density; digestive endocrine tumor.**

Abbreviations: **IR, immunoreactive; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.**

Angiogenesis, the physiologic formation of new blood vessels, is essential for tissue development, reproduction, and wound healing. Unregulated angiogenesis also plays an important role in the pathogenesis of proliferative retinopathy, rheumatoid arthritis, and cancer.[1] In particular, it has been demonstrated that once a tumor reaches about 1 to 2 mm^3 in size, its growth depends strictly on angiogenesis, which also contributes to the metastatic process, facilitating the shedding of tumor cells into blood vessels.[2] Angiogenesis is generally evaluated by microvessel counting. The number of microvessels has been related to metastatic dissemination, tumor aggressiveness, and patient survival in several human tumors, including melanocytic, breast, lung, prostate, cervical, head and neck, testis, ovarian, gastric, pancreatic, and brain malignancies.[3-5] Tumor-induced angiogenesis depends on the production by tumor cells of proangiogenetic growth factors, which overcome other factors that tend to keep existing vessels quiescent and stable. This process involves several molecules, including those that regulate the maintenance and destruction of perivascular milieu (extracellular matrix and perivascular cells), as well as those stimulating endothelial cell division and migration.[6] The best-characterized proangiogenetic factor is vascular endothelial growth factor (VEGF), which acts specifically on vascular endothelium, the principal expressor of VEGF receptors (VEGFRs). VEGF promotes endothelial proliferation and sprouting processes, which lead to the formation of new microvessel-like structures.[6] The production of VEGF by tumor cells is thought to contribute to the onset of tumor-associated angiogenesis.

The VEGF family includes at least 4 molecules. The molecule commonly called "VEGF" corresponds specifically to VEGF-A, the best-known factor. VEGF-B probably plays an important role in vasculogenesis and may also have other functions, including activation of invasive enzymes on endothelial cells. VEGF-C has been reported to be a potential lymphoangiogenetic factor, whereas the role of VEGF-D is less well defined.[3]

Endocrine tumors of the digestive system are a heterogeneous group of neoplasms showing variable

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Supported in part by a grant from the University of Insubria.

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0046-8177/03/3401-0004$30.00/0
doi:10.1053/hupa.2003.56

TABLE 1. Clinicopathologic Features and VEGF, VEGFR1, and VEGFR2 Expression in Gut and Pancreatic Endocrine Tumors

Type	Site	Sex M/F	Average Age (range) (years)	Average Size (range) (cm)	Malignant	Syndrome	MVD	Vascular Invasion	VEGF	VEGFR1	VEGFR2
ECL cell	Stomach	1/3	70 (63-76)	0.5	0/4	0/4	167	0/4	0/4	0/4	2/4
	Stomach	1/1	49 (43-56)	5 (2-8.3)	2/2	0/2	86	0/2	0/2	1/2	1/2
D cell	*	1/4	48 (38-52)	2.8 (1.5-5)	5/5	1/5	126.2	3/4	1/5	1/5	4/5
G cell	Duodenum	1/3	51 (42-62)	0.7 (0.5-1)	0/2	0/3	158.7	0/4	4/4	4/4	2/4
	Pancreas	0/2	56 (40-73)	1.7 (1.5-2)	2/2	2/2	79	2/2	2/2	2/2	2/2
EC cell	Ileum-colon	9/9	59 (39-89)	2.5 (1.5-8)	19/19	1/19	102†	11/18	9/19	12/19	12/18
	Appendix	5/6	42 (14-95)	0.9 (1.4-2.5)	0/11	0/11	67.4	0/11	6/10	6/10	6/10
	Pancreas	0/2	62 (50-75)	2.5 (2-3)	0/2	0/2	176.5	0/2	1/2	2/2	2/2
L cell	Rectum	2/1	35 (24-66)	0.9 (0.3-1.5)	0/3	0/3	51	0/2	2/3	2/3	1/3
GP	Duodenum	1/0	79	6	1/1	0/1	239	1/1	1/1	0/1	1/1
VIPomas	Pancreas	1/1	64 (57-72)	7.5 (5-10)	2/2	2/2	188	1/2	2/2	1/2	2/2
B cell	Pancreas	0/4	52 (50-54)	1.6 (1-2.5)	0/4	4/4	163	0/4	3/4	2/4	3/4
PP cell	Pancreas	0/1	59	8	1/1	0/1	135	1/1	1/1	1/1	1/1
A cell	Pancreas	0/3	63 (60-66)	1.4 (1-2.1)	0/4	0/4	358	0/4	0/3	3/3	2/3
N cell	Pancreas	1/0	67	6.5	0/1	0/1	152	0/1	0/1	0/1	1/1
Undefined cell	Pancreas	2/0	60 (58-62)	2.5 (2-3)	2/2	0/2	136.5	2/2	1/2	0/2	1/2
PDEC	Various**	14/4	64 (52-72)	5.3 (2-10)	18/18	0/18	76.4	18/18	7/18	7/18	12/18

