Targeting of Cytotoxic Somatostatin Analog AN-238 to Somatostatin Receptor Subtypes 5 and/or 3 in Experimental Pancreatic Cancers

Karoly Szepeshazi, Andrew V. Schally, Gabor Halmos, Baodong Sun, Francine Hebert, Balazs Csernus, and Attila Nagy

Endocrine, Polypeptide, and Cancer Institute, Veterans Affairs Medical Center, and Section of Experimental Medicine, Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana 70112

ABSTRACT

Purpose: The expression of somatostatin receptors (SSTRs) allows the localization and treatment of some tumors with radiolabeled SST analogues. We investigated whether SSTRs on human pancreatic cancer lines xenografted into nude mice can be used for targeting of cytotoxic somatostatin analogue AN-238, consisting of 2-pyrrolinodoxorubicin (AN-201) linked to octapeptide carrier RC-121.

Experimental Design: AN-238 and AN-201 were administered i.v. to nude mice bearing SW-1990 pancreatic cancers. Tumor growth reduction and survival were analyzed, and cell proliferation and apoptosis were determined with histological methods. The effects of repeated administration of AN-238 and AN-201 were also evaluated on xenografted Panc-1, MiaPaCa-2, CFPAC-1, Capan-1, and Capan-2 pancreatic cancers. The expression of mRNA for SSTR subtypes 2A, 3, and 5 in tumors was analyzed by reverse transcription-PCR, and binding assays were performed.

Results: All of the cancer models except MiaPaCa-2 displayed functional receptors for SST. SW-1990 expressed mRNA for SSTR subtypes 3 and 5, whereas various patterns of subtypes 2A, 3, and 5 were found in other pancreatic cancers. Repeated administration of AN-238 at 150 nmol/kg significantly inhibited growth of SW-1990 cancers (93% after 45 days; P = 0.016) and other tumors but not MiaPaCa-2. AN-201 was toxic and less effective. The efficacy of AN-238 was consistent with SSTR expression.

Conclusions: Growth of experimental human pancreatic cancers that express SSTRs can be inhibited by cytotoxic somatostatin analogue AN-238.

INTRODUCTION

Pancreatic carcinoma is the second most common gastrointestinal malignancy and the fifth leading cause of cancer-related deaths in the United States (1–3). Carcinoma of the pancreas has a very poor prognosis. Surgery, radiation, and chemotherapy are of limited effectiveness, and the development of new therapies is essential (1, 2).

The role of various hormones, growth factors, and their receptors in pancreatic carcinogenesis has been extensively investigated (4, 5). One of the hormones implicated in the inhibition of growth and malignant transformation of the pancreas is SST (4, 5). SST and its clinically available octapeptide analogues such as octreotide, vapreotide (RC-160), and lanreotide bind to specific SSTRs on cancer cells (5) and can inhibit cell proliferation. Five subtypes of receptors were identified (5–7). SST octapeptides and their radiolabeled analogues bind to subtypes 2, 3, and 5 (SSTR2, SSTR3, and SSTR5; Refs. 8, 9). Thus, radiolabeled SST octapeptides can be used for localization and targeted therapy of tumors expressing these subtypes (8, 10–12).

Although SSTR2 is strongly expressed in the normal pancreas, it seems to vanish during carcinogenesis (13, 14). In contrast, mRNA for SSTR3 and SSTR5 and corresponding functional receptors appear to be expressed in some pancreatic cancers, but the findings on this subject are inconclusive and controversial (13, 15–20). AN-238, a targeted cytotoxic SST analogue developed by us, consists of a potent derivative of DOX, 2-pyrrolino-DOX (AN-201), linked to SST octapeptide analogue RC-121 (21). AN-238 inhibits growth of various experimental tumors (22), including prostatic (23, 24), mammary (25), and renal cancers (26), and brain tumors (27). In this study, we tested the effect of AN-238 in a panel of six human pancreatic cancer models in nude mice using a SW-1990 cell line for a more comprehensive investigation.

