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

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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 epithelial growth factor receptor (EGFR).

Results: All 38 tissue specimens expressed PDGFRα on tumor cells, and 21 of 37 specimens (57%) expressed PDGFRβ 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 PDGFRα 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, 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 chemotherapy 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 PDGFRα 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 pataognomone 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
Expression of Tyrosine Kinase Receptors in EPTs

Malignant EPTs are rare and usually not, but 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 reporting (17).

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.

**Table 1** Expression of tyrosine kinase receptors

<table>
<thead>
<tr>
<th></th>
<th>PDGFRα Tumor</th>
<th>PDGFRα Stroma</th>
<th>PDGFRβ Tumor</th>
<th>PDGFRβ Stroma</th>
<th>c-kit</th>
<th>EGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumors</td>
<td>38/38</td>
<td>21/37* (57%)</td>
<td>28/38 (74%)</td>
<td>36/37* (97%)</td>
<td>35/38 (92%)</td>
<td>21/38 (55%)</td>
</tr>
<tr>
<td>Insulinomas</td>
<td>4/4</td>
<td>3/4 (75%)</td>
<td>0/4</td>
<td>4/4</td>
<td>4/4</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>Gastrinomas</td>
<td>3/3</td>
<td>0/3</td>
<td>3/3</td>
<td>0/3</td>
<td>3/3</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>Glucagonomas</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>VIPomas</td>
<td>2/2</td>
<td>1/2 (50%)</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>ACTHomas</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Nonfunctioning tumors</td>
<td>26/26</td>
<td>16/25* (64%)</td>
<td>20/26 (77%)</td>
<td>25/25</td>
<td>23/26 (88%)</td>
<td>15/26 (58%)</td>
</tr>
<tr>
<td>Well-diff tumors</td>
<td>30/30</td>
<td>15/29* (52%)</td>
<td>24/30 (80%)</td>
<td>28/29* (97%)</td>
<td>27/30 (90%)</td>
<td>17/30 (57%)</td>
</tr>
<tr>
<td>Poorly diff tumors</td>
<td>8/8</td>
<td>6/8 (75%)</td>
<td>4/8 (50%)</td>
<td>8/8</td>
<td>8/8</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>Primary tumors</td>
<td>8/8</td>
<td>5/8 (63%)</td>
<td>4/8 (50%)</td>
<td>8/8</td>
<td>7/8 (88%)</td>
<td>3/8 (38%)</td>
</tr>
<tr>
<td>Metastases</td>
<td>30/30</td>
<td>16/29* (55%)</td>
<td>24/30 (80%)</td>
<td>28/29* (97%)</td>
<td>28/30 (93%)</td>
<td>18/30 (60%)</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>20/20</td>
<td>9/19* (47%)</td>
<td>17/20 (85%)</td>
<td>18/19* (95%)</td>
<td>19/20 (95%)</td>
<td>12/20 (60%)</td>
</tr>
<tr>
<td>Nontreated</td>
<td>18/18</td>
<td>12/18 (67%)</td>
<td>11/18* (61%)</td>
<td>16/18 (89%)</td>
<td>9/18 (50%)</td>
<td></td>
</tr>
</tbody>
</table>

*One sample was not evaluable.

In immunohistochemistry, endogenous peroxidase was blocked with 1% hydrogen peroxide 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.
Stroma.

examples of staining patterns.

tumors (64%) expressed PDGFR

VIPomas, 1 of 1 ACTHoma, and 16 of 25 nonfunctioning/H9251

presses PDGFR

Twenty of 26/H9252

pressed PDGFR

Twenty-eight of 38 tissue specimens (74%) ex-

Stroma.

RESULTS

s exact test for comparison of proportions.

and Fisher

skin for EGFR.

for PDGFR

from placenta for PDGFR

sections from colon cancer stroma/H9251

cation to the sections. As positive controls, we used sections

gens (purchased from Santa Cruz Biotechnology) before appli-

b

) omission of the secondary antibody, and (c

antibodies, (c

hematoxylin for 30 s (Apoteksbo-

counterstained with Mayer

St. Louis, MO) in DMSO as chromogen. The sections were

Previously Untreated Specimens.

No differences were seen in receptor expression when comparing previously medically

Well-differentiated Tumor Tissues versus Poorly Dif-

tegrated Tumor Tissues. No significant differences were

previously medially
treated tumors (chemotherapy and/or biotherapy) and previously

untreated tumors (Table 1).

Metastases versus Primary Tumors. We did not detect
	ny significant differences in tyrosine kinase receptor expres-
sion between specimens derived from primary tumors and me-
tastases (Table 1).

DISCUSSION

We have shown that expression of examined tyrosine ki-

nase receptors is high in malignant EPTs, both on tumor cells

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 un-
treated 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 myeloge-

nous 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 colorec-
tal carcinomas showed similar response rates in tumors with
different levels of EGFR expression (1+ to 3+; Ref. 24). Fur-

thermore, 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 carcino-

mas reduces interstitial hypertension and increases transcapil-

lary transport in tumors by inhibiting normal PDGFRβ. Because
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.

REFERENCES


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