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Immunohistochemical detection and localization of somatostatin receptor subtypes in prostate tissue from patients with bladder outlet obstruction

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Abstract. *Background and aim of the study*: Scant information on the cellular distribution of the five somatostatin receptor (SSTR) subtypes in the normal prostate and in neoplasms of the prostate has been reported in very few studies in which techniques, such as *in situ* hybridization histochemistry, autoradiography, and more recently immunohistochemistry, have been applied. The aim of the study was to examine immunohistochemically the distribution and localization of these 5 subtypes in the various tissue components in normal prostate.

Materials: The study was conducted in 14 surgical specimens of normal prostate tissue from adenomectomy specimens from patients with bladder outlet obstruction. The distribution and localization of the 5 somatostatin receptor (SSTR) subtypes was investigated with an immunohistochemical technique. Specificity of the antibodies against the 5 receptor subtypes was preliminarily investigated.

Results: Close to 90% of secretory cells showed a weak positivity in the cytoplasm, the proportion ranging from 86.3% (SSTR4) to 89.9% (SSTR5). Strong immunoreactivity was seen in a small proportion of cells, ranging from 0.8% (SSTR3) to 3.2% (SSTR1). For the subtypes 1 and 3 the greatest proportion of basal cells showed a moderate intensity (42.5 and 41.4%, respectively), strong immunoreactivity being observed only in 18.1 and 15.8% of cells, respectively. For the subtypes 2, 4 and 5, the majority of cells showed a weak intensity (72.3, 65.7 and 65.1%, respectively). Subtype 1 showed a strong immunoreactivity in the cytoplasm in 60% of the smooth muscle cells. With subtypes 2, 3 and 4 the greatest proportion of cells showed a weak intensity (63.4, 89.8 and 81.7%, respectively). With the subtype 5 the majority of cells (59.8%) were negative. Subtype 1 showed a strong immunoreactivity in the cytoplasm in 98.6% of the endothelial cells. With subtypes 3 and 4 the greatest proportion of cells showed a weak intensity (73.5 and 56.4%, respectively). With the subtype 2 and 5 the majority of cells were negative (59.1 and 50.7%, respectively).

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Conclusions: Our immunohistochemical study on the SSTRs expands our knowledge in the distribution of these subtypes in the various tissue components in the prostate. Such an information may prove useful in developing further non-surgical strategies for the prevention and treatment of benign prostatic hyperplasia and, in particular, of preneoplastic and neoplastic lesions of the prostate.

Keywords: Somatostatin receptors, normal prostate tissue, benign prostatic hyperplasia

1. Introduction

Somatostatin (SST) is a 14- or 28-amino acid peptide that was originally discovered in 1973 as a hypothalamic neuroendocrine hormone [1]. This peptide was first considered to inhibit the secretion of growth hormone from the anterior pituitary gland, but the presence of this peptide hormone was subsequently detected throughout the body, where it inhibits a number of physiological processes through suppression of various peptide hormones, such as pancreatic secretion, neurotransmission in the central nervous system, and gut motility [2].

The actions of somatostatin are mediated by specific somatostatin receptors (SSTR), i.e., SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5. These receptors belong to a family of transmembrane domain G-protein-coupled receptors encoded on five different chromosomes. These receptor subtypes all share common signal pathways such as the inhibition of adenylylcyclase [3], activation of phosphotyrosine phosphatase and others, but some subtypes are also associated with modulation of inward rectifying K^+ channels (SSTR2, 3, 4, 5), to voltage-dependent Ca^{2+} channels (SSTR1, 2), and a Na^+/H^+ exchanger (SSTR1) [3]. The presence of these SSTR subtypes has been demonstrated throughout the body, including the central nervous system [4, 5], gastrointestinal tract [6,7], pancreas [8–10], kidney [11], prostate [12,13] and others [14–17].

