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# Expression of Type 5 Somatostatin Receptor in TSH-secreting Pituitary Adenomas: A Possible Marker for Predicting Long-term Response to Octreotide Therapy

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**Abstract.** In TSH-secreting pituitary adenomas (TSHoma), octreotide (OCT) therapy reduces tumor size and TSH secretion in some cases but not in others. As OCT acts through various types of somatostatin receptors (SSTRs), the different responses of TSHoma to OCT might be explained by the differences of SSTR expression. We therefore studied the expression of subtype-specific SSTR mRNA transcripts in tumor tissues by RT-PCR. Type 2 (SSTR2) mRNA transcripts were detected in all 8 tumors but those of SSTR3 and SSTR5 were demonstrated only in 5 of them. Serum TSH levels were decreased by OCT administration test in all patients but OCT therapy was effective in two patients out of three. SSTR5 mRNA was detected in two tumors from the responder, but not in one tumor that was resistant to OCT. These observations suggest that the temporal decrease of TSH by OCT may be mediated by SSTR2, and that the long term response to OCT therapy may be related with the expression of SSTR5. Therefore, the expression of SSTR5 in TSHoma may be a useful marker for predicting the outcome of the therapy, but further studies with larger numbers of patients are necessary.

*Key words*: TSHoma, Somatostatin receptor, Octreotide

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**TSH**-secreting pituitary adenomas (TSHoma) represent a mere 1% of all pituitary adenomas [1–3]. The increasing number of reported cases these past ten years may be accounted by the use of ultrasensitive immunometric assays for serum TSH determinations. TSHoma is one of the conditions which present inappropriate secretion of TSH (SITSH) [1, 4] and the differential diagnosis consists of resistance of thyroid hormone, elevated or altered binding proteins, assay interference by anti-T3/T4 or heterophile antibodies and medical agents affecting thyroid hormone metabolism. The final diagnosis, therefore, must be confirmed by immunohistochemistry. Surgical removal of the tumor is essential for complete cure, but they are often invasive and fibrous, which hampers complete removal. Therefore, the cure rates are reported to be no more than 40% [1, 5, 6]. As for medical therapy, somatostatin analogues are reported to inhibit tumor growth in addition to TSH secretion and they are used as anti-tumor agents in combination with the surgical or radiological therapy [7]. According to previous reports on the long-term outcome of octreotide (OCT) or lanreotide, the clinical and biochemical cure rates was approximately 73 to 78% and a partial shrinkage of tumors was observed in one third of the cases [5, 7–13]. These data also suggest that some tumors are resistant to OCT or lanreotide but the exact mechanism of resistance is not

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known. We, therefore, studied the expression of somatostatin receptor subtypes in the resected tumor tissues and we also investigated the relationship between the receptor subtype expression and clinical data including the responses to OCT in short term as well as long term.

## Materials and Methods

*Subjects*

One female and seven male patients with TSHoma were studied. Tumor resections were carried out in all patients and the final diagnosis of TSHoma was confirmed by immunohistochemistry. Free thyroxine or triiodothyronine levels of the patients were above reference ranges and TSH concentrations were from 0.478 to 8.23 (median: 3.315) μU/mL. All patients also gave their written informed consent for the diagnostic analysis of tumor tissue for SSTR mRNA expression and mutated thyroid hormone receptor mRNA expression.

*Determination of serum hormone levels*

Serum TSH (μU/mL) was measured by a third generation ECLIA kit from Roche Diagnostic Co. Ltd., Tokyo, Japan. The reference range of TSH level was 0.38 to 4.30 μU/mL. Serum free triiodothyronine ($FT_3$) and free thyroxine ($FT_4$) were measured by commercial kits also from Ortho Clinical Diagnostics Co., Tokyo, Japan. The reference ranges of $FT_3$ and $FT_4$ were 1.95 to 3.55 pg/mL and 0.94 to 1.60 ng/dL, respectively.