Abbreviations: GP, gangliocytic paraganglioma; PDEC, poorly differentiated endocrine carcinoma.

*4 cases were duodenal, and 1 case was pancreatic.

**7 gastric, 2 duodenal, 7 colonic, and 2 pancreatic.

†Ileal and colonic MVDs were 95 and 109, respectively, statistically higher ($P = 0.03$) than that of appendiceal tumors (MVD, 67.4).

hormone production, biological aggressiveness, and behavior that often are not predictable on the basis of histopathologic analysis alone.[7,8] Although several immunohistochemical and molecular parameters (i.e., Ki67 expression, p53 nuclear accumulation, overexpression of oncoproteins and growth factors) have been studied,[7,9-12] the biological mechanisms involved in the pathogenesis, growth, and metastatic spread of digestive endocrine tumors are not well understood. Endocrine tumors of the gut and pancreas are known to be well vascularized, but to the best of our knowledge, no data on angiogenesis and its biological meaning for such tumors are available. To date, although a few studies on VEGF expression in digestive endocrine neoplasms have been reported,[13-15] the roles of VEGF in tumorigenesis, tumor growth, and aggressiveness remain unclear.

In this study we evaluated the expression of VEGF and its receptors (VEGFR1-flt1 and VEGFR2-flk1) in a series of gut and pancreatic endocrine tumors and compared this with expression in related normal endocrine cells. In addition, we investigated whether the immunohistochemical expression of VEGF, VEGFR1, and VEGFR2 was correlated with microvessel density (MVD) or the type and malignancy of tumors.

MATERIALS AND METHODS

Tissues

Surgically resected normal tissues and 84 endocrine tumors of the digestive system (64 of the gut and 20 pancreatic) were fixed in buffered formalin (formaldehyde 4% w/v and acetate buffer 0.05 M, pH 7.4) and embedded in paraffin wax. Clinicopathologic data for the tumors are summarized in Table 1. For histopathologic evaluation, 5-μm thick sections were stained with hematoxylin and eosin (H&E) and Grimelius' silver stains. In addition, specimens of both digestive endocrine tumors and normal tissues were fixed in a mixture of paraformaldehyde and glutaraldehyde for electron microscopy and ultrastructural immunocytochemistry investigations.

Immunohistochemistry

Endocrine tumors were characterized using the antibodies and antisera listed in Table 2. Specific polyclonal and monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were used to study VEGF, VEGFR1, and VEGFR2 expression. The anti-VEGF antibody was a rabbit high-affinity purified polyclonal antibody raised against a peptide mapping at the amino acid terminus of human VEGF. The anti-VEGFR1 (flt-1) was a rabbit high-affinity purified polyclonal antibody directed against a peptide mapping at the carboxy terminus of human flt-1. The anti-VEGFR2 (flk-1) was a mouse monoclonal IgG_1 antibody raised against a recombinant protein corresponding to amino acids 1158 to 1345, mapping at the carboxy terminus of flk-1 of mouse origin, which recognizes the same sequence of human flk-1.

