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Research paper

Somatostatin receptor expression in non-medullary thyroid carcinomas

Kalliopi Pazaitou-Panayiotou[1], Eva Tiensuu Janson[2], Triantafyllia Koletsa[3], Vassiliki Kotoula[3], Mats Stridsberg[4], Georgios Karkavelas[3], Georgia Karayannopoulou[3]

[1]Department of Endocrinology - Endocrine Oncology, Theagenio Cancer Hospital, Thessaloniki, Greece, [2]Department of Medical Sciences, Endocrine Oncology, Uppsala University, Uppsala, Sweden, [3]Department of Pathology, Medical School, Aristotle University of Thessaloniki, Greece, [4]Department of Medical Sciences, Clinical Chemistry, Uppsala University, Uppsala, Sweden

ABSTRACT

OBJECTIVE: Peptide receptor radionuclide therapy (PRRT) is dependent upon binding of radiolabelled peptides to their respective receptor expressing cells. The main objective of this study was to characterize the expression of somatostatin receptor (SSTR) subtypes in non-medullary thyroid cancers in order to be able to recommend the use of PRRT as a treatment option in patients with progressive local or metastatic disease. DESIGN: We constructed tissue microarrays from paraffin blocks prepared from 47 cases of non-medullary thyroid carcinomas and related normal thyroid tissue. Immunohistochemical staining was performed with five different polyclonal SSTR antibodies. RESULTS: SSTR subtypes sst_2 and sst_3 were expressed in all non-medullary thyroid carcinomas, sst_1 and sst_5 in 75%, and sst_4 in 38%. Coexpression of more than three subtypes was detected in 36 of the 47 cases. The expression of SSTR subtypes in normal thyroid tissue was low or absent. CONCLUSIONS: Non-medullary thyroid carcinomas frequently express all SSTR subtypes. This expression provides a basis for further studies with the aim of exploring PRRT as a possible new treatment for iodine-131 refractory metastatic non-medullary thyroid carcinomas.

Key words: Thyroid carcinoma, Somatostatin receptor, Tissue microarray, Immunohistochemistry

INTRODUCTION

Thyroid carcinoma is the most frequent type of endocrine cancer. The prognosis for follicular cell-derived differentiated thyroid carcinoma (DTC) (papillary/follicular thyroid cancer) is generally good.[1] Conventional therapy involves thyroidectomy, thyrotropin hormone suppressive therapy with thyroxin, and iodine-131 administration.[2] Approximately 20% of patients with DTC do not, however, undergo remission but experience progressive local or metastatic disease,[3] and for some of these patients additional radioiodine therapy is ineffective because of the

Address for correspondence:
Georgia Karayannopoulou, MD, Department of Pathology, Medical School, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece,
FAX: +30 2310 999229, e-mail: karayan@med.auth.gr
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loss of their capacity for radioiodine uptake by the dedifferentiated tumour cells.[3,4] New therapeutic approaches are needed for these patients.

Somatostatin receptor scintigraphy (SRS) has become an efficient method for detection of neuroendocrine tumours and radiolabelled somatostatin analogues are also used for tumour targeting therapy.[5] SRS depends upon binding of labelled somatostatin analogues to somatostatin receptor (SSTR) expressing cells and has in a number of studies been used to image DTC.[6] Most studies have been performed with the somatostatin analogue ^{111}In-octreotide, and the results have in most cases been favourable with a sensitivity from 50-100%,[6] although there are reports of much lower SRS positivity.[7,8] The observed differences in uptake during SRS probably depends on the protocol used, the anatomical localization of the tumours, and differences in the expression of the SSTR subtypes (sst_1-sst_5), in particular the sst_2 subtype.[9]

By virtue of its superior diagnostic accuracy as compared to SRS, Gallium-68 somatostatin receptor positron emission tomography/computed tomography (Ga-68 SR-PET/CT) could replace SRS to evaluate the expression of somatostatin receptors in endocrine tumours, Ga-68 SR-PET/CT being one of the newly emerging methods holding potential for the near future. Recently, ^{68}Ga-DOTANOC has been increasingly used in several centres and it was reported to have a more favourable dosimetry and to be more accurate for NET lesion detection than conventional imaging or SRS.[10,11] ^{68}Ga-DOTATOC binds to sst_2 with high affinity and to sst_5 with reasonable affinity, while ^{68}Ga-DOTA-NOC has high affinity to sst_2, sst_3, and sst_5.[10]