*OCT suppression test*

In five patients (Cases 2, 3, 5, 7, and 8), octreotide suppression test was also carried out as a routine clinical evaluation. OCT was from Novartis Co. Ltd, Tokyo, Japan. A dose of 50 μg was injected subcutaneously at 9:00 and blood samples were collected every two-hours for 8 h. The patients were served standardized meals at 9:00, 12:00 and 18:00.

*OCT therapy*

In three patients OCT therapy was carried out. One patient (case 1) received an operation in another hospital, which failed to correct TSH and free thyroxine. OCT therapy was started because of positive staining of the tumor for GH as well as an increased serum GH level. The second patient (case 2) was referred to our hospital for OCT therapy. He insisted on the therapy because of anxiety over surgery. The third patient also requested the therapy before surgical resection for the control of hyperthyroidism. After information of the adverse effects as well as the effectiveness of treatment, these patients agreed to use octreotide at their own expense as the medical insurance in Japan does not cover OCT therapy for TSHoma.

*Determinations of mRNA level in tumors*

Tumor samples were stored at –80°C before extraction. RNA was prepared using a kit (RNAeasy) from Qiagen (Tokyo, Japan) with DNase digestion. All procedures were carried out according to the manufacturer's instruction. The reverse transcription (RT) of mRNA and PCR reaction were also carried out by the standard methods of our laboratory [14]. One μg of each RNA sample was combined with 800 ng of an oligo-$dT_{15}$ primer in 16 μl of RT buffer, heated to 95°C for 2 min, chilled on ice and incubated at 37°C for 30 min for annealing. The samples were then incubated at 37°C for 60 min with 100 U of M-MLV reverse transcriptase (Invitrogen Japan K.K., Tokyo, Japan) in 20 μL of reaction mixture. To one μL of the RT mixture the following was added: 1.0 μL of 10 × reaction buffer, 0.8 μL of 2.5 nM dNTP, 1.0 μL of primer mixture (10 pmol each) and 0.05 μL (0.025 U) Platinum Taq polymerase (Invitrogen Japan K.K.) and the total volume was adjusted to 10 μL with water. The samples were then cycle-incubated by denaturation at 94°C for 30 sec, annealing at 65°C for 30 sec and extension at 72°C for 60 sec. A linear range of mRNA level was identified through different PCR cycles using a serial dilution of a standard RNA with the optimal numbers of cycles being 28 for SSTR and 23 for GAPDH mRNA. Absence of mRNA was also confirmed by a reaction with 40 cycles.

The primers for SSTR1 to 5 are shown in Table 1, and the sequences are the same as given in a previous report [15, 16]. The common primers for both human and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were the same as our previous report [14]. The PCR products were subjected to electrophoresis

**Table 1.** The sequences of PCR primers for somatostatin receptor subtypes

| | | |
|---|---|---|
| SSTR 1 | Sense | 5'-ATGGTGGCCCTCAAGGCCGG-3' |
| | Antisense | 5'-CGCGGTGGCGTAATAGTCAA-3' |
| SSTR 2A | Sense | 5'-TCCTCTGGAATCCGAGTGGG-3' |
| | Antisense | 5'-TTGTCCTGCTTACTGTCACT-3' |
| SSTR 3 | Sense | 5'-TCATCTGCCTCTGCTACCTG-3' |
| | Antisense | 5'-GAGCCCAAAGAAGGCAGGCT-3' |
| SSTR 4 | Sense | 5'-ATCTTCGCAGACACCAGACC-3' |
| | Antisense | 5'-ATCAAGGCTGGTCACGACGA-3' |
| SSTR 5 | Sense | 5'-CCGTCTTCATCATCTACACGG-3' |
| | Antisense | 5'-GGCCAGGTTGACGATGTTGA-3 |

and the amounts of the products were measured by EDAS 290 system (Kodak, New Haven, CT, USA) after ethidium bromide staining. Expression of subtype specific mRNA was expressed as a ratio to GAPDH mRNA [14].