The 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 the sections in 3% hydrogen peroxide for 10 minutes at room temperature. The sections were then incubated with specific antibodies (Table 2) for 18 to 20 hours at 4°C. The avidin-biotin-peroxidase procedure was performed next. Peroxidase activity, indicating the presence of the antigen for which we were looking, was finally revealed by 0.03% 3,3′diaminobenzidine, and nuclei were counterstained with Harris' hematoxylin. Colocalization studies to identify the specific cell types expressing VEGF, VEGFR1, and VEGFR2 were performed using double-label immunostains as previously reported.[12] Sections immunostained for glucagon,

TABLE 2. Antibodies and Antisera Used

Antibodies/Antisera	P/M (clone)	Dilution	Source
Glicentin-glucagon	P	1:2500	Milab, Malamo, Sweden
Glucagon	P	1:1250	Milab, Malamo, Sweden
Pancreatic polypeptide	P	1:4000	Cambridge Research Biochemicals, Cambridge, UK
Insulin	M(AE9D6)	1:200	BioGenex Laboratories, San Ramon, CA
Somatostatin	P	1:500	Dako, Copenhagen, Denmark
Somatostatin	M(YC7)	1:10	Immunochimica Labometrics, Milan, Italy
Secretin	P	1:500	Milab, Malamo, Sweden
Serotonin	M(YC5)	1:50	Biogenesis, Bournemouth, UK
Peptide YY	P	1:1000	Biogenesis, Bournemouth, UK
Vasoactive intestinal peptide	P	1:12000	Milab, Malamo, Sweden
C-terminus gastrin-CCK-cerulein	M(B4)	1:10000	Farmitalia, Milan, Italy
Gastrin 34	P	1:500	Cambridge Research Biochemicals, Cambridge, UK
Neurotensin	P	1:6000	Milab, Malamo, Sweden
CD31	M(JC/704)	1:20	Dako, Copenhagen, Denmark
CD34	M(My10)	1:10	Becton Dickinson, San Jose, CA
Factor VIII-related antigen	P	1:1	Biomeda, Hayward, CA
Vascular endothelial growth factor	P	1:400	Santa Cruz Biotechnology, Santa Cruz, CA
Vascular endothelial growth factor receptor 1	P	1:200	Santa Cruz Biotechnology, Santa Cruz, CA
Vascular endothelial growth factor receptor 2	M(A-3)	1:600	Santa Cruz Biotechnology, Santa Cruz, CA

Abbreviation: P/M, polyclonal/monoclonal.

glucagon-glicentin, and somatostatin (polyclonal) were pretreated with 0.003% subtilisin (protease type XXVII or Nagarse protease; Sigma, St Louis, MO) in 0.05 M Tris-buffered saline, pH 7.4, at room temperature for 10 minutes. Sections immunostained for VEGF, VEGFR1, and CD31 were pretreated with 0.05% trypsin (Sigma) in 0.05 M Tris-buffered saline, pH 7.4, at 37°C for 20 minutes, whereas sections immunostained for VEGFR2 were pretreated with an EDTA buffer, pH 8, in a microwave oven at 700 W for 10 minutes.

Specificity controls consisted of (1) absorption of every antibody with 10 to 20 nmol of related antigen, (2) substitution of the primary antibody with nonimmune serum of the same species at the same dilution, and (3) the use of control tissues with or without the pertinent antigens. The specificity of antibodies directed against VEGF, VEGFR1, and VEGFR2 was also tested previously.[15,16]

Electron Microscopy and Ultrastructural Immunocytochemistry

For ultrastructural analysis, small samples of gastrinomas and normal gut tissues were fixed in a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, for 2 hours at 4°C. They were then postfixed in osmium tetroxide (1% in 0.1 M cacodylate buffer, pH 7.4) for 1 hour at room temperature and embedded in Epon-Araldite (Fluka, Buchs, Switzerland). Ultrathin sections were collected on Formvar-coated nickel, 200-mesh grids, counterstained with uranyl acetate and lead citrate, and observed with an electron microscope (Model CM 120; Philips, The Netherlands).

