Immunohistochemical Determination of Five Somatostatin Receptors in Meningioma Reveals Frequent Overexpression of Somatostatin Receptor Subtype sst<sub>2A</sub><sup>1</sup>

Stefan Schulz, Steffen Ulrich Pauli, Solveig Schulz, Manuela Händel, Knut Dietzmann, Raimond Firsching, and Volker Hölt<sup>2</sup>

Departments of Pharmacology and Toxicology [St. S., M. H., V. H.], Neurosurgery [S. U. P., R. F.], Obstetrics and Gynecology [So. S.], and Neuropathology [K. D.], Otto-von-Guericke-University, 39120 Magdeburg, Germany

ABSTRACT

Meningioma is one of a variety of human tumors that exhibit a very high density of somatostatin receptors and in many cases show a true positive somatostatin receptor scintigraphy. However, the level of expression of individual somatostatin receptor proteins in meningioma has not been investigated. We have recently developed a panel of somatostatin receptor subtype-specific antibodies that effectively stain formalin-fixed, paraffin-embedded tumor tissue (S. Schulz et al., Clin. Cancer Res., 4: 2047–2052, 1998). In the present study, we have used these antibodies to determine the somatostatin receptor status of 40 randomly selected meningiomas. Immunoreactive staining for all somatostatin receptor proteins in meningioma has not been investigated. We have recently developed a panel of somatostatin receptor subtype-specific antibodies that effectively stain formalin-fixed, paraffin-embedded tumor tissue (S. Schulz et al., Clin. Cancer Res., 4: 2047–2052, 1998). In the present study, we have used these antibodies to determine the receptor subtype expression, and somatostatin receptor scintigraphy is of value in the differentiation of meningiomas from other brain tumors. Meningiomas show the highest incidence of somatostatin receptor expression, and somatostatin receptor scintigraphy is of value in the differentiation of meningiomas from other brain tumors (10–13). Treatment of meningioma with somatostatin analogues has also been attempted (14–16).

INTRODUCTION

It is well known that many human tumors can express somatostatin receptors (1). This is the molecular basis for the application of long-acting somatostatin analogues, i.e., octreotide, for therapeutic and diagnostic purposes (2–4). Whereas unlabeled octreotide has been used successfully in the treatment of neuroendocrine malignancies (5–9). Among brain tumors, meningiomas show the highest incidence of somatostatin receptor expression, and somatostatin receptor scintigraphy is of value in the differentiation of meningiomas from other brain tumors (10–13). Treatment of meningioma with somatostatin analogues has also been attempted (14–16).

Recently, five subtypes of somatostatin receptors designated sst<sub>1–5</sub> have been identified (17). Two isofoms of sst<sub>3</sub> have been isolated, sst<sub>2A</sub> and sst<sub>2B</sub>, which differ in size and the sequence of their intracellular COOH-terminal domain (18, 19). All receptors bind natural somatostatin with high affinity but differ in their binding characteristics to various long-acting somatostatin analogues (20). Whereas sst<sub>2A</sub>, sst<sub>3</sub>, and sst<sub>4</sub> exhibit high affinity for the synthetic somatostatin analogues seglitide (MK 678) and octreotide (SMS 201-995), sst<sub>1</sub> and sst<sub>5</sub> do not bind these compounds. There is also evidence for different but not mutually exclusive pathways of intracellular signaling of somatostatin receptor subtypes. Whereas the antiinflammatory action of octreotide has been linked to stimulation of sst<sub>2</sub>-associated tyrosine phosphatases, perturbation of the sst<sub>3</sub> receptor is believed to induce apoptosis in human tumor cells (21–24). Furthermore, the antiproliferative effects of somatostatin analogues seem to require high numbers of somatostatin receptors, whereas the antiinflammatory effects occur in the presence of a relatively low number of receptors. It is therefore crucial to determine the pattern of somatostatin receptor subtype expres-
sion for a specific tumor to select one or more somatostatin analogues for optimal therapeutic effect.