MATERIALS AND METHODS

Materials

SST analogue RC-121 (α-Phe-Cys-Tyr-α-Trp-Lys-Val-Cys-Thr-NH₂) was synthesized in our laboratory (28). The cytotoxic conjugate AN-238 was made by coupling one molecule of 2-pyrrolino-DOX-14-O-hemigluturate to the NH₂ terminus of [Lys(γ-fluorenylmethoxy-carbonyl)₃]RC-121, followed by deprotection and purification (21). AN-201 was synthesized as described (29). For the injection, the cytotoxic compounds were dissolved in 20 μl of 0.01 N acetic acid and diluted with 6% (w/v) aqueous D-mannitol. SST analogue RC-160 (vap-
releasing about 30 μg/day of RC-160 for 2 weeks from an aliquot of 8-mg microgranules. RC-160 microgranules were suspended in an aqueous solvent system containing 2% (v/v) carboxymethylcellulose and 1% (v/v) Tween 80 and injected s.c. Chemicals, unless stated otherwise, were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and Tumors
Male athymic nude mice (Ncr nu/nu), approximately 6 weeks of age on arrival, were obtained from the National Cancer Institute (Frederick Cancer Research and Development Center, Frederick, MD) and maintained under pathogen-limited conditions. The SW-1990 cell line was purchased from the American Type Culture Collection (Manassas, VA) and maintained in culture as described (30). Initially, three male nude mice received injections s.c. with 0.2 ml of SW-1990 human pancreatic cancer cell suspension (10⁶ cells/mouse; Refs. 30, 31). The well-developed tumors resulting after 4 weeks were dissected and mechanically minced, and 2-mm³ pieces of tumor tissue were transplanted s.c. to both flank areas of the experimental animals. Panc-1, MiaPaCa-2, CFPAC-1, Capan-1, and Capan-2 human pancreatic cancer cell lines were also purchased from the American Type Culture Collection and maintained in culture. Transplantation into male nude mice was performed as described above. When tumor growth became apparent, the mice were grouped with about equal average tumor sizes in each group. Body weights and tumor sizes were measured regularly. All of the animal experiments were carried out in accordance with institutional guidelines for animal care.

Experimental Protocol
Three experiments were performed with SW-1990 tumors, and the animals were treated as follows.

Experiment 1. Group 1 (control) received injection vehicle only; group 2 received AN-238; and group 3 received AN-201. There were eight mice in each group. The treatment was started 36 days after transplantation; 200 nmol/kg of the compounds were injected into the jugular vein on days 1 and 36. The experiment was terminated on day 61.

Experiment 2. Group 1 (control) received vehicle used for the cytotoxic compounds only; group 2 received AN-238; group 3 received AN-201; group 4 received RC-121; group 5 received AN-201 + RC-121; group 6 received microgranules releasing about 30 μg of RC-160/day given every 2 weeks; and group 7 received AN-238 after pretreatment with 160 μg of RC-160/20 g of body weight 1 h before injecting AN-238. The groups consisted of 10 mice except for group 7, which had eight animals. The treatment was started 4 days after transplantation of tumors. The cytotoxic compounds and the carrier peptide were administered i.v. at 150 nmol/kg doses on days 1, 15, 51, and 86. This is close to the maximal tolerated dose for AN-238. The first part of the experiment ended after 45 days. At that time, several control animals had large tumors. In the second part of the experiment, the groups receiving RC-121 (group 4) and chronic treatment with RC-160 (group 6) were eliminated, and the control group was reorganized by omitting three mice with large tumors. Thus, with the early death of two control animals on days 40 and 43, the second part of the experiment was started with five mice in the control group having relatively small tumors. On day 45, two mice, one with a large and ulcerated tumor and another that became sick, were left out from group 7. The surviving mice were sacrificed on day 121.

Experiment 3. Experiment 3 was designed as a short study to extend the previous experiment and analyze the effects of a single high dose of the compounds on tumor growth characteristics. Group 1 (control) received vehicle only; group 2 received AN-238; group 3 received AN-201; and group 4 received RC-160 acetate (see below). AN-238 and AN-201 were administered once i.v. at 250 nmol/kg doses. The groups contained eight mice each. The treatment in groups 1–3 was applied 38 days after transplantation of tumors, and the animals were sacrificed 5 days later. The mice in group 4 received injections with 160 μg of RC-160 acetate s.c. 1 h before sacrifice.