Ultrastructural immunocytochemistry was done using the immunogold labeling technique. Small tissue samples were fixed in a solution of 2% paraformaldehyde and 0.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, for 2 hours at 4°C and embedded in London White resin (Polysciences, Warrington, PA). Ultrathin sections were preincubated for 5 minutes with ovoalbumin in 0.01 M Tris-buffered 0.5 M NaCl, pH 7.4, followed by incubation for 12 hours at 4°C with antibodies directed against VEGF, VEGFR1, and VEGFR2. After 3 washes (10 minutes each) in Tris-buffered NaCl to remove nonspecifically bound and unbound antibodies, sections stained with anti-VEGF and anti-VEGFR1 antibodies were incubated for 1 hour with 1:50 diluted gold-tagged goat anti-rabbit Ig antibodies (EY Laboratories, San Mateo, CA), and sections stained for VEGFR2 were incubated with 1:20 diluted gold-tagged horse anti-mouse Ig antibodies (EY Laboratories). Finally, the grids were washed in Tris-buffered NaCl, rinsed in distilled water, dried, and stained for 5 minutes with aqueous uranyl acetate (5%) and for 5 minutes with Reynolds lead citrate. The sections were finally examined with a Philips CM 120 electron microscope.

Evaluation Of Intratumor MVD

Intratumor MVD, representing the number of microvessels present in a well-defined area of the tumor, was evaluated using the criteria described by Weidner.[17] The count was performed on sections immunostained with antibodies directed against endothelial antigens CD31, CD34, and factor VIII–related antigen. Areas of highest vascularization were found by scanning tumor sections at low power (×40 and ×100 total magnification, corresponding to areas of 19.62 mm^2 and 3.141 mm^2, respectively) and selecting those areas with the greatest density of distinct factor VIII–related antigen–, CD31-, and CD34-stained microvessels. After the area of highest vascularization was identified, individual microvessel counts were made at ×200 magnification (0.785 mm^2 area) in 3 different fields using a Leitz Diaplan microscope (Leitz Microscope, Weitzmar, Germany). Any highlighted endothelial cell or endothelial cell cluster clearly separate from adjacent microvessels, tumor cells, and other connective tissue elements was considered a single, countable microvessel. Even those distinct clusters of brown-staining endothelial cells, which might be from the same microvessel snaking its way in and out of the section, were considered distinct, countable, and separate microvessels. The presence of a vessel lumen was not necessary for a structure to be defined as a microvessel, and red cells were not used to define a vessel lumen. According to Bosari et al,[18] vessels of a caliber larger than approximately 8 red blood cells and vessels with a thick muscular wall were excluded from the count. Using the same criteria,

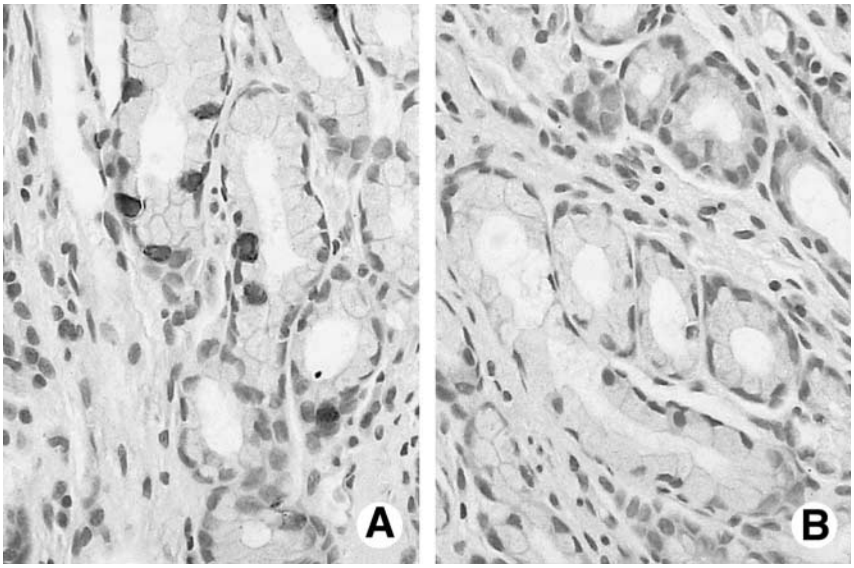

FIGURE 1. (A) VEGF immunoreactivity in several endocrine cells of the antral mucosa. (B) After absorption of the anti-VEGF antibody with the VEGF antigen, the immunoreaction is completely abolished. (Immunoperoxidase with hematoxylin counterstaining.)

the number of microvessels was also counted in normal mucosa of the gut and in pancreatic islets.

