Expression of Molecular Targets for Tyrosine Kinase Receptor Antagonists in Malignant Endocrine Pancreatic Tumors[1]

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ABSTRACT

Purpose: **Molecular targeting with monoclonal antibodies and tyrosine kinase inhibitors is a novel approach to cancer treatment. We have examined the expression of molecular targets in patients with malignant endocrine pancreatic tumors, which is necessary to justify additional studies investigating the potential benefit from such treatment.**

Experimental Design: **Thirty-eight tumor tissues from malignant endocrine pancreatic tumors were examined with immunohistochemistry using specific polyclonal antibodies with regard to the expression pattern of platelet-derived growth factor receptors (PDGFRs) α and β, c-kit, and epidermal growth factor receptor (EGFR).**

Results: **All 38 tissue specimens expressed PDGFRα on tumor cells, and 21 of 37 specimens (57%) expressed PDGFRα in tumor stroma (1 specimen was nonevaluable). Twenty-eight samples (74%) stained positive for PDGFRβ on tumor cells, and 36 of 37 samples (97%) stained positive for PDGFRβ in the stroma (1 specimen was nonevaluable). Thirty-five tumor tissues (92%) stained positive for c-kit, and 21 (55%) stained positive for EGFR on tumor cells. No differences were seen between syndromes or between poorly differentiated or well-differentiated tumors. Previous treatment did not influence expression pattern. Receptor expression pattern varied considerably between individuals.**

Conclusions: **We have found that tyrosine kinase receptors PDGFRs α and β, EGFR, and c-kit are expressed in more than half of the patients with endocrine pancreatic tumors. Because these receptors represent molecular targets for STI571 and ZD1839 (tyrosine kinase inhibitors) and IMC-C225 (a monoclonal antibody), we propose that patients suffering from EPTs might benefit from this new treatment strategy. However, because of great variability in receptor expression pattern, all patients' individual receptor expression should be examined.**

INTRODUCTION

Molecular targeting is becoming increasingly important in modern cancer treatment. Among the most interesting and promising targets today are PDGFRα,[3] PDGFRβ, c-kit, and EGFR, all of which belong to the tyrosine kinase receptor family. Various approaches can be used to block target activity. The most common are Mabs directed toward the tyrosine kinase receptor (1) and TKIs that inhibit receptor phosphorylation (2).

The best-known commercially available Mab directed toward a tyrosine kinase receptor, HER2, is trastuzumab (Herceptin; L. Hoffman-La Roche Ltd., Basel, Switzerland). Treatment with trastuzumab has shown good response rates, both alone and in combination with paclitaxel, in patients with breast cancer overexpressing HER2 (3, 4). Another Mab targeting a different tyrosine kinase receptor, EGFR, is IMC-C225 (cetuximab; Imclone Systems Inc., Somervil, NJ), which has shown promising results in preclinical trials (5, 6). Furthermore, clinical studies have shown enhanced antitumor activity in combination with chemo- and radiotherapy (7, 8).

Several small molecule TKIs are being developed and clinically evaluated. Two of these are STI571 (Glivec; Novartis Pharma, Basel, Switzerland) and ZD1839 (Iressa; Astra Zeneca, Wilmington, DE). STI571 inhibits the activity of PDGFRs α and β, c-kit, and bcr-abl tyrosine kinases (9). Clinically, STI571 has shown good response rates in chronic myelogenous leukemia (10), where the nearly patognomone Philadelphia gene results in a continuously activated bcr-abl fusion protein with tyrosine activity. STI571 has also induced dramatic tumor responses in GISTs that frequently have mutations in the c-kit tyrosine kinase (11, 12). Additionally, Pietras *et al.* (13) have shown that treatment with STI571 inhibits PDGFRβ in tumor stroma, which reduces interstitial hypertension and increases transcapillary transport in s.c. growing rat colon carcinomas. This could lead to a novel strategy to increase drug uptake and hence augment the effectiveness of chemotherapy. The clinical benefit from inhibition of PDGFRα by STI571 is not yet known.

Amplification of EGFR is common in solid tumors and is usually associated with more aggressive tumor growth and poor clinical outcome (14). ZD1839 is a selective inhibitor of EGFR tyrosine kinase, and preliminary data from Phase I and II studies show encouraging antitumor activity (15, 16). In one *in vitro*

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[3] The abbreviations used are: PDGFR, platelet-derived growth factor receptor; Mab, monoclonal antibody; TKI, tyrosine kinase inhibitor; EPT, endocrine pancreatic tumor; EGFR, epidermal growth factor receptor; GIST, gastrointestinal stromal tumor; ACTH, adrenocorticotropic hormone; VIP, vasoactive intestinal peptide.

