Evidence for a selective loss of somatostatin receptor subtype expression in male germ cell tumors of seminoma type

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Somatostatin (SRIF) is a potent antiproliferative signal for both normal and tumoral mammalian cells and an alteration in the SRIF receptor expression pattern has been associated with carcinogenesis. In the present study, the relevance of SRIF signaling to human male germ cell tumors was assessed at the receptor level. The expression of five SRIF receptor (sst1–sst5) mRNAs was estimated by RT–PCR and compared between normal and tumoral testes. All 12 normal testicular tissues studied contained sst3 and sst5 receptor transcripts whereas sst4 was present in almost all (11 of 12). sst1 transcripts were consistently absent while the majority (11/12) of normal samples studied did not contain sst2 mRNA. Parallel assessment of SRIF receptor mRNAs in 10 seminoma testicular germ cell tumors showed expression of a single receptor type, sst5, in all samples analyzed. All seminoma samples were depleted in transcripts corresponding to sst1 and sst2 receptors while either sst3 or sst4 mRNAs were absent in almost all (9 of 10) tumoral samples studied. The comparison of SRIF receptor expression between normal tissue and seminoma tumors thus points to a selective loss of sst3 and sst4 mRNA expression in seminomas. Altogether these data indicate that: (i) normal human testes are putative SRIF targets; (ii) loss of sst3 and sst4 SRIF receptor expression might be associated with seminoma carcinogenesis.

Introduction

Carcinogenesis is commonly related to the abrogation of cell division control by extracellular signals such as mitogens and antiproliferative molecules (1). Besides growth factors and cytokines acting as either positive or negative regulators of cell division, another family of signaling molecules, peptide hormones, are involved in cell proliferation control. Signal transduction of peptide hormone action is generally initiated by activation of specific heptahelical transmembrane receptors. As a corollary, these receptors are possible targets for carcinogenesis and are currently considered as potential tumor markers (2).

Somatostatin (SRIF) is a peptide hormone with potent antiproliferative actions on different cell types under both physiological and pathological conditions. Additionally, SRIF is widely distributed throughout the central nervous system (CNS) and the periphery. In the CNS it fulfills criteria required for neurohormone/neurotransmitter/neuromodulator actions. In the periphery SRIF exercises antisecretory effects in many exocrine and endocrine glands. All these biological actions are mediated by five receptors (sst1–sst5). They have been cloned and are encoded by different genes located on five distinct chromosomes. Almost all mammalian cells and tissues studied express multiple SRIF receptors but the combination of receptor expression is cell and tissue type specific (3).

SRIF receptors have been identified in a variety of human tumors. The most studied among them are those of neuroendocrine (e.g. GH- and TSH-producing pituitary adenomas), endocrine (e.g. enteropancreatic tumors, adrenal pheochromocytomas and medullary thyroid carcinomas) and nervous (astrocytomas, neuroblastomas and meningiomas) origin. All these tumors express more than one of the five cloned SRIF receptors (4–7). Their normal tissue counterparts also display multiple SRIF receptors (2). The cancer etiology is in many instances associated with an alteration in SRIF receptor expression pattern (2,8,9). Moreover, the loss of a particular SRIF receptor (i.e. sst2) has been associated with mechanisms underlying carcinogenesis in the pancreas (8,10). Otherwise, knowledge about SRIF receptor status of a given tumor is currently considered a valuable diagnostic and therapeutic criterion (11). For example, the demonstration of sst2/sst5 receptor expression in different carcinoid tumors provided the rationale for the use of synthetic SRIF agonists such as SMS201995 and BIM23014 in their clinical management (12–14).

Testicular cancers are rare malignancies (3–7/100 000) curable in up to 80% of cases in developed countries. The remaining 20% are resistant to radio- and chemotherapy for reasons that remain unknown. The incidence of testicular cancers has more than doubled over the world in the last 40 years, with a particularly strong tendency in northern and western Europe (15,16). In addition, testicular tumors of germ cell origin, representing 95% of all testicular cancers, are the commonest solid tumor in 15- to 34-year-old men and are the primary cause of death in this age group (16). Germ cell tumors are classified as non-seminomas or seminomas, each accounting for ~50% of germ cell tumors. Pathogenetic mechanisms leading to the appearance of either of them are unknown (15). In particular, the absence of germ cell lines render such mechanisms difficult to study.