In five additional experiments, nude mice bearing Panc-1, MiaPaCa-2, CFPAC-1, Capan-1, and Capan-2 human pancreatic cancers were treated with repeated 150 nmol/kg doses of cytotoxic SST analogue AN-238 and cytotoxic radical AN-201.

TGR was calculated according to the formula: TGR% = 100 – 100 × (T – t)/(C – c), where t = the volume of a treated tumor at the beginning of therapy, T = volume of the same tumor at the end of the experiment, c = volume of a control tumor at the start of treatment, and C = volume of the same tumor at the final measurement. At the end of the experiments, the mice were sacrificed by decapitation under metofane (methoxyflurane; Schering-Plough, Union, NJ) anesthesia.

Histological Examination
The methods used in histological studies in experiments 1 and 3 were as described (30).

Receptor Assay
Binding characteristics of SSTRs on membrane preparations from human pancreatic cancers were determined by ligand competition assays using ¹²⁵I-labeled SST octapeptide RC-160 as reported (32, 33). The LIGAND PC computerized curve-fitting program was used to determine the type of receptor binding, the dissociation constant, and the maximal binding capacity of receptors (32, 33). The receptor-binding affinity of cytotoxic SST analogue AN-238 to tumor membranes was measured in displacement experiments based on competitive inhibition of ¹²⁵I-labeled RC-160 binding using various concentrations of AN-238 (10⁻⁶ to 10⁻¹² M). IC₅₀ value was defined as the concentration of AN-238 causing 50% inhibition of ¹²⁵I-labeled RC-160 binding.
RNA Isolation and Reverse Transcription-PCR

Total RNA was isolated by the guanidinium isothiocyanate-phenol chloroform extraction method using the Stratagene Micro RNA Isolation Kit according to the manufacturer’s instructions (Stratagene, La Jolla, CA). After precipitation, RNA samples were quantified spectrophotometrically at 260 and 280 nm. First-strand cDNA was reverse-transcribed from total RNA with Moloney murine leukemia virus reverse transcriptase. Before reverse transcription, samples for SSTR3 mRNA detection were digested with RNase-free DNase (Ambion, Austin, TX) to eliminate the probability of DNA contamination. Distilled water was used as a negative control. The methods of reverse transcription and PCR amplification were reported (24, 32, 33). All of the reagents used for reverse transcription-PCR were purchased from Stratagene with the exception of Taq DNA polymerase (Intermountain Scientific Corporation, Kaysville, UT).

For amplification of cDNA transcripts, gene-specific primers for human SSTR2A, SSTR3, SSTR5, and glyceraldehyde-3-phosphate dehydrogenase (internal control) were used as described (24, 32, 33). Aliquots of 10 μl of each amplification reaction were electrophoretically separated on 8% polyacrylamide gel, silver stained, and photographed. The bands from the samples were scanned and quantified using an imaging densitometer (Model GS-700; Bio-Rad, Hercules, CA) coupled with the Bio-Rad PC analysis software.

Statistical Methods

The SigmaStat and SigmaPlot software (Jandel Scientific, San Raphael, CA) were used for statistical evaluation of data and the preparation of figures. Tumor volume and body weight changes were evaluated by two-way repeated measures ANOVA, the two factors being the treatment groups and the separate times of assessment, and the groups were compared by Tukey’s multiple comparison method. Survival data in experiment 2 were presented by Kaplan-Meier curves, and the differences were assessed by the log-rank test. All of the other data were evaluated by one-way ANOVA and Dunnett’s method. The Ps given represent results of the multiple comparison analyses.

