High Expression of Growth Factors and Growth Factor Receptors in Ovarian Metastases From Ileal Carcinoids

An Immunohistochemical Study of 2 Cases

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• ***Objective and Design.***—**Ovarian metastatic carcinoids are rare neoplasms that show prominent fibrosis of tumor stroma and are often associated with peritoneal carcinomatosis. We studied formalin-fixed and paraffin-embedded tumor specimens of 2 cases of ovarian metastases from ileal enterochromaffin cell carcinoids immunohistochemically to evaluate whether acidic fibroblast growth factor (aFGF), transforming growth factor-α (TGFα), and their respective receptors (fibroblast growth factor receptor-4 [FGFR4] and epidermal growth factor receptor [EGFR]) may play a role in the pathogenesis of stromal fibroblast reaction and in the mechanism of tumor dissemination.**

Results.—**In both cases, the majority of tumor cells expressed immunoreactivity for aFGF, FGFR4, and TGFα. Immunoreactivity for FGFR4 was detected in stromal cells of both cases, while EGFR-positive stromal cells were found in only 1 case. Immunoreactivity for FGFR4 was also found in peritoneal mesothelial cells.**

Conclusions.—**The coexpression of aFGF and FGFR4 in neoplastic enterochromaffin cells suggests that aFGF may act as an autocrine factor stimulating tumor cell growth. In addition, aFGF and TGFα may stimulate, in a paracrine fashion, the proliferation of FGFR4- and EGFR-immunoreactive stromal fibroblasts. Finally, interaction of aFGF-immunoreactive enterochromaffin cells with FGFR4-bearing mesothelial cells may play a role in the mechanism of serosal implant and spread of tumor cells.**

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Carcinoid tumors of the ovary are rare. The majority are primary, but occasional examples of metastatic carcinoid tumors involving the ovaries have been reported.[1–7] Metastatic ovarian carcinoids account for about 2% of all ovarian metastases.[8,9] The primary tumors are generally located in the small intestine, while they are rarely found in the large bowel, appendix, stomach, pancreas, and lung.[1–6] In the largest series of metastatic ovarian carcinoids reported in the literature, the primary endocrine neoplasms were ileal in 51.4% of cases. The prognosis for the women in that study was poor; in fact, 30% of patients died within 1 year after diagnosis, and 75% died within 5 years.[2]

In this study, we analyzed the clinical and pathologic features of 2 metastatic ovarian carcinoids whose primary tumors were located in the ileum. The importance of growth factors in the progress of tumor metastases, along with the striking finding of extensive ovarian fibrosis, prompted us to study the expression of acidic fibroblast growth factor (aFGF), transforming growth factor-α (TGFα), and their respective receptors (fibroblast growth factor receptor-4 [FGFR4] and epidermal growth factor receptor [EGFR]) in these types of ovarian metastases.

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MATERIALS AND METHODS

Samples of tumors were fixed in buffered formalin (formaldehyde 4% w/v and acetate buffer 0.005 mol/L) and routinely processed in paraffin wax. Sections were stained with hematoxylin-eosin, Grimelius' silver stain for endocrine granules, and van Gieson's stain for the evaluation of fibrous stroma. Histologic architecture was cathegorized according to Soga and Tazawa.[10]

Immunohistochemical stains were performed on 3-μm sections, which were mounted on poly-L-lysine–coated slides and deparaffinized and hydrated through graded alcohols to water. The sections were incubated for 10 minutes with 3% hydrogen peroxide to inhibit endogenous peroxidase. Primary antibody (Table 1) incubations were done at 4°C for 18 hours followed by the avidin-biotin-peroxidase complex (ABC) procedure according to Hsu et al.[11] The immunoreaction was visualized using 3,3'diaminobenzidine tetrahydrochloride as the chromogen. Sections stained for aFGF, FGFR4, TGFα, and the α and β subunits of inhibin/activin were pretreated with 0.01 mol/L citrate buffer, pH 6, for 10 minutes in a microwave oven at 650 W. Sections stained for EGFR were pretreated with pepsin (Sigma Chemical Co, St Louis, Mo; 1 mg/mL, 0.5 acetic acid) for 2 hours at 37°C. Finally, sections stained for calcitonin, CD31, epithelial membrane antigen, and prostatic acid phosphatase were pretreated with 0.05% trypsin in 0.05 mol/L tris-buffered saline, pH 7.4.