SRIF has been found in both normal human testes (17) and testicular cancers of germ cell origin (18). However, previous studies addressed only sst5 SRIF receptor expression in normal human testes (19), whereas a possible alteration in receptor expression has not been assessed in these pathologies before.

In the present work we therefore compared testicular SRIF receptor mRNA expression between samples obtained from normal testes and seminomas using the RT–PCR approach. Our data point to differential expression of sst1–sst5 receptors between the two experimental groups.
Materials and methods

Subjects and tissue samples

Ten patients with testicular cancer of pure seminoma type, not metastatic to supradiaphragmatic nodal or visceral sites, were included in this study. The seminoma samples used were of stage I and II (disease limited to either testis, epididymis, spermatic cord or retroperitoneal lymph nodes, respectively). Biopsies of surrounding testicular tissues with histologically normal appearance were considered as normal controls. In addition, two subjects with histologically normal tests that had been orchiectomized after diagnosis of prostate cancer were also added to the experimental cohort. The patient age ranged from 26 to 56 years (mean 34.5). All testicular samples were collected by the Department of Pathology, Antiquaille Hospital, Lyon, France. Informed consent of the patients was obtained and approved by the Board of the Hospital.

At surgery, tissue samples were divided into a few fragments: they were randomly either frozen and stored in liquid nitrogen for RT–PCR or fixed in 4% paraformaldehyde for histopathological analysis. Clinical diagnoses of seminomas (including serum negativity for α-fetoprotein) were confirmed cytologically based on the presence of immunoreactivity for placental alkaline phosphatase. Only specimens containing 100% normal or seminoma cancer cells were retained for study.

RNA extraction and RT–PCR conditions

TRIzol reagent (Life Technologies, Eragny, France) was used to extract total cellular RNA and DNA from tissue samples. In order to exclude any genomic DNA contamination, 10 μg of each RNA sample was treated for 10 min at 37°C with 0.75 U DNase I (Pharmacia Biotech, Uppsala, Sweden) in the presence of 40 μU RNase inhibitor, RNasin (Promega, Charbonnières, France), 5 mM DTT (Gibco BRL, Cergy Pontoise, France) and buffer 1X (Gibco BRL) in a final volume of 10 μl. The reaction was terminated by heating at 75°C for 10 min. Treated RNA was reverse transcribed with Moloney monkey leukemia virus (MMLV) reverse transcriptase (200 U) in a reaction tube containing 0.5 mM each deoxynucleotide triphosphate (Pharmacia Biotech), 40 U RNasin (Promega), 5 μM hexanucleotide primer (Sigma, l’Isle d’Abeau, France), 10 mM DTT (Gibco BRL) and buffer (Gibco BRL) in a final volume of 20 μl. The mixture was first incubated at 42°C for 1 h and subsequently boiled for 5 min. Then 2 μl of the RT reaction mixture from each sample were diluted into a final volume of 50 μl in 50 mM Tris–HCl buffer (Promega) containing 0.2 mM dNTP (Pharmacia Biotech), 1 U Taq DNA polymerase (Promega) and 0.4 μM each primer (Table I).

In order to be able to compare the expression of sst1–sst5 receptor mRNAs between different subjects we attempted to perform a co-amplification of PCR fragments corresponding to each individual receptor, with β-actin used as an internal standard. A compromise (compared with the parameters that we have previously defined for these primers for human SRIF receptors; refs 20, 21) MgCl2 concentration of 2 mM and annealing temperature of 65°C were chosen for co-amplification. The co-amplification was successful for sst2, sst3 and sst5 receptors since, in addition to the amplification fragment of 661 bp corresponding to β-actin, products of the expected sizes (414, 221 and 154 bp, respectively) for these receptors were observed (see Table I). For the sst1 and sst4 receptors we were unable to perform a satisfactory co-amplification (i.e. with a single PCR product for each set of primers) with a total of four different sets of primers tested (data not shown). For these two receptors, the primer sequences shown in Table I were finally selected and amplifications were performed under the same conditions as for co-amplication (i.e. 65°C, 2 mM MgCl2) but without adding β-actin primers. PCR amplification was carried out using a Trio-thermoblock (Biometra, Kontron Instruments, Champagne au Mont d’Or, France) for 2.5 min of initial denaturation at 94°C, followed by 40 cycles of amplification (94°C for 60 s; 65°C for 60 s; 72°C for 60 s). Final elongation was achieved at 72°C for 5 min. Products of PCR amplification were visualized on 2% agarose gels by ethidium bromide staining. They are shown in Figures 1–5 (for sst1–sst5 receptors, respectively) for six representative normal (N1–N6) and six seminoma (S1–S6) samples; the remaining results are summarized in Table III. However, all exceptions (e.g. a single sst2 mRNA-positive normal sample [corresponding to specimen N6, Table II] out of 12 studied, Figure 2) are systematically shown. All PCR products have been sequenced to confirm the identity (data not shown).

