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ORIGINAL ARTICLE

Importance of Immunohistochemical Detection of Somatostatin Receptors

Attila Zalatnai[1] · Eszter Galambos[1] · Eszter Perjési[1]

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Abstract

The long-acting somatostatin analogs represent important weapons in treatment protocols of patients with neuroendocrine tumors. Because these peptides preferentially bind to the specific somatostatin receptors, the targeted therapy requires detection of them. As one of the national consulting centers, here we present the results of the immunohistochemically positive neuroendocrine neoplasms diagnosed between 2010 and 2014. Twenty-four paraffin-embedded cases (14 females 10 men, 21–79 years) from different localizations were found to express somatostatin-receptor type 2 (SSTR2). None of the patients has received previous hormonal therapy. The immune reactions have shown membranous, cytoplasmic or mixed patterns. There was no correlation between the expression and the chromogranin A levels, the grades or the hormonal activity/inactivity of the given neoplasms. Our results show that the immunohistochemical detection of SSTR2 is a quick, reliable and effective tool that provides useful information to the oncologists for the therapeutic decision. Because the incidence of the neuroendocrine tumors is still low, centralized pathological units are needed to perform such technique.

Keywords Neuroendocrine tumors · Immunohistochemistry · Somatostatin receptors

Introduction

Due to their complex inhibitory potential, long-acting somatostatin-analogs (SSAs) have been incorporated in the therapeutic protocols of neuroendocrine tumors (NETs). They not only interfere with release of several hormones, but other physiological functions are also blocked, such as exocrine secretions, cell proliferation, cell survival or angiogenesis; moreover, they may induce apoptosis [1]. Inhibition of IGF-1, VEGF, anti-inflammatory or anti-nociceptive activities should also be taken into consideration [2]. Using these drugs in majority of neoplasms a stable disease (SD) can be achieved, but recent clinical trials have also revealed their antitumor activity in advanced NETs, leading to delay of progression of the disease [3].

Their far-reaching effects are mediated either by indirect or direct mechanisms. The former inhibiting mechanisms prevail through blocked release of growth factors and trophic hormones, antagonization of EGF-effects, inhibition of angiogenesis, modulation of the immune system, while the direct effects are governed by specific somatostatin receptors (SSTRs) [4].

Somatostatin receptors (SSTR 1–5) belong to the G-protein-coupled transmembrane receptor family, but the different subtypes mediate alternative cellular effects. All the five receptors activate phosphotyrosine phosphatase (PTP) inducing cell cycle arrest [5, 6]. SSTR2 and SSTR3 are the only receptors responsible for stimulation the extrinsic and intrinsic pathways of apoptosis [7]. The central and peripheral (hepatic) GH/IGF-1 axis is primarily suppressed through SSTR2 and SSTR5 [8].

Because the Institute is one of the central pathological consulting centers in Hungary, we have summarized our experience on immunohistochemical characteristics of SSTR2. This receptor was chosen because the used SSAs preferentially bind to SSTR2 and SSTR5.

Attila Zalatnai, Eszter Galambos and Eszter Perjési contributed equally to this work.

✉ Attila Zalatnai
zalatnai@korb1.sote.hu

[1] First Department of Pathology and Experimental Cancer Research, Semmelweis University, Faculty of Medicine, H-1085 Üllői út 26, Budapest, Hungary

Materials and Methods

Between 2010 and 2014 fifty neuroendocrine neoplasms have been diagnosed and immunohistochemically evaluated for SSTR2. All samples represented biopsy or surgically resected materials, none of the patients had received antineoplastic or hormonal therapies earlier. The neuroendocrine nature of the given tumors was evidenced by immunohistochemical positivity of chromogranin A, synaptophysin and CD56, the Grade was assessed based on the mitotic counts and the Ki-67 proliferative index according to the WHO classification [9]. Among them twenty-four positive cases (10 males and 14 females) were found (48%). (Table 1) The median age was 64 years, with a very wide age-distribution (21–79 years). High serum levels of chromogranin A (>98 ng/mL) were measured in 12 patients, among them we found 8 G1, 1 G2 and 3 G3 tumors.