RESULTS

Clinicopathologic Findings

The first patient (case 1), a 73-year-old woman, was admitted to the hospital for abdominal pain, a recent history

Table 1. Antisera and Antibodies Used

Antibody/Antisera	P/M (Clone)	Dilution	Source
Serotonin	M (YC5)	1:50	Biogenesis, Baunemonth, United Kingdom
Substance P	P	1:640	Milab, Malmo, Sweden
Acidic fibroblast growth factor	P	1:100	UBI, Lake Placid, NY
Fibroblast growth factor receptor-4	P	1:100	Santa Cruz Biotechnology Inc, Santa Cruz, Calif
Transforming growth factor-α	P	1:2000	Biogenesis
Epidermal growth factor receptor	M (E30)	1:20	BioGenex, San Ramon, Calif
Calcitonin	P	Undiluted	Ortho Diagnostic System, Raritan, NJ
Prostatic acid phosphatase	P	1:400	Dakopatts A/S, Glostrup, Denmark
CD31	M (JC/70A)	1:20	Dakopatts
α-Smooth muscle actin	M (1A4)	1:10 000	Sigma Immunochemicals, St Louis, Mo
Human inhibin 32-Kd α subunit	M (R1)	1:100	Serotec Ltd, Oxford, England
βA subunit of inhibin/activin	M (E4)	1:100	Serotec

of weight loss and diarrhea, and a slight elevation of CA 125 serum level (64 U/mL; normal 0–35 U/mL). The patient underwent a diagnostic laparotomy that revealed peritoneal carcinomatosis and bilateral ovarian masses. The terminal ileal segment was surrounded by a desmoplastic mass, which also involved the right iliac vein and the ileocolic artery, making surgical resection impossible. An intestinal bypass operation with total hysterectomy and bilateral salpingo-oophorectomy was performed. Postoperatively, the patient was treated with 5-fluorouracil, epirubycin, and streptozotocin, and she is alive with disease 4 months after diagnosis.

The second patient (case 2) was a 69-year-old woman who presented with complaints of vaginal bleeding, pelvic pain, and weight loss. Laboratory investigations showed slightly elevated levels of serum carcinoembryonic antigen, 4 ng/mL (normal <2.5 ng/mL), and CA 125, 40.1 U/mL (normal 0–35 U/mL). Exploratory laparotomy revealed peritoneal carcinomatosis and bilateral ovarian masses. A total abdominal hysterectomy with bilateral salpingo-oophorectomy, omentectomy, and multiple peritoneal biopsies was performed. Pathologic findings of metastatic carcinoids identified a primary ileal tumor, which was surgically resected. The patient died of widespread metastases 37 months after presentation.

At gross examination, both ovaries of case 1 were replaced by white-yellow, multinodular, solid masses of 2.9 and 3.2 cm. Multiple metastases up to 0.5 cm in diameter were identified in the omentum. In case 2, the left ovary was replaced by a solid multinodular tumor measuring 7.5 cm, while the right showed a 3-cm solid mass. Multiple nodular metastases measuring up to 0.8 cm in diameter were identified in the peritoneum and omentum.

Microscopically, in both cases, the tumors showed an insular architecture (type A). A focal glandular-like pattern (type C) (Figure 1) was identified only in case 1. Neoplastic cells showed abundant eosinophilic and finely granular cytoplasm, as well as moderate nuclear atypia with occasional prominent nucleoli. Moderately high mitotic indexes were identified in tumors of both cases (7 and 4 × 10 high-power fields, respectively). The islands of tumor cells were laid in a fibromatous stroma, which was scant in some tumoral areas but prominent in other fields (Figure 1). A few neoplastic emboli were found in small vessels. Careful examination of tumor sections revealed no teratomatous structures.