Positive controls consisted of a reaction with genomic DNA. Negative controls consisted of a reaction performed as described above but without addition of reverse transcriptase as a test of genomic DNA contamination. A second negative control was carried out by replacing cDNA with water.

Results

Clinicopathological data on tissue samples used in the present study are given in Table II. The results shown in Figures 1–5 correspond to specimens N1–N6 for normal tissue and S1–S6 for seminomas that were obtained from cases 1–6 in Table II. Results corresponding to specimens N7–N12 and S7–S10

<p>| Table I. Primers used in PCR reactions |</p>
<table>
<thead>
<tr>
<th>Size (bp)</th>
<th>Upstream</th>
<th>Downstream</th>
</tr>
</thead>
<tbody>
<tr>
<td>sst2 233</td>
<td>414</td>
<td>5'–GCGACGACAATGTTGAGTC662-3</td>
</tr>
<tr>
<td>sst2b 414</td>
<td>221</td>
<td>5'–ATCTCAAACCTGTTGAGTC706-3</td>
</tr>
<tr>
<td>sst3 221</td>
<td>321</td>
<td>5'–TATTGGGTGCTCCGCTT689-3</td>
</tr>
<tr>
<td>sst4 154</td>
<td>5'–CAGGTCGCGCTTTCGTG798-3</td>
<td>5'–CAGGTCGCGCTTTCGTG798-3</td>
</tr>
<tr>
<td>β-actin 661</td>
<td>5'–GCAGGGGTGGTACCC662-3</td>
<td>5'–GCAGGGGTGGTACCC662-3</td>
</tr>
</tbody>
</table>

*Numbers give the position of the first and the last primer nucleotide in the corresponding cDNA sequence.

*<sup>a</sup>These primers amplify both the sst2A and sst2B splice variants of the sst2 receptor.
Normal and tumoral testes SRIF receptors

Fig. 2. All seminomas and almost all normal testicle tissues do not contain sst2 receptor mRNA. Presented results correspond to the samples obtained from the first six subjects (Table II, specimens N1–N6 and S1–S6). All remaining samples (specimens N7–N12 for normal tissues and S7–S10 for seminomas, Table II) were sst2 receptor mRNA negative (data not shown). The only normal sample in which sst2 receptor mRNA could be detected came from case number 6 (specimen N6, Table II; lanes 23–24). The PCR products are indicated by the arrows on the right and the size of amplified fragments is given in base pairs on the left (sst2 = 414; β-actin = 661). Legends are identical to those given in Figure 1.

(Table II) are not presented in the figures; they are summarized in Table III.

RT–PCR assays performed with the selected primers (Table I) and under the chosen experimental conditions (2 mM MgCl2, 65°C, 40 cycles) allowed amplification of fragments of the expected sizes (Table I) in the respective positive controls in which genomic DNA was used as template (lanes 3 and 18 in Figures 1–5). Genomic DNA was used as a positive control for the size of amplified fragments since SRIF receptor genes contain no introns in their coding regions (3). As expected, in SRIF receptor-positive samples, the sizes of the amplified products obtained with cDNA used as template was identical to the sizes of the fragments amplified from genomic DNA (compare for example the size of fragments corresponding to the sst5 receptor amplified from any sample with the size of fragments corresponding to the sst5 receptor obtained with the genomic DNA template in Figure 5). The amplification products obtained from either genomic DNA or cDNA with β-actin primers (internal standards) in co-amplification reactions (Figures 2, 3 and 5) were also identical in size, as predicted, given that the chosen upstream and downstream primers span a region within the same exon. In contrast, no amplification occurred when RT–PCR was performed in the absence of MMLV reverse transcriptase (Figures 1–5, even numbered lanes from 4 to 14 and odd numbered lanes from 19 to 29) or when the template was replaced with water (Figures 1–5, lanes 2 and 17).

