Somatostatin Receptor Subtype 2 Is Expressed by Supratentorial Primitive Neuroectodermal Tumors of Childhood and Can Be Targeted for Somatostatin Receptor Imaging

Michael C. Frühwald,1 Christian H. Rickert,2,3 M. Sue O’Dorisio,4 Mark Madsen,5 Monika Warmuth-Metz,6 Geetika Khanna,5 Werner Paulus,2 Joachim Kühl,7 Heribert Jürgens,1 Peter Schneider,8 and Hermann L. Müller9

1Department of Pediatric Hematology and Oncology, University Children’s Hospital Muenster, Muenster, Germany; 2Institute of Neuropathology, University Clinics Muenster, Muenster, Germany; 3Gerhard Domagk Institute of Pathology, University Clinics Muenster, Muenster, Germany; 4Department of Pediatric Hematology and Oncology, University of Iowa, Iowa City, Iowa; 5Department of Radiology, Division of Nuclear Medicine, University of Iowa, Iowa City, Iowa; 6Institute for Neuroradiology, University Hospitals Wuerzburg, Wuerzburg, Germany; 7University Children’s Hospital Wuerzburg, Wuerzburg, Germany; 8Clinics and Polyclinics for Nuclear Medicine, University of Wuerzburg, Wuerzburg, Germany; and 9Department of Pediatrics, Klinikum Oldenburg gGmbH, Oldenburg, Germany

ABSTRACT

Purpose: Although gliomas predominate among central nervous system (CNS) neoplasms in adulthood, embryonal tumors are the most common malignant brain tumors in children. Despite novel treatment approaches, including improved radiotherapy and high-dose chemotherapy, survival rates remain unsatisfactory. The timely diagnosis of residual or recurrent embryonal CNS tumors and thus the earliest possible time point for intervention is often hampered by inaccuracies of conventional imaging techniques. Novel and refined imaging methodologies are urgently needed.

Experimental Design: We have previously demonstrated the use of somatostatin receptor imaging (SRI) in the diagnosis of recurrent and residual medulloblastomas. Here, we evaluated somatostatin receptor type 2 (sst2) expression using an antibody in an array of CNS tumors of childhood. Eight high-grade gliomas, 4 atypical teratoid/rhabdoid tumors, 7 supratentorial primitive neuroectodermal tumors (stPNET), 1 medullop epithelioma (ME), and 8 ependymomas were screened. Tumors positive in vitro were additionally analyzed in vivo using SRI.

Results: Abundant expression of somatostatin receptor type 2 in stPNET, a ME, and ependymomas warranted in vivo imaging of 7 stPNET, 1 rhabdomyosarcoma, 3 ependymomas, 1 ME, and 1 glioblastoma. Although SRI was positive in 6/7 stPNET, 1 rhabdomyosarcoma, and 1 ME, none of the ependymomas nor the glioblastoma could be imaged using SRI. In selected cases SRI was more sensitive in the detection of relapse than conventional imaging by magnetic resonance imaging and computed tomography.

Conclusions: SRI should be considered in the evaluation of residual or recurrent embryonal CNS tumors, especially stPNET. The strengths of SRI lie in the differentiation of reactive tissue changes versus residual or recurrent tumor, the detection of small lesions, and possibly in the distinction of stPNET from gliomas.

INTRODUCTION

Although neoplasms of glial origin such as glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA) predominate among malignant central nervous system (CNS) tumors during adulthood, embryonal neoplasms are most commonly encountered in childhood (1). Embryonal tumors comprise a heterogeneous group of tumors such as ependymoblastoma, medulloblastoma (MB), medulloepithelioma (ME), atypical teratoid/rhabdoid tumors (AT/RT), and supratentorial primitive neuroectodermal tumors (stPNET; Ref. 2). Because of similarities in histology, stPNET have until recently been grouped with MB (3). It is estimated that stPNET represent 2.5% of all childhood brain tumors, affect children 4 weeks to 10 years of age, and show a predilection of 2:1 for the male gender (4). In an analysis of the German Brain Tumor trial HIT’91, the 3-year progression free survival rate for 63 children with stPNET was 39.1% (5). Treatment for affected patients consists of intensive chemotherapy geared at extending the time to ensuing radiotherapy. Prognostic factors in most studies include tumor location (pineal versus nonpineal) and dissemination along the neuraxis (5, 6).