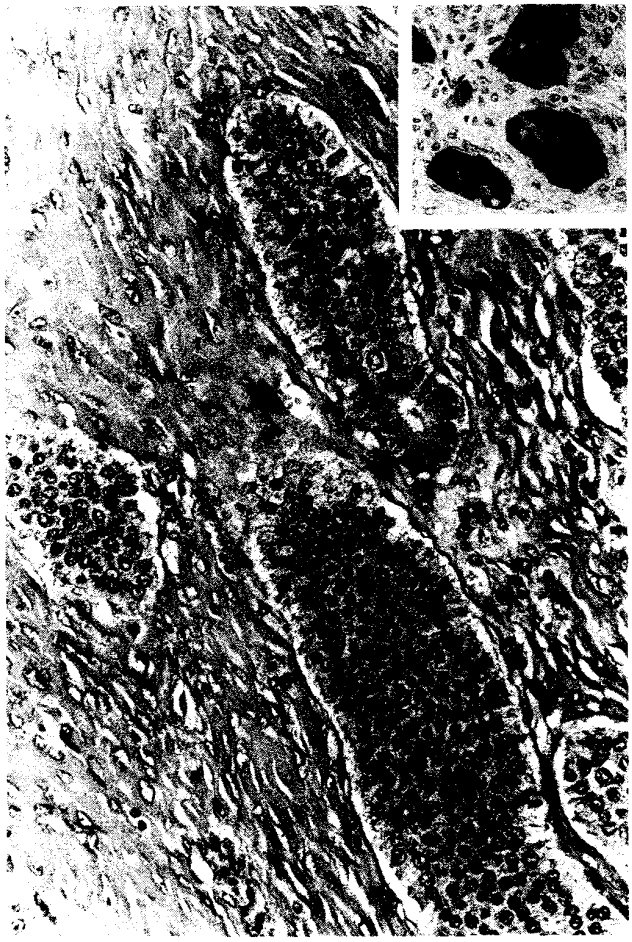

Figure 1. *Case 1. Metastatic ovarian carcinoid showing an insular and glandular-like pattern with abundant fibrous stroma (hematoxylin-eosin, original magnification ×200). Inset, Neoplastic cells are immunoreactive for serotonin.*

Immunohistochemical Findings

The immunohistochemical results are summarized in Table 2. All neoplastic cells of both cases were immunoreactive with serotonin, and 15% to 60% of the neoplastic cells were positive for substance P. Only a few tumor cells (from 2% to 5%) were immunoreactive for prostatic acid phosphatase, but no cells immunoreactive for calcitonin were present in either case. Immunohistochemical staining for aFGF was granular and diffuse in the cytoplasm of several cells (Figure 2). In case 1, aFGF-immunoreactive cells represented about 70% of the tumor cell population and were distributed in all areas, whereas in case 2, they

Table 2. Immunohistochemical Results*

Case No.	GRIM	5HT	SubP	Cal	PAP	aFGF	FGFR4 Tumor Cells	FGFR4 Stromal Cells	TGFα	EGFR Tumor Cells	EGFR Stromal Cells
1	100*	100	60	0	5	70	80	+	60	0	+
2	100	100	15	0	2	10	30	+	60	0	−

* Values are presented as the percentage of positive cells. GRIM indicates Grimelius silver stain; 5HT, serotonin; SubP, substance P; Cal, calcitonin; PAP, prostatic acid phosphatase; aFGF, acidic fibroblast growth factor; FGFR4, fibroblast growth factor receptor-4; TGFα, transforming growth factor-α; EGFR, epidermal growth factor receptor; +, presence of positive cells; and −, absence of positive cells.

