

SSTR2A Protein Expression in Neuroendocrine Neoplasms of the Colorectum

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Background: Expression studies of somatostatin receptor type 2A (SSTR2A) in neuroendocrine neoplasms (NENs) led to the development of clinically relevant diagnostic and therapeutic strategies. However, most of these strategies used in-house-developed antibodies and were focused on lung tumors. We evaluated commercially available SSTR2A antibodies in NENs of the colorectum to observe their subcellular localization and distribution within the resected tumor. **Methods:** The immunohistochemistry of 77 NENs located in the colorectum were studied using a commercially available antibody against SSTR2A. **Results:** Most neuroendocrine tumors (NET) grade (G)1 and G2 expressed the SSTR2A in the cytoplasm with apical or luminal localization. However, all neuroendocrine carcinomas (NEC) G3 were negative for SSTR2A. **Conclusions:** Our data indicate that SSTR2A immunohistochemistry shows cytoplasmic staining with distinct subcellular localization in most NET G1 in the colorectum using a commercially available antibody. Low or no expression of SSTR2A in NET G2 and NEC G3 raises the possibility that SSTR2A may correlate with histologic differentiation and proliferative activity. Further validation studies in large case series are needed.

Key Words: Neuroendocrine neoplasm; Somatostatin receptor 2; Colorectum

Neuroendocrine neoplasms (NENs) originate primarily in the gastro-enteropancreatic (GEP) area (67.5%) and the bronchial tree (25.3%).¹ GEP-NENs belong to a spectrum of lesions with a variable degree of differentiation with various locations and disease stages.² The functional diversity of neuroendocrine cell types and their nonrandom distribution in the gut are probably the reason for the complexity of the tumors derived from them.

In the World Health Organization (WHO) 2010 classification of NENs of the digestive system, diagnoses of neuroendocrine tumors (NET) grade (G)1, NET G2, and neuroendocrine carcinoma (NEC) G3 were applied based on proliferative activity.³ A NET is defined as a well differentiated NEN composed of cells with features similar to those of the normal gut endocrine cells expressing general markers of neuroendocrine differentiation with a low number of mitoses; G1 and G2 are defined according to proliferation fraction and histology (G1: mitotic count, < 2 per 10 high power fields (HPF) and/or ≤ 2% Ki67 index; G2: mitotic count 2-20 per 10 HPF and/or 3-20% Ki67 index).³ A NEC G3 is a poorly differentiated, high grade malignant neoplasm composed of small cells or large to intermedi-

ate cells, diffusely expressing the general markers of neuroendocrine differentiation with marked nuclear atypia, multifocal necrosis, and a high number of mitoses (> 20 per 10 HPF).³

A site specific staging system is combined with grading to improve prognostic strength.³ The American Joint Committee on Cancer (AJCC) has recently published a new tumor, node and metastasis staging manual that includes NENs of all anatomic sites; the staging criteria for both systems rely predominantly on the size of the tumor and the extent of invasion into similar landmarks as used for the staging of non-NEC of the same site.⁴

Several neuroendocrine markers and hormonal receptors with diagnostic, prognostic, and therapeutic implications have been described in NENs. Among them are somatostatin receptors (SSTRs), which are a family of five widely distributed G protein coupled receptors that mediate different intracellular signaling pathways involved in cell proliferation, differentiation, and angiogenesis.⁵ For therapeutic purposes, synthetic somatostatin analogues have been produced, with octreotide and lanreotide being the most widely employed in clinical practice. Their high receptor binding affinities (especially for SSTR2, SSTR3, and

SSTR5) are the bases for both diagnostic (scintigraphy or positron emission tomography scans) and therapeutic procedures.⁶

GEP-NENs express SSTRs in 80-100% of cases.⁷ Expression of SSTRs depends on the tumor stage of differentiation; well differentiated GEP-NENs express higher levels and more types of SSTRs (most frequently SSTR2, followed by SSTR1, SSTR5, and SSTR3) than poorly differentiated tumors.⁸ Various methods to detect SSTR in tumor specimens were reported, including reverse transcription polymerase chain reaction, *in situ* hybridization (ISH), immunoblotting, and immunohistochemistry (IHC).² IHC is a desirable alternative to *in vitro* SSTR evaluation because it is a cheap and reproducible procedure and quick results can be obtained from formalin fixed tissue in routine pathology laboratories.⁹ Commercially available antibodies specific to the different receptor subtypes are currently available, but still need to be validated in clinical practice.² SSTR2A is the most important receptor subtype because of its high over-expression in NENs, as demonstrated by mRNA expression, and its high affinity for the clinically available somatostatin analogs.¹⁰ Although SSTR2A has been detected in NENs by IHC, there are no systematic data on receptor status and expression patterns of SSTR2A in NENs of colorectum where surgical and/or biopsy material were available.¹¹ In this study, we evaluated commercially available SSTR2A antibodies in NENs occurring in the colorectum, to observe their subcellular localization and distribution within the resected tumor.