The early detection of residual or relapse tumor should prompt timely intervention and contribute to improved survival. Several investigators have analyzed the value of postoperative surveillance imaging in malignant CNS tumors of childhood, mainly MB (7–9). Although no improvement in survival could be documented, it was still concluded that surveillance will gain importance with the evolution of new effective treatment modalities. Guidelines for the categorization of histological entities for staging and surveillance of childhood brain tumors have been developed (10). The time to detection of a relapse or residual tumor and the sensitivity of imaging modalities may be
substantially improved as recent methodological advances become Standard of Care (11).

We have demonstrated that MB express high levels of the somatostatin receptor subtype sst2, and can be imaged using scintigraphic somatostatin receptor imaging (SRI) (12). We were able to differentiate nonspecific tissue changes such as inflammation or scar formation from vital tumor tissue. In some cases, SRI proved more sensitive in the detection of residual and relapse tumors than conventional imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) and proved a valuable asset in the follow-up of affected patients (12). Evaluating somatostatin receptors in MB and stPNET by reverse transcription-PCR and autoradiography, we found that both groups primarily express sst2. Receptor densities on stPNET were above those in other tissues that could easily be studied by SRI (13).

Until recently, the determination whether a tumor type can be studied by SRI depended on the availability of sophisticated techniques such as radioreceptor-autoradiography. In this study, we evaluated a series of childhood brain tumors using a recently developed antibody against sst2. After determination of the expression status in stPNET, AT/RT, and gliomas in vitro, a series of children were evaluated in vivo using SRI.

**MATERIALS AND METHODS**

**Patients.** Patients ranged in age from 1 month to 17 years. Typical age distributions for the corresponding tumor entities were observed (e.g., infants for AT/RT). Twenty-five patients were male and 9 female. The clinical data on these and the ones whose tumor were studied by immunohistochemistry (IHC) alone are given in Tables 1 and 2. The tumor tissue of 29 children was studied by sst2-IHC. Thirteen patients were additionally studied by SRI. Informed consent was obtained from the legal guardians and if age permitted from the patients as well. All procedures were approved by the respective Institutional Review Boards.

**Tumor Tissue.** Tumor material was from patients treated at three different institutions (University clinics of Muenster and Wuerzburg, and University of Iowa). Tissue had been obtained at the time of surgery (primary or relapse). Tissue samples were processed for routine histopathological examination. Pathological diagnosis was made by local neuropathologists (W. Paulus, Institute of Neuropathology, Muenster, W. Roggendorf, Institute of Neuropathology, Wuerzburg, and P. Kirby, University of Iowa) and in the cases from Germany confirmed by the reference neuropathology panel of the GPOH (University Clinics Bonn, Bonn, Germany). None of the patients had previously been treated with somatostatin or any of the currently available analogs of the peptide.

**Immunohistochemical studies** were performed on archival paraffin-embedded material from 7 stPNET, 1 ME, 4 AT/RT, 3 AA, 5 GBM, 4 ependymomas grade 2 (EP II), 4 anaplastic ependymomas (AE), 5 GBM, 4 ependymomas grade 2 (EP II), 4 anaplastic ependymomas (AE), and 1 parenchymal cerebral rhabdomyosarcoma.
Table 2  Somatostatin receptor imaging in children with malignant central nervous system tumors