SRIF receptor mRNAs were detected in all normal and tumor testicle samples studied. Normal tissues express mRNAs for more than one receptor type (Figures 3–5). In contrast, in seminomas, only expression of sst5 receptor mRNA was consistently observed (Figure 5).

Concerning individual receptor transcripts, sst1 was absent in all normal samples (corresponding to specimens N1–N12 obtained from cases 1–12 in Table II; results corresponding to the first six specimens are shown in Figure 1) whereas sst2 mRNA was absent in 11 of 12 samples. The only normal testicle sample positive for sst2 receptor expression came from case number 6 (specimen N6, Table II and Figure 2, lane 24). However, the selected sst1 and sst2 primers amplified fragments of the expected size from genomic DNA used as a positive control (Figures 1 and 2, lanes 3 and 18). Moreover, in co-amplification reactions performed with sst2 and β-actin primers, a product corresponding to the latter was systematically obtained in all tumor testicle samples studied. Normal tissues express mRNAs for more than one receptor type (Figures 3–5). In contrast, in seminomas, only expression of sst5 receptor mRNA was consistently observed (Figure 5).

Of the expected size from genomic DNA used as a positive control (Figures 1 and 2, lanes 3 and 18). Moreover, in co-amplification reactions performed with sst2 and β-actin primers, a product corresponding to the latter was systematically found (Figure 2). All normal testicle samples tested contained sst3 and sst5 mRNA. The sizes of fragments amplified from cDNA were identical to those obtained with genomic DNA (positive control) used as template; fragments corresponding to β-actin were co-amplified in all cases (Figures 3 and 5). Similarly, 11 of 12 normal testicle samples (specimens N1 and N3–N12 in Table II, of which the first six, N1–N6, are shown in Figure 4) display sst4 transcripts; the sole sst4 mRNA-negative sample (N2) corresponds to case number 2 (Table II and Figure 4, lane 7).

No amplification product was obtained with primers specific...
Table II. Clinicopathological data on testicular samples used

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Specimen</th>
<th>Histological subtype</th>
<th>Tumor size (cm)</th>
<th>Extension</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>S1</td>
<td>Seminoma with important scintiotrophoblastic contingent, absence of other invasions</td>
<td>2×1.8×2</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>N1</td>
<td>Normal</td>
<td>2×1.8×2</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>S2</td>
<td>Pure intratesticular seminoma with extension to rete testis and severe atrophy</td>
<td>5×4×3</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>N2</td>
<td>Normal</td>
<td>5×4×3</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>S3</td>
<td>Pure seminoma developed in atrophic testis</td>
<td>12×9×9</td>
<td>NA</td>
<td>IIIC</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>N3</td>
<td>Normal</td>
<td>12×9×9</td>
<td>NA</td>
<td>IIIC</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>S4</td>
<td>Pure intratesticular seminoma</td>
<td>5.4×3.8×2.5</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>N4</td>
<td>Normal</td>
<td>5.4×3.8×2.5</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>S5</td>
<td>Pure intratesticular seminoma with sparse carcinoma in situ lesions</td>
<td>1.8×2×2</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>N5</td>
<td>Normal</td>
<td>1.8×2×2</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>S6</td>
<td>Seminoma with necrosis and dystrophy</td>
<td>12×9×9</td>
<td>NA</td>
<td>IIIC</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>N6</td>
<td>Normal</td>
<td>12×9×9</td>
<td>NA</td>
<td>IIIC</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>S7</td>
<td>Seminoma in atrophic testis with intratubular neoplasia</td>
<td>NA</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>N7</td>
<td>Normal</td>
<td>NA</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
<td>S8</td>
<td>Pure intratesticular seminoma</td>
<td>3.5×2.5×3</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>16</td>
<td>25</td>
<td>N8</td>
<td>Normal</td>
<td>3.5×2.5×3</td>
<td>pT1</td>
<td>I</td>
</tr>
<tr>
<td>17</td>
<td>25</td>
<td>S9</td>
<td>Pure intratesticular seminoma</td>
<td>6×5×5</td>
<td>pT1</td>
<td>NA</td>
</tr>
<tr>
<td>18</td>
<td>25</td>
<td>N9</td>
<td>Normal</td>
<td>6×5×5</td>
<td>pT1</td>
<td>NA</td>
</tr>
<tr>
<td>19</td>
<td>25</td>
<td>S10</td>
<td>Pure intratesticular seminoma</td>
<td>5×4×3.5</td>
<td>pT1</td>
<td>NA</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>N10</td>
<td>Normal</td>
<td>5×4×3.5</td>
<td>pT1</td>
<td>NA</td>
</tr>
</tbody>
</table>