MATERIALS AND METHODS

Case selection

A series of 77 NENs of the colorectum (encountered from 2004-2008), treated in Samsung Medical Center was selected

according to the following criteria: 1) revised histopathological diagnosis, 2) tissue material available for pathological review, and 3) IHC; all surgical samples were fixed in formalin. All cases have been reviewed and diagnosed according to the 2010 WHO classification of endocrine tumors. They series included 69 NET G1, 6 NET G2, and 2 NEC G3 from the colorectum. In all cases, clinicopathologic information including sex, age, tumor location, surgical procedure, primary tumor size, nodal status, as well as stage and site of distant metastases, was collected (Table 1). All cases with lymph node metastasis and distant metastasis were pathologically confirmed.

Immunohistochemical staining

Four- μ m thick sections were cut from paraffin blocks, followed by deparaffinization and rehydration. An SSTR2A antibody (1 : 200, AB9486, Millipore, Bedford, MA, USA) was applied after heat-induced antigen retrieval with Bond Epitope Retrieval Solution (Leica Microsystems, Wetzlar, Germany).¹² This rabbit antibody against SSTR2A was raised against a synthetic peptide corresponding to the 2nd extracellular domain of human SSTR2. Immunostaining was performed using the Leica BOND-MAXTM system (Leica Biosystems) with diaminobenzidine as a chromogen.

Evaluation of staining pattern

The IHC results were semi-quantitatively analyzed. The proportion of positive cells and the staining intensity were assessed. The staining intensity was scored as follows: 3+ (dark staining easily visible with low power magnification), 2+ (medium staining visible with low power magnification), 1+ (pale staining not easily seen with low power magnification), and 0 (absence of immunoreactivity).

Table 1. Clinicopathologic findings in patients with colorectal neuroendocrine neoplasms

WHO 2010 classification	No. of cases	Age (mean, yr)	Sex		Size (mean, cm)	AJCC prognostic group		Cases with LN metastasis	Cases with distant metastasis
			Male	Female					
NET G1	69	56	43	26	0.6	Stage I	69	0	0
NET G2	6	56	3	3	2.3	Stage IIA	1	4	2
						Stage IIIA	1		
						Stage IIIB	2		
						Stage IV	2		
						Stage IIB	1		
NEC	2	51	2	0	6.8	Stage IIIB	1	1	0
						Stage IIIB	1		

WHO, World Health Organization; AJCC, American Joint Committee on Cancer; LN, lymph node; NET, neuroendocrine tumor; G, grade; NEC, neuroendocrine carcinoma.

RESULTS

Positive immunostaining for SSTR2A was found in 67 of 69 (97%) NET G1, and 1 of 6 (17%) NET G2 (Table 2). Of note, in the vast majority of positive tumors, SSTR2A was localized in the cytoplasm of tumor cells. In cases scoring 1+ ($n=8$), the staining was relatively non-specific with diffuse cytoplasmic localization. In cases scoring 2+ ($n=46$) and 3+ cases ($n=14$), specific apical or luminal localization was observed (Fig. 1). In positive cases, the staining was diffuse and homogenous throughout the tumor.

Table 2. Results of immunohistochemistry for somatostatin receptor type 2A in the neuroendocrine neoplasms of the colorectum

WHO 2010 classification	Staining intensity			
	Negative	Positive		
	0	1+	2+	3+
NET G1	2	7	46	14
NET G2	5	1		
NEC	2			

WHO, World Health Organization; NET, neuroendocrine tumor; G, grade; NEC, neuroendocrine carcinoma.

In 9 (11.7%) colorectal NENs, SSTR2A was totally negative; 2 were NET G1, 5 were NET G2; and the remaining 2 were NEC G3 (Fig. 2). According to the AJCC staging system, all 69 NET G1 were stage I. Six NET G2 patients were composed of 1 stage IIA, 1 stage IIIA, 2 stage IIIB,

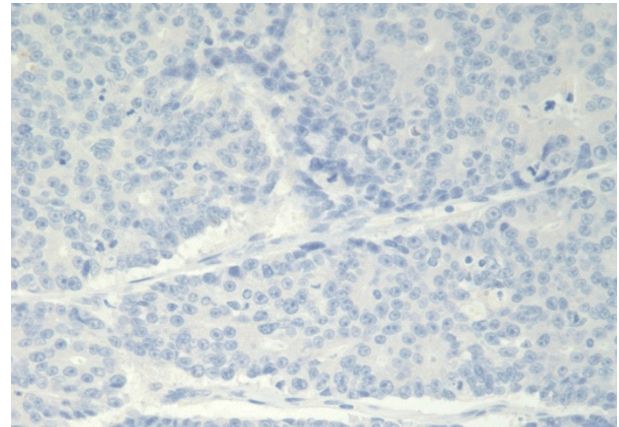


Fig. 2. Neuroendocrine carcinoma, poorly differentiated, and high grade are totally negative for somatostatin receptor type 2A.

