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Neuroendocrine neoplasms and somatostatin receptor subtypes expression

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Abstract

Neuroendocrine neoplasms (NENs) show wide spectrum of clinical course — from benign biological potential to recurrences and rapidly progressive disease. Somatostatin analogs that bind to somatostatin receptor are part of the therapy; detection and evaluation of activation of somatostatin receptor subtypes are part of the process of new therapy induction. When using RT-PCR method and immunohistochemistry, it is possible to detect more than two SSTR subtypes in majority or all neuroendocrine neoplasms regardless tumor origin. Generally with some exceptions, from the viewpoint of tumor grade — apart the site of origin, there is a tendency to decrease the percentage of SSTRs expression; 100% (G1, 2)–85.7% (G3) for SSTR 1; 81.8% (G1, 2)–61.9% (G3) for SSTR 2; 54.5% (G1, 2)–52.4% (G3) for SSTR 3; 9% (G1, 2)–4.8% (G3) for SSTR 5. Different studies indicate significant differences in the expression of SSTR 1 and 2A and 2B between NEC G3 small cell type and non-small cell type. Further research on SSTRs expression in NEN could serve as base to development and improvement of somatostatin analogs' pharmacotherapy in patients with unsatisfactory course.

KEY words: somatostatin, somatostatin receptor, SSTR, neuroendocrine neoplasm, NEN, expression

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Introduction

Somatostatin analogs take a role in pharmacotherapy from the early 1980s [1]. Somatostatin binds to all somatostatin receptor subtypes (SSTR 1–5), in contrast to octreotide (first synthesized somatostatin analog) and lanreotide, which bind only with a high affinity to SSTR 2 and SSTR 5 [2, 3]. Expression of somatostatin receptors by endocrine tumors is essential for the use of the somatostatin analogs in pharmacotherapy. In 1973, somatostatin was isolated from sheep hypothalami in the search for the hormone responsible for stimulating the release of GH from the anterior pituitary [4].

Many physiological functions in the GI tract (e.g. motility, gastric acid production, pancreatic enzyme secretion, bile secretion) are inhibited by somatostatin. It also acts as a secretory pan-inhibitor, by suppressing secretion from the anterior pituitary gland (e.g. GH, prolactin, TRH, ACTH) [5–7], gastrointestinal tract (e.g. cholecystokinin, gastrin, secretin, vasoactive intestinal peptide), endocrine pancreas (e.g. glucagon, insulin, and pancreatic polypeptide) [8], thyroid (e.g. triiodothyronine, thyroxin, and calcitonin), kidney and adrenals (e.g. renin aldosterone system) [9]. Control of the cell proliferation in normal tissues and tumors was as well recorded [10–13].

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Two biologically active forms of somatostatin: somatostatin-14 and somatostatin-28 act through high affinity G protein-coupled membrane receptors. Proteolytic processing of larger precursor molecules (prepro-somatostatin and pro-somatostatin) forms the somatostatin [14, 15]. Five somatostatin receptor (SSTR) subtype genes have been cloned and described — SSTR 1, SSTR 2, SSTR 3, SSTR 4 and SSTR 5 [16]. The genes which encode the five SSTR subtypes are localized on different chromosomes [17]. Alternative splicing can generate two forms of the SSTR2 receptor (SSTR 2A and SSTR 2B) [18, 19].

Somatostatin influence on cells has a wide spectrum, through binding to its SSTR subtype(s), and activation of the second messenger system. These systems include (1) inhibition of adenylate cyclase activity and (2) activity of calcium channels, as well as (3) stimulation of phosphotyrosine phosphatase or (4) MAPK (mitogen-activated protein) kinase activity [17, 20, 21]. The inhibitory effects of somatostatin on adenylate cyclase activity and on the influx of calcium are linked to inhibition of secretion processes.

Activation of SSTR 1, 2, 4 and 5 promotes cell cycle arrest (via modulation of MAPK pathway) [22, 23]. Interestingly, SSTR 2 and SSTR 3 trigger apoptosis (accompanied by p53 and Bax protein activation) [24–26].