For sst1 and sst2 when seminoma cDNA was used as template (corresponding to specimens S1–S10 in Table II; results corresponding to the first six samples are shown in Figures 1 and 2). Nevertheless, the internal standard (a fragment corresponding to β-actin) for co-amplification of sst2/β-actin was visualized in all samples (Figure 2, lanes 11, 13, 15, 26, 28 and 30). All of 10 seminoma samples tested expressed sst5 receptor mRNA (Figure 5, lanes 11, 13, 15, 26, 28 and 30, corresponding to specimens S1–S6 in Table II). Indeed, a 154 bp long product was obtained in PCR assays using either genomic or cDNA as template.

The most striking difference between normal and tumoral samples was seen in relation to the expression of sst3 and sst4 receptor transcripts. They were lost in 90% of tumors, contrasting with their presence in >90% of normal samples (shown in Figures 3 and 4 for the first six specimens presented in Table II). The absence of PCR products corresponding to these two receptors is not due to inappropriate experimental conditions since fragments of the expected size were amplified from a genomic DNA (positive control) template for both sst3 and sst4 (Figures 3 and 4, lanes 3 and 18). In addition, in co-amplification reactions of sst3/β-actin all seminoma samples displayed the PCR products corresponding to β-actin (Figure 3, lanes 11, 13, 15, 26, 28 and 30). The only seminoma samples expressing either sst3 (Figure 3, lane 28) or sst4 (Figure 4, lane 11) receptor mRNAs came from two different subjects corresponding to specimens S5 and S1 obtained from cases number 5 and number 1, respectively (Table II).

The expression of different SRIF receptors is summarized in Table III.

Table III. SRIF receptor mRNA expression in normal and tumoral human testicular samples

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>Normal Positive</th>
<th>Normal Negative</th>
<th>Normal Total</th>
<th>Seminoma Positive</th>
<th>Seminoma Negative</th>
<th>Seminoma Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>sst1</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>sst2</td>
<td>1</td>
<td>11</td>
<td>12</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>sst3</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>sst4</td>
<td>11</td>
<td>1</td>
<td>12</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>sst5</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

Fig. 5. Both normal testes and seminomas express sst5 receptor mRNA. Data corresponding to the first six cases described in Table II (specimens N1–N6 and S1–S6) are given in lanes 4–9 and 19–24 (normal tissues) and lanes 10–15 and 25–30 (seminomas). All remaining samples presented in Table II (specimens N7–N12 and S7–S10) and not shown in this figure were also sst5 receptor mRNA positive. The PCR products are indicated by the arrows on the right and the size of amplified fragments is given in base pairs on the left (sst5 = 154; β-actin = 661). Legends are identical to those given in Figure 1.

Discussion

The results presented in this paper point to a selective loss of sst3 and sst4 receptor expression in testicular germ cell cancer of seminoma type. However, sst3 and sst4 transcripts are each
expressed in one of 10 seminoma samples tested. Since there was no amplification in RT–PCR reactions in which either template or MMLV reverse transcriptase was omitted (negative controls), the observed fragments corresponding to sst3 and sst4 receptors obtained with these two seminomas could not be the result of amplification of contaminating genomic DNA sequences. They are rather due to the well-documented inter-individual variability in SRIF receptor expression by tumors (2). Alternatively, these two samples might have come from two subjects in whom the cancerization process was less advanced than in the remaining nine subjects considered in our study. Indeed, the two cases (case number 5 for sst3 and case number 1 for sst4) are the only two patients in our experimental cohort who displayed a tumor stage I seminoma with a maximal tumor diameter <2 cm.