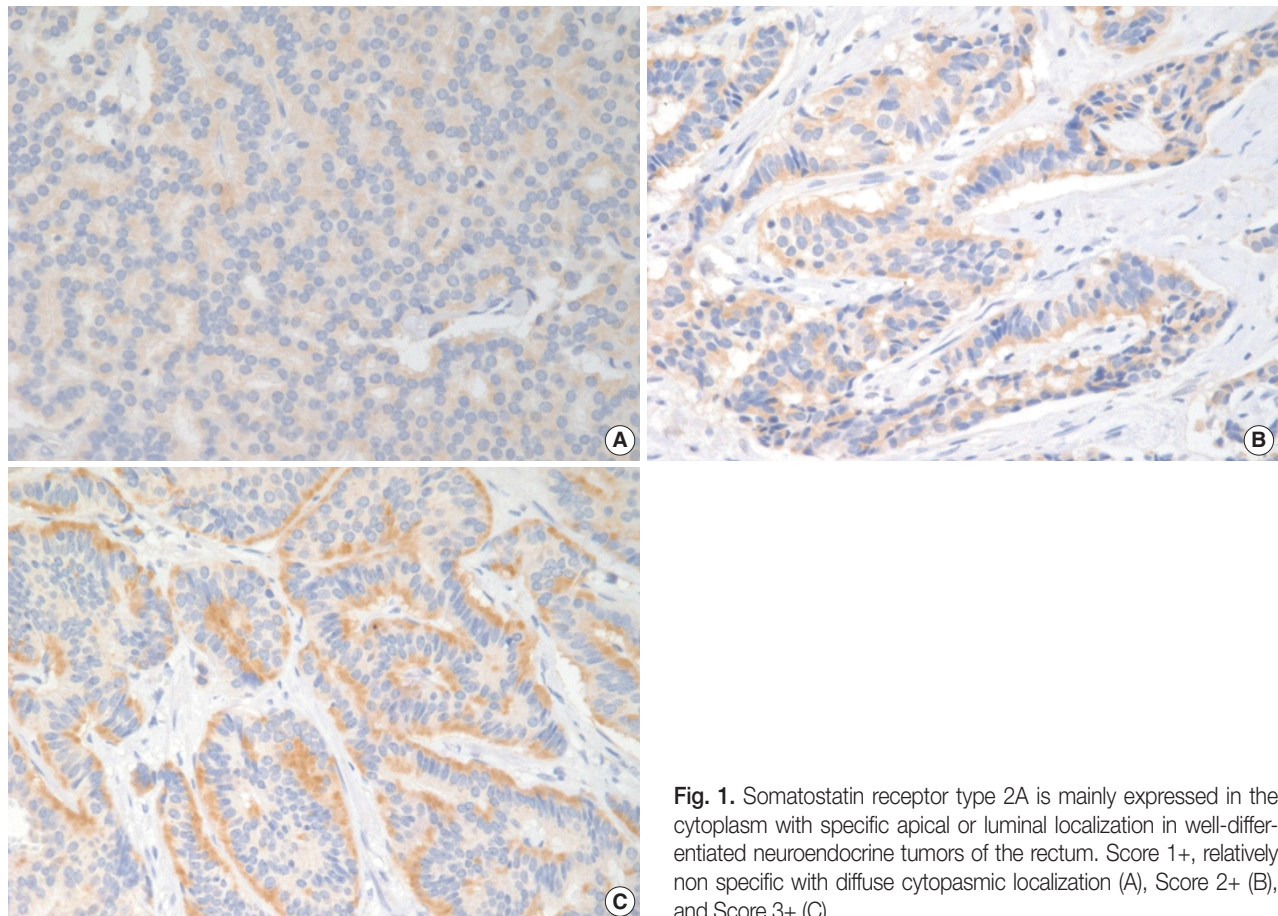


Fig. 1. Somatostatin receptor type 2A is mainly expressed in the cytoplasm with specific apical or luminal localization in well-differentiated neuroendocrine tumors of the rectum. Score 1+, relatively non specific with diffuse cytoplasmic localization (A), Score 2+ (B), and Score 3+ (C).

and 2 stage IV. Among 6 NET G2 cases, only one was immunoreactive for SSTR2A and this case was clinically stage IIIB. Two NEC G3 patients were stage IIB and stage IIIB each.