A high density of SSTRs are expressed in tumors, arising from tissues which are target for the somatostatin [27–31].

Many tumors express the SSTR: e.g. pituitary adenomas, pancreatic endocrine tumors, carcinoids, paragangliomas, pheochromocytomas, small cell lung cancers, medullary thyroid carcinomas, breast cancers and malignant lymphomas [28, 32].

Multiple SSTR subtypes are coexpressed by the majority of the SSTR-positive tumors and essential variation in their expression between the different tumor types and among tumors of the same type were described [33–36].

SSTR 2 predominance is generally found in more than 80% of the endocrine pancreatic and endocrine GI tract tumors [29, 31, 37–39].

Different methods have been used for identification SSTR subtypes expression in tissue samples. The most relevant are: the specific mRNA of SSTR detection (e.g. in situ hybridization, RNase protection assays and RT-PCR), using radiolabeled somatostatin analogs and immunohistochemistry. IHC based on SSTR-specific antibodies seems to be widely available and cost effective method which identifies SSTR subtypes — it is worthy to mention that many researchers noticed that this method needs the precise standardization [40–48].

Many articles bring information about SSTR expression using the nuclear medicine methods — for this knowledge we send the reader to the references [49–54].

Pituitary adenoma

Pituitary adenomas (PA) in the majority have a benign biological potential. These tumors are classified based on the hormones that are produced by the neoplastic cells (GH, prolactin, adrenocorticotropin, thyrotropin, follitropin, or luteinizing hormone) — some of them can secrete two hormones (GH and prolactin as the most common combination), and rarely, pituitary adenomas are plurihormonal. Clinically non-functioning adenomas (CNFA) lack the typical hypersecretion of the functional pituitary adenomas and do not produce clinical symptoms of hormone excess.

Recent data based on mRNA and immunohistochemistry analysis showed that in (GH) adenomas SSTR 2, SSTR 5, SSTR 3 and SSTR 1 transcripts were expressed in descending level order. Macroprolactinomas revealed significant amount of SSTR 1 transcripts. Corticotroph adenomas showed expression of SSTR 2 and SSTR 1 transcripts, but levels of SSTR 5 transcripts were very low. Patients with CNFA dominantly expressed SSTR 3 and SSTR 2mRNA [55], other data demonstrate expression of SSTR 1-5 by all CNFA, but with very low/missing levels of SSTR 4 and 5 [56–58]. High relative expression (ratio to β-glucuronidase mRNA > 1) of SSTR 1 was found in 7.5 %, SSTR 2 in 7 %, SSTR 3 in 4 % and SSTR 5 in 0.5 % of tumors. Among histological adenoma types in null cell adenomas high levels of SSTR 1 and SSTR 2 were observed, accompanied with low levels of SSTR 3. In gonadotrophs adenomas, expression of SSTR 3, SSTR 2 and SSTR 1 in descending order was demonstrated. Silent ACTH adenomas showed expression of SSTR 2, SSTR 1, SSTR 5 and low level of SSTR 3 [56]. In recent years, scientist investigate coexpression of the SSTRs and other receptors in many neuroendocrine neoplasms (e.g. dopamine 2) [55, 56].

Gastro-intestinal tract and Pancreas

Neuroendocrine neoplasms (NEN) in the digestive system and pancreas are divided into neuroendocrine tumors (NET; low to intermediate grade, G1 and G2 respectively) and neuroendocrine carcinomas (NEC; poorly differentiated — high grade, G3). Among

NECs, large cell NEC and small cell NEC can be recognized. Mixed adenoneuroendocrine carcinomas (MANEC) are neoplasms with both high grade neuroendocrine and adenocarcinoma features. Classification criteria of NENs are common to all GI and pancreatic neuroendocrine neoplasms. NENs can be functional or non-functional [59].