<table>
<thead>
<tr>
<th>No.</th>
<th>Identifier</th>
<th>Dx</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Somatostatin receptor imaging</th>
<th>Volumea</th>
<th>sst₂ IHCb</th>
<th>Remark</th>
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<tr>
<td>1</td>
<td>Sc1</td>
<td>stPNET</td>
<td>1st/2</td>
<td>M</td>
<td>Positive at suspected relapse</td>
<td>30.4</td>
<td>++</td>
<td>Clinical relapse</td>
</tr>
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<td>2</td>
<td>Sc2</td>
<td>stPNET</td>
<td>8</td>
<td>M</td>
<td>Questionable for residual tumor</td>
<td>~3</td>
<td>n.d.</td>
<td>dod</td>
</tr>
<tr>
<td>3</td>
<td>Sc3</td>
<td>stPNET</td>
<td>10</td>
<td>F</td>
<td>Positive at relapse</td>
<td>2.34</td>
<td>++ + +</td>
<td>Clinical progression</td>
</tr>
<tr>
<td>4</td>
<td>Sc4</td>
<td>stPNET</td>
<td>6</td>
<td>M</td>
<td>Positive for residual tumor</td>
<td>~3.5</td>
<td>n.d.</td>
<td>Clinical progression</td>
</tr>
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<td>stPNET</td>
<td>4</td>
<td>M</td>
<td>Positive at diagnosis and progression</td>
<td>1.93</td>
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<td>stPNET</td>
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<td>F</td>
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<td>n.d.</td>
<td>Clinical progression</td>
</tr>
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<td>stPNET</td>
<td>3</td>
<td>M</td>
<td>Positive at relapse</td>
<td>6.35</td>
<td>n.d.</td>
<td>Clinical progression</td>
</tr>
<tr>
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<td>ME</td>
<td>8/12</td>
<td>M</td>
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<td>4.89</td>
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<td>cRMS</td>
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<td>M</td>
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<td>18.5</td>
<td>+ +</td>
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<td>GBM</td>
<td>3</td>
<td>M</td>
<td>Questionable at diagnosis</td>
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<td>n.d.</td>
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<td>AE</td>
<td>6</td>
<td>F</td>
<td>Questionable for residual tumor</td>
<td>10.92</td>
<td>n.d.</td>
<td>MRI + and clinical progression</td>
</tr>
</tbody>
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Somatostatin Receptor sst₂-IHC. IHC analysis was performed using a monoclonal antibody to sst₂. The preparation of the antibody and its application in the study of neuroblastomas has been described previously (14). Tumor tissues were fixed in 10% buffered formalin for 24 h, routinely processed, and paraffin-embedded for histology. IHC was performed on 2-μm sections for sst2 at a dilution of 1:2000. The avidin-biotin-complex method with 3,3′-diaminobenzidine as a chromogen was used for the detection of the reaction product.

To classify staining of tumor cells by the antibody, we developed a scoring system. Tumors in which <10% of the tumor cells stained positive were evaluated as negative (−), tumors with 11–20% scored (+), with 21–30% as (+ +), with 31–50% as (+ + +), and all those with >50% of positive staining as (+ + + + +).

SRI. A total of 13 children was studied by scintigraphy and some of them repeated (Table 2). For imaging the radiopharmaceutical In-111-pentetreotide, Octreoscan (Mallinkrodt, Petten, the Netherlands) was injected i.v. using a dose of 3 MMBq/kg body weight. The dose ranged from 30 to 100 MBBq. In-111-pentetreotide is a diethylenetriaminepentaacetic acid (DTPA)-bearing analogue of the octapeptide somatostatin analogue octreotide coupled to 111In. Octreotide binds with high affinity the somatostatin receptor subtypes sst₂ and sst₅ (15). Single-photon emission computed tomography images were acquired 4 h and in some cases at longer intervals after injection employing a Siemens Diacam gamma camera with a medium energy, parallel-hole collimator with energy peaks of 173 and 247 keV (window 20%). In 9 cases, integrity of the blood-brain barrier was additionally evaluated using Tc-99m-DTPA as a tracer. In all these cases, single-photon emission computed tomography was done in a dual-isotope technique (medium energy collimator, 173 keV peak for In-111 and 140 keV peak for Tc-99m, window 15%). Imaging was performed 4 h after injection of the tracer with In-111 and 2 h after injection with Tc-99m. A Butterworth filter with a frequency number of 0.6, and an order of six was selected for reconstruction of both scans in a 64 × 64-matrix. In 4 cases (patients Sc 5, 7, 8, and 12), coregistration of MRI with SRI was performed. All scintigraphic imaging results were assessed without prior knowledge of other imaging modalities by two independent observers. SRI films were classified as true positive and negative or false positive or negative in comparison to the results of CT, MRI, and/or tumor histology and CSF evaluation. In all cases, radiotracer uptake in the tumor was compared with uptake in the skull. Images in which uptake in the tumor was above uptake in the skull (normal bone marrow) were registered as positive. To ascertain the potential resolution of SRI versus MRI/CT, volumes of suspicious lesions on MRI and CT were determined using the approximation formula for an ellipsoid (coronal × sagittal × axial/2). Infants and children not tolerating the procedure received a mild sedation using oral chloralhydurate 1 h before SRI. Informed consent was obtained from legal guardians (in most cases the parents) at least 24 h before the imaging and associated sedation procedure.