In addition, our data show sst3, sst4 and sst5 SRIF receptor mRNA expression in normal human testes. Besides, expression of SRIF receptor mRNA is variable to some extent in normal testes as well. For example, the sst2 transcript was present in one normal sample whereas it was lacking in 11 other samples tested. The opposite was observed for sst4 receptor mRNA. In contrast, expression of sst5 and absence of sst1 receptor mRNA expression appear much less polymorphous in both normal and seminoma samples.

Given the heterogeneous SRIF receptor expression in different tumor types (for a review see ref. 2), it is not possible to extrapolate the loss of sst3 and sst4 receptor mRNA expression observed here in testicular cancer to the general pathogenetic mechanisms of carcinogenesis. Nevertheless, at this point it is tempting to speculate on an emerging common tendency concerning these receptors. Thus, as in seminomas, in all tumors in which the expression pattern of SRIF receptors has been estimated, the sst3 receptor was rarely expressed or absent. Indeed, small cell lung carcinoma (22), pancreatic, colorectal (8) and prostate (9) cancers as well as pituitary adenoma (4) lack this receptor type. In addition, in accordance with our data, the sst4 transcript was not found in pituitary adenoma (4) and medullary thyroid carcinoma (7). However, it seems clear that, if relevant, such an absence or loss of sst3 and sst4 receptor expression cannot be considered as the sole mechanism involved in carcinogenesis. For example, in pancreatic carcinomas which lack the sst3 SRIF receptor, carcinogenesis was formally associated with loss of sst2 receptor expression. In an elegant study, Delesque and colleagues demonstrated that re-expression of the sst2 receptor reverses tumorigenicity (10). Further studies on additional tumor types are now needed in order to extend these findings.

The physiological significance of the presence of SRIF receptors in normal human testis as well as the pathological implications of their observed loss in seminoma remains to be elucidated. However, it is of interest to note the observations made in a previous work in which SRIF analog (SMS201995) injection was used as a tool to measure growth hormone secretion recovery in healthy adult males during physical exercise. This study reported an unexpected, rapid (2 h after SMS201995 injection) rise in serum testosterone level in both rest and exercise trials. Such an increase in testosterone secretion occurred without a simultaneous increase in LH secretion, prompting the authors to suggest that SRIF can modulate testosterone secretion at the testicular level (23). Our data concerning sst3 and sst5 SRIF receptor expression in normal human testes are compatible with the actions of SMS201995 in vivo. Indeed, this analog acts on human sst2, sst3 and sst5 receptors (24). Similar conclusions on SRIF-mediated regulation of testosterone secretion have also been documented in animal models (25). The presence of SRIF and its receptors in human (17; present study) and animal (26,27) testes therefore supports the existence of auto/paracrine loops controlling local testosterone secretion. In the light of this and in order to advance our understanding of SRIF testicular functions, a systematic evaluation of male gonadal parameters as part of a routine survey of patients suffering from neuroendocrine tumors and undergoing long-term treatment with stable SRIF analogs might produce precious information.

Otherwise, concerning direct SRIF effects on germ cell proliferation, our data on the loss of SRIF receptor expression in seminomas (characterized by uncontrolled, high rate germ cell proliferation) are compatible with the proposed anti-proliferative actions of this peptide. However, the possible negative regulation of germ cell proliferation by SRIF, such as has been documented in other cell types (3), remains to be demonstrated explicitly in human and animal testicular models. The establishment of germ cell lines as paradigms to study physio-pathological actions of SRIF in mammalian testes will probably help our understanding of underlying mechanisms.

In conclusion, our data indicate that a routine RT–PCR assessment of SRIF receptor expression in testicular biopsies may turn out to have important diagnostic and therapeutic implications. However, further studies at the international level are now required in order to confirm on a larger scale the data reported here on a relatively modest tumor cohort issuing from a low (but increasing) frequency of seminoma. If confirmed by other studies, our data would suggest that a comparison of SRIF receptor expression patterns in tumoral and surrounding normal testicular tissues revealing a loss of particular receptor types might help to establish seminoma diagnosis. Knowledge of receptor expression patterns could also be used to develop clinical trials for testing synthetic SRIF analogs in germ cell tumor therapies. Our results show that seminomas express the sst5 receptor, which can be targeted with high affinity by stable SRIF agonists (e.g. SMS201995). This analogy is already used in the clinical treatment of a number of tumors (for a review see ref. 2). It is now worthwhile to compare the potential therapeutic benefit of SRIF analogs with those provided by classical radio- and chemotherapies of seminomas.

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