Statistical Analysis

Expression of VEGF, VEGFR1, and VEGFR2 among the various tumor types was evaluated statistically using Fisher's exact test. The relationship between VEGF, VEGFR1, VEGFR2 expression and vessel density was evaluated using Wilcoxon's rank sum test.

RESULTS

Normal Tissues

VEGF immunoreactivity was found in several endocrine cells of the antral and duodenal mucosa, but not in the other portions of the gut (Fig 1). The immunoreactivity was cytoplasmic, granular, and intense. Colocalization studies indicated that gastrin-producing G cells were VEGF immunoreactive, whereas the other normal endocrine cells of the gut, including somatostatin-, motilin-, secretin-, and serotonin-producing enterochromaffin cells, were negative. Ultrastructural immunocytochemical analysis demonstrated that VEGF was localized within the secretory granules of G cells, but not in the other types of normal endocrine cells of the antral and duodenal mucosa (Fig 2). In the pancreas, an intense VEGF immunoreactivity was seen in some islet cells. VEGF-IR cells were numerous in the islets of the PP-rich part of the pancreatic head (Fig 3), where double-label immunohistochemistry demonstrated that they corresponded to PP cells. VEGF-IR cells were rare in the other islets of the head and in the islets of the body and tail. In addition to endocrine cells, VEGF immunoreactivity was observed in some endothelial cells, in ganglion cells of the myenteric plexus, and in acinar cells of the pancreas. Here the intensity of VEGF staining was weaker than that observed in endocrine cells.

VEGFR1 immunoreactivity was observed in rare endocrine cells of the antroduodenal mucosa and of the pancreatic islets, identified morphologically. The pattern of immunoreactivity was cytoplasmic and granular, although increased staining at the membrane level was observed in some cells. No VEGFR2 immunoreactivity was observed in normal endocrine cells distributed along the gut or in the pancreatic islets. Endothelial cells in all gut and pancreatic samples examined were positively stained by both the anti-VEGFR1 and VEGFR2 antibodies. The mean number of microvessels in normal gut mucosa and in pancreatic islets is reported in Table 3.

Endocrine Tumors

VEGF-IR cells were identified in 40 of 83 (48%) endocrine tumors. With the exception of ECL-cell, A-cell, and neurotensin (N)-cell neoplasms, VEGF-IR cells were observed in all of the neoplasms investigated but with some differences among the different functional types of tumors (Table 1). In addition, the percentage of positive cells and the intensity of the immunostaining also varied among various types. In particular, all G-cell tumors, whether or not associated with the Zollinger-Ellison syndrome, were VEGF positive (Fig 4) and had the highest percentage of VEGF-positive cells (mean percentage of positive cells, 64%). Sixteen of 31 (51.6%) EC-cell tumors of the gut were also positive (Fig 5), but their mean percentage of positive cells was lower (38%) than that of gastrinomas. Among pancreatic endocrine tumors, 3 of 4 insulinomas, 1 of 1 PP-cell tumor, 2 of 2 VIPomas were VEGF positive (Fig 6). VEGF expression was not significantly correlated with the presence of endocrine symptoms, tumor diameter, metastatic disease, MVD, or vascular invasion. Electron microscope immunocytochemical analysis performed on 2 cases of gastrin cell tumors showed that, as in normal G cells, the VEGF immunoreactivity was localized within the secretory granules (Fig 4).