Table 1 Expression of tyrosine kinase receptors

	PDGFRα		PDGFRβ			
	Tumor	Stroma	Tumor	Stroma	c-kit	EGFR
All tumors	38/38	21/37[a] (57%)	28/38 (74%)	36/37[a] (97%)	35/38 (92%)	21/38 (55%)
insulinomas	4/4	3/4 (75%)	0/4	4/4	4/4	1/4 (25%)
gastrinomas	3/3	0/3	3/3	2/3 (67%)	3/3	1/3 (33%)
glucagonomas	2/2	0/2	2/2	2/2	2/2	2/2
VIPomas	2/2	1/2 (50%)	2/2	2/2	2/2	1/2 (50%)
ACTHoma	1/1	1/1	1/1	1/1	1/1	1/1
nonfunctioning tumors	26/26	16/25[a] (64%)	20/26 (77%)	25/25	23/26 (88%)	15/26 (58%)
Well-diff tumors	30/30	15/29[a] (52%)	24/30 (80%)	28/29[a] (97%)	27/30 (90%)	17/30 (57%)
Poorly diff tumors	8/8	6/8 (75%)	4/8 (50%)	8/8	8/8	4/8 (50%)
Primary tumors	8/8	5/8 (63%)	4/8 (50%)	8/8	7/8 (88%)	3/8 (38%)
Metastases	30/30	16/29[a] (55%)	24/30 (80%)	28/29[a] (97%)	28/30 (93%)	18/30 (60%)
Prior therapy	20/20	9/19[a] (47%)	17/20 (85%)	18/19[a] (95%)	19/20 (95%)	12/20 (60%)
Nontreated	18/18	12/18 (67%)	11/18 (61%)	18/18	16/18 (89%)	9/18 (50%)

[a] One sample was not evaluable.

study, potentiation of cytotoxic drug activity has also been reported (17).

Malignant EPTs are rare and usually, but not always, have an indolent growth pattern. According to their hormone-related symptoms, tumors are divided into different subgroups: insulinomas; gastrinomas; glucagonomas; VIPomas; somatostatinomas; and nonfunctioning tumors (18). Most often, patients present with distant metastases, and treatment is thus palliative. The median life expectancy is 4–4.5 years, despite advanced stage at diagnosis. First-line medical antitumoral therapy is streptozotocin combined with 5-fluorouracil or doxorubicin. Biotherapy has also shown good response rates when administered as a combination of IFN and somatostatin analogues (19). At best, treatment of these tumors is aimed at prolongation of life while maintaining quality of life.

Chaudry *et al.* (20) have previously shown, using immunohistochemistry, that tumor tissue from five patients with EPTs expressed PDGFRβ in tumor stroma but not on tumor cells. Wulbrand *et al.* (21) have examined the expression of EGFR in 10 insulinomas, 9 gastrinomas, and 9 nonfunctioning tumors and found that the receptor was expressed almost exclusively in gastrinomas (9 of 9 gastrinomas). To our knowledge, no studies examining the expression of c-kit in malignant EPTs have been published yet.

In searching for new treatment strategies for malignant EPTs, we have examined tumor tissue with regard to the expression of molecular targets that can be treated with currently available Mabs directed toward tyrosine kinase receptors or with TKIs.

PATIENTS AND METHODS

Patients. Tumor tissue was obtained from 38 patients (19 men and 19 women) with malignant EPTs (Table 1). Median age at diagnosis was 55.5 years (range, 24–72 years). Thirty patients had well-differentiated tumors (18 patients had nonfunctioning tumors, 3 had gastrinomas, 2 had glucagonomas, 4 had insulinomas, 2 had VIPomas, and 1 had ACTHoma). Eight patients had poorly differentiated tumors that were all nonfunctioning. All patients had malignant disease (35 patients had liver metastases, 15 patients had lymph node metastases, 6 patients had bone metastases, and 2 patients had brain metastases). Twenty patients had received treatment with chemotherapy and/or biotherapy before the collection of tumor tissue. Of these, 15 patients had received chemotherapy (most commonly streptozotocin and fluorouracil), and 10 had received biotherapy (IFN-α and/or somatostatin analogues). Thirty-five of 38 patients were examined with somatostatin receptor scintigraphy (Octreoscan), and of these, 31 (89%) showed pathological uptake.