The routine pathological diagnosis was supplemented with immunohistochemical determination of SSTR2. Four μm sections were cut from the formalin-fixed, paraplast-embedded blocks and antigen retrieval was performed in dewaxed slides (0.1 M citrate buffer at pH 6.0 using pressure-assisted microwave device at 95 °C, for 50 min). After rinsing in phosphate buffer saline (PBS) the endogenous peroxidase activity was blocked by 0.3% hydrogen peroxidase (H_2O_2) followed by PBS again. The non-specific binding was blocked with normal horse serum for 20 min, and the section were overnight incubated with polyclonal anti-human somatostatin receptor-2 (SSTR2) antibody (MBL Intl., Woburn, MA; Cat. No. MC-1001) at −4 °C. According to the supplier, the antibody reacts with the C-terminal domain, without differentiating the splice variants (SSTR2 A-B) of the receptor. After washing with PBS the ImmPress Polymer Universal Detection Kit (Vector Laboratories, Burlingame, CA; Cat. No. MP-7500) was applied for 30 min in room temperature. Immune reaction was visualized by freshly prepared 3,3-diaminobenzidene (DAB) until the required intensity was achieved. The background was counterstained with hematoxylin. In some cases the intensity of the reaction was amplified by using 2 drops of nickel solution provided in the ImmPRESS Kit.

For positive controls normal human pancreata were used, where the Langerhans islands reacted strongly (Fig. 1a.) Positivity was accepted if more than 10% of the tumors cells reacted, and the strength was 2+ or 3 + .

Results

The antibody properly identified the SSTR2 in the neuroendocrine tumors. Positive staining was seen along the cell

Table 1 Clinocopathological characteristics of the SSTR2-positive neuroendocrine neoplasms

Sex	Age	Localization	Grade	Chromogranin A level
female	70	pancreas, VIPoma	G1	normal
female	70	stomach + lymph node metastasis	G1	high
male	21	small bowel + hepatic metastasis	G1	high
male	71	lung + liver + bone metastasis	G1	high
male	64	lung + multiple metastases	G2	normal
male	66	lung	G1	high
female	42	lung	G2	normal
male	52	lung + lymph node metastasis	G3	normal
female	64	small bowel + lymph node metastasis	G1	no data
female	67	lung	G2	normal
male	43	paraganglioma	G2	no data
female	70	pancreas + multiple metastases	G1	high
female	53	bowel + multiple metastases	G1	high
female	67	large bowel + multiple metastases	G2	no data
male	65	lung + brain metastasis	G3	high
male	34	lung + lymph node metastasis	G2	no data
female	65	gastrointestinal	G1	normal
male	62	rectum + lymph node metastasis	G1	high
male	43	liver + lymph node metastasis	G3	normal
female	63	lung + multiple metastases	G2	high
female	63	lung	G1	high
female	79	lung	G1	no data
female	52	breast + bone metastasis	G3	high
female	64	oesophagus	G3	high

