Somatostatin receptor expression in thymic tumors

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1. ABSTRACT

Detection of thymic tumors by [¹¹¹In-DTPA⁰]octreotide scintigraphy and the antitumor effect exerted by somatostatin (SS) analogs suggest significant expression of SS receptors (SSRs) in these tumors. We measured SSR subtype (sst) expression by immunohistochemistry (IHC) in 14 thymic tumors previously studied by SSR scintigraphy (SRS). Scintigraphy showed significant [¹¹¹In-DTPA⁰]octreotide uptake in 13/14 tumors (tumor-to-background ratios: 1.4- to 6-fold). By IHC, 4 tumors were positive for sst₂A and sst₃; two for sst₂A and five tumors for sst₃. Three tumors were completely negative. Overall, 11/14 (approximately 78% of cases) expressed at least one SSR subtype. Staining was highly heterogeneous: sst₂A was confined to malignant epithelial cells or within stromal structures, and sst₃ was predominantly associated with thymocytes. SRS provides immediate visualization of primary and metastatic lesions, while IHC reveals SSR subtype expression. This joined approach could help to select SS analogs beneficial for therapy.

2. INTRODUCTION

High somatostatin receptors (SSRs) expression have great relevance in neuroendocrine human tumors (1-3). Imaging by [¹¹¹In-DTPA⁰]octreotide scintigraphy is a well established in vivo method used to detect SSR-expressing tumors (4), whereas therapy with SS analogs (SSAs) is effective in selected SSR-positive neuroendocrine tumors (3,5). Thus, in vivo and in vitro identification of SSR provides the rationale for the therapeutic use of SSAs. Five different SSRs have been identified so far, of which sst₂ is the most widely expressed. Moreover, sst₂ has the highest affinity for the SSA octreotide compared with the other SSAs, and high tissue sst₂ expression is mostly responsible for in vivo visualization of SSRs with SSR scintigraphy (SRS) and for the clinical response to octreotide. Therefore, in vitro identification of SSRs is clinically relevant. After in vitro identification of SSR in tumor tissue by ligand-binding techniques and RT-PCR, SSA can be used for diagnosis and therapy (6-8). Thanks to the development of antibodies specific for the different SSR subtypes, SSR expression can
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Table 1. Comprehensive view of patients’ characteristics and related somatostatin receptor scintigraphy and immunohistochemical findings

<table>
<thead>
<tr>
<th>N.</th>
<th>Sex/Age</th>
<th>Surgery</th>
<th>Histotype</th>
<th>Stage</th>
<th>Associated syndrome</th>
<th>SRS</th>
<th>Sites of uptake</th>
<th>T-to-B ratios</th>
<th>Immunohistochemistry (IHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>f/57</td>
<td>B</td>
<td>B2</td>
<td>IVB</td>
<td>PRCA</td>
<td>Med</td>
<td>3.1</td>
<td>+ TE</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>2</td>
<td>m/27</td>
<td>Thy</td>
<td>B2</td>
<td>II</td>
<td>MG</td>
<td>Med</td>
<td>1.4</td>
<td>+ TE</td>
<td>sst&lt;sub&gt;3&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>3</td>
<td>f/48</td>
<td>Thy</td>
<td>C</td>
<td>IVA</td>
<td></td>
<td>Med, lung</td>
<td>6.3</td>
<td>+ TE</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>4</td>
<td>M/59</td>
<td>B</td>
<td>B2</td>
<td>IVB</td>
<td></td>
<td>Med, pleura</td>
<td>2.1/1.4</td>
<td>+ TE</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>5</td>
<td>f/56</td>
<td>Thy</td>
<td>B2+B3</td>
<td>IVA</td>
<td></td>
<td>Med</td>
<td>6.2</td>
<td>-</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>6</td>
<td>m/52</td>
<td>Thy</td>
<td>B2</td>
<td>IVA</td>
<td>MG</td>
<td>Med</td>
<td>3.3</td>
<td>-</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>7</td>
<td>m/53</td>
<td>Thy</td>
<td>B2</td>
<td>IVA</td>
<td></td>
<td>Med</td>
<td>3.2/1.7</td>
<td>-</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>8</td>
<td>m/23</td>
<td>B</td>
<td>SCNC</td>
<td>IVB</td>
<td></td>
<td>Med, cervical lymph</td>
<td>1.5/1.5</td>
<td>-</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>9</td>
<td>m/72</td>
<td>Thy</td>
<td>B2</td>
<td>III</td>
<td></td>
<td>Med</td>
<td>1.4</td>
<td>n.e.</td>
<td>sst&lt;sub&gt;2&lt;/sub&gt; + TH, ± TE</td>
</tr>
<tr>
<td>10</td>
<td>f/68</td>
<td>B</td>
<td>C</td>
<td>III</td>
<td>My</td>
<td>Med, lung</td>
<td>4.2/3.3</td>
<td>Scattered TE</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>f/67</td>
<td>B</td>
<td>SCNC</td>
<td>IVA</td>
<td></td>
<td>Med</td>
<td>3.4</td>
<td>+ TE</td>
<td>n.e.</td>
</tr>
<tr>
<td>12</td>
<td>m/48</td>
<td>Thy</td>
<td>B3</td>
<td>IVA</td>
<td></td>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>f/74</td>
<td>Thy</td>
<td>B1</td>
<td>IVB</td>
<td>MG</td>
<td>Med</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>f/60</td>
<td>B</td>
<td>C</td>
<td>IVA</td>
<td></td>
<td>Med</td>
<td>2.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: f, female; m, male; B, biopsy; Thy, thymectomy; SCNC, small cell neuroendocrine carcinoma; PRCA, Pure Red Cell Aplasia; MG, Myasthenia Gravis; My, Myeloradiculopathy; Med, mediastinum lymph lymphnode; T-to-B, Tumor to Background ratio; n.e., not evaluated; TE, tumor epitheliali cells; TH, thymocytes.