Tissue Samples. Tissue samples were obtained by either surgery or ultrasound-guided needle biopsy. Eight tumor specimens came from primary tumors in the pancreas, and 30 tumor specimens came from metastases (26 from liver metastases and 4 from lymph node metastases; Table 1). The diagnosis was histopathologically verified, and all tumor samples stained positive for chromogranin A. The material obtained was frozen in liquid nitrogen and kept at −86°C. Five-μm-thick sections were cut, dried for 5 min, and then fixed in ice-cold acetone for 10 min. The sections were then dried for 15 min and stored at −20°C. Before staining, the sections were thawed and rinsed in PBS.

Immunohistochemistry. Endogenous peroxidase was blocked with 1% hydrogen peroxidase in PBS for 30 min. Avidin-binding protein was blocked by incubating the sections sequentially with avidin and biotin in Blocking Kit (Vector Laboratories, Burlingame, CA). Between incubations, the sections were washed in PBS, and excess liquid was carefully wiped away from around the specimen. To avoid nonspecific background staining, the sections were incubated with normal goat serum in a 1:5 dilution in PBS for 30 min before applying the primary antibody. Polyclonal antibodies against PDGFRα and PDGFRβ, c-kit, and EGFR were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against chromogranin A were kindly provided by Dr. M. Stridsberg (University Hospital, Uppsala, Sweden). The dilutions of the primary antibodies (in PBS with 1% BSA) were 1:400 for PDGFRα, 1:100 for PDGFRβ, 1:250 for c-kit, and 1:150 for EGFR. Incubation was performed at room temperature for 90 min. Antirabbit serum diluted 1:200 was used as biotinylated secondary antibody. The immunoreaction was visualized with an Elite

kit (Vector Laboratories). We used 0.02% hydrogen peroxidase as catalyst and 3-amino-9-ethylcarbazol (Sigma Chemical Co., St. Louis, MO) in DMSO as chromogen. The sections were counterstained with Mayer's hematoxylin for 30 s (Apoteksbolaget).

The control stainings included (*a*) omission of the primary antibodies, (*b*) omission of the secondary antibody, and (*c*) preincubation of the primary antibodies with the relevant antigens (purchased from Santa Cruz Biotechnology) before application to the sections. As positive controls, we used sections from placenta for PDGFRα, sections from colon cancer stroma for PDGFRβ, sections from GIST for c-kit, and sections from skin for EGFR.

To be considered positive, >50% of the tumor or stroma cells had to show positive staining. Three different authors have independently assessed the stainings.

Statistics. Statistical analyses were performed using χ^2 and Fisher's exact test for comparison of proportions.

RESULTS

The results are summarized in Table 1. Fig. 1 shows two examples of staining patterns.

Expression of PDGFRα on Tumor Cells and in the Stroma. All 38 tissues examined for expression of PDGFRα on tumor cells stained positive.

Twenty-one of 37 tumor samples (57%) were positive for PDGFRα in the stroma. One sample was not evaluable. Three of 4 insulinomas, none of the gastrinomas or glucagonomas, 1 of 2 VIPomas, 1 of 1 ACTHoma, and 16 of 25 nonfunctioning tumors (64%) expressed PDGFRα in the tumor stroma.

Expression of PDGFRβ on Tumor Cells and in the Stroma. Twenty-eight of 38 tissue specimens (74%) expressed PDGFRβ on tumor cells. None of the 4 insulinomas but all of the gastrinomas, glucagonomas, and VIPomas and the ACTHoma stained positive for the receptor. Twenty of 26 nonfunctioning tumors (77%) expressed PDGFRβ.

Thirty-six of 37 tumor specimens (97%; the exception was a gastrinoma) examined for expression of PDGFRβ in the tumor stroma stained positive. One specimen, from a nonfunctioning tumor, was not evaluable.

Expression of c-kit on Tumor Cells. Thirty-five of 38 tissue specimens (92%) expressed c-kit on tumor cells. All tumor specimens were positive except for three nonfunctioning tumors.

Expression of EGFR on Tumor Cells. Twenty-one of 38 tumor samples (55%) expressed EGFR on tumor cells. One of four insulinomas, one of three gastrinomas, two of two glucagonomas, one of two VIPomas, and one of one ACTHoma expressed the receptor. Nonfunctioning tumors stained positive in 15 of 26 patients (58%).

