Expression and Function of Somatostatin Receptors in Peripheral Nerve Sheath Tumors

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Abstract

Although somatostatin receptors have been detected in many normal and neoplastic tissues, little is known of their expression and function in peripheral nerve tumors. In the present study, we examined the expression of all 5 somatostatin receptor subtypes (sst1–5) in 3 normal peripheral nerves, 3 traumatic neuramas, 27 schwannomas, 18 neurofibromas, and 177 malignant peripheral nerve sheath tumors (MPNSTs) by immunohistochemistry as well as by Western blot and reverse transcriptase–polymerase chain reaction investigations in 2 normal peripheral nerves, one neurofibroma, 5 schwannomas, and 5 MNPsTs. Immunoreactive somatostatin receptors were not detectable in normal peripheral nerve and in noneploic Schwann cell proliferations. In contrast, sst2A mRNA and protein was present in 89% of schwannomas. This receptor subtype was less frequently detected in neurofibromas (22%) and MNPsTs (15%). Interestingly, sst4 was seen in 32% of MPNSTs and was almost exclusively expressed in this malignant tumor type. In support of a role in Schwann cell tumor growth control by somatostatin was the observation of induced internalization of sst2A and inhibition of cell proliferation in an NF1-associated MPNST cell line. Moreover, administration of an sst2A-selective agonist resulted in induction of MPNST cell apoptosis. We conclude that peripheral nerve sheath tumors often express at least one functional somatostatin receptor. Furthermore, our findings suggest a potential clinical role for somatostatin receptor agonists in tumor imaging and/or treatment of schwannomas and MPNSTs.

Key Words: Malignant peripheral nerve sheath tumor (MPNST), Neurofibroma, NF1, Peripheral nerve, Schwannoma, Somatostatin receptor.

INTRODUCTION

The neuropeptide somatostatin exerts a variety of effects in the central and peripheral nervous system. Two biologically active forms have been identified in mammals, the cyclic tetradecapeptide somatostatin-14 (SS14) and the N terminally extended somatostatin-28 (SS28), both of which are derived from a common precursor molecule (1). The physiological effects of somatostatin are mediated through a family of membrane-spanning G-protein-coupled receptors (2). Five subtypes of somatostatin receptors designated sst1–5 have been identified (2). Four (sst1, sst3, sst4, and sst5) of the somatostatin receptor genes lack introns, whereas sst2 is alternatively spliced into sst2A and sst2B. All five receptors bind natural somatostatin with high affinity, but differ in their binding characteristics to various long-acting somatostatin analogs (3). Whereas sst2, sst3, and sst5 exhibit high affinity for the synthetic somatostatin analog octreotide and lanreotide, sst1 and sst4 do not bind these compounds.

Somatostatin receptors can be found in many human tumors, including benign and malignant tumors of the central nervous system (CNS) (4–6). This is the molecular basis for the clinical application of long-acting somatostatin analogs to tumor imaging and treatment. Interestingly, recent studies have shown that the spectrum of somatostatin receptor subtype expression varies among different intracranial neoplasms. Whereas meningiomas frequently overexpress the sst2A subtype (4, 5), astrocytic neoplasms express certain receptor subtypes in relation to their degree of differentiation with a high proportion of sst and sst, immunopositivity in malignant gliomas (6). These observations suggest that the transition from a benign to a malignant phenotype may, in certain tumors, be accompanied by a change in somatostatin receptor expression.

All 5 somatostatin receptors are known to have antiproliferative effects on tumor cells, either by cell cycle inhibition or stimulation of apoptosis. Sst1, sst2, sst4, and sst5 induce G1 cell-cycle growth arrest, whereas sst3 is proapoptotic through induction of p53 and Bax (7–9). Sst1, sst3, and sst5 are also known to exert antiangiogenic effects (10).
Most studies have focused on intracranial tumors. Somatostatin receptor expression in peripheral nerve sheath tumors is largely unknown. Recently, Stafford et al (11) reported preferential immunoexpression of sst, in vestibular schwannomas, whereas other receptor subtypes (sst1, sst3, and sst5) were less frequently expressed in these tumors. Interestingly, earlier receptor-binding studies had suggested that schwannomas do not contain somatostatin receptors (4).