be studied by immunohistochemistry (IHC) on paraffin-embedded tissues (9,10). Preliminary reports indicated that IHC is a reliable method with which to evaluate SSR expression in carcinoids and other SSR-positive tumors (11-14). IHC is easier to perform and more practical for clinical use than RT-PCR or in situ hybridization methods. Furthermore, IHC reveals the exact cell localization of a given antigen-protein and can be applied on fixed material.

SSRs are differentially, but widely, expressed in normal and abnormal lymphoid tissue and immune cells, including thymic tumors (15,16). Moreover, SSR expression in thymic epithelial tumors could indicate a neoplasm originating from an organ characterized by neuroendocrine cells.

We previously demonstrated that thymic tumors can be visualized in vivo by SRS and have very high tumor-to-background (T-to-B) ratios (17). Moreover, patients with thymic tumors have been reported to respond to SSA-based therapies (18-20). SSR expression was not found in thymic carcinoids and in normal thymuses (21). However, more recently, with a combination of in vitro techniques demonstrated the expression of sst<sub>1</sub>, sst<sub>2</sub> and sst<sub>3</sub> in a B1 (cortical) thymoma, which was previously detected by SRS (22), and of sst<sub>2</sub> and sst<sub>3</sub> in an A (medullary) thymoma also previously imaged with SRS (23).

The aim of the present study was to verify sst<sub>2A</sub> and sst<sub>3</sub> expression in a series of 14 thymic tumors previously imaged with SRS to verify the concordance, if any, between in vitro and in vivo results.

3. MATERIALS AND METHODS

3.1 Patients

Fourteen patients (7 f and 7 m, age-range: 23-74 yrs) with a diagnosis of thymic tumors were evaluated. According to the Masaoka et al classification (24), 1 patient was at stage II, 2 patients at stage III, 7 patients were at stage IVA and 4 patients were at stage IVB. Myasthenia gravis was the most common associated paraneoplastic syndrome in this series, whereas pure red cell aplasia was present in only one patient. One patient had also myeloradiculopathy. Six patients underwent only biopsy because of unresectable primitive tumor at presentation, whereas the others underwent thymectomy. Tumors were classified according to the 1999 WHO classification (25): 1 B1 thymoma, 6 B2 thymomas, 1 B3 thymoma, 1 combined B2+B3 thymoma, 3 C thymoma, 2 small cell neuroendocrine carcinoma (SCNC). The patients’ profiles are shown in Table 1. Ethical committee approved the study and all patients gave their informed consent.

3.2. Scintigraphic studies

Scintigraphy with [111In-DTPA<sup>6</sup>]octreotide (Mallinkrodt, Petten, The Netherlands) was performed as previously reported (17). Briefly, planar and tomographic images were collected 24 hrs after the i.v. injection of 180-222 MBq of radioligand. Labeling was performed according to the manufacturer’s instructions; injected preparations had <2% of uncoupled [111In]. Images were obtained with a γ-camera (Siemens, Erlangen, Germany) equipped with a medium energy collimator (Photopeaks were set at 172 and 242 keV, 10% window). Tumor-to-background ratios were obtained with the regions of interest (ROIs) method. A ROI was manually drawn around the area/s of radioligand uptake; a similar ROI was placed on the patient’s chest where no uptake was detectable. In each patient, the T-to-B ratio was obtained in the planar or tomographic images of the chest 24 hrs after the injection of the radioligand by dividing the counts in the thymic tumor by those in the background area.