VEGFR1 immunoreactivity was found in 44 of 82 (53.6%) endocrine tumors, mainly in insulinomas and G cell, EC cell, L cell, and A cell neoplasms. Among these, the strongest immunostaining and the highest percentage of VEGFR1-IR cells were observed in gastrinomas (Fig 4). VEGFR2-IR cells were observed in 55 of 82 (67%) tumors investigated, without any significant predilection for a specific tumor type (Fig 4). As for VEGF expression, the positivity of both VEGFR1 and VEGFR2 was not correlated with MVD, vascular invasion, or tumor malignancy. In addition to tumor cells, vascular endothelial cells expressed both VEGFRs. At the ultrastructural level, we were unable to obtain convincing and specific immunoreactivities with the antibodies directed against VEGFR1 and VEGFR2. Thirty-four tumors coexpressed VEGF and at least 1 of 2 receptors: 8 tumors coexpressed VEGF and VEGFR1, 7 tumors coexpressed VEGF and VEGFR2, and 19 neoplasms coexpressed VEGF, VEGFR1, and VEGFR2.

Unlike the expression of VEGF and of its receptors, we observed a relationship between malignancy and MVD for intestinal EC-cell tumors. In particular, EC-cell neoplasms of the ileocolonic region, which were all malignant, had a statistically greater ($P = 0.03$) MVD (mean value, 102) than that of appendiceal ones, which were all benign (mean value, 67.4). On the other hand, considering all 84 tumors without differentiating them in terms of type and site, no relationship between MVD and malignancy was observed.

The mean value of MVD in endocrine tumors is reported in Table 3. No significant differences in MVD were observed between normal and neoplastic tissues in gastric, duodenal, and colonic mucosa. In contrast, ileal endocrine tumors had a significantly higher number of vessels than the normal ileal mucosa ($P < 0.05$). In the pancreas we found the opposite; the number of capillaries in the normal islets was statistically higher ($P = 0.01$) than that of pancreatic endocrine tumors.

DISCUSSION

In this study we have investigated the expression of VEGF, VEGFR1, and VEGFR2 in normal endocrine cells of the gut and pancreas and in a large series of endocrine tumors of the digestive system. We observed strong VEGF immunoreactivity in several endocrine cells of the antral and duodenal mucosa, corresponding to gastrin-producing G cells. Our observations were