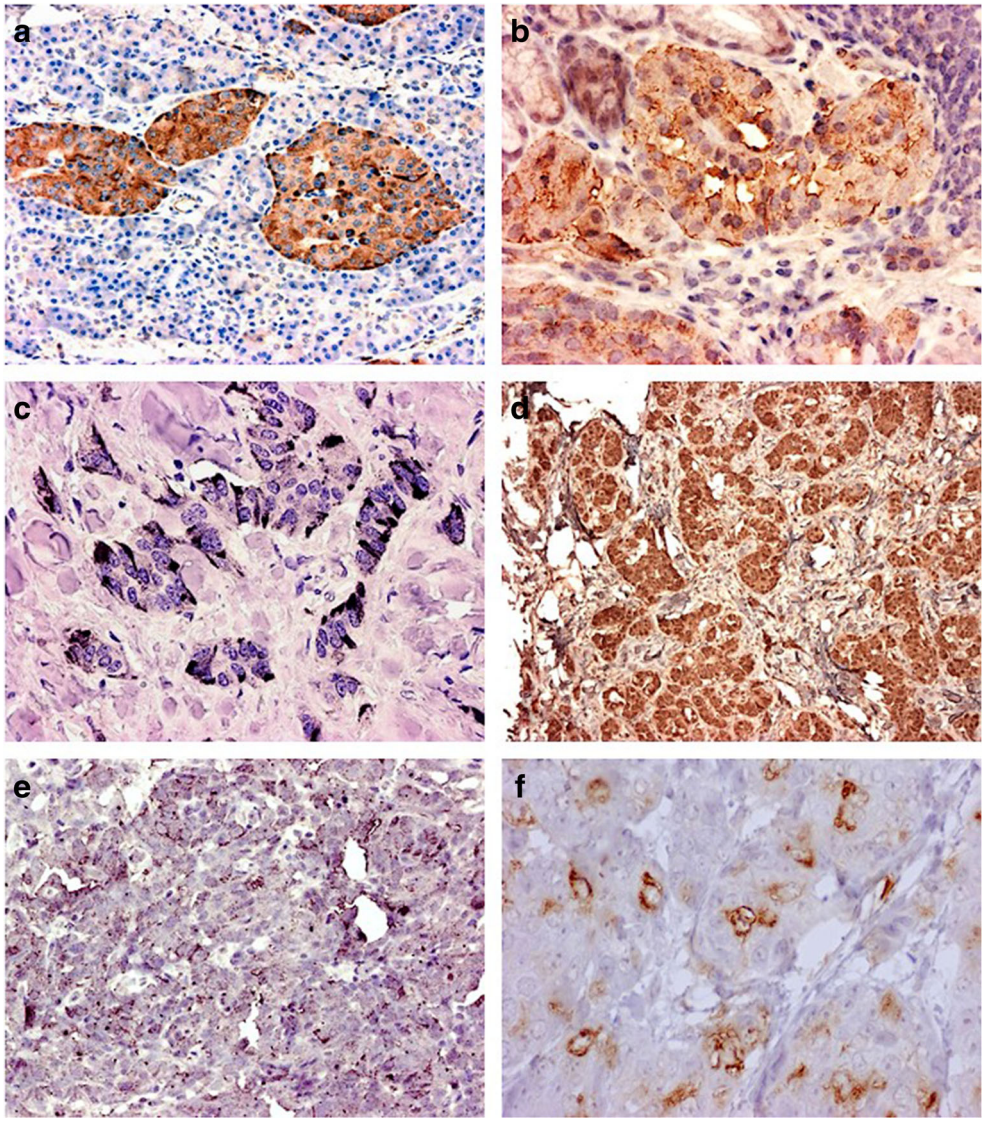

Fig. 1 Various appearances of SSTR2 receptors: A: Positive control. The pancreatic Langerhans islands are strongly stained (×200); B-F: Neuroendocrine neoplasms. B: Mainly membrane positive reaction (×400); C: Cytoplasmic positivity (Nickel-enhanced reaction, ×200); D: Mixed staining pattern (×100); E: Finely granular appearance (Nickel-enhanced reaction, ×200); F: Coarse granular reaction (×400)

membrane, in the cytoplasm, or in both locations (Fig. 1b-d). When the reaction was localized in the cytoplasm, its pattern was diffuse or finely granular (Fig. 1c-e), but in some cases the appearance was coarsely granular (Fig. 1f). No nuclear expression was observed, and the stromal elements were similarly negative. The staining intensities were unrelated to the degree of differentiation, the serum chromogranin A levels, the gender or the age, and neuroendocrine neoplasms deriving from different organs were equally positive. No differences were noted between the hormonally active (e.g. VIPoma), and the inactive (e.g. colorectal, pancreatic, esophageal, breast, bronchial) tumors.

The expression patterns proved to be variable. In most of the cases the neoplastic tissue was evenly positive. Due to the heterogeneity of the tumors, however, some areas expressed just a weak reaction. In other cases, the reaction was strong, but confined to small percent of tumor cells. This feature was especially seen in poorly differentiated ones. Because there is no universally accepted guide about the proportion of the positive cells, we have arbitrary chosen a cut-off level as 10%.