The expression of somatostatin receptors in human tumors has previously been detected using binding autoradiography, in situ hybridization, or reverse transcription-PCR. However, the diagnostic value of these methods is limited because the subtype selectivity of ligands available for binding autoradiography is not high enough to discriminate between individual somatostatin receptors. Moreover, it is often uncertain whether transcripts detected in reverse transcription-PCR originate from tumor cells or from adjacent normal tissue. Progress on this front has been hampered by the lack of specific antibodies for immunohistochemical detection of somatostatin receptor proteins. We have recently generated antibodies that exert selective specificity for the somatostatin receptor subtypes sst1, sst2A, sst2B, and sst3 (25). We have also developed an immunohistochemical protocol that allows efficient staining of formalin-fixed, paraffin-embedded human tumor tissue using these antibodies (25). The need for the development of specific anti-somatostatin receptor antibodies is exemplified by the fact that at the same time, several other laboratories have reported very similar protocols for the detection of sst2A in human endocrine tumors (26–28). In the present study, we have generated antibodies directed against the COOH-terminal sequences of sst4 and sst5 and determined the complete somatostatin receptor status of 40 meningiomas, one of a variety of human tumors known to exhibit particularly high levels of somatostatin binding sites.

**MATERIALS AND METHODS**

**Patients, Tumors, and Tissue Preparation.** Meningiomas from 40 patients were studied. All patients were initially treated by surgical tumor resection between 1996 and 1998 at the Department of Neurosurgery, Otto-von-Guericke University (Magdeburg, Germany). Pertinent data from patient histories (age, gender, diagnosis, and histological grade) are given in Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Histological grade</th>
<th>Histological grade</th>
<th>Histological grade</th>
<th>Histological grade</th>
<th>Histological grade</th>
<th>Histological grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>m</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>3</td>
<td>74</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>6</td>
<td>61</td>
<td>m</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>7</td>
<td>86</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>8</td>
<td>68</td>
<td>f</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>9</td>
<td>68</td>
<td>f</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>10</td>
<td>67</td>
<td>m</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>11</td>
<td>47</td>
<td>f</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>12</td>
<td>65</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>13</td>
<td>73</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>14</td>
<td>64</td>
<td>m</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>15</td>
<td>47</td>
<td>m</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>16</td>
<td>42</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>17</td>
<td>62</td>
<td>f</td>
<td>MC</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>18</td>
<td>80</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>19</td>
<td>61</td>
<td>m</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>20</td>
<td>74</td>
<td>f</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>21</td>
<td>58</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>22</td>
<td>61</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>23</td>
<td>65</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>24</td>
<td>60</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>25</td>
<td>82</td>
<td>f</td>
<td>A</td>
<td>G2</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>26</td>
<td>44</td>
<td>m</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>27</td>
<td>46</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>28</td>
<td>56</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>29</td>
<td>51</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>30</td>
<td>51</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>31</td>
<td>64</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>32</td>
<td>64</td>
<td>f</td>
<td>F</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>33</td>
<td>61</td>
<td>f</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>34</td>
<td>75</td>
<td>f</td>
<td>PS</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>35</td>
<td>62</td>
<td>f</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>36</td>
<td>64</td>
<td>m</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>37</td>
<td>30</td>
<td>f</td>
<td>M</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>38</td>
<td>46</td>
<td>f</td>
<td>T</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>39</td>
<td>64</td>
<td>f</td>
<td>A</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>40</td>
<td>64</td>
<td>f</td>
<td>A</td>
<td>G1</td>
<td>−−</td>
<td>+−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
</tbody>
</table>

*T, transitional (fibroblastic and meningothelial) meningioma; F, fibroblastic meningioma; M, meningothelial meningioma; MC, microcystic meningioma; A, atypical meningioma; PS, psammon body rich meningioma.

*b 111, strong; 11, moderate; 1, weak; 2, negative immunostaining.
Table 1. Tumor specimens were fixed in phosphate-buffered 4% formalin for a minimum of 24 h. After dehydration through graded percentages of ethanol and xylene, the tissue was embedded in paraffin wax. In addition, 16 specimens were frozen immediately in liquid N\textsubscript{2} and stored at −70°C until analysis.