RESULTS

StPNET Consistently Display Expression of sst₂

The antibody used for our studies has previously been used in stably transfected cells, expressing sst₂ and, in primary neuroblastomas, expressing the receptor (14). We validated this antibody in normal human cerebellum obtained at autopsy. As in previous studies, we found abundant staining of the granular cell layer in normal human cerebellum (Ref. 16; data not shown).

To screen childhood CNS malignancies justifying evaluation by in vivo SRI, we collected histological entities available in the neuropathology archives of the Institute for Neuropathology/University Clinics of Muenster. The main focus was embryonal CNS malignancies but also malignant gliomas. We retrieved 29 samples for sst₂-IHC (Table 1). Staining patterns were cytoplasmic mostly but also membranous and rarely nuclear (Fig. 1). As expected from our previous studies, all StPNET stained positive for sst₂ (13). Receptor positivity in
stPNET was among the highest of all evaluated tumor entities ranging between 30 and 80% of immunopositive cells. Of the 4 studied AT/RT, only 1 was positive. Among the glial tumors, 3 of 4 GBM showed a rather low receptor density with one exception, which had 80% of cells staining for sst₂. Of 3 AA, 1 had virtually absent immunopositive cells (~5%), whereas 2 others were positive with 40 and 80%, respectively. All but 1 of 4 EP II were negative for immunostaining. The one with positive staining had a very high density of immunopositive cells (90%). Among the AE, 2 of 4 exhibited a high rate of immunopositive cells (both 80%), whereas both other tumors had <5% of stained tumor cells. The one studied cerebral rhabdomyosarcoma, a very rare entity, had a receptor density of ~25% stained tumor cells (Fig. 2). The ME had a density of sst₂ immunopositive cells comparable with stPNET.

Patient Sc 1. Pineal stPNET in an 18-month-old boy. sst₂-IHC: performed on primary tumor material ~30% immunopositive cells. SRI: performed after therapy (HIT-SKK '92 vincristin, cyclophosphamide, carboplatinum, etoposide, and i.v. methotrexate) when the boy developed signs of irritability and strabism. Comparison SRI versus MRI/CT: SRI detected two nodules of high radiotracer uptake. MRI confirmed the diagnosis, tumor volume 30.4 cm³. The patient has succumbed to disease.

Patient Sc 2. stPNET in an 8-year-old boy. sst₂-IHC: no tumor material. SRI: performed after primary surgery. Comparison SRI versus MRI/CT: SRI without clear radiotracer uptake. On early postoperative CT, no differentiation of vital tumor versus reactive tissue changes possible, estimated volume 3 cm³ (CT). On follow-up, MRI residual/recurrence was evident. The patient has succumbed to disease.

Patient Sc 3. Ten-year-old girl with a stPNET. sst₂-IHC: on primary tumor 50–60% immunopositive cells. SRI: studied at suspected relapse after chemotherapy (HIT '91, ifosfamide, HD-methotrexate, cisplatinum, and cytarabine) and radiotherapy (54 Gy). Comparison SRI versus MRI/CT: MRI and SRI imaging in excellent concordance demonstrating progressive disease, lesion on MRI 8.3 cm³ (Fig. 2).

Tumors Expressing High Levels of sst₂ Can Be Visualized by SRI

Seven patients with stPNET were evaluated by SRI and conventional imaging techniques such as CT and/or MRI. In 4 cases, information of the in vitro sst₂ status of the tumors was obtained in addition to evaluation of the receptor status in vivo by SRI.
Patient Sc 4. Six-year-old boy with stPNET. 
stt<sub>2</sub>-IHC: primary tumor tissue previously tested by receptor-autoradiography yielding a high density of receptors (17). SRI: after neurosurgical complete resection SRI (48 h after operation) showed a higher uptake compared with the study using $^{99m}$Tc-DTPA, suggesting receptor-positive tumor tissue. Comparison SRI versus MRI/CT: postoperative CT was read as potential residual disease with a recommendation of close neuroradiologi-
cal monitoring, volume of suspected tissue change ~3 cm³. SRI is clearly positive (Fig. 3A). The patient has died of progressive disease.