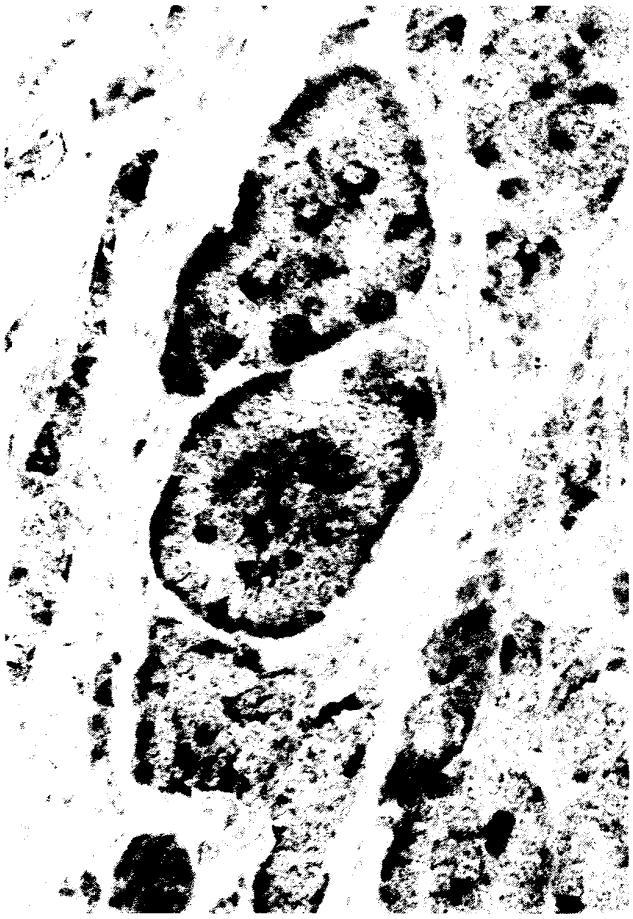

Figure 2. *Case 1. Positive immunostaining of neoplastic cells for acidic fibroblast growth factor (ABC technique with hematoxylin counterstain, original magnification ×200).*

Figure 3. *Case 1. Fibroblast growth factor receptor-4 immunoreactivity in the majority of epithelial neoplastic cells and in some stromal cells of case 1 (ABC technique with hematoxylin counterstain, original magnification ×200).*

represented about 10% of neoplastic cells and were scattered randomly. A cytoplasmic-diffuse FGFR4 immunoreactivity, which displayed a more intense reaction at the membrane level in some cells, was found in 80% (case 1) and 30% (case 2) of epithelial neoplastic cells (Figure 3). In 60% of cells of both neoplasms, an intense cytoplasmic, granular-diffuse staining for TGFα was expressed (Figure 4), whereas no neoplastic cell displayed any EGFR immunoreactivity. Immunoreactivity for FGFR4 was detected in stromal cells of both cases, while EGFR-positive stromal cells were found in only 1 case. Furthermore, mesothelial cells of the ovarian and salpingeal surface were immunoreactive for FGFR4.

A discrete number of stromal smooth muscle cells, identified with anti-α-smooth muscle–specific actin antibodies, was found within the stroma of metastatic carcinoids, whereas they were absent in the normal residual ovaries. Immunoreactivities for α and βA subunits of inhibin/activin were found in polygonal luteinic-like cells of the residual ovarian stroma, but not in the stroma interposed between tumor nests. CD31 immunostaining of endothelial cells confirmed the presence of neoplastic emboli in case 1.

COMMENT

Fewer than 2% of ovarian metastases are represented by metastatic carcinoids, whose primary tumors are more