**Generation of Anti-peptide Antiserum.** Production and characterization of anti-sst\textsubscript{1} (4819), anti-sst\textsubscript{2A} (6291), anti-sst\textsubscript{2B} (4820), and anti-sst\textsubscript{3} (4823) antiserum has been described previously. In this study, polyclonal antiserum was generated against the COOH-terminal tails of sst\textsubscript{2} and sst\textsubscript{3}. The identity of the peptides was CQEPVQAEPGCKQVPFTKTTTF, which corresponds to residues 362–384 of the mouse sst\textsubscript{4} receptor, and QEA\textsubscript{PRTA}AAANGLMQTSK, which corresponds to residues 345–364 of the human sst\textsubscript{3} receptor. Peptides were custom-synthesized by Gramsch Laboratories (Schwabhausen, Germany), purified by high-performance liquid chromatography, and coupled via an NH\textsubscript{2}-terminally added cysteine and a succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate linker to keyhole limpet hemocyanin. The conjugates were mixed 1:1 with Freund’s adjuvant and injected into groups of two rabbits (6001–6002 for anti-sst\textsubscript{4} and 6005–6006 for anti-sst\textsubscript{3}, antiserum production). Animals were injected at 4-week intervals, and serum was obtained 2 weeks after immunizations beginning with the second injection.

**Immunodot-Blot Analysis.** The specificity of the antisera as well as possible cross-reactivity with other somatostatin receptor subtypes was initially tested in dot-blot assays. Serial dilutions of the unconjugated peptides corresponding to the COOH-terminal sequences of sst\textsubscript{1}, sst\textsubscript{2A}, sst\textsubscript{2B}, sst\textsubscript{3}, sst\textsubscript{4}, and sst\textsubscript{5} were blotted onto nitrocellulose membranes. The identity of the peptides was: (a) CRNGTCTSRITL, which corresponds to residues 382–391 of the human sst\textsubscript{1} receptor; (b) ETQRTLLNGDLQTSI, which corresponds to residues 355–369 of the human sst\textsubscript{2A} receptor; (c) FRNNKNRRKK, which corresponds to residues 348–356 of the human sst\textsubscript{2B} receptor; (d) CQERPPSRVA, which corresponds to residues 355–369 of the human sst\textsubscript{3} receptor; (e) CQERQPEKPGRKRIPLRTTTF, which corresponds to residues 366–388 of the human sst\textsubscript{4} receptor; (f) CQEQPVQAEPGCKQVPFTKTTTF, which corresponds to residues 362–384 of the mouse sst\textsubscript{4} receptor; and (g) QEA\textsubscript{PRTA}AAANGLMQTSK, which corresponds to residues 345–364 of the human sst\textsubscript{5} receptor. Membranes were then incubated with the antisera at dilutions ranging from 1:1,000 to 1:20,000 for 60 min at room temperature. Blots were developed using the enhanced chemiluminescence method (Amersham, Braunschweig, Germany). Specimens were examined using a Leica TCS-NT laser scanning confocal microscope equipped with a krypton/argon laser. Cyanin 3.18 was imaged with 568 nm excitation and 570–630 nm bandpass emission filters.

**Western Blot Analysis.** Membranes were prepared from stably transfected HEK-293 cells as well as 16 meningiomas, and glycoproteins were partially purified using wheat germ lectinin-agarose (Vector Laboratories) essentially as described previously. Briefly, tissue was lysed in homogenization buffer (5 mM EDTA, 3 mM EGTA, 250 mM sucrose, and 10 mM Tris-HCl (pH 7.6) containing 1 mM phenylmethylsulfonyl fluoride, 1 μM pepstatin, 10 μg/ml leupeptin, and 2 μg/ml aprotinin). The homogenate was spun at 500 × g for 5 min at 4°C to remove unbroken cells and nuclei. Membranes were then pelleted at 20,000 × g for 30 min at 4°C. Membranes were then dissolved in lysis buffer (150 mM NaCl, 5 mM EDTA, 3 mM EGTA, and 20 mM HEPES (pH 7.4) containing 4 mg/ml dodecyl-β-maltoside and protease inhibitors as described above) and incubated with 150 μl of wheat germ lectinin-agarose beads for 90 min at 4°C. Beads were washed five times in lysis buffer, and adsorbed glycoproteins were eluted with SDS-sample buffer for 60 min at 37°C. The protein content was determined using the BCA method according to the instructions of the manufacturer (Pierce), and aliquots of each sample containing equal amounts of protein were subjected to 8% SDS-PAGE and immunoblotted onto nitrocellulose. Another aliquot of each sample was run on a duplicate gel that was then stained with Coomassie Blue, and equal loading was verified by densitometric analysis as described above. Blots were incubated with anti-sst\subscript{1} (4819), anti-sst\subscript{2A} (6291), anti-sst\subscript{2B} (4820), anti-sst\subscript{3} (4823), anti-sst\subscript{4} (6002), or anti-sst\subscript{5} (6006) antibodies either crude at a dilution of 1:20,000 or after affinity purification at a concentration of 1 μg/ml overnight at 4°C. Blots were developed using peroxidase-conjugated secondary antibodies purchased either from Sigma (A-9169;1:5,000 dilution) or from Amersham (NA 934; 1:5,000 dilution) and enhanced chemiluminescence. Densitometric analysis of Western blots exposed in the linear range of the X-ray