RESULTS

Effects of Therapy on Growth of SW-1990 Pancreatic Cancers. In experiment 1, cytotoxic SST analogue AN-238 given twice at 200 nmol/kg inhibited powerfully the growth of SW-1990 cancers as shown by a significant decrease in tumor volume (P = 0.017) and weight (P = 0.039) on day 61 (Fig. 1A and Table 1). Cytotoxic radical AN-201 had a slight initial effect, but the second treatment with AN-201 given on day 36 was ineffective. The mice tolerated well the two injections of 200 nmol/kg of both compounds. Histologically, AgNOR counts were significantly lower in the group treated with AN-238 as compared with the control (P = 0.001) or with those receiving AN-201 (P = 0.009). AN-238 also significantly increased the number of apoptotic cells in tumors (P = 0.03) and the ratio of apoptotic:mitotic indices (P = 0.013; Table 1).

In the first part of experiment 2 (days 0–45; Fig. 1B and Table 1), 150 nmol/kg of AN-238 given on days 1 and 15...
significantly reduced tumor growth \((P = 0.016)\). Equimolar amounts of AN-201 or the mixture of AN-201 and the carrier RC-121 caused some growth inhibition, whereas RC-121 alone was ineffective. Pretreatment with RC-160 reduced the efficacy of AN-238, resulting in an inhibition similar to that caused by AN-201. Microgranules of RC-160 were ineffective. In the second part of experiment 2 (days 50–121; Fig. 1C), 150 nmol/kg of AN-238 administered on days 51 and 86 further inhibited growth of SW-1990 cancers \((P = 0.022\) on day 107). The cytotoxic radical AN-201 had no effect on tumor growth, and it was toxic. Pretreatment with RC-160 decreased the effect of AN-238; tumor volume on day 107 was reduced by 69% compared with 81% for AN-238 alone. This difference was not significant possibly because of the elimination of a mouse with a large tumor from the group pretreated with RC-160. The median survival of mice derived from the Kaplan-Meier curve (Fig. 2) was 102 days in the group treated with AN-238, 85 days after treatment with AN-201, and 67 days in the control group (Table 1); however, the differences among the groups were statistically not significant (log-rank test).

In experiment 3, the effects of a single, higher dose of the cytotoxic SST analogue on relatively large SW-1990 cancers were analyzed in a short study. Administration of 250 nmol/kg of AN-238 resulted in a shrinkage of tumors, whereas AN-201 had only a slight effect (Table 1 and Fig. 1B inset). Treatment with AN-238 also caused a significant decrease in AgNOR numbers compared with controls \((P = 0.002)\) or AN-201 \((P = 0.036)\). Apoptosis in tumors was significantly enhanced by AN-238 \((P = 0.014)\) and AN-201 \((P = 0.001)\). The ratio of apoptotic:mitotic indices was increased by AN-238 \((P = 0.040)\) but not by AN-201.

**Receptor-binding Assays on SW-1990 Cancers.** Radiolabeled SST octapeptide RC-160 was bound to a single class of high-affinity, low-capacity binding sites on membrane fractions of control SW-1990 tumors (Table 2). One h after s.c. administration of 160 \(\mu\)g of RC-160/20 g body weight to mice