3.3. Tissue samples

The samples of 14 histologically proven thymomas were collected and used for IHC. All samples were formalin-fixed, paraffin-embedded. Fixation was performed for 24-36 hours.

3.4. Immunohistochemical localization of sst<sub>2</sub> and sst<sub>3</sub> receptors

IHC was performed on 5-µm paraffin-embedded sections as previously reported (22). Negative controls for
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4. RESULTS

4.1. Somatostatin receptor scintigraphy

SRS was positive in 13 out of 14 patients. In 4 cases, there were at least two sites of abnormal radioligand uptake in the chest, whereas in the other 9 patients there was a single area of uptake. The degree of $^{111}$In-DTPA-octreotide uptake varied greatly among the patients. The SRS results and T-to-B ratios are shown in Table 1.

4.2. Immunohistochemical detection of sst$_2$ and sst$_3$ receptors

Expression of sst$_2A$ and sst$_3$ or their co-expression was clearly detectable by IHC in 11 out of 14 thymic tumors, whereas 3 tumors were completely negative. Immunostaining was possible for both SSR subtypes in 12 cases, and for only one subtype in 2 cases because of a limited amount of tissue and antibody. sst$_2$ was expressed in more than half the patients studied (9/14) while sst$_{2A}$ was clearly detectable in 4 cases and on scattered cells in other 2 cases. The results are reported in Table 1. The B1 tumor had no SSR staining; 3/5 B2 tumors were positive for sst$_{2A}$ and all were positive for sst$_3$. The B2/3 tumor was positive only for sst$_1$, whereas the B3 tumor was positive only for sst$_{2A}$. One C tumor was negative for both receptors, one was positive only for sst$_{2A}$, one was positive for both, and one SCNC was positive for sst$_{2A}$ whereas the other was positive for sst$_1$. The immunostaining was weak to moderate. However, the distribution of both subtypes was highly heterogeneous.

IHC revealed the exact location the cellular distribution of SSR within these thymic tumors. The sst$_{2A}$ was homogeneously expressed throughout the tumor in only 2 cases (n. 3 and 11) whereas immunoreactivity was highly heterogeneous in the remaining sections and was confined to limited areas in the tissue samples (n. 2 and 4), or on scattered cells (n. 1 and 10). sst$_3$ was expressed throughout the tumor in both cell types, but was mainly associated with thymocytes. After absorption with specific peptide antigen, the binding of these antisera was completely prevented on adjacent sections taken from all patients, which demonstrates the specificity of sst$_{2A}$ and sst$_3$ immunostaining.

The expression of sst$_{2A}$ was predominantly associated to neoplastic epithelial cells, whereas the expression of sst$_3$ was found mainly associated to normal reactive thymocytes. In 4 thymic tumor biopsies (n. 1-4), both sst$_{2A}$ and sst$_3$ were localized. In detail, in 2 cases (n. 3 and 4) sst$_{2A}$ immunoreactivity was present on tumor cells, whereas in the other 2 (n. 1 and in vivo) 2) immunoreactivity was detected on few scattered cells in 1 (n. 1), and on the stromal compartment of the medullary region in the remaining case (n. 2). One of the 2 cases with immunohistochemical co-expression of sst$_{2A}$ and sst$_3$ (case...
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3) had the highest level of $[^{111}\text{In-DTPA}]$octreotide uptake in vivo. Figure 1 shows the co-expression of sst$_{2}$ and sst$_{3}$ in case 3. In 5 cases (n. 5-9), only sst$_{2}$ was detectable. The sst$_{3}$ receptor was not co-expressed in 4 of these biopsies and not measurable in the remaining one (n. 9) due to the limited amount of sample available. In 2 cases (n. 10 and 11), only sst$_{3}$ was expressed. sst$_{1}$ was undetectable in one of these cases and not measurable in the other case (see above). In 3 cases (n.12-14) neither sst$_{2A}$ nor sst$_{3}$ were detectable.

4.3. Comparative analysis of in vitro and in vivo results

We next compared the results of immunostaining with the T-to-B ratios measured at SRS. The higher T-to-B ratios in vivo corresponded to the expression of at least one of the two SSR subtypes in vitro. In particular, the highest ratio was found in a patient with widespread sst$_{2A}$ and sst$_{3}$ co-expression. The expression of both subtypes and sst$_{2A}$ alone occurred within tumors with a high T-to-B ratio.

5. DISCUSSION

In normal thymuses, sst$_{3}$ and sst$_{2A}$ mRNAs have been identified in thymic tissue and isolated thymic epithelial cells, whereas sst$_{1}$ mRNA has been demonstrated in thymic tissue only and in mature isolated thymocytes (26,27). However, the evidence that thymic tumors may express SSRs is poor (23). Reubi et al demonstrated SSRs in normal thymuses using autoradiography, and a lack of binding sites in 4 epithelial thymic tumors using autoradiography with radiolabeled SSAs (21). However, this finding appeared to be in contrast with the evidence that tumors originating from SSR-positive tissues generally express SSRs. The identification by IHC of sst$_{2A}$ and/or sst$_{3}$ in most of our 14 thymic tumors provides the first confirmation, and in a larger series, that SSRs are expressed in this rare human malignancy.