Receptor patterns in the other common nerve sheath tumors of the peripheral nervous system (PNS), neurofibromas, and malignant peripheral nerve sheath tumors (MPNSTs) are largely unknown. The occurrence of plexiform neurofibromas is a diagnostic hallmark of neurofibromatosis type 1 (NF1; von Recklinghausen's disease). In this autosomal-dominant disorder, single or multiple localized as well as plexiform neurofibromas occur. The latter possesses a significant risk of malignant progression to MPNST (12, 13). Although sporadic MPNSTs account for approximately 10% of all soft tissue sarcomas, roughly 25% to 50% of all MPNSTs occur in the setting of NF1 (14). MPNSTs are highly aggressive tumors; approximately 60% of patients die of their disease. Metastasis is a common complication, especially to the lung (14).

To date, patterns of somatostatin receptor expression in neurofibromas and MPNSTs have not been studied. Furthermore, the functional effect of sst expression, for example, tumor cell proliferation in schwannomas, neurofibromas, and MPNSTs, is unknown. The characterization of somatostatin receptors in PNS tumors could provide a rationale for using somatostatin analogs for both enhanced diagnostic tumor imaging and therapy, because somatostatin may have substantial antiproliferative effects on tumor cells. In the present study, we therefore studied the expression of sst receptor types at the mRNA and protein level in cells and tissues from common PNS tumors in an effort to determine their functional relevance.

**MATERIALS AND METHODS**

**Tumor Samples**

Paraffin-embedded biopsy samples from 10 neurofibromas, 27 vestibular schwannomas, and eight MPNSTs (including one malignant triton tumor) were used for immunohistochemical studies, together with sections of a tissue microarray (TMA) block containing 169 MPNSTs and 8 neurofibroma samples. The NF1 status was known in all cases. To determine the distribution of somatostatin receptors in nontumoral peripheral nerve tissue, we studied 2 sural nerve biopsies, one sample of the vestibular division of the eighth cranial nerve derived at autopsy and 3 traumatic neuromas. For reverse transcriptase–polymerase chain reaction (RT-PCR) and Western blot studies, autopsy and 3 traumatic neuromas. For reverse transcriptase–polymerase chain reaction (RT-PCR) and Western blot studies, autopsy and 3 traumatic neuromas. For reverse transcriptase–polymerase chain reaction (RT-PCR) and Western blot studies, autopsy and 3 traumatic neuromas. For reverse transcriptase–polymerase chain reaction (RT-PCR) and Western blot studies, autopsy and 3 traumatic neuromas.

**Cell Culture**

The MPNST cell line S462, derived from an NF1-associated MPNST, has been previously characterized in detail (15). Cells were grown in DMEM (Gibco, Gaithersburg, MD) medium with 10% FCS (Gibco), supplemented with 100 μg/mL antibiotics (penicillin/streptomycin mix) (Gibco) and 2 mM glutamine (Gibco), at 37°C and in 5% CO2. As previously described (16), Schwann cells were obtained after in vivo predegeneration of sciatic nerves from adult male Wistar rats (Charles River, Germany). Aliquots of cell suspension (2 mL) were plated onto poly-L-lysine-coated Petri dishes at a final density of 1.6 × 10⁶ cells per 8 cm² and were cultured in DMEM containing 10% fetal calf serum, 50 U/mL penicillin, and 50 μg/mL streptomycin (Sigma, St. Louis, MO) in a humidified atmosphere at 37°C and 5% CO2. After 2 days in vitro, tissue debris, including myelin components, were discarded, leaving behind only adherent cells.