Cloning of five SSTR subtypes has led to the development of subtype-selective agonists. Among those, SSTR2-specific SST analogs octreotide (OCT) and lantreotide have attracted significant attention in the past several years. They have been used as new diagnostic and treatment modalities for various endocrine disorders and as adjunctive treatment for a variety of benign and malignant tumors [18–25]. The antiproliferative and antiangiogenic properties of OCT have been exploited in several clinical trials [26,27].

It is very important to determine which subtypes of the SSTRs exert their effects on which cells of the human body. It is nearly impossible to obtain precise data on the distribution and location of SSTR subtypes when employing molecular methods, such as Northern blot, reverse-transcriptase polymerase chain reaction (RT-PCR), and real-time PCR which treat the tissue as a mass [12,28–33].

To date scant information on the cellular distribution of these receptor subtypes in the normal prostate and in neoplasms of the prostate has been reported in very few studies in which techniques, such as *in situ* hybridization histochemistry, autoradiography, and more recently immunohistochemistry, have been applied [12, 13,29,30,34].

The aim of this study was to examine immunohistochemically the distribution and localization of the five SSTR subtypes in prostate tissue from adenomectomy specimens from patients with bladder outlet obstruction.

2. Materials and methods

2.1. Research protocol

Patients candidate for open surgery adenomectomy for bladder outlet obstruction due to benign prostatic hyperplasia were eligible for the study. The criteria for inclusion were: stage III benign prostatic hyperplasia requiring surgery, total PSA less than 10 ng/ml and free/total PSA ratio > 0.20, digital rectal examination negative, trans-rectal ultrasound negative for prostate areas suspicious for cancer. Additional criteria for inclusion were: absence of upper urinary tract pathology; absence of intractable urinary infection; normal renal and liver function; absence of tumors other than cutaneous basal cell carcinoma; hepatitis A, B and C markers negative; HIV serology negative; and absence of previous hormonal treatments, including finasteride and dutasteride. The research protocol was approved by the Ethics Committee of Institution of the individual authors. This study was designed as the initial step in a larger investigation on tissue distribution and localization of the 5 somatostatin receptor subtypes in preneoplastic and neoplastic lesions of the prostate, with the aim of developing tissue targets for non-surgical treatment strategies.

2.2. Human tissues

The study was conducted in the normal tissue from 14 cases of adenomectomy specimens from patients with bladder outlet obstruction due to benign prostatic hyperplasia. A signed consent was obtained from each patient. The surgical intervention was made in the urology service of the urologists authors of this paper. The specimens were fixed in 10% buffered formalin for 24–48 h. Pathology evaluation of all cases was done at the Section of Pathological Anatomy of the United Hospitals and Polytechnic University of the Marche Region, Ancona, Italy. Even though a complete sampling procedure was not applied, a generous sampling of each specimen was done. The material was then embedded in paraffin and tissue section of 5 microns of thickness were stained with hematoxylin and eosin. There were no cases with prostate cancer or high grade prostatic intraepithelial neoplasia. Acute and chronic inflammation was seen focally in 4 cases.

2.3. Antibody against somatostatin receptor subtypes

For immunohistochemistry, all rabbit polyclonal anti-SSTR subtype antibodies were commercially obtained from Chemicon® International Inc. (Temecula, CA, USA). Dilution of the antibodies used is shown in Table 1. Positive control experiments included normal human pancreas and/or anterior pituitary gland obtained from surgery and autopsy, respectively. Negative controls were used for the tested antibodies; the primary antibody was replaced by rabbit non-immune serum.

2.4. Immunohistochemistry

Ten percent formalin-fixed, paraffin-embedded tissue blocks were serially cut into 5 μm thick sections which were mounted on silane-coated slides. The sections were then dewaxed in xylene and rehydrated through a graded series of ethanol. Antigen retrieval was done by microwave treatment for 20 min at 98°C using 0.01 M citric acid buffer pH 6.0. Endogenous peroxidase activity was quenched by incubating the sections in 3% hydrogen peroxide for 10 min at room temperature. Non-specific binding sites were blocked through pre-incubation with 1% albumin bovine in PBS for 20 min at room temperature. Reacted tissue sections were then incubated with the antibodies for each SSTR subtype for 18 h at 4°C. Antigen-antibody complex was subsequently visualized using the Envision™ Detection System kit peroxidase/DAB (DAKO, Glustrop, Denmark) and counterstained with hematoxylin.