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**Table 1** The effect of cytotoxic SST analog AN-238, cytotoxic radical AN-201, carrier molecule RC-121, and SST analog RC-160 on the growth characteristics of SW-1990 cancers xenografted into nude mice and the survival of animals.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor volume on day 1 (mm(^3))</th>
<th>TGR %</th>
<th>Tumor weight (mg)</th>
<th>Median survival in days</th>
<th>Apoptotic index</th>
<th>Ratio of apoptotic:mitotic indices</th>
<th>AgNOR count</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34</td>
<td>0(^b)</td>
<td>859 ± 170</td>
<td>3.5 ± 1.0</td>
<td>0.83 ± 0.25</td>
<td>5.92 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>AN-238</td>
<td>28</td>
<td>72(^c)</td>
<td>378 ± 127</td>
<td>6.9 ± 0.8(^c)</td>
<td>2.88 ± 0.71(^c)</td>
<td>4.33 ± 1.0(^d)</td>
<td></td>
</tr>
<tr>
<td>AN-201</td>
<td>35</td>
<td>8(^b)</td>
<td>1108 ± 989</td>
<td>4.7 ± 0.8</td>
<td>2.94 ± 1.04</td>
<td>5.00 ± 0.09(^c)</td>
<td></td>
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<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>39</td>
<td>0(^c)</td>
<td>693 ± 294</td>
<td>2.2 ± 0.2</td>
<td>0.41 ± 0.19</td>
<td>6.08 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>AN-238</td>
<td>26</td>
<td>93(^e)</td>
<td>332 ± 199</td>
<td>4.9 ± 0.8(^c)</td>
<td>2.57 ± 0.84(^c)</td>
<td>5.20 ± 0.08(^d)</td>
<td></td>
</tr>
<tr>
<td>AN-201</td>
<td>33</td>
<td>68(^e)</td>
<td>605 ± 256</td>
<td>5.8 ± 0.6(^c)</td>
<td>1.39 ± 0.29</td>
<td>5.65 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>RC-121</td>
<td>44</td>
<td>11(^c)</td>
<td>458 ± 217</td>
<td>1.4 ± 0.1</td>
<td>6.08 ± 0.11</td>
<td>5.65 ± 0.19</td>
<td></td>
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<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>366</td>
<td>0(^c)</td>
<td>693 ± 294</td>
<td>2.2 ± 0.2</td>
<td>0.41 ± 0.19</td>
<td>6.08 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>AN-238</td>
<td>416</td>
<td>129(^c)</td>
<td>332 ± 199</td>
<td>4.9 ± 0.8(^c)</td>
<td>2.57 ± 0.84(^c)</td>
<td>5.20 ± 0.08(^d)</td>
<td></td>
</tr>
<tr>
<td>AN-201</td>
<td>398</td>
<td>55(^c)</td>
<td>605 ± 256</td>
<td>5.8 ± 0.6(^c)</td>
<td>1.39 ± 0.29</td>
<td>5.65 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>RC-160</td>
<td>270</td>
<td>1(^c)</td>
<td>458 ± 217</td>
<td>1.4 ± 0.1</td>
<td>6.08 ± 0.11</td>
<td>5.65 ± 0.19</td>
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</tr>
</tbody>
</table>

\(^a\) Tumor weight and histological data are means ± SE.

\(^b\) Day 60.

\(^c\) \(P < 0.05\) vs. control (the exact Ps are given in the text).

\(^d\) \(P < 0.01\) vs. control (the exact Ps are given in the text).

\(^e\) Day 45.

\(^f\) Day 107.

\(^g\) ND, not determined.

\(^h\) Day 5.
bearing SW-1990 cancers, the concentration of receptors on the tumors was decreased by 63% (P = 0.042) without a change in the affinity. AN-238 exhibited high affinity to SSTRs on SW-1990 tumor membranes, displacing the specific binding of the radioligand at IC50 value of 3.11 ± 0.1 nm. Specificity studies showed that the binding of radiolabeled RC-160 was strongly displaced by SST14 but only slightly by vasoactive intestinal peptide.

Expression of mRNA for SSTRs in SW-1990 Cancers. mRNAs for SSTR3 and SSTR5 were present in all of the peptide. Displaced by SST14 but only slightly by vasoactive intestinal peptide.

Findings in Five Other Pancreatic Cancer Lines. A single class of high-affinity, low-capacity binding sites for RC-160 was strongly displaced by SST14 but only slightly by vasoactive intestinal peptide.

Expression of mRNA for SSTRs in SW-1990 Cancers. mRNAs for SSTR3 and SSTR5 were present in all of the samples. In experiment 3, the expression of mRNA for SSTR5 was reduced by 53% after the injection of 160 μg of RC-160 1 h before sacrifice compared with that of controls (P = 0.007). No mRNA for SSTR2A was detected in any samples of SW-1990 cancers.

Findings in Five Other Pancreatic Cancer Lines. A single class of high-affinity, low-capacity binding sites for RC-160 was detected in four additional pancreatic cancer lines studied (Table 2) but not in the MiaPaCa-2 line. All of the five human pancreatic cancer models examined expressed mRNA for at least one of the three SSTR subtypes tested. Most tumors contained mRNA for two or three SSTR subtypes, the most frequent combination being SSTR2A/3 followed by SSTR2A/5 and SSTR2A/3/5.