Well-differentiated Tumor Tissues *versus* Poorly Differentiated Tumor Tissues. No significant differences were seen between well-differentiated and poorly differentiated tumor tissues with regard to receptor expression (Table 1).

Previously Medically Treated Tumor Specimens *versus* Previously Untreated Specimens. No differences were seen in receptor expression when comparing previously medically treated tumors (chemotherapy and/or biotherapy) and previously untreated tumors (Table 1).

Metastases *versus* Primary Tumors. We did not detect any significant differences in tyrosine kinase receptor expression between specimens derived from primary tumors and metastases (Table 1).

DISCUSSION

We have shown that expression of examined tyrosine kinase receptors is high in malignant EPTs, both on tumor cells and in tumor stroma. Tumor tissues stained positive for stromal PDGFRβ and c-kit in >90% of the samples and stained positive for EGFR in more than half of the samples. We could not detect any differences in receptor expression between tumor tissues from previously medically treated patients and those from untreated patients, between different tumor subgroups, or between poorly differentiated and well-differentiated tumors. Metastases expressed PDGFRβ and EGFR on tumor cells to a higher extent than did primary tumors (80% *versus* 50% for PDGFRβ and 60% *versus* 37.5% for EGFR), but the differences were not significant. Individual expression patterns varied greatly.

Our results differ from those shown by Chaudry *et al.* (20) regarding expression of PDGFRβ on tumor cells: expression was absent in all EPTs examined by Chaudry but present in 74% of our tumors. Wulbrand *et al.* (21) could only find EGFR expression in gastrinomas, whereas we found expression of EGFR in 55% of our tumors, not only in gastrinomas. In Chaudry's paper, it is difficult to know whether the tumors are malignant or benign, and in Wulbrand's paper, it seems as though most tumors are benign. We included only malignant tumors in our study. Perhaps there is a difference in receptor expression, depending upon whether the tumor is malignant or not.

In a recent paper, Sawyers (22) suggests that a TKI will be effective only if it inhibits a target whose function is essential for maintenance of the cancer phenotype. This is supported by studies showing that breast cancer patients respond to treatment with trastuzumab only if HER2 is overexpressed (3+; Ref. 23) and that patients with GISTs expressing a mutated c-kit respond to a greater extent to treatment with STI571 than those not carrying the mutation (12). Furthermore, in chronic myelogenous leukemia, the target for STI571 is the bcr-abl fusion protein with tyrosine kinase activity that is the product of the Philadelphia gene expressed in 95% of the patients (10).

On the other hand, a Phase II study evaluating IMC-C225 in combination with irinotecan in irinotecan-refractory colorectal carcinomas showed similar response rates in tumors with different levels of EGFR expression (1+ to 3+; Ref. 24). Furthermore, it is not yet clear whether EGFR needs to be overexpressed on the tumor cells for the treatment with ZD1839 to be effective. The level of EGFR required in the tumor to obtain clinical benefit still needs to be determined (25).

Pietras *et al.* (13) have suggested a possible alternative use of STI571 to increase drug uptake and hence improve the effectiveness of cancer chemotherapy. They have shown that treatment with STI571 in rats with s.c. growing colon carcinomas reduces interstitial hypertension and increases transcapillary transport in tumors by inhibiting normal PDGFRβ. Because