**Immunohistochemical and Immunocytochemical Methods**

For immunohistochemistry, 4-μm-thick paraffin sections were deparaffinized with xylene for 15 minutes and dehydrated through a series of graded alcohols. Sections were pretreated in a microwave oven in 0.01 M sodium acetate buffer (pH 6.0) for 3 × 10 minutes. Endogenous peroxidase activity was blocked by 30-minute incubation in 0.3% H₂O₂ in methanol. The sections were then gently rinsed with TBS buffer and incubated with bovine serum albumin for 30 minutes to reduce nonspecific antibody binding. Sections were then individually incubated with affinity-purified anti-sst₁ (rabbit no. 4819), anti-sst₂₂ (rabbit no. 6291), anti-sst₃ (rabbit no. 4823), anti-sst₄ (rabbit no. 4802), and anti-sst₅ (rabbit no. 6006) antibodies at concentrations of 1 to 2 μg/mL. These polyclonal antisera were generated against the carboxy terminal tails of somatostatin receptors in the following way. Peptides were custom-synthesized with reference to the human somatostatin sequences by Gramsch Laboratories (Schwabhausen, Germany) purified by high-performance liquid chromatography and coupled through an NH₂-terminally added cysteine and a succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate linker to keyhole limpet hemocyanin (Sigma). Rabbits (Zykahybrid) were immunized subcutaneously with the respective peptide conjugates emulsified in complete Freund’s adjuvant at 1:1 (vol/vol). They were given booster injections every 4 weeks, and serum was obtained 2 weeks after immunizations beginning with the second injection. The titer of the generated antibodies was checked by enzyme-linked immunosorbent assay. Antiser specificity was monitored using immunodot-blot analysis with serial dilutions of the unconjugated peptides corresponding to the COOH-terminal sequences of sst₁, sst₂₂, sst₃, sst₄, and sst₅ (5, 17). The identity of the peptides was as follows: CRNGTCTSRITTL corresponding to residues 382–391 of human sst₁; ETQRTLLNGDLQTSICQQEALQPEPGRKRIPLTRTTTF corresponding to residues 355–369 of human sst₂₂; C Q E R -

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Concentrations ranging from 1 to 10 μg/mL of homologous or heterologous peptides for 2 hours at room temperature. Normal human pancreatic tissue served as a positive control in each staining batch. The signal was detected using the streptavidin-biotin-peroxidase complex method according to the manufacturer’s recommendation (DAKO, Hamburg, Germany). DAB (3,3′-diaminobenzidine hydrochloride containing 0.08% hydrogen peroxide) served as the chromogen. Sections were then counterstained with hematoxylin.

For immunocytochemistry of in vitro specimens, cells were grown for 24 hours (MPNSTs) or 7 days (Schwann cells) on poly-L-lysine-coated slides. The cells were fixed for 40 minutes in 4% paraformaldehyde/0.2% picric acid in phosphate buffer at pH 6.9 at room temperature. They were then washed several times in 10 mM Tris-Cl at pH 7.4, 10 mM phosphate buffer, 137 mM NaCl, and 0.05% thimerosal at pH 7.4 (TPBS). Specimens were then 1) incubated for 3 minutes in 50% methanol and then another 3 minutes in 100% methanol, 2) washed several times in TPBS, and 3) preincubated with TPBS and 3% normal goat serum for 1 hour at room temperature. Cells were subsequently incubated overnight with anti-sst1, -sst2A, -sst3, -sst4, and -sst5 antibodies at a concentration of 1 μg/mL in TPBS and normal goat serum. Bound primary antibody was detected using a Cy3-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA; 1:50). Slides were then mounted in DPX (Fluka, Neu-Ulm, Germany), coverslipped, and examined using a Leica TCS-SL laser scanning confocal microscope (Heidelberg, Germany). Schwann cells were examined on a fluorescence microscope (Axioskop; Zeiss, Jena, Germany) equipped with phase-contrast fluorescence and rhodamine optics.

Apoptotic MPNST cells were detected using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) in situ cell death detection kit (Roche Diagnostics, Germany). Schwann cells were examined on a fluorescence microscope (Axioskop; Zeiss, Jena, Germany). DAB (3,3′-diaminobenzidine hydrochloride containing 0.08% hydrogen peroxide) served as the chromogen. Sections were then counterstained with hematoxylin.