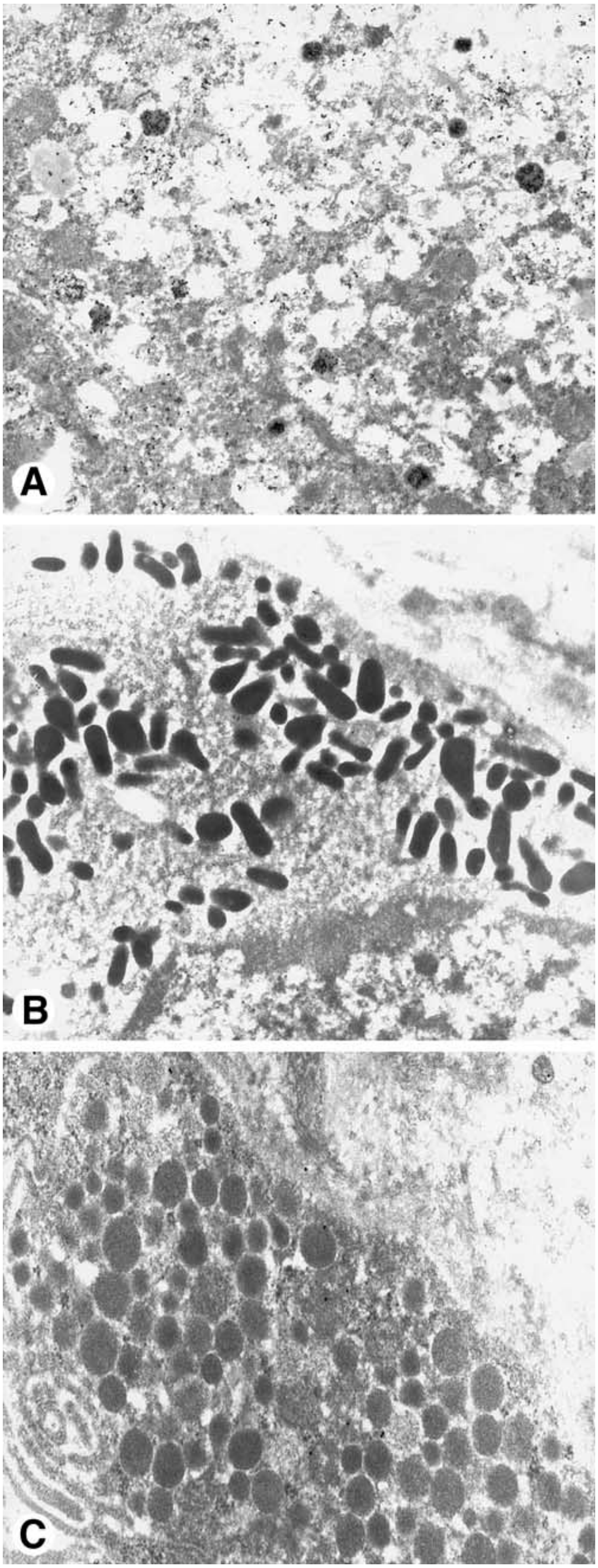

FIGURE 2. Ultrastructural immunocytochemistry demonstrates that VEGF immunoreactivity is localized in the secretory granules of G-cells (A), whereas secretory granules of EC-cells (B) and D-cells (C) are completely negative. (Original magnification (A, B) ×18,400, (C) ×29,900.)

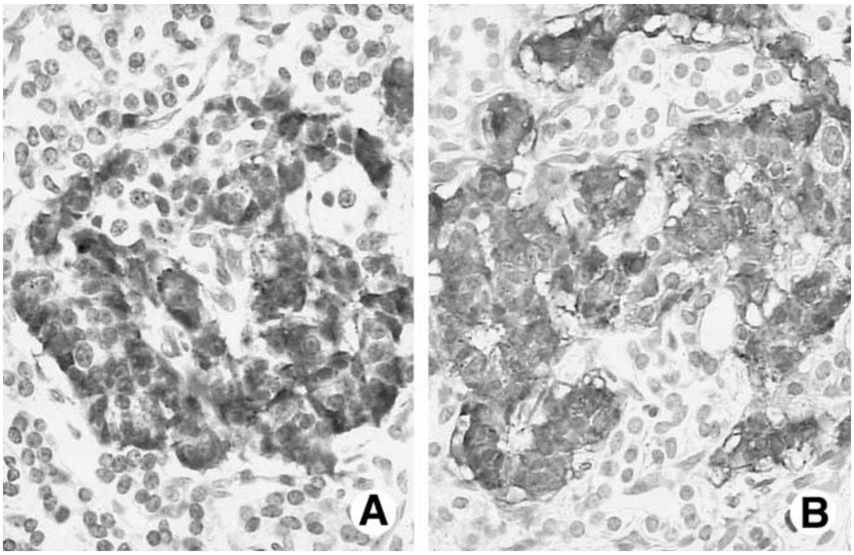

FIGURE 3. VEGF immunoreactivity in almost all cells of an islet localized in the PP-rich lobe (A), where the islets show a trabecular structure and are mainly composed by PP-cells as demonstrated by strong immunoreactivity for PP (B). (Immunoperoxidase with hematoxylin counterstaining.)

confirmed by the immunoelectron microscopic localization of VEGF in secretory granules of G cells but not in those of other endocrine cell types of the gut. In the pancreas we observed strong VEGF immunoreactivity in PP cells of the islets, whereas B, A, and D cells were negative. The expression of VEGF in normal endocrine cells of the gut and pancreas has been poorly investigated, and the reported results are different and contradictory in some studies relating to the antibodies used and the series and species investigated.[13-15,19,20,22-24]

The significance of VEGF expression in endocrine cells of the gut and pancreas is not clear. The fact that it is present in the secretory granules along with the hormone, as demonstrated in the present study, may suggest that VEGF is released together with the hormone. VEGF may contribute to the maintenance of the endothelial cell subsets associated with endocrine tissues, especially considering that VEGF has been demonstrated to induce fenestration in neovessels[25] and that islet capillaries are lined by fenestrated endothelial cells.[26]

TABLE 3. Mean Value of Microvessels in Normal Tissues and in Endocrine Tumors

	Normal		Tumors	
Site	Mean	95% Confidence Interval	Mean	95% Confidence Interval
Stomach*	128.5	117.62-140.1	117.69	111.86-123.74
Duodenum*	140	124.00-157.39	130.54	123.88-137.47
Ileum†	67.66	58.67-77.64	95.14	90.10-100.39
Colon*	66.66	57.74-76.57	84	79.26-88.94
Pancreatic islets§	279.4	264-294	174	168-179

*The difference is not statistically significant.
†The difference is statistically significant ($P < 0.05$).
§The difference is statistically significant ($P = 0.01$).