AN-238 given 4–5 times at 150 nmol/kg significantly inhibited growth of Panc-1 (P = 0.033), CFPAC-1 (P = 0.018), Capan-1 (P = 0.017), and Capan-2 (P = 0.028) cancers but had no effect on MiaPaCa-2 tumors (Fig. 3). AN-201 was also very effective in CFPAC-1 (P = 0.021), Capan-1 (P = 0.025), and Capan-2 (P = 0.011) cancers, but it was more toxic than AN-238. Treatment with each compound led to a significant fall in the body weight of mice, with AN-201 causing a greater decrease (Fig. 3). The effects of AN-238 and AN-201 were about equal in Capan-2 tumors that had the lowest concentration of SSTRs. We did not find a correlation (P = 0.549) between tumor growth rate and expression of mRNA for SSTR2A in these five pancreatic cancer models.

### DISCUSSION

High levels of SSTRs have been demonstrated in various tumors of neuroendocrine origin including cancers of the endocrine pancreas. Nonendocrine tumors also express SSTRs, but the levels and frequency are lower, and the pattern of subtypes differs from neuroendocrine tumors (34–36).

The findings on the expression of mRNAs for the five different SSTRs and binding of SST and its octapeptide analogues to cancers of the exocrine pancreas are the subject of some controversy (13–20). Buscail et al. (13, 17) reported that 98% of samples of normal and cancerous human pancreas showed mRNA for SSTRs, more than half of them expressing two or more subtypes. Normal pancreata contain mRNAs for all of the subtypes except for SSTR3, but in pancreatic cancers only mRNA for SSTR1, 4, and 5 is detected and the expression of SSTR-2 is lost in most tumors (13). Fisher et al. (15, 18) found mRNAs for SSTR1, 2, and 5 in human pancreatic cancers but could not detect mRNAs for SSTR3 and 4 (15, 18). They also indicated that the expression of receptor mRNA in cells does not imply the presence of functional receptors on the cell surface. In contrast, Raderer et al. (16) found mRNA for SSTR3 and SSTR4 in three human pancreatic cancer specimens and four cell lines. They also reported the detection of the primary and metastatic lesions in three patients using radiolabeled lanreotide (DOTALAN) but not Octreoscan (16). However, in another study (19) they could not demonstrate significant binding of either analogue to tumor samples. Similarly, Reubi et al. (20) could not find SSTR binding in human pancreatic cancers using autoradiography with labeled [Tyr3]octreotide or SST-28. Previously (37, 38), SST binding sites were detected in human pancreatic cancer samples by radioreceptor assays. The binding of labeled SST-14 could be displaced by RC-160 and RC-121, but much less by octreotide (38).

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### Table 2: Binding characteristics and mRNA expression of SSTRs in various human pancreatic cancers in nude mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>AN-238</th>
<th>AN-201</th>
<th>1 h after RC-160</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW-1990</td>
<td>5.32 ± 1.16</td>
<td>0/3</td>
<td>0/3</td>
<td>5.77 ± 0.26</td>
</tr>
<tr>
<td>CFPAC-1</td>
<td>10.9 ± 1.17</td>
<td>4/6</td>
<td>5/6</td>
<td>86 ± 0.32</td>
</tr>
<tr>
<td>Capan-1</td>
<td>7.08 ± 0.36</td>
<td>2/4</td>
<td>5/6</td>
<td>83 ± 0.46</td>
</tr>
<tr>
<td>Capan-2</td>
<td>5.89 ± 0.52</td>
<td>1/6</td>
<td>2/3</td>
<td>32 ± 0.52</td>
</tr>
<tr>
<td>Panc-1</td>
<td>4.03 ± 0.51</td>
<td>2(1)/4</td>
<td>3(3)/6</td>
<td>0.52 ± 225</td>
</tr>
<tr>
<td>MiaPaCa-2</td>
<td>NB</td>
<td>5(1)/6</td>
<td>3/6</td>
<td>0.56 ± 447</td>
</tr>
</tbody>
</table>

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*Binding data are means ± SE of two-three experiments, each done in duplicate or triplicate. mRNA data show SSTR subtype-positive tumors (plus weak positive samples in parentheses). Total number of tumors analyzed. Except for SW-1990, all of the data represent control tumors.

