Differential Expression of Somatostatin and Dopamine Receptor Subtype Genes in Adrenocorticotropic (ACTH)-secreting Pituitary Tumors and Silent Corticotroph Adenomas

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Abstract. Somatostatin analogs and dopamine agonists are clinically used for medical therapy of functioning pituitary tumors, such as growth hormone- and prolactin-secreting tumors, however, their effects on ACTH-secreting tumors are controversial. This study was aimed to determine whether somatostatin receptor (SSTR) subtype (1–5) and dopamine receptor type 2 (D2R) are differentially expressed in pituitary tumors causing Cushing’s disease (CD), silent corticotroph adenoma (SCA), and non-functioning pituitary tumor (NFT). Tissue specimens were obtained from 35 pituitary tumors during transsphenoidal surgery. The steady-state mRNA levels of SSTR1–5 and D2R genes were determined by real-time reverse-transcription polymerase chain reaction. Both SSTR1 and 2 mRNA levels in SCA were greater than CD, while SSTR1 mRNA levels, but not SSTR2, in SCA were also greater than NFT. SSTR5 mRNA levels in CD were greater than SCA, but did not differ between NFT and SCA. SSTR4 mRNA expression was undetectable. D2R mRNA levels were markedly lower in CD and SCA than in NFT. The present study suggests that somatostatin analogs more selective for SSTR5 and for SSTR1 and/or 2 may have the therapeutic potential for medical treatment of CD and SCA, respectively, whereas clinical application of dopamine agonists selective for D2R is very limited in either CD or SCA.

Key words: Cushing’s disease, Silent corticotroph adenoma, Non-functioning pituitary tumor, SSTR, D2R, Real-time RT-PCR

CUSHING’S disease (CD) is a hypercortisolism secondary to an adrenocorticotropic hormone (ACTH)-secreting pituitary tumor, which leads to high mortality rate related to infection and cardiovascular complication [1]. On the other hand, silent corticotroph adenoma (SCA) is defined as a pituitary adenoma with positive immunoreactivity for ACTH without any signs or symptoms of Cushing’s syndrome. Although both CD and SCA are considered to be derived from corticotroph adenoma, their clinicopathological profiles appear to be entirely different from each other. We have recently reported that genes related to proopiomelanocortin (POMC) transcription, secretion, and processing are differentially expressed between CD and SCA, suggesting that differential gene expression profiles are partly responsible for their different endocrinological and clinical features [2].

Somatostatin receptors (SSTR) are widely distributed in various neuroendocrine cells; there are five members of SSTR, subtype [1-5]. Somatostatin analogs have been shown to directly inhibit growth hormone (GH) secretion and tumorigenesis in GH-secreting pituitary tumors through SSTR2 and/or SSTR5 [3-6]. However, gene expression profile of SSTR subtype in pituitary corticotroph adenoma causing CD and SCA has not been fully understood. Dopamine agonists, on the other hand, have been widely used for the medical treatment of prolactin (PRL)-secreting pituitary tumors. Although several studies have documented the therapeutic usefulness of dopamine agonists in some cases of CD [7, 8], gene expression profile of
plasma ACTH and serum cortisol concentrations, and their lack of diurnal rhythm, suppression after high-dose (8 mg) dexamethasone suppression test, and/or hyperresponse of ACTH to corticotropin-releasing hormone (CRH), and the presence of a pituitary tumor on a diagnostic magnetic resonance image, and demonstration of the positive ACTH immunoreactivity in the pituitary tumor. SCA was diagnosed with the positive ACTH immunoreactivity of the pituitary tumor without any stigmata of Cushing’s syndrome. Based on the results from the clinical, endocrinological, and immunohistochemical studies, 12 patients were diagnosed with CD, 8 with SCA, and 15 with NFT.

**Patients and Methods**

**Patients**

Thirty-five patients (13 male and 22 female; mean age, 47.8 ± 2.6 years) who underwent transsphenoidal surgery (TSS) for the removal of pituitary tumors, were studied. The protocol was approved by the Ethical Committees of each institute. Informed consent was obtained from each patient before surgery. CD was diagnosed on the basis of signs and symptoms of Cushing’s syndrome and endocrine data; increased dopamine receptor subtype in corticotroph adenoma has not been characterized yet.

Therefore, the present study was aimed to examine whether somatostatin receptor subtype [1–5] and dopamine receptor type 2 (D2R) were differentially expressed in pituitary tumors with CD and SCA in comparison with those in NFT.