**Western Blot Analysis**

Membranes were prepared from five schwannomas, three MPNSTs, rat Schwann cells, and MPNST cells. Glycoproteins were partially purified using wheat germ lectin-agarose (Vector Laboratories, Burlingame, CA), essentially as described previously (5, 18, 18). Briefly, tissue was lysed in homogenization buffer (5 mM EDTA, 3 mM EGTA, 250 mM sucrose, and 10 mM Tris-Cl at 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 5000 × g for 5 minutes at 4°C to remove unbroken cells and nuclei. Membranes were then pelleted at 20,000 × g for 30 minutes at 4°C. Membranes were dissolved in lysis buffer (150 mM NaCl, 5 mM EDTA, 3 mM EGTA, and 20 mM HEPES at pH 7.4) containing 4 mg/mL dodecyl-β-maltoside and proteinase inhibitors as described previously and were incubated with 120 μL of wheat germ lectin-agarose beads for 90 minutes at 4°C. The beads were washed 5 times in lysis buffer, the adsorbed glycoproteins then being eluted with SDS-sample buffer for 60 minutes at 37°C. Aliquots of each sample were subjected to 8% SDS-PAGE and were immuno-

**Reverse Transcriptase–Polymerase Chain Reaction Analysis**

Total RNA was extracted from frozen tissue samples using TRIzol reagent (Gibco) according to the manufacturer’s instructions. First-strand cDNA was prepared from 500 ng RNA with avian myeloblastosis virus (AMV) reverse transcriptase. Ten percent of the cDNA was used for subsequent PCR reactions. The following previously described primer pairs (19) detecting sst from rat and human origin were used: sst1 F: 5′-TATCTGGCTGTGCTACGTC-3′, R: 5′-GATGACCGAGCACGTGACT-3′; sst2 (detects both sst2A and sst2B) F: 5′-ATCTGGGGCTTGGTACACAG-3′, R: 5′-CTTCTTCTCTTCTTAGAGAGCCC-3′; sst3 F: 5′-TACGTCAACGTCATATCC-3′, R: 5′-AGCCTCAGATECTGACGC-3′; sst4 F: 5′-CGCTGGAGAAAGAATAATCAC-3′, R: 5′-CCACCCTTTGCTCTTGAAG-3′; sst5 F: 5′-GGTTTTGGAGAGAGGAATTC-3′, R: 5′-GGCCAGGTGACGATCGTGA-3′. Beta-2-microglobulin was amplified as the control. The products were separated on silver-stained 8% polyacrylamide gels.

**MTT Assay**

The effect of somatostatin administration on MPNST cell viability was determined using S462 cells. Three thousand cells were plated in 96-well plates. Serum-containing medium was replaced after 24 hours by serum-free medium to which they were exposed for another 24 hours before treatment with somatostatin (SS14) for another 24 hours. After a total incubation time of 3 days, all media were replaced by DMEM containing 1.5 mg/mL MTT salt (Sigma). The plates were then further incubated at 37°C to allow tetrazolium reduction to a blue formazan dye. After 2 hours, the medium was replaced by DMSO, which leads to cell lysis and solubilization of the formazan dye. The absorbance at 562 nm (reference wavelength 620 nm) was read using an Anthos-2010-ELISA-Reader (Anthos, Krefeld, Germany). After subtraction of a background control (cell-free medium), the number of viable cells was compared with untreated controls, which were set at 100%.

**3H-Thymidine Proliferation Assay**

Cells were seeded into 96-well plates at a density of 50,000 cells/well, each containing 200 μL cell culture medium, and were incubated at 37°C and 5% CO2 with the additions indicated in the figure legends. After 72 hours, cultures were pulsed for 6 hours with [3H]-methylthymidine (0.2 μCi per well; Amersham-Biosciences, Freiburg, Germany). Thereafter, incorporated radioactivity was measured by scintillation counting (20).
Statistical Analysis

The associations between sst1, sst2A, and sst4 expression in MPNSTs and patient survival were tested using the Kaplan-Meier procedure. Statistical significance was tested using the logrank test; a p value < 0.05 was considered statistically significant.