There are several reports on the expression of SSTRs in thyroid tumours,[12,13] most of which have employed mRNA analysis by e.g. RT-PCR. Klagge and collaborators[12] investigated the mRNA expression of $sst_{1\text{-}5}$ in benign and malignant epithelial thyroid tumours and detected a predominant expression of sst_2 and sst_5, a weak expression of sst_1 and sst_3, and expression of sst_4 in only a few papillary thyroid carcinomas.

Immunohistochemistry (IHC), which in contrast to RT-PCR can reveal the cellular and subcellular pattern of expression, has been used in only a few instances to investigate SSTR expression in thyroid cancers. Sancak and coworkers[14] have determined the expression of sst_2, and Papotti[15] analyzed the localization of all subtypes in medullary carcinomas and found a rich but heterogenous expression of all subtypes with the exception of sst_4.

We have characterized the expression of SSTR subtypes in non-medullary thyroid carcinomas aiming to provide a basis for future development of imaging and therapy with somatostatin analogues for those patients with thyroid cancer who fail to respond to treatment by conventional therapies.

MATERIALS AND METHODS

Tissue samples

Paraffin block samples from 47 patients (15 males, 32 females) with thyroid carcinomas were selected blindly from archive material at the Pathology Department, Medical School, Aristotle University of Thessaloniki (AUTH), Greece. The samples included cases with known follicular cell thyroid cancer. Patients with medullary thyroid carcinoma were excluded from the present study. Thirty-three of the patients had a history of total thyroidectomy and 14 had a total thyroidectomy and cervical lymph node dissection.

Clinical and pathological data

The samples encompassed 38 papillary thyroid carcinomas (25 classical type, 13 follicular variant), four follicular, two anaplastic and three Hürthle cell thyroid carcinomas. The mean age of the patients was 49 years (range 8-85) and the male/female ratio was approximately 1:2. Eleven of the tumours measured ≤1cm at their greatest diameter, and the remaining 36 measured >1cm. Fifteen out of 47 patients presented lymph node metastases (six men and nine women) and twelve showed invasion of the thyroid parenchyma or extrathyroid soft tissue invasion. The 46 samples from the non-neoplastic thyroid tissue, obtained from areas adjacent to the carcinomas, consisted of 39 nodular goiters, six Hashimoto / lymphocytic thyroiditis, and two normal thyroid tissue.

Tissue microarray (TMA) construction

Hematoxylin and eosin-stained sections from the 47 specimens were examined and areas with adequate

material of neoplasms were marked. TMA construction was performed with a tissue arraying instrument (Beecher Instruments, Silver Springs, MD) at the Department of Pathology of the AUTH. Tissue cylinders with a diameter of 0.6 mm were punched from the marked areas of each "donor" paraffin block and transferred into the recipient block. Each case was represented by 7 cores from the thyroid carcinoma (TC) and 5 cores from non-neoplastic thyroid tissue (NNTT) adjacent to the carcinoma. At the two edges of the recipient block, 8 cores from skin tissue and 5 cores from intestinal carcinoma were inserted for orientation of the block. The TMA was subsequently cut into three μm sections.

Immunohistochemistry (IHC)

The immunohistochemical staining was performed at the Department of Endocrine Oncology, Uppsala University Hospital, Uppsala, Sweden. Sections were deparaffinized in xylene and rehydrated using decreasing concentrations of ethanol. Before immunostaining, the sections were treated in a microwave oven at 700 W for 10 minutes and 350 W for 15 minutes in 50 mM Tris buffer (pH 8.0). Endogenous peroxidase was blocked with 1% hydrogen peroxidase in PBS for 30 minutes. Avidin-binding protein was blocked by incubating the sections sequentially with avidin and biotin in Blocking Kit (Vector Laboratories, Burlingame, CA, USA). Between incubations, the sections were washed in PBS and excess liquid was carefully wiped away from around the specimen. To avoid non-specific background staining, the sections were incubated with normal goat serum in a dilution 1:5 in PBS for 30 minutes before applying the primary antibody. The production and specificity of the five different polyclonal SSTR specific antibodies have been described before.[16] The primary antibodies were used in the following dilutions; sst_1 1:3500, sst_2 1:3500, sst_3 1:2500, sst_4 1:3000, and sst_5 1:5000. Anti-rabbit serum diluted 1:200 was used as biotinylated secondary antibody. The immunoreaction was visualized with an Elite kit (Vector Laboratories). Diaminobenzidine tetrahydrochloride (Vector Laboratories) was used as chromogen. The sections were counterstained for 30 seconds with Mayer's hematoxylin (Apoteksbolaget, Sweden).