## Results

*Subtype-specific mRNA for SSTRs in tumor tissues*

As shown in Fig. 1, the relative amount of SSTR2 mRNA was variable but was detectable in all tumor tissues from the eight patients. However, the transcripts for types 3 and 5 receptors were expressed only in 5 different tumors. The amount of SSTR2 had no correlation with the amount of SSTR5 or with that of SSTR3. In addition to these mRNA transcripts, SSTR 1 mRNA was also detectable in 7 tumors (except case 7) and SSTR4 mRNA was expressed in 5 tumors (cases 1, 2, 3, 5 and 6).

*OCT suppression test*

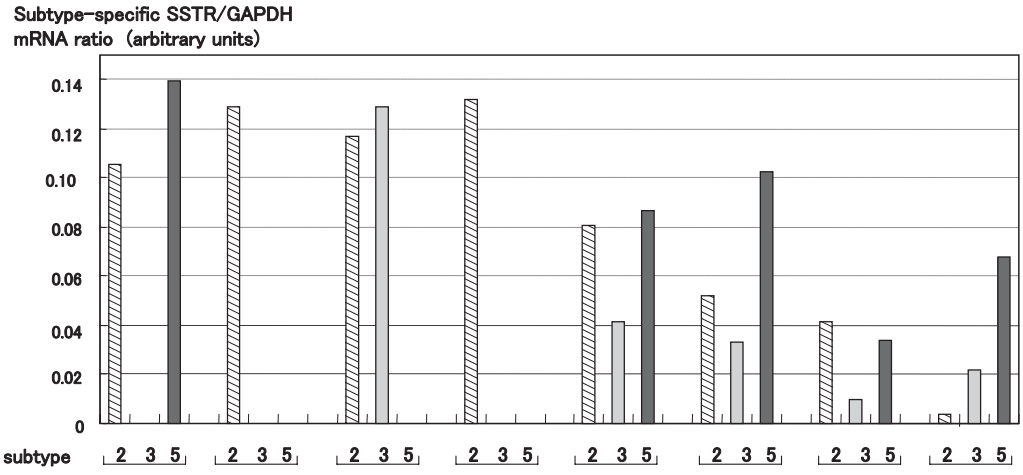
As shown in Fig. 1, OCT decreased serum TSH levels in all patients and SSTR2 mRNA was detectable in all tumors. OCT suppressed serum TSH levels to more than 50% only in cases 5 and 7. Interestingly, SSTR5 mRNA was detectable in the tumors from these patients. However, SSTR5 mRNA was also detectable in one tumor less responsive to OCT (case 8).

*Clinical outcome of octreotide therapy*

Three patients (cases 1, 2 and 7) were treated with OCT before surgical resection of the tumors as described in Materials and Methods. One patient (case 1) was treated with OCT 300 μg/day for 4 months for the reduction of tumor tissue as well as control of thyroid hormone. Serum TSH and $FT_4$ levels were decreased to normal range and the size of the tumor was also decreased (40% volume reduction). The second patient (case 2) was treated with OCT 100 μg/day for 10 months. TSH was slightly suppressed for the first 6 months by the therapy but the TSH levels increased after 6 months. The tumor size was not changed for the first 6 months but increased after 6 months by MRI studies. Therefore, surgical removal of the tumor was carried out. The third patient (case 7) was treated with OCT 100 μg/day for 2 months before surgical removal for control of hyperthyroidism. It decreased serum TSH and $FT_4$ levels to normal range but tumor volume was not decreased. It should be worth mentioning that SSTR5 mRNA was expressed only in two tumors (cases 1 and 7) which showed fair therapeutic response to OCT. The transcript for SSTR5 mRNA was not detectable in the tumor resistant to OCT therapy in spite of the fact that all tumors expressed SSTR2 mRNA.