2.5. Evaluation of immunohistochemistry

At least 4,000 cells were counted by one of us (DM) in contiguous 400× microscopic fields in each case, separately for benign secretory epithelium, basal cells, smooth muscle cells of the stroma and endothelial cells. Immunostaining was evaluated for the following three cell components: cytoplasm, membrane and nucleus. Staining intensity was classified as negative, weak (+), moderate (++) or strong (+++). In each case the percentage of cells with negative, weak, moderate or strong immunoreactivity was calculated and reported in this study. For each groups the mean and standard deviation were then evaluated.

2.6. Chromogranin A immunostaining

To determine the co-localization of SSTR subtypes and chromogranin A, immunostaining for chromogranin A on serial tissue sections was done as previously described [35].

Table 1
Characteristics of the polyclonal antibodies used in immunohistochemistry

Antibody	Type	Immunogen* i.e., synthetic peptide from:	Dilution	Source
SSTR1	Rabbit	C-terminal domain of human SSTR1	1/300	Chemicon International Inc., USA
SSTR2	Rabbit	2nd extracellular domain of human SSTR2	1/100	Chemicon International Inc., USA
SSTR3	Rabbit	C-terminal domain of human SSTR3	1/300	Chemicon International Inc., USA
SSTR4	Rabbit	N-terminal extracellular domain of human SSTR4	1/400	Chemicon International Inc., USA
SSTR5	Rabbit	C-terminal domain of human SSTR5	1/200	Chemicon International Inc., USA
"Visualization System"	ENVISION™	–	Ready-to-use	Dako, Denmark

*From manufacturer's data sheet.

2.7. Assessment of antibody specificity

In order to evaluate the specificity of the five rabbit polyclonal anti-SSTR antibodies employed in this study, we performed western blot (WB) experiments on a prostate tissue extract. Briefly, a prostate tissue sample was homogenized at 10% (wt/vol) in Tris-buffered saline (TBS; 0.05 M Tris/0.2 M NaCl, pH 7.4) containing 1% Nonidet P-40 and 1% sodium deoxycholate (DOC). Homogenate was clarified at 500*g* for 15 min at 4°C. Protein concentration in the supernatant was determined by Bio-Rad protein assay. Six individual 100 μg aliquots of prostate homogenate were mixed with Laemmli buffer, heated to 100°C for 10 min, then run on a 10% SDS/PAGE gel and transferred onto a nitrocellulose membrane. The membrane was blocked with 5% (wt/vol) nonfat dry milk in TBS containing 0.1% Tween 20 (TBST) for 1 h at room temperature, then cut in six vertical parts separated by color molecular markers. Each individual membrane was then independently probed overnight at 4°C with one of the five anti-SSTR reagents at a concentration of 2.5 μg/ml in TBS; the sixth membrane was incubated with non immune rabbit IgG (Santa Cruz Biotechnology, Heidelberg, Germany) at the same concentration. After five washes in TBST, membranes were incubated for 45 min at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, Heidelberg, Germany) diluted 1:5,000 in TBS. Membranes were then washed five times in TBST and visualized with enhanced chemiluminescence reagent (Pierce) by Chemi-Doc (Bio-Rad Laboratories, Hercules, CA, USA) analysis system.

3. Results

Table 2 summarizes the clinical features of the 14 patients.