IHC evaluation

A scale involving percentage of positive cells for both membranous and cytoplasmic localization of stains was applied. We considered a case as negative when the percentage of positive cells was <5% of the total number of cells, focal positive when it was >5-20% and diffuse for these cases having >20% positive cells. Immunostains were evaluated independently by two persons (ETJ, GK).

Statistical methods

Variables analyzed included age, sex, tumour size, invasion of thyroid parenchyma or extrathyroidal extension, lymph node metastases, and SSTRs expression. Calculations were carried out using SPSS v14**.** For comparisons between different categories of immunohistochemistry results, the Pearson's chi square (x^2) test was used. Results were considered statistically significant when p value was less than 0.05.

RESULTS

The immunohistochemical analysis showed that SSTR subtypes were commonly expressed in non-medullary thyroid carcinoma cells (Table 1). All analyzed samples displayed positive immunostaining for both sst_2 and sst_3 and, although the staining was heterogenous, nearly all samples were scored as "diffuse", i.e. >20% of the cells showed immunoreactivity. In addition, sst_1 and sst_5 were detected in the majority of the samples, whereas sst_4 appeared to be infrequently expressed. Co-expression of several receptor subtypes was very common in the analyzed carcinomas and expression of three or more subtypes was detected in 36 of the 47 cases (Figure 1A-E). In the non-neoplastic thyroid tissues the expression of SSTR was quite low. No expression at all of sst_1 or sst_4 could be detected, whereas sst_2 was expressed in 13%, sst_3 in 24%, and sst_5 in only 2% of the 46 samples. Thus, all SSTR subtypes showed a higher expression in non-medullary thyroid carcinomas than in the normal thyroid tissue (Figure 2).

Cellular localization of somatostatin receptors

SSTRs expression was observed mainly in the cytoplasm of neoplastic cells (Figure 3A-B), although membranous localization was apparent for all subtypes in the thyroid carcinomas (Figure 3C-D). Among the

Table 1. Immunohistochemical expression of somatostatin receptor subtypes (sst1-5) in 47 non-medullary thyroid carcinomas