## Discussion

Previous reports that TSHomas expressed somatostatin receptors support OCT therapy for TSH-dependent hyperthyroidism [8, 17–21]. According to these reports, OCT suppressed TSH levels in more than 90% of patients with TSHomas and it also decreased serum thyroid hormone levels to normal ranges in 70% of the patients. In addition to the inhibitory effects on TSH production and secretion, it also possesses an antitumor effect and reduced tumor size in approximately 50% of patients [1, 6, 8, 9, 11, 22]. These findings also suggest that some tumors are sensitive to the agent but others are not. However, the exact mechanism of the difference of response of TSHoma to the agent has not been reported so far. As for GH-secreting pituitary adenoma (GHoma), consistent expression of mRNA for both SSTR2 and SSTR5 and less abundant mRNA expression for SSTR1 and SSTR3 were reported [23–27]. The mRNA for SSTR4 was not demonstrated in these tumor tissues by these reports. Somatostatin binds to all five SSTR subtypes with high affinity. However, OCT and lanreotide bind to SSTR2 with high affinity and bind to SSTR3 and SSTR5 with relative low af-




| Case # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Basal TSH( $\mu$ U/ml) | 0.478 | 4.19 | 2.44 | 1.32 | 4.83 | 2.12 | 8.23 | 4.26 |
| Nadir TSH( $\mu$ U/ml) | | 2.55 | 1.66 | | 1.79 | | 2.80 | 2.53 |
| Suppression rate(%) | | 39.1 | 31.1 | | 62.9 | | 66.0 | 40.6 |
| Therapeutic response | fair | poor | | | | | fair | |

**Fig. 1.** Expression of Type 2, 3 and 5 somatostatin receptor mRNA in TSH-secreting pituitary adenomas and the responses to octreotide suppression tests and therapy. RNA extraction and RT-PCR were conducted as described in Materials and Methods and the amounts of PCR products were estimated by image analysis system after electrophoresis with ethidium bromide staining. The relative amounts of the mRNA transcripts were expressed as a ratio to GAPDH mRNA. The values are representative of one from three experiments. The subtypes of SSTR and the case numbers are as indicated. The responses to octreotide suppression test are also indicated. Octreotide suppression test was carried out in 5 patients as described in Materials and Methods. TSH levels at the start of the test (basal) and the lowest level after octreotide administration (nadir) are indicated below the case number. Suppression rate was calculated from the TSH values as follows; suppression rate (%) = [(basal – nadir)/basal] × 100. The responses to octreotide therapy are also indicated. Octreotide decreased thyroid hormone levels to normal range in cases 1 and 7 (fair response) but thyroid hormone levels and tumor volume were increased in case 2 (poor response).

finity. OCT was reported to inhibit GH secretion by SSTR2 and 5 mediated pathways. Therefore, the decrease in expression of these subtypes in tumors induced resistant to OCT therapy [28–30]. These findings also suggested the clinical significance of SSTR2 and SSTR5 expression in the response to OCT. However, a study failed to demonstrate the predictive value of SSTR 2 and 5 expressions [31]. More recently, a mutation of SSTR5 in tumor tissue was reported in one acromegalic patient who was resistant to somatostatin analog treatment [32] and the finding suggests the importance of SSTR5 for predicting the response. Regarding TSHoma, the relationship between its response to OCT and expression of subtype-specific SSTRs in tumor tissues has yet to be clarified. Our findings on OCT suppression test and expression of SSTR2 in tumor tissues suggest that OCT may inhibit TSH secretion in all tumors which express SSTR2 and that the expression of the type 5 receptor may enhance the inhibitory effects of OCT. As for tumor growth, the finding that SSTR5 mRNA was not expressed in one tumor which was operated because of enlargement in spite of OCT therapy suggests the importance of SSTR5 expression for the control of growth. The fact that OCT treatment suppressed both TSH and thyroid hormone levels for the first 6 months may also suggest following two possibilities. First, SSTR2 expression alone might be sufficient to suppress tumor growth in addition to TSH secretion in short term. Second, SSTR5 expression might be lost during the therapy, allowing the tumor to resume growth. In spite of these possibilities, analysis of SSTR5 expression in TSHoma may be useful for predicting the effect of OCT therapy regarding tumor growth as well as control of hyperthyroidism in

long term. However, additional studies with a larger number of cases are necessary for the establishment of exact role of SSTR5 in OCT therapy for TSHoma.

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