**Patient Sc 5.** Four-year-old boy with a pineal stPNET. sst₂-IHC: performed on primary tumor tissue with a receptor density of 50%. SRI: studied at diagnosis and during follow-up 15 months after initial SRI. Comparison SRI versus MRI/CT: MRI and SRI were coregistered. MRI confirmed the positive findings of residual tumor after the operation and then progressive disease after chemotherapy and radiotherapy. Furthermore, the suspicion of hippocampal and cerebellar metastases based on SRI were confirmed by MRI, nodules with 1.93 cm³.

**Patient Sc 6.** Two-year-old girl with a stPNET. sst₂-IHC: no tissue. SRI: imaged at suspected relapse. Comparison SRI versus MRI/CT: MRI coregistered with SRI showed clear uptake, tumor volume 19.2 cm³.

**Patient Sc 7.** Three-year-old boy with a stPNET. sst₂-IHC: no tissue. SRI: imaging performed at suspected relapse. Comparison SRI versus MRI/CT: MRI coregistered with SRI showed clear uptake, tumor volume 6.33 cm³.

**Patient Sc 8.** Eight-month-old boy with a ME. Therapy consisted of chemotherapy plus confocal radiotherapy. sst₂-IHC: no tissue. SRI: negative. SRI is currently used in the follow-up evaluation of this patient, tumor volume 19.2 cm³.

**Fig. 3** Somatostatin receptor imaging (SRI) in the early detection of central nervous system tumor relapse. A, postsurgical imaging in a 6-year-old boy (patient Sc 4). Preoperative T2-MRI (left) shows a parieto-occipital lesion. Postoperative contrast computed tomography reveals several nodules that could not be differentiated from postoperative tissue alterations. Postoperative SRI (top panel) with an enhancing lesion indicating vital tumor tissue. ⁹⁹Tc-DTPA imaging is weaker than SRI but still positive, indicating blood brain barrier disruption. B, top row: sequential T1-MRI images in an 8-month-old male (patient Sc 8). The first three magnetic resonance images (MRIs) were read as postsurgical tissue change. The MRI furthest to the right was performed when the patient presented with clinical symptoms and was read as tumor relapse. Middle panel: coregistered SRI shows a gradual increase of tracer uptake suggesting a relapse 2–3 months before confirmation by clinical signs and positive MRI. Bottom panel: comparison of SRI uptake in tumor and skull demonstrates a progression from a ratio of 1.01 tumor/skull to 2.1.
IHC: high receptor density with 70–80% of immunopositive cells. SRI: treatment response was followed by sequential coregistrations of MRI with SRI. Comparison SRI versus MRI/CT: SRI 3 days after neurosurgical resection demonstrated minimal uptake of radiotracer. At early follow-up (2 months after surgery), a suspicious lesion was seen on SRI but not on MRI (MRI interpreted as postradiation change, suspicious lesion 4.89 cm³). Two months later, SRI was read as increasing uptake and suspicion of residual disease, whereas MRI was still perceived as therapy associated tissue change. Another month later, the child presented with clumsiness and irritability. MRI confirmed the suspicion offered by SRI 4 months earlier, and the diagnosis of progression was made. At this time, SRI was highly positive. Volumes of suspected lesions increased from 4.9 to 5.8 to 7.4 to 16.3 cm³. MRI was first read as truly positive at 7.4 cm³ (Fig. 3B).

Patient Sc 9. Two-year-old boy with a temporal rhabdomyosarcoma. sst₂-IHC: up to 30% of positive cells. SRI: studied before and after initial surgery. Comparison SRI versus MRI/CT: excellent concordance between preoperative SRI and MRI (tumor volume, 18.5 cm³). Postoperative imaging by CT and by SRI confirmed the neurosurgeons assessment of a complete neurosurgical resection.