3 The abbreviations used are: TPBS, 10 mM Tris, 10 mM phosphate buffer, 137 mM NaCl, and 0.05% thimerosal (pH 7.4); NGS, normal goat serum.
Somatostatin Receptor Status in Meningioma

To the COOH-terminal regions of hsst1, hsst2A, hsst2B, hsst3, msst4, hsst4, or hsst5 were blotted onto nitrocellulose membranes and incubated enhanced chemiluminescence method. Note that both the anti-sst4 and antisera at a dilution of 1:2000. Membranes were developed using the anti-sst5 antisera. Serial dilutions (0 – 2000 ng) of the peptides corresponding to the human sst4 receptor. In addition, the anti-sst4 antibodies detected the sequence corresponding to the mouse as well as the sequence corresponding to their cognate receptor but did not detect the peptides corresponding to other somatostatin receptors. In addition, the anti-sst4 antibodies detected the sequence corresponding to the mouse as well as the sequence corresponding to the human sst4 receptor.

Fig. 1 Immunodot-blot analysis of the specificity of anti-sst4 and anti-sst5 antisera. Serial dilutions (0–2000 ng) of the peptides corresponding to the COOH-terminal regions of hsst1, hsst2A, hsst2B, hsst3, msst4, hsst4, or hsst5 were blotted onto nitrocellulose membranes and incubated either with anti-sst4 (6002; top panel) or anti-sst5 (6006; bottom panel) antisera at a dilution of 1:2000. Membranes were developed using the enhanced chemiluminescence method. Note that both the anti-sst4 and the anti-sst5 antisera selectively detected the peptide corresponding to their cognate receptor but did not detect the peptides corresponding to other somatostatin receptors. In addition, the anti-sst4 antibodies detected the sequence corresponding to the mouse as well as the sequence corresponding to the human sst4 receptor.

Immunohistochemistry. Seven-μm sections were cut and floated onto positively charged slides (SuperFrost®/Plus; Menzel, Braunschweig, Germany) for immunohistochemical staining. Sections were dewaxed three times in xylene and rehydrated in a graded series of ethanol. After rinsing in TPBS, sections were incubated in methanol containing 0.3% H2O2 for 30 min at room temperature. Sections were transferred into TPBS and subsequently microwaved in 10 mM citric acid (pH 6.0) for 20 min at 600 W. Specimens were then allowed to cool to room temperature, washed in TPBS, and preincubated in TPBS containing 3% NGS for 1 h at room temperature. Sections were then incubated either with anti-sst1 (4819), anti-sst2A (6291), anti-sst2B (4820), anti-sst3 (4823), anti-sst4 (6002), or anti-sst5 (6006) antibodies at a dilution of 1:500 (crude) or at a concentration of 1 μg/ml (affinity pure) in TPBS containing 1% NGS overnight. Primary antibody staining was detected using the biotin amplification procedure as described previously (25, 30, 32, 33). Briefly, tissue sections were transferred to biotinylated goat antirabbit IgG or biotinylated goat anti-guinea pig IgG (1:200; Vector Laboratories) for 1 h, incubated in AB solution (reagents from Vector Laboratories ABC Elite kit; 25 μl of A and 25 μl of B) for 60 min, and incubated in biotinylated tyramine (1:250 dilution; prepared as described in Ref. 32) for 30 min, followed by a final incubation in AB solution (12.5 μl of A and 12.5 of μl B). Tissue was rinsed and stained with 3,3′-diaminobenzidine-glucose oxidase for 30 min. All incubation steps were carried out at room temperature. The cell nuclei were lightly counterstained with hematoxylin. Sections were then dehydrated through several concentrations of alcohol, cleared in xylol, and coverslipped with DPX. For immunohistochemical controls, the primary antibody was either omitted, replaced by preimmune sera, or adsorbed with several concentrations (range, 1–10 μg/ml) of homologous or heterologous peptides for 2 h at room temperature. A tumor known to stain positively was included in each batch of staining as a positive control.