DISCUSSION

In the present study, we used a commercially available antibody against SSTR2A and systematically analyzed its presence, cellular localization, and distribution in a large series of colorectal NENs consisting of low to high-grade tumors. We have shown that most NENs express the SSTR2A protein. However, this is the first study using commercially available SSTR2A antibody.

Many somatostatin target tissues, as well as many NENs often originating in these target tissues, have been shown to express SSTRs.^{13,14} The expression of SSTR in NENs has been widely investigated and has led to the development of clinically relevant diagnostic and therapeutic strategies.⁵ Although the presence of SSTR mRNAs has been examined in various tumors using costly methods such as ISH, there is little data characterizing the expression pattern of individual SSTR subtypes in NENs at the translation level.¹¹ SSTR2A is the first SSTR subtype to be assessed in a diagnostic pathology laboratory with IHC. Among the SSTR subtypes, SSTR2A is the most important because it is predominantly targeted by synthetic somatostatin analogs in current clinical practice. IHC may become more important for other SSTR subtypes when a new generation of somatostatin analogs, which have high affinities for multiple SSTR subtypes, become available for clinical use in the future. The availability of an adequate, commercially available SSTR2A antibody for IHC allows for the possibility of routine SSTR2A assessment in formalin fixed tumor tissue in pathology laboratories. In fact, SSTR2A IHC may represent an alternative to receptor autoradiography when no fresh frozen tumor tissue is available on the basis of a good matching of immunohistochemical and autoradiographic results, with respect not only to SSTR2A expression in tumors in general, but in particular, the proportion of SSTR2A-positive tumor cells and SSTR2A density.⁹

In a recent study by Janson *et al.*,¹⁵ their own SSTR2 antibodies were stained along the cytoplasmic membrane in human NENs. In contrast, our study, which used a commercially available antibody, found that SSTR2A IHC revealed cytoplasmic expression. These observations are consistent with the previous SSTR2A expression in cultured cells; internalized SSTR2A were

localized in specific compartments including the late endosomes, rather than diffusely in the cytoplasm.¹⁶ These observations suggest that in well differentiated, SSTR2A may be functionally integrated in the apical portion of the cytoplasm and may represent either neosynthesized or internalized receptors, as suggested previously.^{17,18} It would, however, be difficult to consider all the cytoplasmic receptors detected by IHC in our study to be truly internalized receptors, as there is not always enough local somatostatin to act as an agonist trigger.¹⁹ The membranous staining pattern observed in a previous study using an in-house-developed antibody, R2-88, also showed subcellular localization in the cytoplasm. In many tumors, SSTR2A IHC reveals not only cell membrane staining, which was consistent with the established cellular membrane bound expression of SSTR2A, but also cytoplasmic staining, in many cases in the presence of positive autoradiography.⁹

In previous studies of receptor autoradiography, a low percentage of NENs were shown to be receptor-negative.⁸ It is intriguing to note that most of the negative cases were NENs of the lung, whereas only one ileal NEN was negative.⁸ Another characteristic of SSTR-negative NENs is their higher grade of dedifferentiation or even anaplastic appearance.⁸ SSTR expression is not related to tumor stage or outcome, but is related to tumor grade, with high-grade NEC G3 having a reduced SSTR content.²⁰ In our study, high grade NET, NEC, and 5 of 6 NET G2, were also completely negative for SSTR2A. These findings suggest that only well differentiated and low grade NETs retain the capacity to express SSTRs. All malignant NETs with node metastasis or distant metastasis were negative for SSTR2A.

In a previous study, IHC for SSTR2A revealed heterogeneous distribution, i.e., small groups of SSTR2A-negative tumor cells amid receptor positive tumor cells.⁹ However, in our study, immunoreactivity was homogenous throughout the tumors. Determinations of which patterns are correct will stem from clinical studies on large series of NENs treated with somatostatin analogues. As SSTR-targeted diagnoses and therapies increase, the number of tumors that need to be assessed for their SSTR expression will increase.

In conclusion, our data indicate that IHC represents a reliable and useful method for characterizing SSTR2A expression in NENs, particularly those of colorectal origin. By performing the first study using a commercially available antibody, we found cytoplasmic staining with distinct subcellular localization in most NENs in the colorectum. No or low expression of SSTR2A in intermediate and high grade NET raises the possibility that SSTR2A IHC may be related to proliferative activity and

differentiation of the tumor.

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