Case	age	sex	diagnosis	Size (cm)	sst1	sst2	sst3	sst4	sst5
1	25	F	PTC	0.5	-	+d	+d	+f	+f
2	21	F	PTC	2.7	-	+d	+d	-	+d
3	44	F	PTC	0.7	-	na	na	-	na
4	29	F	PTC	2.8	-	+d	+d	-	+d
5	40	F	PTC	0.8	-	na	na	-	na
6	40	M	PTC	1.0	na	na	+d	+f	+d
7	54	F	PTC	1.8	+d	+d	+d	na	na
8	74	F	PTC	2.5	+d	+d	+d	+d	+d
9	42	F	PTC	0.5	-	+d	na	+d	na
10	46	F	PTC	1.1	+d	+d	+d	+d	+d
11	47	F	PTC	1.5	+d	+d	+d	+d	+d
12	60	F	PTC	0.9	+d	+d	+d	+d	+d
13	51	F	PTC	3.2	+d	+d	+d	+f	+d
14	13	F	FTC	3.5	+d	+d	+d	-	+d
15	20	M	FTC	2.7	+d	+d	+d	-	-
16	70	M	FTC	3.8	+d	+d	+d	-	-
17	70	F	ATC	4	+d	+d	+f	-	-
18	56	M	FTC	6.0	na	na	na	-	na
19	85	F	ATC	5.0	+d	na	na	-	na
20	64	F	HCC	3.5	+d	+d	+d	-	+d
21	62	F	HCC	2.6	+d	+d	+d	+f	+d
22	66	F	HCC	4.0	+d	+d	+d	+f	-
23	62	M	PTC	3.0	+d	+d	+d	-	+d
24	47	F	PTC	3.0	+d	+d	+d	-	+d
25	60	M	PTC	0.2	+d	+d	+d	+f	+d
26	48	F	PTC	0.7	na	na	na	-	na
27	70	M	PTC	5.4	+d	na	+f	-	na
28	60	F	PTC	5.5	na	na	na	-	na
29	24	F	PTC	3.0	+d	+d	+d	-	+d
30	61	M	PTC	1.8	+d	+d	+d	+f	+d
31	32	F	PTC	2.5	+d	+d	+d	+f	+d
32	8	M	PTC	4	+d	+d	+d	-	+d
33	65	F	PTC	1.5	-	+d	+d	-	+d
34	57	M	PTC	0.5	+d	+f	+f	+f	na
35	50	F	PTC	1.4	+d	+d	+d	+f	+f
36	65	M	PTC	1.3	+d	+d	+d	-	+f
37	36	F	PTC	2.2	-	+d	+d	-	+f
38	54	F	PTC	7.5	+d	+d	+d	+f	-
39	60	F	PTC	1.6	-	+d	+d	-	+d
40	58	F	PTC	3.6	-	+d	+d	-	-
41	28	M	PTC	3.2	-	+d	+d	-	-
42	52	F	PTC	0.4	+d	+d	+d	-	-
43	57	M	PTC	1.3	+d	+d	+d	+f	+f
44	64	F	PTC	2.0	+d	+d	+d	-	-
45	64	F	PTC	1.8	+d	+d	+d	+f	+f
46	21	M	PTC	3.0	+d	+d	+d	-	+f
47	57	M	PTC	0.7	+d	+d	+d	+f	+f

M: male, F: female, PTC: papillary thyroid carcinoma, FTC: follicular thyroid carcinoma, ATC: anaplastic thyroid carcinoma, HCC: Hürthle cell carcinoma, -: negative, na: not available tissue cores, +d: diffuse positive, +f: focal positive.