Grossly NETs have tendency to be small, circumscribed, and in GI tract they can be covered by a flattened mucosa [60] or have the gross appearance of gastric polyps [61]. Microscopically, the predominant pattern of growth may be microglandular, trabecular or insular. The nuclei are regular and normochromatic, mitoses are scanty, necrosis is usually absent, and vascularization is florid. Focal mucin positivity may be present. Immunohistochemically, GI NENs are positive for neuron-specific enolase, chromogranin, synaptophysin, and keratin. NECs may form a large, sometimes fungating mass deeply infiltrating the GI wall and often metastatic to lymph nodes and liver. They are composed of large, poorly formed trabeculae, nests or sheets of anaplastic round, polyhedral to spindle cells, small to fairly large in size, and immunoreactive for general neuroendocrine markers including chromogranin A, synaptophysin, neural cell adhesion molecule (NCAM1/CD56) or neuron-specific enolase [59].

Immunohistochemistry revealed that SSTR 1, SSTR 2A, 3 and 5 are expressed in gastrinomas, insulinomas and carcinoid tumors. Immunostaining of the specific antibodies is non-homogenous and shows differences between SSTR subtypes and between commercial antibodies. Generally immunoreactivity for SSTR 2 is localized in the plasma membrane in contrast to SSTR 1, 3 and 5 which stain mainly the cytoplasm.

SSTR 1 was expressed in 30% of gastrinomas, 31% of insulinomas ad 37% of NETs. SSTR 2a showed expression in 100% of gastrinomas, 58% of insulinomas and 86% of NETs. Immunostaining for SSTR 3 was observed in 79% of gastrinomas, 78% of insulinomas and 71% of NETs. SSTR 5 immunoreactivity was detected in of 76% gastrinomas, 78% of insulinomas and 83% of NETs [62]. Other researchers also indicate SSTR 2 as the commonest SSTR expressed, which is accompanied by co-expression of SSTR 3 and 5 in 32% and 24% of cases, respectively [63].

From the viewpoint of tumor localization: 85.5% of pancreatic, 100% of gastric, 70% of intestinal, 85.7% of appendiceal and 100% of rectal NENs show SSTR 2. SSTR 5 expression in present in: 61.9% of pancreatic, 37.5% of gastric, 70% of intestinal, 71.5% of appendiceal and 66.6% of rectal NENs. According to the tumor grade, SSTR 2 is expressed in 91% of grade1, 82.8% of grade 2 and 100% of grade 3 GI NENs. SSTR 5 show expression in 81.8%, 60% and 0% of grade 1, 2 and 3, respectively [64].

Lung

The pulmonary neuroendocrine tumors include typical carcinoid (TC), atypical carcinoid (AC), small cell carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC). These tumors are characterized by similar microscopic appearance, like other neuroendocrine tumors in the body — they have organoid nesting, palisading and trabecular pattern with mitotic activity and presence of necrosis as the distinguishing features (TC: < 2 mitoses per 2 mm^2 and no evidence of necrosis. AC: 2–10 mitoses per 2 mm^2 and/or focal necrosis).
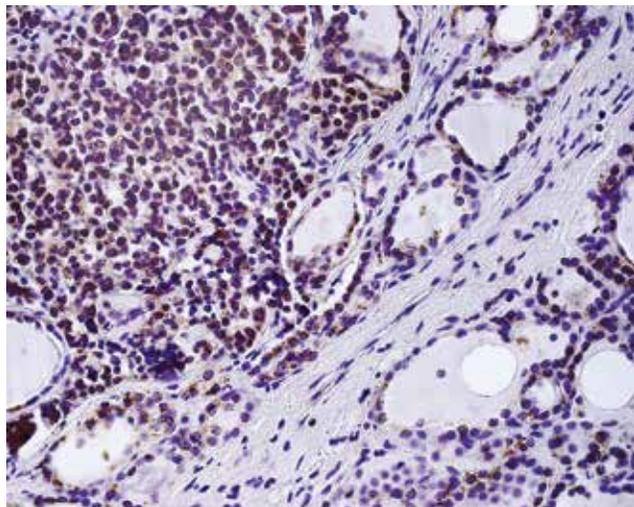
Figure 1. Medullary thyroid carcinoma — SSTR 1. Medullary thyroid carcinoma (upper left) show weak SSTR 1 expression
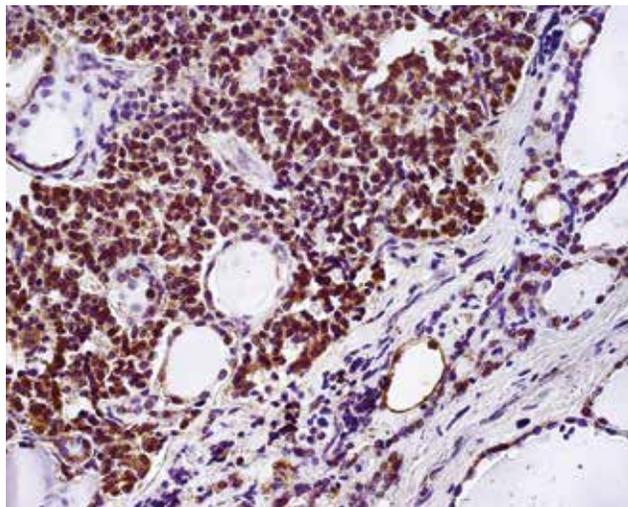