Table 2
Preoperative patients' characteristics

	Mean	SD°	Range
Age of the patients	67.0	4.8	61–76
Prostate volume (ml)	102	35.6	55–162
Total serum PSA (ng/ml)	4.8	2.10	1.5–8.4
Serum testosterone (ng/ml)§	4.3	0.93	3.4–6.0
Serum chromogranin A (ng/ml)*	45.9	6.1	35–59

°SD, standard deviation, §normal value $>$ 2.7 ng/ml, *normal value 19–95 ng/ml.

3.1. Secretory cells of the ducts and acini

None of the cases was negative. Immunoreactivity was primarily detected in the cytoplasm, in the perinuclear region and cell membrane in all SSTR subtypes. In all the cases and for all the receptor subtypes, close to 90% of cells showed a weak positivity in the cytoplasm, the proportion ranging from 86.3% (SSTR4) to 89.9% (SSTR5). Strong immunoreactivity was seen in a small proportion of cells, ranging from 0.8% (SSTR3) to 3.2% (SSTR1). A distinct positivity in the cell membrane was seen only with the subtypes 3 and 4. For the former subtype 65.4% of cells showed a moderate intensity. It was 23.5% for the latter. Nuclear staining was seen with subtypes 4 and 5. It was always weak, and seen in 26% of the former and 58.8% of the latter. The results are summarized in Table 3.

3.2. Basal cells of the ducts and acini

Immunoreactivity was primarily detected in the cytoplasm in all SSTR subtypes. For the subtypes 1 and 3 the greatest proportion of cells showed a moderate intensity (42.5 and 41.4%, respectively), strong immunoreactivity being observed only in 18.1 and 15.8% of cells, respectively. For the subtypes 2, 4 and 5, the majority of cells showed a weak intensity (72.3, 65.7 and 65.1%, respectively), whereas a strong immunoreactivity was present in a small proportion of cells, ranging from 0.9% (SSTR2) to 3.2% (SSTR4). The results are summarized in Table 4.

Figure 1A–D is an example of weak, moderate and strong immunoreactivity in the basal and secretory cells. A distinct positivity in the cell membrane is seen in Fig. 1D.

Figure 1E shows a few scattered cells with strong immunoreactivity for SSTR1. On serial sections, cells in the same location are chromogranin A positive. There was no co-localization between strongly stained cells for the other four SSTRs and chromogranin A.

3.3. Smooth muscle cells

Subtype 1 showed a strong immunoreactivity in the cytoplasm in 60% of the cells (Fig. 1F). With subtypes 2, 3 and 4 the greatest proportion of cells showed a weak intensity (63.4, 89.8 and 81.7%, respectively). With the subtype 5 the majority of cells (59.8%) were negative. There were no cases with a distinct positivity in the cell membrane. Nuclear staining was seen only

Table 3
Secretory cells of the ducts and acini (mean and standard deviation of cells for all cases)

	Intensity of staining			
	Negative	Positive +	Positive ++	Positive +++
SSTR1				
C	0	89.8 ± 5.6	7 ± 4.9	3.2 ± 2.3
M	100	0	0	0
N	100	0	0	0
SSTR2				
C	0.5 ± 0.3	88.2 ± 2.3	9.7 ± 2.6	1.5 ± 0.6
M	100	0	0	0
N	100	0	0	0
SSTR3				
C	0.2 ± 0.3	88.9 ± 2.3	9.9 ± 2.3	0.8 ± 0.6
M	14.3 ± 10	21.3 ± 15	65.4 ± 17	0
N	100	0	0	0
SSTR4				
C	0.4 ± 0.8	86.3 ± 4.5	12.3 ± 4.6	1 ± 0.5
M	55 ± 6.4	21.7 ± 7	23.5 ± 8.6	0
N	74.1 ± 18.2	26 ± 19	0	0
SSTR5				
C	0	89.9 ± 4	9.1 ± 4	0.9 ± 0.7
M	100	0	0	0
N	41.9 ± 16.6	58.8 ± 17	0	0

C: cytoplasm, M: cell membrane, N: nucleus.