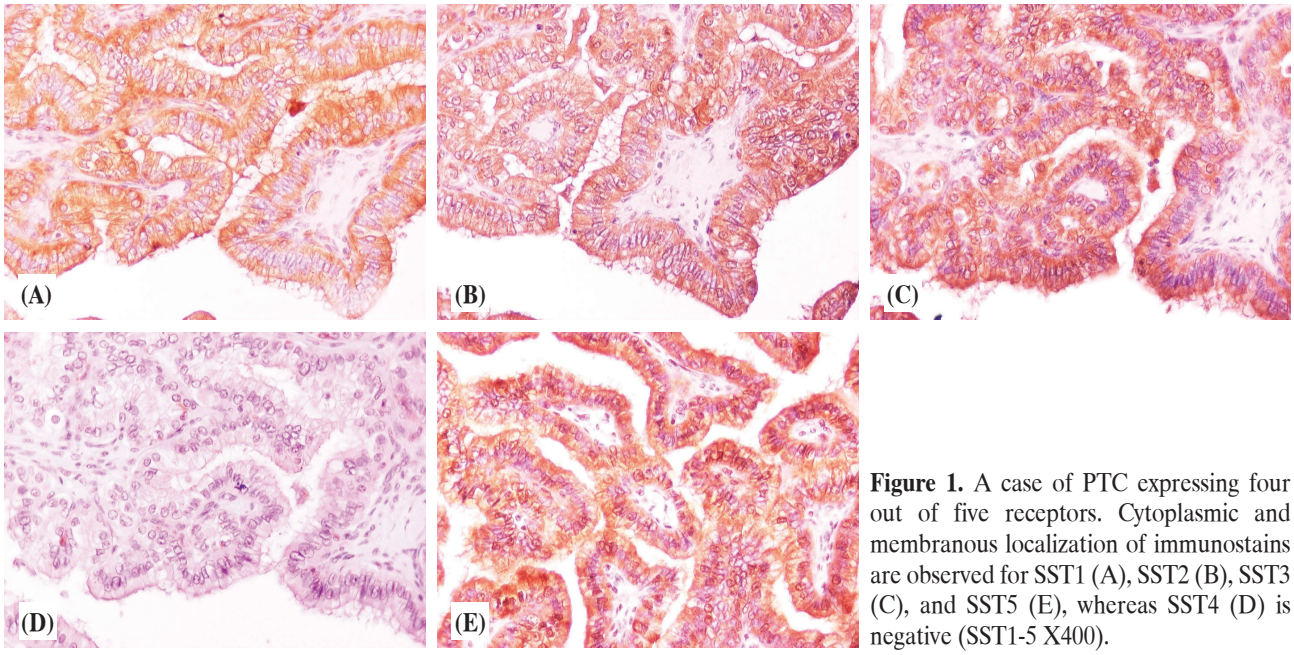

Figure 1. A case of PTC expressing four out of five receptors. Cytoplasmic and membranous localization of immunostains are observed for SST1 (A), SST2 (B), SST3 (C), and SST5 (E), whereas SST4 (D) is negative (SST1-5 X400).

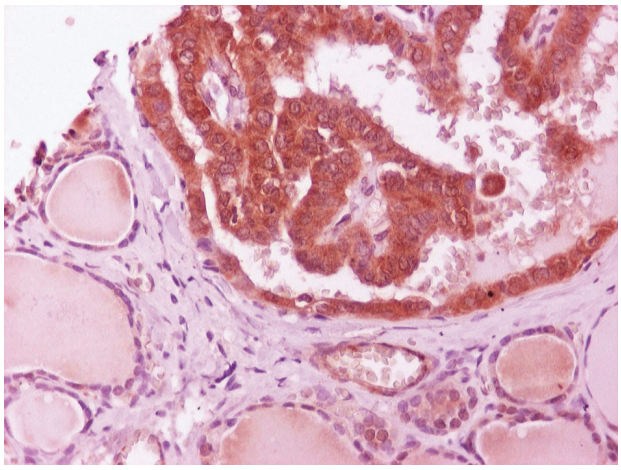

Figure 2. Overexpression of SST3 in thyroid carcinoma compared to adjacent normal tissue (SST3 X400).

different subtypes, sst_2 and sst_5 were most frequently localized to the plasma membrane (Table 2). In the non-neoplastic tissues no membranous localization of any SSTR could be detected.

Correlation of SSTR expression to clinicopathological parameters

Statistical analysis was meaningful to perform only on the total collection of samples and the PTC subgroup, but no significant correlation was found between SSTR subtype expression and tumour size or any other clinicopathological parameter. Furthermore, there was no correlation between sst_2, sst_3, and sst_5 cytoplasmic or membranous expression in thyroid carcinomas and the expression in the corresponding non-neoplastic tissue (x^2, sst_2 cytoplasmic: $p= 0.506$, sst_2 membranous: $p= 0.228$, sst_3 cytoplasmic: $p= 0.656$, sst_3 membranous: $p= 0.581$, sst_5 cytoplasmic: $p= 0.244$, sst5 membranous: $p= 0.140$). Although the number of Hürthle cell carcinomas was low, one may note that all three cases express at least four of the five SSTR subtypes.

DISCUSSION

In this study, we demonstrated that there is a high expression of all types of SSTRs in human non-medullary thyroid carcinoma tissue, while the expression of SSTRs was low in the non-neoplastic thyroid tissue obtained from the same operation material. The sst_2 and sst_3 subtypes appeared to be the most abundantly expressed, whereas sst_4 was expressed in only a small number of tumours. In normal thyroid tissue the expression of SSTR subtypes was restricted to sst_2, sst_3, and sst_5, which is in complete agreement with results from a study on the distribution of SSTRs in humans.[17,18] The SSTR expression was located both in the cytoplasm and at the plasma membrane of the thyroid cells, also in agreement with earlier findings.[17,18]