Patients Sc 10–13. Furthermore we studied four high-grade gliomas by SRI. The relapse in 2 ependymomas that had <5% of sst₂-positive tumor cells on IHC did not enhance on SRI (patient Sc 11, 3.36 cm³ and patient Sc 12, 3.6 cm³). One GBM (3 years) with a low density of sst₂ on IHC (10%) had questionable radiotracer uptake, and SRI was thus graded as non informative (volume on MRI 9 cm³). The same was true for the AE of a 6-year-old girl (patient Sc 13, 10.92 cm³). SRI was questionable whereas MRI and the clinical course confirmed the diagnosis of a progressing tumor.

DISCUSSION

Despite their rarity, embryonal tumors of the CNS represent a substantial dilemma in pediatric neuro- oncology. Although treatment results have been promising for tumors such as standard risk MB, high-risk MB or AT/RT, and stPNET can rarely be cured. Survival of recurrent stPNET has only been reported in high-dose chemotherapy trials (18, 19). Because of their histological resemblance, stPNET and MB were until recently differentiated by their localization (4). Molecular studies have recently demonstrated differences between MB and stPNET. For instance, genetic and epigenetic lesions of the short arm of chromosome 17 (17p), frequently detected in MB, are rare in stPNET (20, 21). Only sparse data have appeared on stPNET and mostly in conjunction with MB. This has mainly been due to a less than clear differentiation between tumor types and to the rarity of stPNET. Calculating from a CCG study stPNET occur in ~1 in 10 million children (6). It is thus not surprising that among the cases for our study only 6 could be evaluated by IHC and seven by SRI.

The presence of sst receptors on tumor tissue has until recently been assessed by the use of radioactively labeled peptide analogues (22). A major drawback of this approach is the requirement for fresh tissue and a technically demanding procedure. As a consequence, antibodies for each sst have been developed. Cervera et al. (23) demonstrated the use of using receptor subtype-specific antibodies by comparing IHC to autoradiography in the same tumor samples. We used an antibody generated in the laboratories of one of us and observed membranous, cytoplasmic, and nuclear staining patterns (Ref. 14; Fig. 1). Guyotat et al. (24), in a study on ependymomas, and Reubi et al. (25), in an analysis of neuroblastomas and pheochromocytomas, found cytoplasmic staining using sst₂ antibodies. These authors discovered that autoradiography demonstrated membranous receptors in tumors that exhibited a cytoplasmic staining pattern on IHC and that the cytoplasmic staining may represent internalized receptors. In fact, as expertly reviewed by Hofland et al. (26), sst₂ internalization occurs within 60 min after binding of ligands. We thus argue that in our samples, immunostaining is in part due to membrane-bound and otherwise due to internalized receptors. We cannot conclude whether the receptors are functionally active in the expressing tumors; however, our own studies on MB cell lines and other investigators work on glioma cells demonstrate that intracellular transduction pathways are activated upon ligand binding (13, 27).

The most important finding of our study is the consistent expression of sst₂ in stPNET and the consecutive ability to image these tumors by SRI. sst₂ is expressed in 100% of stPNET, whether analyzed by autoradiography, reverse transcription-PCR, or IHC (13). Receptor densities are above those reported for other malignancies, which can easily be imaged by SRI. In only 1 stPNET, SRI could not clearly image the tumor (patient Sc 2). At suspected residual tumor, neither conventional imaging by postoperative CT nor SRI could clearly differentiate between therapy-associated tissue changes and vital tumor tissue. Follow-up MRI months later proved the presence of vital tumor tissue. In 5 cases, SRI was in full accordance with other imaging modalities (patients Sc 1, 3, 5, 6, and 7; Fig. 2). In 1 case (patient Sc 4), SRI was more reliable than postoperative CT in that it yielded a high radiotracer-uptake (Fig. 3A). Early postoperative MRI imaging, preferably within the first 72 h after surgery, is well suited for the differentiation of vital enhancing tumor tissue versus blood or scar tissue without the hazards associated with the use of radioactivity. However, in facilities where postoperative MRI may not be available or not possible because of other causes, SRI may be available. It is not so much the situation of residual tumor, however, where the strength of the methodology is seated. In patient Sc 8 for instance, coregistration of MRI along with SRI showed advantages of SRI (Fig. 3B). Although MRI at 4 months after resection was read as postradiation changes, SRI was strongly suspicious for recurrent tumor. At 5 months, SRI yielded an even stronger positive signal, and MRI confirmed the diagnosis as well as the clinical course with the onset of symptoms such as irritability and ataxia. Thus, in addition to a perfect correlation between receptor expression in vitro and imaging by SRI in vivo, there was an excellent correlation between conventional imaging techniques and SRI for stPNET. As outlined, these correlations may be very useful in the diagnosis of early recurrent or residual tumors that cannot be imaged by other modalities.