Table 4
Basal cells of the ducts and acini (mean and standard deviation of cells for all cases)

Intensity of staining in the cytoplasm	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Negative	4.7 ± 5	15.7 ± 8	3.1 ± 3.1	5.7 ± 2.7	9.5 ± 7.5
Positive +	35 ± 30.2	72.3 ± 21	39.4 ± 34	65.7 ± 23.4	65.1 ± 21.8
Positive ++	42.5 ± 27.5	11 ± 21	41.4 ± 30.2	25.4 ± 25.7	23.9 ± 23
Positive +++	18.1 ± 15.8	0.9 ± 2.2	15.8 ± 9	3.2 ± 2.7	1.4 ± 2.1

with subtypes 4 and 5, it was always weak and it was observed in a minority of cells (27.1 and 45.2%, respectively). The results are summarized in Table 5.

3.4. Endothelial cells

Subtype 1 showed a strong immunoreactivity in the cytoplasm in 98.6% of the cells (Fig. 1F). With subtypes 3 and 4 the greatest proportion of cells showed a weak intensity (73.5 and 56.4%, respectively). With the subtype 2 and 5 the majority of cells were negative (59.1 and 50.7%, respectively). There were no cases with a distinct positivity in the cell membrane. Nuclear staining was not seen. The results are summarized in Table 6.

3.5. Assessment of antibody specificity

WB analysis of prostate tissue performed with the panel of five polyclonal anti-SSTR antibodies yielded single bands as previously described by Helboe et al. [36].

4. Discussion

Most of the previous studies were based on molecular methods, such as Northern blot, reverse-transcriptase polymerase chain reaction, and real-time PCR, which treat the tissue as a mass [12,28–33]. This means that the type of information produced does not allow the derive data on SSTR cellular location.