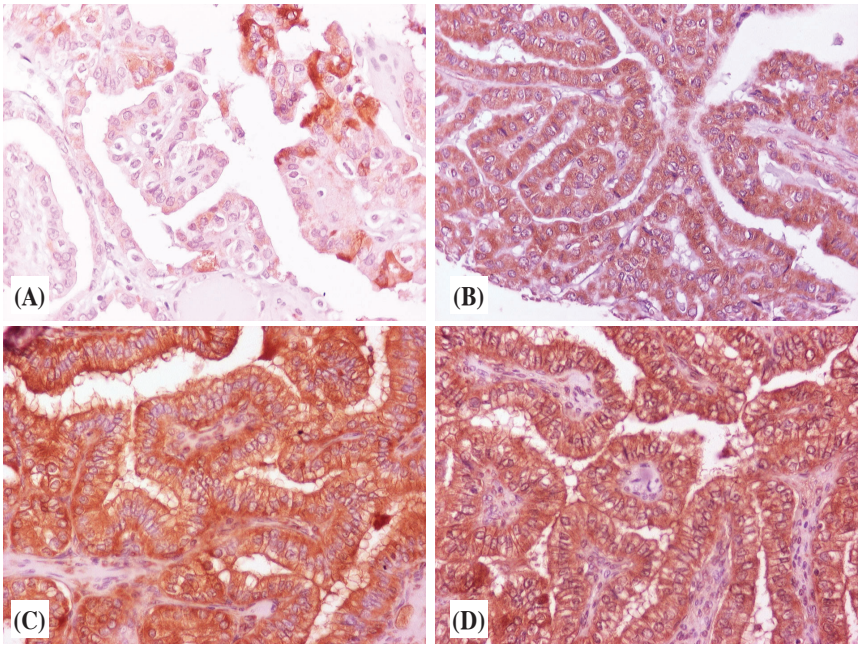

Figure 3. Focal (SST4, A) and diffuse (SST3, B) cytoplasmic expression (IHC, ×400) of SSTRs. Membranous localization of immunostains is seen as well (C: SST1 ×400, D: SST2 ×400).

Table 2. Localization of somatostatin receptor subtypes in non-medullary thyroid carcinomas

Receptor subtype	Cytoplasmic		Membranous	
	>20 (%)	5 -20 (%)	>20 (%)	5 - 20 (%)
sst_1	74.4	0	0	30
sst_2	97.4	2.6	43	38
sst_3	92.5	7.5	7	24
sst_4	10.9	30.4	0	12
sst_5	52.6	21.6	34	21

The results from the immunohistochemical analysis of thyroid tumours obtained here are mainly in line with results obtained by Klagge et al. using RT-PCR.[12] This group reported the predominant expression of sst_2 and sst_5 mRNA, a weaker expression of sst_1 and sst_3, and sst_4 expression only in a few PCTs. The similarities between our results on non-medullary thyroid carcinomas and the results from Papotti et al[15] on medullary thyroid carcinomas are also substantial. In their study, approximately 50% of the medullary thyroid carcinomas showed immunoreactivity for one or several of the subtypes sst_1, sst_2, sst_3 or sst_5, and only 4% were stained positive with antibodies to sst_4. In agreement with our results, Papotti et al. did not find any significant correlation between SSTR expression and sex, age, tumour size or tumour stage. Taken together, thyroid carcinomas, of both medullary and non-medullary types, commonly express one or several of the SSTR subtypes (sst_1, sst_2, sst_3, and sst_5).[19]

The apparent prominent expression of SSTRs in Hürthle cell thyroid carcinomas is in agreement with positive imaging results with SRS in non-medullary thyroid cancer.[20] Pathological uptake was observed in Hürthle cell carcinomas with 95% positive examinations in patients with thyroglobulin levels exceeding 10 ng/ml. The analogue ^{90}Y-DOTATOC was, however, ineffective in the treatment of three cases of metastasizing Hürthle cell carcinomas.

SRS is not commonly used in the diagnostic work-up of patients with non-medullary thyroid carcinoma. Our results indicate that treatment with PRRT might be explored as a possible option for patients with metastatic non-medullary thyroid carcinomas refractory to conventional treatment. The development of new somatostatin analogues and new radionuclides used for treatment may provide new agents for this. However, further studies are needed to determine if PRRT can be used as an alternative for this group of patients.

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