Assessment of Staining Patterns. Immunohistochemical staining patterns were assessed as described previously (25), and all slides were evaluated by the same investigator. Briefly, the presence or absence of staining and the depth of color were noted, as well as the number of cells showing a positive reaction and whether or not the staining was localized to the plasma membrane. The depth of color was recorded as pale, medium, or dark according to how easily it was seen. The tumors were then categorized as weak, moderate, or strong staining according to the following criteria: (a) strong (+ + +), dark staining at the plasma membrane that is easily visible with a low-power objective; (b) moderate (+ +), medium staining that is visible with a low-power objective; (c) weak (+), pale staining that is not easily seen under a low-power objective; and (d) negative (−), tumors that show none of the above.

Statistical Evaluation. Data were analyzed by using the SAS statistical program package (SAS Institute, Cary, NC). Data grouped into categories were analyzed for correlations with the χ2 test, Fisher’s exact test, and Spearman test.

RESULTS

Characterization of Antibodies. Specificity of the antisera was monitored using immunodot-blot analysis. After four booster injections, one anti-sst4 antiserum and one rabbit anti-
sst 5 antiserum developed a titer against their immunizing peptides. As shown in Fig. 1, the antisera 6002 (anti-sst 4 ) and 6006 (anti-sst 5 ) specifically detected quantities as low as 25 ng of their cognate peptide but did not detect the peptides corresponding to other somatostatin receptor subtypes. Moreover, antisera 6002, which was raised against the COOH terminus of the mouse sst 4 receptor, detected not only the sequence of the mouse but also the corresponding sequence of the human sst 4 .

Somatostatin receptor antisera were further characterized using immunofluorescent staining of stably transfected HEK-293 cells. When HEK-293 cells stably expressing human sst 1, sst 2A, sst 3, sst 4, or sst 5 (vertical columns) were immunofluorescence stained with either anti-sst 1 (4819), anti-sst 2A (6291), anti-sst 3 (4823), anti-sst 4 (6002), or anti-sst 5 (6006) antiserum (horizontal columns). Note that prominent immunofluorescence localized at the level of the plasma membrane was seen only in HEK-293 cells bearing their cognate somatostatin receptor and not in HEK-293 cells transfected with other somatostatin receptors. Scale bar, 10 μm.

sst 1 antiserum developed a titer against their immunizing peptides. As shown in Fig. 1, the antisera 6002 (anti-sst 4 ) and 6006 (anti-sst 5 ) specifically detected quantities as low as 25 ng of their cognate peptide but did not detect the peptides corresponding to other somatostatin receptor subtypes. Moreover, antisera 6002, which was raised against the COOH terminus of the mouse sst 4 receptor, detected not only the sequence of the mouse but also the corresponding sequence of the human sst 4 .

Somatostatin receptor antisera were further characterized using immunofluorescent staining of stably transfected HEK-293 cells. When HEK-293 cells stably expressing human sst 1, sst 2A, sst 3, sst 4, or sst 5 were stained with either anti-sst 1 (4819), anti-sst 2A (6291), anti-sst 3 (4823), anti-sst 4 (6002), or anti-sst 5 (6006) antiserum, prominent immunofluorescence localized at the level of the plasma membrane was seen only in HEK-293 cells bearing their cognate somatostatin receptor (Fig. 2) and not in HEK-293 cells transfected with other somatostatin receptors. This staining was completely blocked by preincubation of the antisera with homologous but not heterologous peptides (data not shown).

Antisera were then subjected to immunohistochemical staining of a panel of human tumor tissues including primary breast cancer, carcinoid tumor, pituitary adenoma, and meningioma. The antisera 4819 (anti-sst 1 ), 6291 (anti-sst 2A ), 4820 (anti-sst 2A ), 4823 (anti-sst 3 ), 6002 (anti-sst 4 ), and 6006 (anti-sst 5 ) yielded prominent staining that was predominantly localized to the plasma membrane of the tumor cells and were used throughout this study. Immunostaining for each antiserum was completely abolished by preabsorption with 10 μg/ml immunizing peptides.

Somatostatin Receptor Immunohistochemical Staining in Meningioma. A series of 40 meningiomas was stained immunohistochemically with polyclonal anti-sst 1 (4819), anti-sst 2A (6291), anti-sst 3 (4823), anti-sst 4 (6002), and anti-sst 5 (6006) antisera. Scale bar, 10 μm.