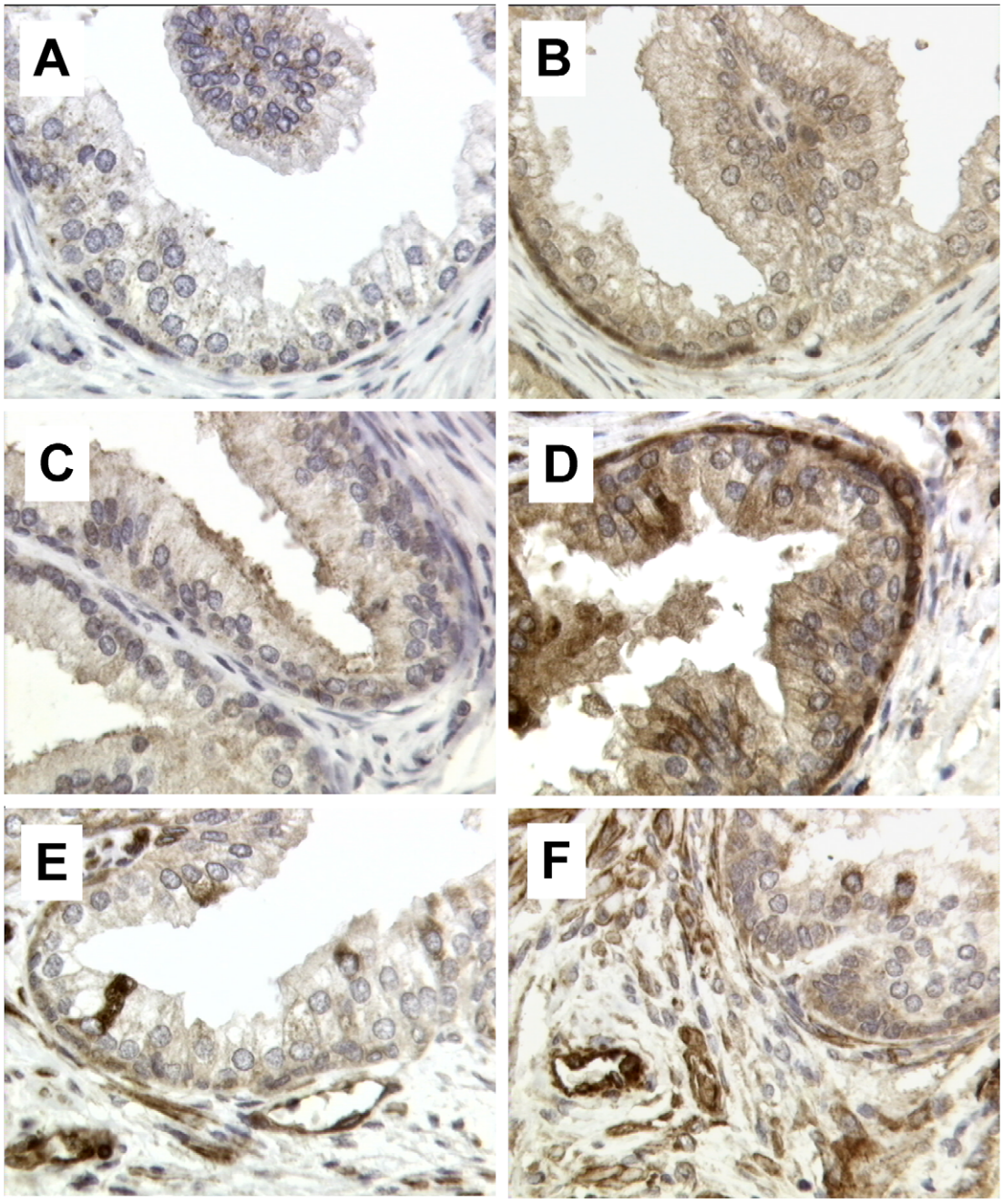

Fig. 1. (A) Weak immunoreactivity for SSTR2 in the basal cells; (B) moderate immunoreactivity for SSTR3 in the basal cells; (C) moderate immunoreactivity for SSTR5 in the secretory cells; (D) strong immunoreactivity for SSTR4 in the secretory and basal cells. A distinct positivity in the cell membrane is seen; (E) a few scattered neuroendocrine cells with strong immunoreactivity for SSTR1 (on serial sections cells in the same location are chromogranin A positive); (F) strong immunoreactivity for SSTR1 in the stromal and endothelial cells.

Molecular techniques such as *in situ* hybridization histochemistry and autoradiography have been used in a limited number of studies [12,13,29,30,37]. The former basically investigates SSTR mRNA expression in cryostat sections. The latter also utilizes cryostat sections and is based on radioligands, i.e., ^{125}I-labeled somatostatin ligands, such as octreotide. The studies often dealt with only some of the subtypes, therefore

Table 5
Smooth muscle cells (mean and standard deviation of cells for all cases)

Intensity of staining in the cytoplasm	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Negative	0	0	5.6 ± 7.6	8.7 ± 9	59.8 ± 39
Positive +	6.3 ± 8.2	63.4 ± 39	89.8 ± 10	81.7 ± 9	38.9 ± 37
Positive ++	33 ± 34	35.7 ± 38	4.6 ± 8.6	10 ± 12	1.3 ± 3
Positive +++	60 ± 40	0.9 ± 2.7	0	0	0

Table 6
Endothelial cells (mean and standard deviation of cells for all cases)

Intensity of staining in the cytoplasm	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
Negative	0	59.1 ± 42	16.1 ± 29	35.7 ± 39	50.7 ± 44
Positive +	0	23.6 ± 30	73.5 ± 34	56.4 ± 40	47.1 ± 42
Positive ++	1.4 ± 3.6	17.3 ± 35	4.6 ± 8.1	7.8 ± 26	2.1 ± 4.6
Positive +++	98.6 ± 3.6	0	5.7 ± 21	0	0

Table 7
Studies on the expression and localization of the SSTRs in human prostate tissue*