Figure 2. Medullary thyroid carcinoma — SSTR 2a. Medullary thyroid carcinoma (upper left) show SSTR 2a expression

LCNEC is differentiated from SCLC by larger cell size, abundant cytoplasm and prominent nucleoli. Mitotic activity is high (10–11 mitoses/10 hpf). Prominent nuclear molding and deposition of hematoxylin-stained material (DNA) in blood vessel walls are characteristic for SCLC. These tumors are positive for chromogranin, synaptophysin and NCAM (CD56), about 50% of LCNECs also express TTF-1.

TC and AC occur in significantly younger patients than SCLC and LCNEC. They present as firm and well-demarcated tumors, with predilection to bronchi (frequently endobronchial growth). TC is evenly distributed in the lung parenchyma, while AC arises more commonly in small peripheral bronchi. Clinical symptoms are related to bronchial obstruction (cough and hemoptysis as the most common), Cushing's syndrome and carcinoid syndrome are rare.

SCLC has tendency to occur in central location with locoregional spread, commonly accompanied by paraneoplastic syndromes. On macroscopy it presents as a white-tan, soft and friable mass with extensive necrosis and frequent nodal involvement.

LCNEC occurs preferentially as peripheral mass, but may also involve subsegmental or large bronchi. It spreads on visceral pleura and chest wall. Typically LCNECs do not produce clinical symptoms of hormone excess [65–70].

Typical carcinoids (TC) show immunoexpression of SSTR 1, 2A, 2B, 3, 4, and 5 in 79%, 96%, 66%, 49%, 5%, and 0%, respectively. Atypical carcinoids (AC) expression of SSTR 1, 2A, 2B, 3, 4, and 5 was observed in 77%, 77%, 77%, 33%, 0% and 0%, respectively. In Large cell NECs (LCNEC), immunoexpression of SSTR 1, 2A, 2B, 3, 4, and 5 was observed in 60%, 60%, 30%, 40%, 0%, and 15% of cases, respectively. Small-cell carcinomas (SCLC) show immunoexpression of SSTR 1, 2A, 2B, 3, 4, and 5 in 27%, 69%, 24%, 15%, 0%, and 0% of cases, respectively.

Tsuta et al. demonstrated that SSTR 1, 2A, 2B, 3 and 4 have a tendency toward decreased expression in well to poorly differentiated NECs [71].

Other study of TC and AC [58] also indicates SSTR 2A as a most frequently expressed (72% of cases), followed by SSTR 1 (63%),

SSTR 5 (40%) and SSTR 3 (20%), whereas researchers did not record SSTR 4 expression.