RESULTS

Somatostatin Receptor Expression in Nonneoplastic Peripheral Nerve Tissue

Because the expression pattern of somatostatin receptor subtypes in normal human peripheral nerves and nontumoral lesions has been determined only for the sst2A in peripheral nerves of the gastrointestinal tract (21), we first investigated sural nerve biopsies and samples from traumatic neuromas for sst subtype expression by immunohistochemistry and RT-PCR. Transcripts of all five receptors were found by RT-PCR in sural nerves (Fig. 1). However, none of the 5 receptors could be detected by immunohistochemistry in the 2 sural nerves, the sample from the eighth cranial nerve, or in the 3 traumatic neuroma samples (Table 1). Western blot analysis also did not show sst protein in the 2 normal sural nerve samples studied.

Distribution of Somatostatin Receptor Expression in Peripheral Nervous System Tumors

Vestibular schwannomas showed mRNA transcripts for all 5 receptor subtypes (Fig. 1). Immunohistochemical studies revealed that 24 of 27 (89%) schwannomas examined strongly expressed the sst2A receptor subtype (Fig. 2A, B). Immunoreactivity was found in both hypercellular Antoni A and hypocellular Antoni B areas. The specificity of the immunoreaction was demonstrated by preabsorption experiments (Fig. 2A, inset). In addition, the presence of sst2A in schwannomas was confirmed by Western blotting (Fig. 2C), with the expected appearance of the receptor (22). The other 4 receptor subtypes were not detectable by immunohistochemistry (Table 1).

Among 18 neurofibromas (9 from the TMA), the predominant receptor subtype was sst2A. Staining was typically patchy and the intensity was weaker than in schwannomas (Fig. 3A). Moreover, weak sst1, sst3, and sst4 expression was detected in single cases (Table 1). RT-PCR detected sst1, sst2, and sst4 transcripts in one neurofibroma (Table 1). Western blotting failed to detect sst2A expression in one neurofibroma (not shown), most likely as a result of the small amounts of tissue available from this tumor.

In MPNSTs, RT-PCR showed the presence of sst1, sst2, sst3, and sst4 transcripts (Fig. 1). Immunohistochemical studies of the 177 MPNSTs (169 derived from the TMA; Fig. 3B) showed that the detection rate of sst2A (Fig. 3C) was lower than in neurofibromas and schwannomas. Interestingly, a significant proportion of the samples were immunopositive for the sst4 receptor subtype (Table 1; Fig. 3D). Furthermore, the sst1 receptor subtype was detectable in some MPNSTs (Fig. 3E). Compared with sst2A expression seen in schwannomas, the intensity of sst1, sst2A, and sst4 staining in MPNSTs was only moderate in all immunopositive cases.
Functional Relevance of Somatostatin Receptor Expression in Peripheral Nervous System Tumor Cells

To determine the functional role of somatostatin expression in PNS tumors, which is mainly indicated by receptor internalization after somatostatin administration, we first evaluated the expression of receptor types in cultured MPNST cells and in rat Schwann cell cultures. As shown in Figure 4A, RT-PCR detected mainly sst2 transcripts in rat Schwann cells and cultured S462 MPNST cells. Immunofluorescence staining showed strong membranous sst2A expression in Schwann cells (Fig. 4B) and in cultured MPNST cells (Fig. 4C). In contrast, MPNST cells demonstrated only occasional weak membranous immunoreactivity for sst4. No specific staining was seen for the remaining somatostatin receptor subtypes in either cultured Schwann or MPNST cells.

We next determined whether somatostatin administration resulted in sst receptor endocytosis in cultured Schwann and S462 MPNST cells. As shown in Figure 4B and 4C, the administration of somatostatin promoted endocytosis of sst2A in both cell types. In contrast, no somatostatin-induced receptor endocytosis was observed in sst2-immunopositive S462 cells (not shown).