**Table 1. Clinical features of patients with pituitary tumors**

<table>
<thead>
<tr>
<th></th>
<th>ACTH-secreting pituitary tumor</th>
<th>Silent corticotroph adenoma</th>
<th>Non-functioning pituitary tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>12</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>2/10*</td>
<td>1/7*</td>
<td>10/5</td>
</tr>
<tr>
<td>Age (year-old)</td>
<td>38.6±4.6**</td>
<td>52.1±3.4</td>
<td>52.9±4.6</td>
</tr>
<tr>
<td>Tumor Size (mm)</td>
<td>11.8±2.2**</td>
<td>42.8±12.2</td>
<td>30.2±2.0</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>172.2±30.6*</td>
<td>29.9±4.5</td>
<td>29.9±5.3</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>33.5±4.8*</td>
<td>9.9±1.6</td>
<td>13.4±1.4</td>
</tr>
</tbody>
</table>

*p < 0.01 and, ** p < 0.05 vs non-functioning pituitary tumors

**Table 2. PCR primers used for real-time RT-PCR**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Forward Sequences</th>
<th>Reverse Sequences</th>
<th>PCR product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSTR1</td>
<td>AGATCGTCAACCTGCTAAACAGAC</td>
<td>GGGCTCTGGACTGTTAAATGATTA</td>
<td>128bp</td>
</tr>
<tr>
<td>SSTR2</td>
<td>AGACCGGAGCTAGGGATT</td>
<td>CAGGCCAGCCAGAGACTTTA</td>
<td>101bp</td>
</tr>
<tr>
<td>SSTR3</td>
<td>GAGAGGGAGCCAGAGATGA</td>
<td>GGGGCTTCGTTCTCGTCTC</td>
<td>158bp</td>
</tr>
<tr>
<td>SSTR4</td>
<td>CACCCAGGTCTTCGTTCTCA</td>
<td>ATGGGGAGGTGCAACCGAG</td>
<td>150bp</td>
</tr>
<tr>
<td>SSTR5</td>
<td>CGCCGTCTTCTACATCTACA</td>
<td>TCCACCAGAGCAGGAGCTCA</td>
<td>87bp</td>
</tr>
<tr>
<td>D2R</td>
<td>AGACCCACAACTACCTGAT</td>
<td>GCTGAATTTCCACTCCTACC</td>
<td>113bp</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GCTGAGAAGGGGAAGGCTGT</td>
<td>TCTCCATGGGTGGAAGACG</td>
<td>136bp</td>
</tr>
</tbody>
</table>

Abbreviations used: SSTR: somatostatin receptor, D2R: dopamine receptor type 2, GAPDH: glyceraldehyde-3-phosphate dehydrogenase
both the plasma ACTH and serum cortisol levels in CD patients were significantly \( p < 0.01 \) higher than those in SCA and NFT patients.

**Quantification of mRNA**

A portion of the tumor tissue specimens obtained during TSS from each pituitary tumor was immediately frozen with RNALater (Ambion Inc., US) in liquid nitrogen and stored at \(-80^\circ\text{C}\) until analysis. The remaining tissue specimens were subjected to histological and immunohistochemical analyses. Total RNA was extracted from the pituitary tumors (30–200 mg) by using the TRIzol reagent protocol (Life Technologies, Inc., Gaithersburg, MD). The total RNA (5 µg) was reverse transcribed with a first-strand synthesis kit (GE Healthcare UK). The mRNA levels of SSTR1-5 and D2R were quantified with real-time reverse transcription-polymerase chain reaction (RT-PCR) by using fluorescent SYBR green technology (LightCycler: Roche Molecular Biochemicals, Mannheim, Germany). The PCR primers of the genes were synthesized by Greiner Bio-one (Tokyo, Japan), and their sequences are shown in Table 2. In the SYBR green real-time PCR method, fluorescence data were quantitatively analyzed using a serial dilution of the control samples included in each reaction in order to generate a standard curve. To compare the relative expression of each gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous internal control and the relative levels of each mRNA to that of GAPDH were calculated.

**Statistical analysis**

Data were expressed as means ± SEM. Differences between groups were examined for statistical significance with an unpaired \( t \) test or analysis of variance (ANOVA) with Dunn’s post hoc test, if appropriate, and \( p \) values less than 0.05 were considered statistically significant.

**Results**

The steady-state mRNA levels of 5 different receptor subtypes [1-5] of SSTR in CD, SCA, and NFT are shown in Fig. 1. The SSTR1 mRNA levels in SCA were about 200-fold \( p < 0.01 \) and 17-fold \( p < 0.01 \) greater than those in NFT and CD, respectively; there was no significant difference between NFT and CD. The SSTR2 mRNA levels in CD were about 5-fold lower \( p < 0.01 \) than those in SCA; there was no significant difference between NFT and SCA. The SSTR3 mRNA levels did not differ among the three tumors, while the SSTR5 mRNA levels in CD were about 14-fold \( p < 0.01 \) greater than those in SCA; there was no significant difference between NFT and
SCA. SSTR4 mRNA expression was barely detected in any pituitary tumors (data not shown).

The steady-state mRNA levels of D2R in CD and SCA were significantly \( (p < 0.01) \) and markedly lower (about 1/200-300) than those in NFT (Fig. 2).