![Fig. 2 Characterization of anti-sst antisera using stably transfected HEK-293 cells. HEK-293 cells stably transfected to express either sst 1, sst 2A, sst 3, sst 4, or sst 5 (vertical columns) were immunofluorescence stained with either anti-sst 1 (4819), anti-sst 2A (6291), anti-sst 3 (4823), anti-sst 4 (6002), or anti-sst 5 (6006) antiserum (horizontal columns). Note that prominent immunofluorescence localized at the level of the plasma membrane was seen only in HEK-293 cells bearing their cognate somatostatin receptor and not in HEK-293 cells transfected with other somatostatin receptors. Scale bar, 10 μm.](#)
was present in 10 tumors (25%), unequivocal staining for sst4 was present in 11 tumors (28%), and unequivocal staining for sst5 was present in 4 tumors (11%). Interestingly, the majority of sst2A-positive tumors showed moderate to strong immunostaining. In contrast, none of the other somatostatin receptor subtypes revealed a particularly strong staining, indicating that sst2A is the predominant somatostatin receptor subtype expressed in meningioma. In the vast majority of positively stained tumors, somatostatin receptor immunoreactivity was uniformly present on nearly all tumor cells. Both the level and the pattern of expression of somatostatin receptor subtypes varied greatly between individual tumors. No staining for sst2A or other somatostatin receptor subtypes was observed in normal meninges. Thus, sst2A overexpression in meningiomas appears to reflect a tumor-specific phenotype. Somatostatin receptor staining patterns were analyzed for correlation of each subtype with patient age, gender, diagnosis, and histological grade. No correlations among these data groups were found. The lack of correlation of somatostatin receptor subtype expression and patient age, gender, diagnosis, and histopathological grade indicates that somatostatin receptor subtype expression was regulated independently of these variables.

**Correlation between sst2A-immunoreactive Staining and sst2A Protein Expression.** The clinical utility of octreotide depends on the number of sst2A receptors on the tumor cells. However, to what extent sst2A-immunoreactive staining intensity translates into sst2A protein expression is uncertain. Thus, we have collected 16 surgically removed meningiomas and analyzed immunoreactive sst2A receptors in paraffin sections as well as in immunoblots. sst2A immunohistochemical staining was evaluated according to the criteria described in “Materials and Methods.” Typical staining patterns are shown in Fig. 4. On Western blots, the sst2A receptor was readily detectable as a broad band migrating at M₉ 70,000 (Fig. 5). In some tumor lysates, an additional band was detected at M₉ 110,000. However, this band appeared to originate from nonspecific binding of the secondary antibody (Sigma) because it was neither detected with a secondary antibody from a different manufacturer (Amersham) nor completely blocked by preincubation with antigenic peptide. As shown in Fig. 6, top panel, the level of sst2A protein expression varied greatly between individual tumors. In fact, eight tumors (50%) revealed particularly high levels of immunoreactive sst2A receptors. In the remaining tumors, the sst2A-specific band was either weak or not detectable. Similar immunoreactive staining for sst2A was scored moderate to strong in 7 of the 16 tumors (44%). Nine tumors were scored negative or weak. Interestingly, there was an excellent correlation (P < 0.001; r = 0.8622) between the densitometric analysis of sst2A band intensity and the sst2A-immunoreactive staining score (Fig. 6, bottom panel). No other somatostatin receptors were unequivocally detected by Western blot analysis in these 16 tumors.

**DISCUSSION**

In the present study, we have determined the pattern of somatostatin receptor protein expression in human meningioma using a panel of somatostatin receptor subtype-selective antibodies that have been characterized extensively. Several lines of evidence indicate that these antisera specifically detect their
Fig. 4 sst2A immunohistochemical staining in meningioma. Left panels, staining patterns for sst2A in typical meningiomas displaying either strong (+++), moderate (++), weak (+), or negative (−) staining. Right panels, corresponding peptide adsorption controls. Sections were dewaxed, treated with methanol-H2O2, microwaved in citric acid, and incubated with anti-sst2A (6291) antibodies at a dilution of 1:500. Sections were then sequentially treated with biotinylated antirabbit IgG, AB solution, biotinylated tyramine, and AB solution. Sections were then developed in 3,3’-diaminobenzidine-glucose oxidase and lightly counterstained with hematoxylin. The immunoreactive score was determined according to the criteria described in “Materials and Methods.” For adsorption controls, primary antibodies were preincubated with the immunizing peptide (10 μg/ml). Scale bar, 10 μm.
Somatostatin Receptor Status in Meningioma