Authors	Year	Technique	Receptors	Localization	Type of tissue
J.C. Reubi et al.	1995	Autoradiography, *in situ* hybridization	SSTR1 SSTR2 SSTR3	Smooth muscle, endothelium	Benign prostatic hyperplasia and prostate cancer
G. Halmos et al.	2000	RT-PCR	SSTR1 SSTR2 SSTR5	Epithelial cells	Prostate cancer
N. Dizeyi et al.	2002	IHC, RT-PCR, Western blot	SSTR1 SSTR2 SSTR3 SSTR4 SSTR5	Epithelial cells, stromal cells, endothelium	Normal, benign prostatic hyperplasia, prostate cancer
J. Hansson et al.	2002	*In situ* hybridization	SSTR2 SSTR4	Epithelial cells, stromal cells	Benign prostatic hyperplasia, HGPIN, prostate cancer
M. Valente et al.	2007	IHC	SSTR2A SSTR3 SSTR5	Epithelial cells	Cancer**
R. Montironi et al.	2008 (present study)	IHC	SSTR1 SSTR2 SSTR3 SSTR4 SSTR5	Secretory cells, basal cells, smooth muscle stromal cells, endothelial cells	Benign prostatic hyperplasia

*See text for abbreviation. **3 cases of adenocarcinomas with neuroendocrine differentiation of the breast and prostate.

information being only partial. For instance, Reubi et al. investigated SSTRl, SSTR2, and SSTR3 only in [33] (Table 7). The type of information that these two techniques can give is not always comparable to that obtained with immunohistochemical analysis in formalin-fixed and paraffin-embedded tissue in which the architecture and the cytology in the background is well preserved. In addition, the immunohistochemical technique is much easier to apply than *in situ* hybridization histochemistry and autoradiography.

When considering localization in our immunohistochemical investigation, although SSTRs are membrane-associated receptors, a significant amount of staining was seen within the cytoplasm, in the peri-

nuclear region and some nuclear staining in many immunoreactive cells. The interpretation, based on molecular studies suggesting nuclear accumulation of SST analogs mediated by SSTRs [20,37,38], is that, after binding their ligand, SSTR-ligand complexes undergo cellular internalization with progressive intracytoplasmic and intranuclear translocation. This hypothesis is not supported by the studies made by Le Romancer et al. [39]. This group suggested that the nuclear translocation of SST analogs is mediated by p86-Ku and not by SSTRs.

The present immunohistochemistry-based study demonstrated at the microscopic level the degree of expression and localization of the five SSTR subtypes in the normal prostate tissue obtained from patients with clinical diagnosis of benign prostatic hyperplasia and no clinical evidence of prostate cancer. In particular, the five subtypes were seen in the secretory and basal cells lining the ducts and acini as well as in the stromal and endothelial cells.

Dizeyi et al. [29] published a recent study on immunohistochemical expression and localization of the five SSTR subtypes in normal and pathological prostate tissue obtained from radical prostatectomy specimens. They demonstrated the presence of SSTR1 and SSTR3 in tumoral and non-tumoral epithelial cells as well as in the stromal compartment, whereas SSTR4 was found to be confined to epithelial cells, and SSTR5 was not detectable. In particular, they showed the presence of SSTR1 in a subpopulation of cancerous and neurondocrine cells, whereas SSTR2 was found in the stroma, peritumoral blood vessels and tumor cells. Receptor subtype 3 was demonstrated to be present on the cell membrane of BPH and malignant areas. A strong immunoreaction of SSTR4 was found in tumor cells, as compared with a less intense immunoreaction in adjacent BPH areas. SSTR subtype 5 was not detectable.

There are similarities between our investigation and that by Dizeyi et al. [29]. In particular, in both studies it was shown that there was no co-localization of NE cells (detected with an antibody against chromogranin A) with SSTRs, except for SSTR1.