**Discussion**

In this study, we clearly showed that SSTR1–5 and D2R genes are differentially expressed in ACTH-secreting pituitary tumors causing CD and SCA in comparison with those in NFT. Both SSTR1 and SSTR2 mRNA levels in SCA were robustly increased compared to that in CD, whereas SSTR5 mRNA levels in CD were increased compared to that in SCA. In contrast, D2R mRNA expression was markedly decreased in both CD and SCA than that in NFT.

It has been reported that somatostatin has an anti-proliferative effect in pancreatic cancer cells via activation of tyrosin phosphatase through SSTR1 [10, 11], and an inhibitory effect on secretion of \( \alpha \)-subunit and chromogranin A from human non-functioning pituitary tumors [12]. However, its effect on cell viability of human non-functioning pituitary adenomas is equivocal [13]. A robust increase in SSTR1 expression as well as a modest increase in SSTR2 expression in SCA over CD as shown in this study suggests that any somatostatin analogs more selective for SSTR1 and 2 may be a potential therapeutic drug for medical treatment to inhibit tumor growth of SCA with aggressive and invasive characteristics. However, further study is needed to ascertain our assumption.

Our data demonstrates that ACTH-secreting pituitary tumors causing CD expressed SSTR2 mRNA by 5-fold less and SSTR5 mRNA by 14-fold more than SCA, respectively. Our data are in accordance with those of recent studies showing that most ACTH-secreting pituitary tumors causing CD expressed SSTR5 more abundantly than SSTR2 by RT-PCR and immunohistochemistry [14-16]. In fact, it has been reported that in ACTH-secreting pituitary tumors, the relative copy number of SSTR5 gene corrected by a housekeeping gene (hypoxanthine-phosphoribosyl-transferase) was much higher than that of SSTR2 gene [16].

Although octreotide with higher affinity to SSTR2, has been shown to have neither an inhibitory effect on basal or CRH-induced ACTH release in patients with CD, nor the anti-proliferative or anti-apoptotic effects on a mouse ACTH-secreting pituitary tumor cell line, AtT-20 cells [17], SST analogs with affinity to both SSTR2 and SSTR5 inhibited accumulation of intracellular cAMP and reduced ACTH secretion by inhibiting intracellular Ca\(^{2+} \) in AtT-20 cells [18, 19]. Taken together, it is suggested that clinical efficacy of SSTR2-selective somatostatin analogs, such as octreotide and lanreotide, seems to be limited in CD.

On the other hand, SOM230 (pasireotide), a somatostatin analog with a higher affinity to SSTR5, is more potent than octreotide in suppressing ACTH release from human corticotroph adenomas and mouse AtT-20 cells which express SSTR5 more than SSTR2 in vitro as well as in rats in vivo [16, 20, 21]. Furthermore, SOM230 has an inhibitory effect on cell proliferation in human corticotroph adenoma cells in vitro [15]. Taken together, it is suggested that somatostatin analogs more selective for SSTR5 such as pasireotide may be a novel potential drug for medical treatment of CD in terms of inhibition of ACTH secretion and tumor growth in ACTH-secreting pituitary tumors with the robust increase in SSTR5 expression in ACTH-secreting pituitary tumors as demonstrated in this study and others [14, 15].

Our data complement the previous studies [15, 22] showing that SSTR3 and SSTR 4 mRNA are expressed extremely low or undetectable in ACTH-secreting pituitary tumors, suggesting that both SSTR3 and 4 have little, if any, functional roles in ACTH-secreting pituitary tumors.

The mechanism(s) responsible for the differential SSTR subtype gene expression between ACTH-
secretion of pituitary tumors (CD) and SCA remains unknown.
Glucocorticoids have been shown to regulate SSTR expression in corticotroph cells [20, 23]; dexamethasone treatment induced downregulation of SSTR1–4 mRNA expression, but upregulation of SSTR5 mRNA expression in pituitary tissues in vitro and in vivo [23]. Therefore, it is possible to speculate that glucocorticoids excess may play differential roles of SSTR subtype in the upregulation of SSTR5 and downregulation of SSTR1 and 2 in ACTH-secreting pituitary tumors causing CD.

We clearly demonstrated that D2R mRNA expression in the ACTH-secreting pituitary tumors was far lower than those in the NFTs. Dopamine agonists have been reported to be effective in some, but not all, patients with CD by suppressing ACTH secretion [7, 8]. Short-term treatment with bromocriptine or cabergoline has been reported to induce significant inhibition and normalization of cortisol secretion in about 40% of CD patients [24, 25]. However, the effectiveness of bromocriptine treatment for CD is controversial with a success rate ranging between 0–50% [26]. Moreover, normalization of cortisol secretion and/or tumor shrinkage after long-term treatment with bromocriptine were only sporadically reported, suggesting that only a subset of CD patients respond to chronic bromocriptine treatment [26-28]. Thus, it is suggested that clinical application of dopamine agonists selective for D2R is very limited in either CD or SCA.

**Acknowledgments**

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**References**