In some cases we could evaluate both the primary tumor and the metastasis from lymph node, liver or bone, and the positive reactions were identical indicating a well-retained receptor status.

Discussion

The long-acting somatostatin analogs represent a major medical treatment option in neuroendocrine neoplasms. These peptides suppress not just the hormone release but they also exert antiproliferative and antiangiogenic effects [10]. Although these analogs may also act through indirect mechanisms, the treatment success mainly depends on the specific receptors. In vivo the somatostatin receptor scintigraphies (e.g. octreoscan) can visualize primary tumors and metastases

expressing SSTR subtypes 2, 3 or 5, but their presence may be detected by Northern blot, in situ hybridization or reverse transcriptase-polymerase chain reaction (RT-PCR). The receptors can also be demonstrated in circulating tumor cells in the peripheral blood [11]. Immunohistochemical determination of SSTR2 is a useful and practical method, allowing specific localization of the receptor in the given tumor, and offering precise information to diagnostic or therapeutic decisions. For research studies, it is applicable in retrospective archival materials, too. Comparative studies have identified SSTR2 by SRS and IHC with a high rate (70–83%) of concordance [12–14], but in around 50% the immunohistochemical expression was not reinforced by receptor scintigraphy [13]. Nevertheless, in clinical practice SRS remains a method of choice in diagnostic/follow up management.

Apart from its diagnostic importance, detection of SSTR2 in neuroendocrine tumors does have a prognostic value [15]. Chinese studies claimed that the positive expression of SSTR2 and SSTR5 predicted improved survival, especially in Grade 1–2 tumors [16], but other studies could not reinforce this conclusion [15, 17]. Investigating tissue microarrays from 279 patients, *Brunner et al*. found that SSTR2 was an independent prognostic factor in NET patients [18]. High immunohistochemical expression of this receptor was associated with longer overall survival (OS), and it proved to be a stronger prognostic indicator than the Ki-67 score [15, 17]. Among SSA-treated metastatic small bowel NET patients the SSTR2 expression was associated with longer progression free survival (PFS) and OS [17].

In basal conditions somatostatin receptor type 2 is located at the cell membrane, but some stimulatory effects (e..g. somatostatin or somatostatin agonists) result in internalization to the cytoplasm [19, 20]. This process seems to be rather rapid: following 5 min of stimulation, the membrane positivity was accompanied by perinuclear expression, and after 15–30 min SSTR2 was mainly localized around the nuclei [19]. In our cases, the patients had not received any hormonal therapy before biopsy or resection, still, the localization of the immune reactions were variable. The explanation is not clear but based on international experience, is not unusual.

There is no single, universally accepted SSTR antibody. Like other immunohistochemical products, several manufacturers produce primaries for the same antigen/epitope. Before choosing the given SSTR antibody we have compared various brands, and evaluated the staining properties. Some of them were polyclonal, monoclonal, the positive reactions were localized in membranes, cytoplasm or both. In our hands the aforementioned antibody proved to be the most reliable one, the same batch is used, and we have a 10-year good experience.

The SSTR-density in neuroendocrine neoplasms is variable, not “yes-or-no”. Among others, it differs in the given tissues, strongly depends on the stage of the disease, and because of heterogeneity of tumors, their distribution is not homogeneous, therefore the too small biopsy materials may be false negative.

Although the incidence of neuroendocrine tumors is slowly increasing, these neoplasms are still relatively rare. In Hungary, for example, approximately 200–400 cases are diagnosed yearly. Therefore, centralized units are needed to detect the presence of somatostatin receptors. The Institute is one of these centers, here we present the 10-year experience. Our results show that the immunohistochemical method is a quick, effective and reliable tool, providing important information for the therapeutic decision and predicting the amenability of a tumor.

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Compliance with ethical standards

Conflict-of-interest The authors declare no conflict of interest.

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