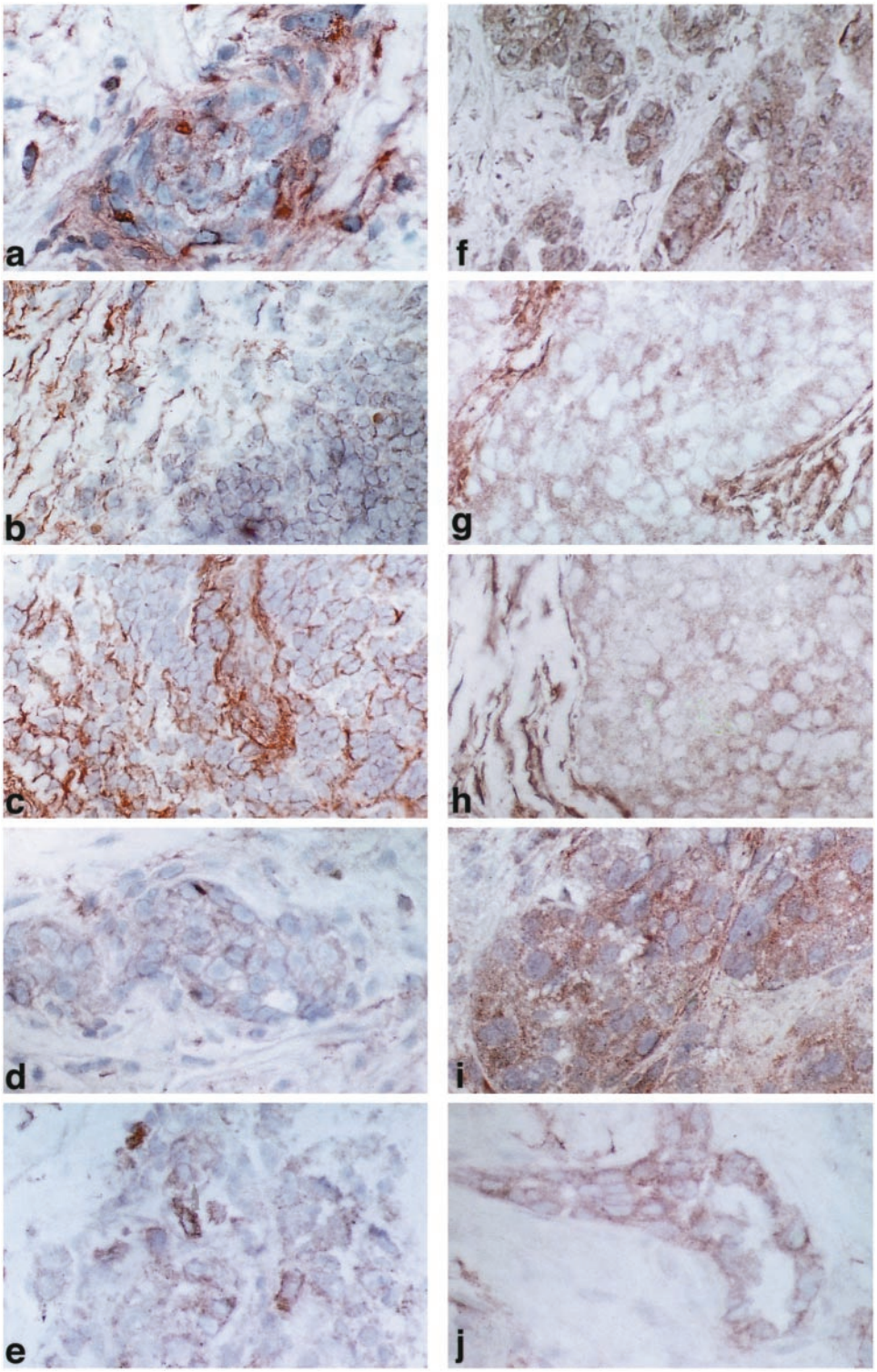

Fig. 1 Immunohistochemical stainings for chromogranin A, PDGFRα, PDGFRβ, c-kit, and EGFR, using subtype-specific polyclonal antibodies, in two patients with nonfunctioning tumors (*a*−*e* and *f*−*j*, respectively). Shown are positive stainings for chromogranin A (*a* and *f*) and PDGFRα (*b* and *g*) on tumor cells and in stroma, positive staining for PDGFRβ (*c* and *h*) on tumor cells and in stroma, and positive stainings for c-kit (*d* and *i*) and EGFR (*e* and *j*). ×400.

PDGFRβ is expressed in the stroma of most solid tumors, this method of using STI571 could be widely used.

It seems that TKIs can be used in several different ways: to inhibit a target whose function is essential for maintenance of the cancer phenotype; to potentiate the effect of chemo- and radiotherapy; and to increase the effect of cytotoxic drugs by enhanced drug uptake.

We will continue our research of tyrosine kinase receptors

by examining further whether there are any gene amplifications or mutations that can be targeted by the TKIs. Because streptozotocin plus 5-fluorouracil or doxorubicin is the first-line medical treatment in EPTs, it is tempting to add STI571, which might increase therapeutic response without increasing side effects. EPTs express EGFR in over more than of the patients, and hopefully, treatment with IMC-C225 or ZD1839, as single drugs or in combination with chemotherapy, can be of value.

We conclude that EPTs express PDGFR, c-kit, and EGFR, all of which can be targeted by currently available TKIs. We think that the use of tyrosine kinase receptor inhibitors, both as single drugs and in combination with chemo- and radiotherapy, will result in new treatment strategies for malignant EPTs.

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