The volumes of the suspected lesions ranged in size from 1.93 to 30.4 cm³. However, whereas the smallest lesion (patient Sc 5; 1.93 cm³) could be visualized by SRI, larger lesions could...
not when sst$_3$ receptor densities were too low (patient Sc 10; 3.36 cm$^3$). Furthermore, in the few cases studied by SRI and Tc-DTPA to assess blood-brain barrier permeability, we found an excellent correlation. In all cases where Tc-DTPA showed disruption of the blood brain barrier, SRI was positive and showed a larger region of enhancement. SRI enhancement is highly specific for sst$_3$-bearing tissue and not likely due to nonspecific extravasation into surrounding brain edema. This has previously been demonstrated for gliomas and meningomas but also for MB (12, 28, 29). Meewes et al. (29), for instance, showed less intense uptake in TC-DTPA images than in SRI. Furthermore, in patients Sc 1, 3, 4, 5, 8, and 9, we found a perfect correlation between in vitro receptor status and in vivo imaging using SRI.

As we were able to image lesions as small as 1.93 cm$^3$, SRI may help to image sst$_2$-positive stPNETs in regions with high background enhancement on MRI (i.e., choroid plexus, inflammatory tissue). Specific uptake in sst$_2$-bearing tissues should prove more pronounced in the sst$_2$-positive tissues than the negative tissues or those with low activity.

Currently, the differential diagnosis between malignant glioma and stPNET is close to impossible by MRI or CT so that surgical sampling is performed. As we and others have shown, malignant gliomas are almost always negative on SRI. Thus, a supratentorially located tumor with MRI and CT features characteristic of a malignant process (perifocal edema, mixed enhancement), which enhances on SRI, is very likely a stPNET. This may be of major importance as novel therapeutic strategies, including neoadjuvant chemotherapy, are used to make the tumor amenable to a complete neurosurgical resection.

Up to 40% of supratentorially located PNET exhibit metastases at diagnosis, and an even higher number cannot be reliably resected (5). Furthermore, conventional treatment strategies have been rather disappointing with low survival rates for primary tumors and numbers close to zero for recurrent tumors. The presence of peptide receptors that can be targeted by a radiolabeled ligand indicate therapeutic alternatives. In fact, somatostatin analogues might be used i.v. because stPNET almost inevitably demonstrate a disrupted blood-tumor barrier. As many of these tumors require access to the CSF via an implanted catheter direct application into the CSF is possible. Although therapy with somatostatin and its congeners has been disappointing in most solid cancers, Kouroumalis et al. (30) raised some hope. These authors treated patients with hepatocellular carcinoma and found additive beneficial effects when octreotide was combined with conventional chemotherapy. With the development of subtype-specific sst ligands but also of ligands with high affinity for all five sst subtypes, it may be possible to influence the proliferation of solid tumors such as stPNET (15). Some researchers have linked somatostatin analogues to cytotoxic agents. Kiaris et al. (31) used a doxorubicin derivative linked to the somatostatin analogue RC-160 developed by Nagy et al. (31) to efficiently suppress the growth of glioma xenograft tumors in mice. Very high potential lies in the approach of peptide receptor radionuclide therapy. Somatostatin that can be labeled with either high energy $^{111}$In or the $\beta$-emitter $^{90}$Y or the $\alpha$ emitter $^{177}$Lu have been developed. Vaidyanathan et al. (32) used novel iodinated somatostatin analogues that could be targeted to MB xenografts. In a pilot study using brachytherapy Merlo et al. (33) used a $^{90}$Y-labeled somatostatin analogue to control the growth of a series of gliomas. These authors found this approach not only safe but also efficient in the locoregional control of the progression in low to intermediate grade gliomas. Similar approaches may well be taken for stPNET.