In our series of 40 randomly selected meningiomas, sst2A was clearly the predominant somatostatin receptor subtype. The sst2A receptor was not only the most frequently detected receptor but did not stain wild-type cells or cells transfected with other somatostatin receptors. Third, in Western blots, the antiserum detected a band of the appropriate molecular weight only in those HEK-293 cells that expressed their cognate receptor and not in cells that expressed other somatostatin receptor subtypes. Fourth, the anti-somatostatin receptor antibodies effectively stained formalin-fixed, paraffin-embedded tissue from a variety of human tumors including meningioma, primary breast cancer, and carcinoid tumor. Staining of all antisera was completely neutralized by preincubation with homologous peptide and not with heterologous peptides. Finally, the COOH-terminal peptides are likely to have served as somatostatin receptor-specific immunogens because these peptides were found to have minimal homologies to other peptide sequences when aligned to current entries in the European Molecular Biology Laboratory databases using BLASTp or FASTA.

In our series of 40 randomly selected meningiomas, sst2A was clearly the predominant somatostatin receptor subtype. The sst2A receptor was not only the most frequently detected receptor but also yielded the most prominent staining of all somatostatin receptor subtypes. It is believed that the antiproliferative effects of somatostatin analogues require high numbers of somatostatin receptors, whereas the antihormonal effects occur in the presence of a relatively low number of receptors. Thus, in a clinical setting, it may prove useful to determine both the presence and the level of sst2A expression on the tumor cell. However, it is uncertain to what extent sst2A-immunoreactive staining intensity may translate into sst2A protein expression on the tumor cells. Therefore, we conducted a prospective study using 16 surgically removed meningiomas. Interestingly, there was an excellent correlation (P < 0.001) between sst2A-immunoreactive staining and sst2A protein expression. The correlation coefficient and statistical significance were determined using the Spearman test.

It has been well documented that many cases of meningioma show a particularly high tracer uptake during somatostatin receptor scintigraphy using [111In-DTPA-D-Phe1]octreotide (10–13). Octreotide, which binds preferentially to sst2 and sst5, has also been implicated in the treatment of meningioma (14–16). Moreover, at the mRNA level, several studies have detected a particularly high expression of sst2A in the majority of men-
ingiomas (7, 9). Thus, our observation that sst2A protein is frequently overexpressed in human meningioma corresponds well to these findings and would thus explain the high rate of true positive somatostatin receptor scintigraphy of this tumor.

What are the implications of immunohistochemical somatostatin receptor determination for the treatment of meningioma? Surgical removal of the tumor is clearly the first option; however, some cases of unresectable tumor or recurrent disease exist that demand further attention. Knowledge of the somatostatin receptor status of these tumors may help in identifying those cases that may possibly respond to therapy with octreotide or other sst2A-selective ligands. It should be noted, however, that the effectiveness of octreotide in the treatment of meningioma is expected to be limited. Although some cases of successful treatment of meningioma with octreotide have been reported (14–16), somatostatin analogues have also been shown to stimulate the growth of cultured human meningioma cells in vitro (34, 35). Nevertheless, novel nonpeptide agonists for all somatostatin receptors as well as cytotoxic and radiolabeled somatostatin analogues are currently being developed and may provide further options for treatment (36–41).

In conclusion, we have generated and extensively characterized subtype-selective antibodies for all five somatostatin receptors. We demonstrate that these antibodies are well suited for an immunohistochemical procedure that allowed us to provide precise information about the somatostatin receptor protein expression in a given tumor specimen. With the development of subtype-selective ligands, it will be of particular importance to establish patterns of somatostatin receptor expression for each tumor to select one or more somatostatin analogues for an optimal therapeutic effect.

ACKNOWLEDGMENTS

We thank M. Albrecht, D. Nüß, and D. Wibornado for skillful technical assistance and Dr. F-W. Röhl for help with statistical analysis.

REFERENCES


Immunohistochemical Determination of Five Somatostatin Receptors in Meningioma Reveals Frequent Overexpression of Somatostatin Receptor Subtype sst\textsubscript{2A}

Stefan Schulz, Steffen Ulrich Pauli, Solveig Schulz, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/5/1865

Cited articles
This article cites 40 articles, 13 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/5/1865.full#ref-list-1

Citing articles
This article has been cited by 24 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/6/5/1865.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.