Previous experiments have suggested that both Schwann and MPNST cells are responsive to somatostatin through the sst2A receptor. To determine whether receptor activation influences tumor cell proliferation, we measured cell viability and cell proliferation in S462 MPNST cells after somatostatin administration. As shown in Figure 5, somatostatin reduced cell proliferation as measured by [H]-thymidine-incorporation very effectively (Fig. 5A). Furthermore, a significant reduction of the cell viability was seen after somatostatin administration in the MTT assay (Fig. 5B). Finally, to further elucidate the effect of somatostatin on cell viability in S462 MPNST cells, we applied a selective agonist for the sst2A receptor (L-779,976) and found a high number of TUNEL-positive apoptotic S462 cells (Fig. 5C).

Association Among sst Expression, NF1 Status, and Clinicopathologic Parameters

As shown in Table 2, there was a slightly higher frequency of MPNSTs positive for sst1, sst2A, or sst4 in the non-NF1 group as compared with the NF1-related group. However, these differences were not statistically significant.

In our series, 4 NF1 cases with associated tumors underwent progression from neurofibroma to MPNST. In 2 of these, there was no expression of sst1, sst2A, or sst4 in the non-NF1 group as compared with the NF1-related group. However, these differences were not statistically significant.

In our series, 4 NF1 cases with associated tumors underwent progression from neurofibroma to MPNST. In 2 of these, there was no expression of sst1, sst2A, or sst4 in the non-NF1 group as compared with the NF1-related group. However, these differences were not statistically significant.

In the other 2 cases, sst expression was seen in the MPNST sample but not in the precursor neurofibromas; one of these 2 tumors showed weak sst1 expression in both the neurofibroma and

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**TABLE 1.** Immunoexpression of Somatostatin Receptor Subtypes (sst) in Peripheral Nerve Sheath Tumors and Nonneoplastic Peripheral Nerve Tissue

<table>
<thead>
<tr>
<th>Immunoexpression</th>
<th>sst1</th>
<th>sst2A</th>
<th>sst3</th>
<th>sst4</th>
<th>sst5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal peripheral nerve (n = 3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Traumatic neurona (n = 3)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Schwannoma (n = 27)</td>
<td>—</td>
<td>24 (89%)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Neurofibroma (n = 18)</td>
<td>1 (6%)</td>
<td>4 (22%)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>—</td>
</tr>
<tr>
<td>Malignant peripheral nerve sheath tumor (MPNST) (n = 177)</td>
<td>16 (9%)</td>
<td>27 (15%)</td>
<td>—</td>
<td>57 (32%)</td>
<td>—</td>
</tr>
</tbody>
</table>

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**FIGURE 2.** Immunoexpression of sst2A in human schwannomas. (A) Hypocellular (Antoni B type) tissue shows membranous sst2A expression (insert: preabsorption experiment completely abolishes the immunoreaction) (200×). (B) Antoni A-area with closely packed tumor cells showing strong membranous sst2A expression (400×). (C) Western blot demonstration of sst2A in a schwannoma. Three meningiomas served as positive controls. No sst2A is detected in 3 malignant peripheral nerve sheath tumor samples.

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Lastly, we studied whether the presence or absence of sst receptor expression affected survival in a total of 81 MPNST patients with available follow-up data. However, we found no significant survival differences in relation to the expression of sst1, sst2A, or sst4. This was true of all the MPNST cases, as well as stratified into NF1 associated and sporadic subsets.

**DISCUSSION**

In the present study, we have shown that the sst2A receptor subtype is the predominantly expressed somatostatin receptor subtype in tumors of the peripheral nervous system and that the sst2A receptor is functionally active in these tumors. Additionally, we found a shift in receptor expression patterns among schwannomas, neurofibromas, and MPNSTs, the latter most commonly expressing sst4.