Little is known about the expression of VEGF receptors in normal endocrine cells of the gut and pancreas. Both VEGFR1 and VEGFR2 mRNAs have been found to be present in islet endothelial cells, whereas endocrine cells seemed to be negative.[13,27] No VEGFR2 immunoreactivity was seen in normal endocrine cells of the gut and pancreas, whereas only rare and scattered endocrine cells were VEGFR1 positive. Taken together, these results suggest that in normal conditions, VEGF/VEGFRs binding is not an important mechanism in the modulation of biological functions of gut and pancreatic endocrine cells, but, as has been reported earlier, it may play a role in inducing vessel permeability and modulating other endothelial functions.

Our results proving that VEGF and its receptors are widely expressed in endocrine tumors of the gut and pancreas add new information to the previous investigations on this subject.[14,15] In the study of Terris et al,[15] VEGF expression was found in 25 gut carcinoids, represented mainly by midgut tumors (16 cases), and in 16 pancreatic endocrine tumors, represented mainly by nonfunctioning neoplasms (9 cases). In addition, Kuroda et al[14] reported VEGF expression in 3 of 7 insulinomas but no expression in other types of pancreatic endocrine neoplasms, including 1 A-cell, 3 D-cell, and 1 G-cell tumors. Our data confirm that EC-cell tumors, probably corresponding to the midgut carcinoids described by Terris et al,[15] do express VEGF, although we also observed a strong VEGF immunoreactivity in G-cell tumors, both duodenal and pancreatic, and in rectal L-cell carcinoids. Our results regarding VEGF expression in pancreatic endocrine tumors (Table 4) are also in agreement with those previously reported;[14,15] in our study, however, pancreatic tumor types that had not previously been examined for VEGF expression, such

FIGURE 4. Duodenal gastrinoma. (A) VEGF immunoreactivity in the cytoplasm of numerous cells. (B) As in normal cells, the immunostaining is localized in the secretory granules. (Original magnification ×29,900.) The same tumor is also positive for VEGFR1 (C) and VEGFR2 (D). (Immunoperoxidase with hematoxylin counterstaining.)

as EC-cell tumors, neurotensin-cell tumors, VIPomas, and poorly differentiated endocrine carcinomas, were also analyzed.

The expression of VEGF by gut and pancreatic endocrine tumors supports the hypothesis that this growth factor may be involved in tumor angiogenesis. It assumes a particular interest considering that these tumors are generally well vascularized. However, we have not found any significant relationship between VEGF expression and MVD. Although this result may seem contradictory, it is not really surprising. The fact that VEGF has no correlation with MVD does not rule out a crucial role of VEGF as a key factor in tumor angiogenesis. Angiogenesis is a very complicated biological mechanism in which several growth factors cooperate, with different power, to stimulate vascular proliferation. Although VEGF seems to be the most important proangiogenetic factor, it has recently been demonstrated that VEGF induction of angiogenesis is potentiated by the synergistic action of other growth factors, including acidic and basic fibroblast growth factors[28,29] and hepatocyte growth factor.[30] In this context, it is of interest to recall that endocrine tumors of the digestive system, and in particular EC-cell tumors of the midgut, are known to produce several proangiogenetic factors, including acidic and basic fibroblast growth factors, transforming growth factor-α, and hepatocyte growth factor.[9,11,31] Thus the relationship between VEGF and MVD that we have investigated may be highly influenced by the presence of other growth factors.

The localization of the two VEGF receptors in digestive endocrine tumors has not been previously reported. We found VEGFR1 expression in 44 of 82