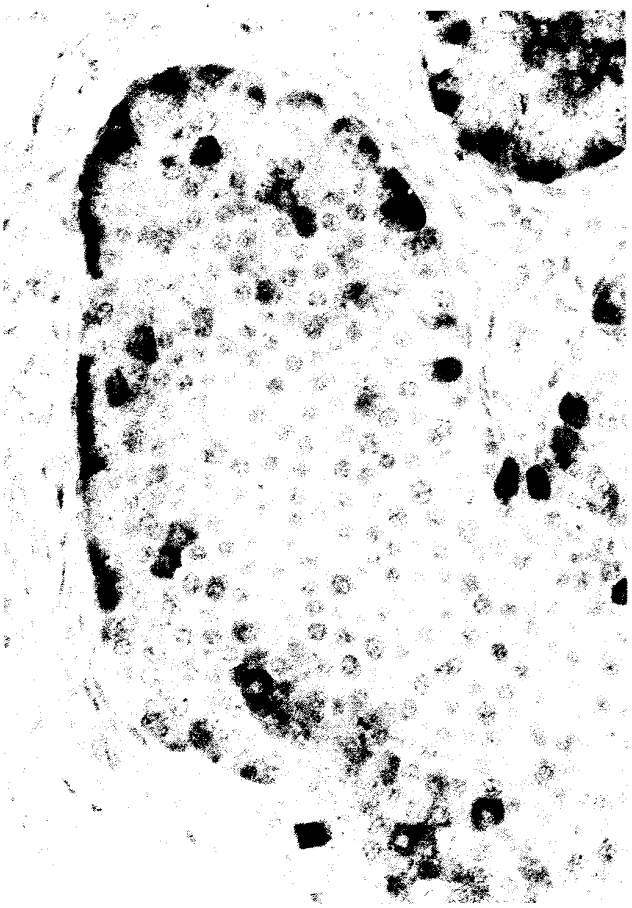

Figure 4. *Case 1. Immunoreactivity for transforming growth factor-α in neoplastic epithelial cells (ABC technique with hematoxylin counterstain, original magnification ×200).*

frequently located in the ileum.[8,9] Robboy et al[2] reported that the majority (65.7%) of ovarian metastases from ileal carcinoids in their study were associated with peritoneal carcinomatosis. This association has also been found in our 2 cases and in a case recently reported by Vasseur et al.[12] The mechanism of abdominal extension from ileal carcinoids is unknown. On the basis of our results, it may be speculated that the interactions between aFGF and FGFR4 may play a role in serosal colonization. In fact, neoplastic cells of ileal carcinoids express aFGF, whereas mesothelial cells are positive for FGFR4, one of its high-affinity transmembrane receptors. It is possible that aFGF-positive cells of ileal carcinoids may easily stick to FGFR4-bearing mesothelial cells. A similar interaction between growth factor and relative receptor has been suggested to explain the pathogenesis of neural invasion in pancreatic carcinoma, in which the interaction was between EGFR present in nerves and TGFα expressed by cancer cells.[13]

A prominent feature of enterochromaffin cell carcinoids metastatic to the ovary is the fibrous stroma, which is particularly abundant in some areas. It is well known that intestinal enterochromaffin cell carcinoids are associated with significant stromal fibrosis, and these tumors produce several growth factors that may stimulate the proliferation of stromal fibroblasts and smooth muscle cells.[14–20] Recent studies on gut enterochromaffin cell carcinoids have shown that aFGF colocalizes with serotonin in tumor cells, and that aFGF expression positively correlates with the amount of fibrous stroma.[19] The demonstration, by immunohistochemical analysis in the 2 cases here reported, of cytoplasmic aFGF immunoreactivity in neoplastic endocrine cells and of FGFR4 expression in stromal cells favors the hypothesis that aFGF may stimulate stromal cell proliferation. This hypothesis, however, has to be confirmed by cell biology studies showing that tumor stromal cells isolated in vitro are activated by aFGF and that this action of aFGF is exerted by binding to surface receptors having intrinsic tyrosine kinase activity. To our knowledge, the expression of growth factors and growth factor receptors in ovarian metastases has not been previously reported in the English literature. However, it is interesting to recall that some cancers of the gatrointestinal tract, which can give origin to ovarian metastases showing a prominent fibrous stroma, have been found to express growth factors, including aFGF.[21] Studies on the expression of FGFR4 or other subtypes of fibroblast growth factor receptors in stromal cells of these types of ovarian metastases are therefore needed to verify a possible role of aFGF in the pathogenesis of the desmoplastic stromal reaction.

In the two ovarian metastases examined in this study, the stroma contained, in addition to fibroblasts, a discrete number of smooth muscle cells. However, it lacked inhibin/activin–immunoreactive cells, which were detected in normal residual ovarian stroma. Interestingly, this combination of stromal cells reflects that found in primary ileal enterochromaffin cell carcinoids,[19] but is lacking in normal ovarian stroma.

An additional growth factor that may contribute to stromal reaction around metastatic ovarian carcinoids is TGFα, which was detected in the tumor cells of our cases. This factor may bind to EGFR present in stromal fibroblasts in a way similar to that of aFGF.

On the basis of our results, the establishment of an autocrine growth response in metastatic ovarian carcinoids, which may regulate tumor growth, seems possible for aFGF because tumor cells simultaneously express aFGF and FGFR4. This response can probably be excluded for TGFα, however, since tumor cells lack the TGFα receptor (ie, EGFR).

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