We were unable to identify correlation between tumor histotype and SSR pattern probably because of the relatively small number of cases and of their considerable heterogeneity. However, B2 thymomas, which was the most represented in this series of tumors, expressed at least one SSR subtype. Despite their “classical” neuroendocrine phenotype, SCNC expressed only one SSR. This pattern of SSR expression supports the concept of that thymic tumors share a common neuroendocrine phenotype (28,29).

Expression of sst$_{2}$ was predominantly associated to thymocytes, whereas sst$_{2A}$ expression was confined to malignant epithelial cells and/or within the thymic stroma (endothelium, accessory bone marrow-derived cells). Indeed, thymomas are prevalently constituted by thymic epithelial cells mixed with benign thymocytes (T lymphocyte precursors) and stromal cells (30). There are architectural differences, i.e. prevalence of cortical areas and deficiency of medullary areas, between thymic tumors and normal thymus (31). There is a strong compartmentalization in the distribution of SSRs within the normal thymus (21,26). The same was reported for other neuropeptide receptors within thymus (32). This not casually determined distribution of receptors could be particularly important in modulating the physiological processes occurring in this organ. Different SSRs are selectively expressed in different regions and perhaps on specific epithelial cell types in the human thymus (26). Our results indicate that this complex distribution of SSR is preserved also in thymic tumors. If confirmed, this differential expression might indicate that the SS/SSR system plays a role in thymic disturbances and their relative clinical syndromes.

Although we did not find a clear correlation between histotype and SSR expression, a comparison of the scintigraphic results and immunohistochemical findings indicates that the higher T-to-B ratios in vivo correspond to the expression of at least one of the two SSR subtypes in vitro. In particular, the highest ratio occurred in one of the two patients with widespread co-expression of sst$_{2A}$ and sst$_{1}$. sst$_{3}$ has recently been implicated in the uptake of $[^{111}\text{In-DTPA}]$octreotide in vivo in pheochromocytomas not expressing sst$_{2A}$ (33). We found a link between histotype and SSR expression also in one of our cases with negative SSRs, in which IHC failed to detect SSR in the tumor tissue. However, T-to-B ratios were high in other two patients with tumor lacking SSR expression in vitro. Although the sensitivity of this technique or the sample fixation procedures might account for this discrepancy, the presence of another SSR subtype, namely sst$_{2}$, cannot be ruled out. Moreover, the in vivo visualization of cases found to be sst$_{2}$/sst$_{3}$-negative might also result from aspecific or specific uptake in other adjacent tissues (i.e. inflammatory tissues).

$[^{111}\text{In-DTPA}]$octreotide binds with high affinity to sst$_{2}$ and with lower affinity to sst$_{3}$ and sst$_{5}$. We have evaluated 2 of these SSRs and both receptors appear to be involved in the uptake of the radiopharmaceutical. In 3 cases a high uptake in vivo corresponded to the selective expression of sst$_{2}$ within the tumor, confirming that, despite the lower affinity for $[^{111}\text{In-DTPA}]$octreotide, sst$_{2}$ may play a role or be directly involved in the uptake of the radiotracer (26,33). Apart from the affinity, additional biological events related to membrane receptor turnover or receptor cross-talk might play a role in determining the in vivo uptake of radiolabeled SSAs. In fact, given the high rate of agonist-dependent internalization described in cells transfected with this receptor subtype (34), sst$_{2}$ may be involved in this process. This hypothesis is in line with recent data about neuropeptide receptor trafficking and cross talk in cells co-expressing different SSR subtypes that derive from receptor dimerization (35,36).

In conclusion, sst$_{2}$ and sst$_{3}$ appear to be highly and heterogeneously expressed in thymic tumors, without correlation with epithelial rather than neuroendocrine histotype. The immunohistochemical detection of these SSR subtypes concords with scintigraphic results obtained with $[^{111}\text{In-DTPA}]$octreotide. Thus, SRS provides immediate visualization of primary and metastatic sites of tumor, whereas IHC can be used to define more precisely the pattern of SSR subtype expression. This combined approach provides diagnostic information and the results
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may be useful in deciding which SSA is the most appropriate for the treatment of a given patient.

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7. REFERENCES


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**Key Words**: Somatostatin Receptors, Somatostatin, Thymic Tumors, Neuroendocrine Tumors, Octreotide, Immunohistochemistry

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