**Kd** dissociation constant; **Bmax** maximal binding capacity of receptors; NB, no binding.

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In addition to differences in techniques, other factors could have caused these conflicting results. The receptor status changes during carcinogenesis, and differences can be detected between normal tissue, premalignant transformations, and fully developed cancers (13). Moreover, even normal organs are complex; SSTR2A was found in human islet cells but not in acinar structures (39). Some tissue components, such as stromal elements, present in or close to tumors may also express SSTRs (40).

In our study, mRNAs for both SSTR3 and SSTR5 were expressed in every SW-1990 cancer sample, but mRNA for SSTR2A was absent. AN-238 showed strong binding to SW-1990 cancers, as verified by displacement binding assays. Binding of labeled RC-160 to SW-1990 could be strongly displaced by SST-14, but only a slight displacement was observed by vasoactive intestinal peptide, which binds to SSTR3 (41). These findings indicate that SSTR5 is probably more important for binding of AN-238 than SSTR3.

Other pancreatic cancer lines tested in this study expressed mRNA for SSTR2A and, in some cases, for SSTR3 and SSTR5. There were variations in subtypes, even within one tumor type. However, every sample of each tumor line expressed at least one of the SSTR subtypes investigated, and only MiaPaCa-2 showed no binding of labeled RC-160. Putting together the effects of treatment with the SSTR subtypes expressed on tumors, it appears that in addition to SSTR2 or in its absence, SSTR5 is the most important receptor for targeting of octapeptide analogues. However, SSTR3 can also be involved in binding of AN-238.

AN-238 powerfully inhibited the growth of SW-1990 cancers in nude mice; its inhibitory effect was considerably stronger than that of radical AN-201, which was also more toxic. The carrier peptide alone was without activity, and the unconjugated mixture of AN-201 and RC-121 had the same effect as AN-201 alone. Pretreatment of mice with RC-160, before administration of AN-238, decreased its efficacy and increased the toxicity. Presumably, RC-160 competitively blocked the binding of AN-
2860 SSTR-targeted Cytotoxic Therapy of Pancreatic Cancer

238 to the receptors. Accordingly, the binding capacity of SSTRs in SW-1990 cancers and mRNA expression for SSTR5 in experiment 3 were markedly decreased 1 h after administration of a high dose of RC-160. This is in accordance with results showing that short exposure to octreotide down-regulates subtype 2 receptors in tumors, whereas prolonged treatment results in up-regulation (42). If the receptors are blocked, AN-238 is hydrolyzed by carboxylesterases in the circulation, releasing AN-201 that causes toxic effects (43). Previous studies (23, 25, 27) showed that treatment with AN-238 had no effect on binding characteristics of SSTRs.

Poor therapeutic results with octapeptide SST analogues in Phase I/II clinical trials (44, 45) are likely attributable to the loss of gene expression for SSTR2 in pancreatic cancers (13) and relatively low levels of SSTR3 and SSTR5 (15). Our findings on SW-1990 cancers, which also express mRNA for SSTR3 and SSTR5 but not for SSTR2, demonstrate the differences in efficacy between a “straight” analogue such as RC-160 and cytotoxic analogue AN-238. Thus, it is possible that very potent cytotoxic SST analogues such as AN-238 would be targeted even to tumors with a low concentration of SSTRs, producing effective clinical responses (46).

Our results suggest that patients with exocrine pancreatic cancers expressing receptor subtype 5 and/or 3 might benefit from treatment with cytotoxic SST analogue AN-238. Clinical trials with AN-238 are planned after toxicity studies are completed. Scintigraphic imaging with Octreoscan (8) or DOTALAN (16) may help in the selection of patients for targeted therapy with AN-238.

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