**TABLE 2. Relation Between NF1 Status and Immunoexpression of sst1, sst2A, and sst4 in Malignant Peripheral Nerve Sheath Tumors (MPNSTs)**

<table>
<thead>
<tr>
<th>Immunoexpression</th>
<th>sst1</th>
<th>sst2A</th>
<th>sst4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPNST (NF1-associated) (n = 41)</td>
<td>2 (4.9%)</td>
<td>5 (12.2%)</td>
<td>10 (24.4%)</td>
</tr>
<tr>
<td>MPNST (sporadic) (n = 136)</td>
<td>14 (10.3%)</td>
<td>22 (16.2%)</td>
<td>47 (34.6%)</td>
</tr>
</tbody>
</table>

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Schwannomas often arise from peripheral or cranial nerves in the head and neck region. Both sensorimotor and autonomic nerves may be affected. In contrast, visceral schwannomas are rare (23). To date, only sst2A expression has been found in visceral nerves of the myenteric plexus of the gut. These studies showed that sst2 receptor is detectable by autoradiography and immunohistochemistry (21). Other receptor subtypes have not been investigated (24). In our study, we found no immunoreactive sst receptor subtypes in normal sural or cranial nerves. This suggests differences in the sst expression pattern between peripheral nerves with different electrophysiological characteristics. An additional explanation might be that the amount of sst protein is below immunohistochemical detection level, because RT-PCR studies showed the presence of sst transcripts in normal nerve samples.

The vast majority of schwannomas analyzed in our study showed strong sst2A expression, and functional studies using rat Schwann cells suggested that this receptor is functionally active in this tumor. Although schwannomas are benign and show no propensity to metastasize, they may recur when incompletely excised and are sometimes treated with irradiation (25). Whether the administration of somatostatin analogs represents another therapeutic option should be further investigated in the future. The presence of sst2A may also be of use in radioimaging. Our immunohistochemical data differ from those of at least one previous study investigating sst1, sst2A, sst3, and sst5 expression in vestibular schwannomas (11). The authors reported that these 4 receptors are expressed within as many as 50% (sst1 and sst2A), 75% (sst3), and 90% (sst2A) of schwannomas. Although these discrepancies might be the result of the application of different antibodies, those used in our current study have previously shown to be highly specific and sensitive (5, 6, 17).

Another large and sometimes problematic group of PNS tumors are neurofibromas. They arise either as sporadic tumors or in the setting of NF1, in which plexiform neurofibromas involving sizable peripheral nerves show a significant
tendency to progression to MPNST. The predominant somatostatin receptor subtype found in our series of neurofibromas was sst2A, but its frequency of expression was much lower than in schwannomas. Interestingly, we rarely detected other somatostatin receptors in neurofibromas. This difference in the pattern of sst expression may be related to the differing cellular composition of schwannomas and neurofibromas, the latter containing fibroblasts and perineurial-like cells in addition to neoplastic Schwann cells. Unfortunately, as a result of the lack of neurofibroma cell cultures, we could not assess the functional significance of sst2A in such tumors.

The pattern of somatostatin receptor expression in MPNSTs differed markedly from that seen in schwannomas or neurofibromas. We found a significant proportion (32%) of these tumors to be immunopositive for sst4. Interestingly, among 4 NF1-associated cases in which both neurofibroma and MPNST coexisted, the only difference in sst expression between them was positivity of sst4 in the MPNST component. These observations suggest that a shift in receptor expression occurs in a subset of cases during the course of malignant progression. It is of note, however, that cell culture studies using NF1-derived MPNSTs failed to demonstrate the presence of the sst4 receptor mRNA, precluding further functional studies. In contrast, although the sst2A receptor was detectable in only 15% of the tumors, cell culture studies indicated that this receptor is nonetheless functionally active in MPNST cells. Moreover, most likely through the sst2A receptor, somatostatin administration was able to significantly reduce cell proliferation and to evoke tumor cell apoptosis. These data suggest that among MPNSTs, there may be a subgroup that responds therapeutically to synthetic somatostatin analogs such as octreotide or lanreotide. Because MPNSTs are known to metastasize, frequently to the lung, metastases may similarly be targeted by such radiolabeled somatostatin analogs.

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