Several questions not answered by our rather small cohort need to be addressed in future studies on more patients. Although we were able to ascertain that a positive in vitro status of sst$_2$ expression correlates well with the imaging of tumors at diagnosis and relapse as well as in residual tumors and metastases (patients Sc 7 and 8), we could not establish the limit of resolution of SRI. However, as mentioned above, studies on meningiomas and gliomas estimate that lesions need to be at least 1 cm$^3$ in size to be reliably imaged (28, 29). Second, patients with suspected relapse may in the future undergo an array of imaging procedures such as diffusion weighted MRI, MRI spectroscopy, and nuclear medicine technologies (e.g., SRI). The advantage of our approach may be that tumors can be tested in vitro to ascertain that in vivo imaging by SRI is worthwhile. In our study on MB, we tested if imaging after 72 h yields any additional information. For the cohort of stPNET, we chose to abandon this route because no additional information but a gradual decay could be noted and because most children are in a critical postoperation phase not warranting any unnecessary procedures. Follow-up of our patients has not been long enough to warrant investigation as to whether early diagnosis has a real clinical impact on survival or more realistically quality of life.

Some of the brain tumors evaluated in our study have previously not been assessed for sst$_2$ expression. AT/RT have gained major attention as a clinical as well as a biological enigma (34). In three of our samples, staining was faint to absent. In contrast, another specimen had 30% immunopositive cells mainly of the neuroectodermal component. It remains speculative whether tumors that showed no sst$_2$ staining represent a subvariant of AT/RT.

The studied rhabdomyosarcoma had an intermediate receptor density of $\sim$30% immunopositive cells. The studied ME showed an even stronger expression of the sst$_2$ protein. SRI was highly positive in both tumor entities. Both tumor types are thought to be derived from primitive neuroepithelium. The rather abundant expression of sst$_2$ on these and on stPNET and MB may be the basis for a unifying concept.

The data on sst expression in glial tumors are conflicting. Although Reubi et al. (35) detected sst in only 1 of 20 GBM by autoradiography, others (36, 37) found specific binding of somatostatin analogues on GBM. Lamszus et al. (38) and Feindt et al. (27) detected sst$_2$ mRNA in GBM and concluded that sst$_2$ expression is not abundant but present on GBM. Using subtype-specific antibodies for sst$_1$ and sst$_3$, as well as autoradiography and reverse transcription-PCR, Cervera et al. (23) determined microglia and proliferating vessels as the main source for sst receptors in GBM. In our analysis, all of the examined GBM showed some degree of sst immunopositivity. This contradictory finding may in part be reconciled by the different nature of GBM in children and adults, e.g., TP53 mutations, which are often found in adult GBM, are sparse in childhood GBM (39).
Among the evaluated AA we, as most other authors, found a varying pattern.

We found sst2 expression on most of the ependymomas; as had Guyotat before (24). In our specimen, which differed from the ones studied by Guyotat et al. (24) in that they were derived from children (1–15 years), immunostaining for sst2 was absent in >50% (3 of 4 EP II and 2 of 4 AE). However, in 3 tumors (2 AE and 1 EPII), we found rather dense receptor distribution (80% in 2 AE and 90% in an EP II). From our limited numbers, AE showed higher sst2 expression than did lower grade tumors. This is confirmed by Guyotat et al. (24) in adult ependymomas, where AE had higher sst2 levels than EP II, which had again higher levels than EP I. The question whether ependymomas of childhood express sst2 protein will need to be evaluated in larger cooperative studies. In contrast to Guyotat, who was able to image all ependymomas, SRI was not helpful in the diagnosis of ependymoma recurrence in our patients. Our data are supported by other investigators, who could not image ependymomas by SRI (27, 40).

In conclusion, we have demonstrated that stPNET and some other rare neoplasias of the CNS during childhood express levels of sst2 that warrant scintigraphic imaging; if the blood-brain barrier is disrupted. In selected cases, SRI was more sensitive for the detection of small lesions than MRI and CT. SRI thus holds great promise as an asset in the imaging of residual and/or recurrent stPNET. Our cohort is not yet large enough to precisely determine sensitivity and specificity of SRI in children with stPNET. More patients with this rare disease can only be studied in a multi-institutional fashion.

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REFERENCES


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