83% of TCs and ACs are positive for at least one SSTR subtype, these which are negative for SSTR 2A, show expression of another SSTR in nearly half cases (48%) — among them 35% are positive for SSTR 1, 11% for SSTR 3 and 11% for SSTR 5.

There is no difference in distribution of SSTR 1–5 compared with TNM parameters [72].

Paraganglioma/pheochromocytoma

Pheochromocytoma (PCC) and paraganglioma (PGL) are neuroendocrine tumors derived from adrenal chromaffin cells and extra-adrenal paraganglia, respectively. Hereditary tumors are frequently diagnosed before 40 years of age, while sporadic tumors are usually discovered in patients aged 40 to 50 years. Clinical presentation results from effects of secreted catecholamines, including: hypertension, tachycardia, pallor, headache, and anxiety; but up to 25% of pheochromocytomas are asymptomatic. Macroscopically tumors are variable in size and weight (up to > 2000 g), with round to oval and sharply circumscribed presentation. Cut surface shows a dusky red-brown mass with possible marked hemorrhage and necrosis. Tumor cells vary in size and shape and show round nuclei, prominent nucleoli and granular amphiphilic to basophilic cytoplasm. They are arranged in well-defined nests (Zellballen), surrounded by the sustentacular cells. Cell nests can be separated by thin strands of fibrovascular stroma.

Patients with MEN II syndrome may demonstrate tumors accompanied by adrenal medullary hyperplasia, occasionally multiple or bilateral [73–76].

Among SSTRs, type 2 and 1 are the most commonly expressed subtypes in PCCs and PGLs. Expression of SSTR 3 and SSTR 5 is more frequent in PCCs than PGLs. More precise data show SSTR expression level in PCCs: 95%, 100%, 56% and 54% for SSTR 1, 2, 3 and 5, respectively. SSTR 1, 2, 3 and 5 are expressed in PGLs in: 92%, 100%, 31% and 31%, respectively. Researchers did
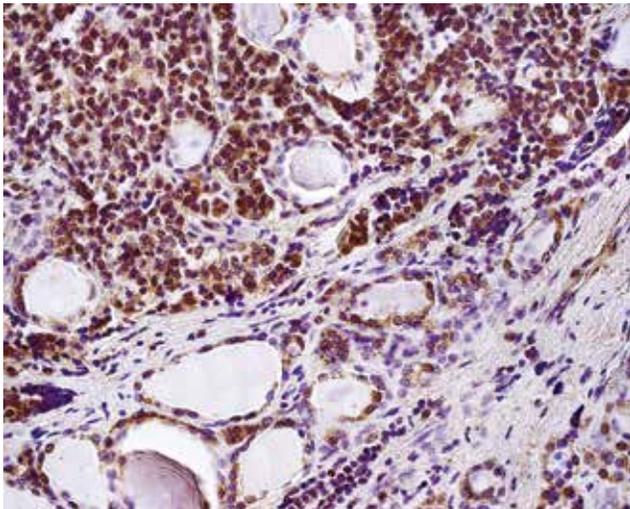
Figure 3. Medullary thyroid carcinoma — SSTR 5. Medullary thyroid carcinoma (upper left) and adjacent parenchyma show SSTR 5 expression

not indicate significant differences in SSTRs expression between sporadic and hereditary tumor types [77].

Merkel cell carcinoma

Merkel cell carcinoma is an uncommon neoplasm of the skin with neuroendocrine differentiation, which involves commonly head and extremities. It is usually small (from 0.8 cm to 4 cm), pink, solitary nodule, rarely with multiple nodules presentation. Tumor is composed of small, round, blue cells with scant cytoplasm and irregular nuclei with uniformly distributed chromatin. Nucleoli are inconspicuous, frequent mitotic figures and individually necrotic tumor cells are commonly visible. Tumor cells are positive for chromogranin, synaptophysin, neuron-specific enolase and cytokeratin 20 [78–83].