There are differences in results between our study and that Dizeyi et al. [29] probably due to the types of antibodies used (in Dizeyi's study the antibodies were a private source and not commercially available) as well as to the fixation quality and time of the specimens. Our study also differs in that we collected information on the differential expression of the five receptor subtypes in the secretory cells but also in the basal cells.

The current study showed that the five SSTR subtypes are also expressed in the smooth muscle cells of the prostatic stroma. Subtype 1 was the most intensely expressed receptor and its location was in the cytoplasm. Subtypes 2, 3 and 4 were also seen in the cytoplasm with their expression always weak. Close to 60% of the cells were negative for subtype 5. Our findings are similar to those obtained by Reubi et al. [33] in their molecular study of SSTRl, SSTR2 and SSTR3. A possible role of the SSTRs in the smooth muscle could be to influence the release of various growth factors (i.e., nerve growth factor) known to be synthesized in the stroma. As several of these growth factors act in a paracrine manner on the glandular part of the prostate to regulate prostate growth [29], somatostatin could indirectly regulate biological events in the prostatic gland through a stromal action. Another possible role would be a regulatory mechanism dependent on the SSTR action on the prostate mediated by the pituitary.

The current study also showed the presence of the five SSTRs in the endothelial cells. In particular, subtype 1 showed a strong immunoreactivity in the cytoplasm in 98.6% of this type of cells. This type of information confirms that provided previously by Curtis et al. [40] and Badway et al. [41] who independently demonstrated SSTR1 and SSTR2 expression in endothelial cells. The presence of the SSTR subtypes in endothelial cells in prostate tissue was also reported by Dizeyi et al. [29]. This group mentioned that peritumoral vessels were intensely stained by the SSTR2 antiserum. In their molecular study in prostate cancer, Reubi et al. [13,42] observed that many peritumoral vessels, in particular veins, contain a high density of SSTRs. SSTR-positive veins were also occasionally identified in samples of prostate tissue distant from the prostate tumour as well as in the prostate of the patient with bladder cancer. Precise actions of somatostatin in endothelial cells have not yet been clarified, but somatostatin may be involved in regulating endothelial cell homeostasis and anti-inflammatory effects [43].

An interesting study on the immunohistochemical expression of somatostatin receptor subtypes 2A in a series of 107 neuroendocrine tumors from different organs was published by Dr. Valente and his coworkers [34] after the completion of our present investigation. Our study and that by Dr. Valente et al. are not fully comparable because we investigated epithelial and non-epithelial components of the transition zone of the prostate from patients with bladder outlet obstruction, whereas the other group dealt with

the epithelial component of neuroendocrine tumours, mostly not from the prostate. That study included only 3 cases of adenocarcinomas with neuroendocrine differentiation of the breast and prostate. However, there are differences in the epithelial localization between our study and that study, being cytoplasmic in our and membranous and cytoplasmic in the other. The differences in tissue localization of this receptor subtype could be related to differences in the type of tissue and the type of antibody used in the two studies. Dr. Valente et al. made a proposal of a scoring system that correlated with somatostatin receptor scintigraphy. It was behind the scope of our small study to propose a scoring system.

The main limitation of our study is that immunohistochemistry was not associated with a molecular investigation. This type of study was done to some extent by others, including Hansson et al. [12] in human BPH and prostatic cancer, and Klisovic et al. [20] in human ocular tissue. The former group investigated SSTR2 and SSTR4 only. The latter investigated SSTR1 and SSTR2 in particular. Both groups found good correlation between immunohistochemical distribution of SSTRs with their gene expression.

5. Conclusions

The current study showed that the expression and location of five SSTR subtypes can be determined in tissue section of normal prostate tissue. Our findings confirm the type of information obtained previously by others when molecular techniques were mostly used and at the same time expands our knowledge in the location of these subtypes in the various tissue components of the prostate. Such an information may prove useful in developing further non-surgical strategies for the prevention and treatment of benign prostatic hyperplasia and, in particular, of preneoplastic and neoplastic lesions of the prostate.

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