Overall, 76.5% of Merkel cell carcinomas express at least one of SSTRs. SSTR 2A and SSTR 5 are expressed in 59.2% and in 44.9% of tumors, respectively. Expression of SSTRs is not associated with clinical characteristics, Ki67 proliferative index and survival [84].

Neuroblastoma

Neuroblastoma is the most common solid tumor of young children (90% occur < 5 years); it shows minimal to none maturation and is composed of small round blue cells arranged in nodular aggregates separated by fibrovascular septa. Homer-Wright pseudorosettes can be visible. Macroscopically tumor forms large mass with a gray cut surface, often accompanied by hemorrhage and necrosis. Neuroblastoma cells are positive for neurofilament, synaptophysin, chromogranin and neuron-specific enolase [85–90].

Neuroblastomas show expression in 66.7%, 81.5%, 9.3%, 37% and 0% for SSTR 1, 2, 3, 4 and 5 respectively. The favorable histology group of NBs are characterized by higher SSTRs expression than the unfavorable histology group — the researchers indicate significant differences in SSTR 1, 2 and 4 expression. SSTR subtypes expression in normal tissues from the adrenal medulla and sympathetic ganglia are similar to those of ganglioneuromas and ganglioneuroblastomas, with rates being 100% for SSTR 1, approximately 90% for SSTR 2, approximately 40% for SSTR 3, 100% for SSTR 4 and 0% for SSTR 5 [91].

Medullary thyroid carcinoma

Medullary thyroid carcinoma is a malignant entity derived from C cells and accounts for up to 5% of thyroid malignancies. Tumor can be sporadic (80%) or hereditary (20%, related to familial medullary thyroid carcinoma, MEN IIA and MEN IIB). Elevated level of calcitonin in serum is a useful parameter to monitor residua, recurrent or metastatic disease. Macroscopically tumor occurs often as circumscribed, tan-yellow mass with firm consistency. Most common localization is upper and middle third of lobe (which corresponds to predominant C cells localization); in hereditary types multifocal nodules may occur. Medullary thyroid carcinoma presents variety of histologic patterns: solid, sheet-like, lobular, trabecular and insular. Tumor cells are round, polygonal, fusiform and commonly mixed cell types are visible, with salt-pepper chromatin pattern appearance. Stromal deposition of amyloid occurs in about 80% of cases. Immunohistochemistry reveals positive reaction to calcitonin, chromogranin, synaptophysin, carcinoembryonic antigen and TTF 1. Congo red stains amyloid material and shows birefringence in polarized light [92–95].

Researchers demonstrated SSTRs by IHC in medullary thyroid carcinoma in 1970s and 1980s, later works showed that 85% of tumors express at least one SSTR; 49%, 43%, 47%, 4% and 57% tumors are positive for SSTR 1, 2, 3, 4 and 5, respectively. There is no correlation between SSTR expression and clinical data, TNM and histological type [96].

Others studies analyzed SSTR expression in other thyroid malignancies — they also showed that SSTR 5 is commonly expressed in thyroid neoplastic and non-neoplastic entities (86.2–100%) [97–98].

Summary

Using RT-PCR method and immunohistochemistry it is possible to detect more than two SSTR subtypes in majority or all neuroendocrine neoplasms regardless tumor origin. Generally with some exceptions, from the viewpoint of tumor grade — apart the site of origin, there is a tendency to decrease the percentage of SSTRs expression; 100% (G1, 2)–85.7% (G3) for SSTR 1; 81.8% (G1, 2)–61.9% (G3) for SSTR 2; 54.5% (G1, 2)–52.4% (G3) for SSTR 3; 9% (G1, 2)–4.8% (G3) for SSTR 5. Different studies indicate significant difference in the expression of SSTR 1 and 2A and 2B between NEC G3 small cell type and non-small cell type [71, 99].

Further research on SSTRs expression in NEN could serve as a base for development and improvement of somatostatin analogs' pharmacotherapy in patients with unsatisfactory course.

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