ORIGINAL ARTICLE

Immunohistochemical expression of somatostatin type 2A receptor in neuroendocrine carcinoma of uterine cervix

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Abstract

Introduction Somatostatin and its analogues inhibit the growth of various kinds of endocrine and exocrine cells via the somatostatin receptor (SSTR). Expression of SSTR2A has been reported in many well-differentiated neuroendocrine tumors and small numbers of poorly differentiated neuroendocrine carcinomas. SSTR2A-positive neuroendocrine tumors may be treatable by SS analogues. Uterine cervical neuroendocrine carcinoma is generally associated with poor prognosis.

Materials and methods We examined the expression of SSTR2A and other neuroendocrine markers [neural cell adhesion molecule (NCAM), chromogranin A, and synaptophysin] by immunohistochemical methods in seven neuroendocrine tumors of the uterine cervix: five small cell carcinomas and two large cell neuroendocrine carcinomas.

Results SSTR2A was expressed in one small cell carcinoma and two neuroendocrine large cell carcinomas. The cell membrane of cells in these carcinomas showed strong immunohistochemical reactivity for SSTR2A. SSTR2A-positive cases are also frequently positive for the expression of chromogranin A. Treatment with SSTR2A may be effective for endocrine tumors. The results indicate the usefulness of SSTR2A analogs for the treatment of some uterine neuroendocrine carcinomas.

Keywords Somatostatin receptor2A (SSTR2A) · Neuroendocrine tumor · Uterine cervix · Immunohistochemistry

Introduction

Well-differentiated neuroendocrine tumors, such as carcinoid tumors and pancreatic endocrine tumors, generally have a good prognosis. However, poorly differentiated endocrine carcinomas, such as small cell lung cancer, medullary carcinoma, and uterine neuroendocrine carcinomas have distant metastases in the liver, lung, or bone; neither chemotherapy nor irradiation therapy is very effective in treating them. Neuroendocrine carcinoma of the uterine cervix has been described as the tumor with the worst prognosis. The mean survival time for patients with this carcinoma has been reported to be 32.3 months [1].

Somatostatin has a broad spectrum of biological actions; exerts suppressive effects on multiple organs, including the brain, pituitary, gut, exocrine and endocrine pancreas, adrenals, thyroid, and kidneys; and appears to be an endogenous growth inhibitor [2].

The diverse biological effects of somatostatin are mediated by a family of G protein-coupled receptors [namely, somatostatin receptors (SSTRs)] that are encoded by five non-allelic genes located on separate chromosomes. The receptors can be further divided into 5 subtypes. This review focuses on the molecular pharmacology and function of these receptors, with particular emphasis on the

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ligand-binding domain, subtype-selective analogs, agonist-dependent receptor regulation, desensitization responses, subtype-specific effector coupling, and the signal transduction pathways responsible for inhibiting cell secretion and cell growth or induction of apoptosis [3].

Although there have been investigations on the localization of SSTR by autoradiography, PCR, and in situ hybridization (ISH) [4–6], few studies have examined the cellular localization of the receptor subtype proteins in neuroendocrine tumors, especially in metastatic or highly malignant neuroendocrine tumors [2, 7, 8].

Uterine cervical endocrine carcinoma is rare. Therefore, there are few reports on the expression of SSTR2A in uterine cervical endocrine carcinoma.

In the present study, we examined the expression of SSTR2A in uterine cervical neuroendocrine cancers.

Materials and methods

The majority of the specimens investigated were surgically obtained, and the findings were noted in the records of the Tokai University Hospital. The seven neuroendocrine carcinomas of uterine cervix examined included five small cell carcinomas and two large cell neuroendocrine carcinomas. Two of the former carcinomas were accompanied with squamous cell carcinoma and adenosquamous cell carcinoma. In five of all the seven cases, there was a distant metastasis and death from the disease within 2 years after the operation, except in two current cases (Table 1).

Immunohistochemistry

The tissues were fixed by immersion in 4% buffered formaldehyde and embedded in paraffin according to standard procedures. These tissues were subjected to the following studies. For immunohistochemical staining, 4-μm sections were deparaffinized and rehydrated. Blocking of endogenous peroxidase was done by pretreating the sections by methanol containing 10% H_2O_2 for 30 min. The primary antibodies that were used, the procedure for their dilution, and the method of antigen retrieval from the sections are described in Table 2. The immunohistochemical staining for SSTR2A was carried out by using the Envision/HRP kit (DAKO). The nuclei were slightly counterstained with hematoxylin. To reveal the colocalization of immunoreactive SSTR2A and other neuroendocrine markers (namely, neural cell adhesion molecule (NCAM), chromogranin A, synaptophysin), serial sections were cut, and pairs of adjacent sections were examined (Table 3).

For the positive control specimens of SSTR2A, we used normal pancreas tissues obtained by autopsy, fixed by 10% buffered formalin, and embedded in paraffin.

Evaluation of immunostaining

The results of immunostaining for SSTR2A and other neuroendocrine markers were evaluated. When less than 25% cells were positively stained, the results were classified as +; when 25–50% cells were stained positively, they were

Table 1 Profile of present cases

Case	Age (years)	Histopathology	pTNM stage	Metastatic organs	Outcome (post-operative months)
1	73	Small + squamous	T3aN1	Liver, lung, brain	DOD 18M
2	23	Small	T2aN1	Liver, bone	DOD 11M
3	37	Large cell neuroendocrine	T1b2N1	Thyroid, breast, bone, peritoneum	DOD 21M
4	55	Large cell neuroendocrine	T2aN0	Liver, lung, bone	DOD 12M
5	51	Small	T2bN0	Liver, adrenal, bone, peritonrum	DOD 16M
6	56	Small + adenosquamous	T1b1N0	Ascites	AWD*
7	50	Small	T3bN1MA	–	AWD*

DOD death of disease, *AWD* alive with disease

* Postoperative observation is less than 6 months

Table 2 Primary antibodies and method of antigen retrieval

	Code or clone	Manufacturer	Dilution	Antigen retrieval
SSTR2A	ss-800	Gramsch	1:500	Boil used by citrate buffer/pH 6.0
NCAM	1B6	Novo castra	1:50	Boil used by citrate buffer/pH 6.0
Chromogranin A	Poly	Invitrogen	1:1	–
Synaptophysin	N1566	DAKO	1:50	1% trypsin

Table 3 Result of immuno-staining

Case	Endocrine component				Non-endocrine components			
	SSTR2A	NCAM	CGA	SYN	SSTR2A	NCAM	CGA	SYN
1	−	−	+	+++	−	−	−	−
2	−	+++	+	+++				
3	+++	+++	++	+++				
4	+	−	+++	+++				
5	−	++	−	+				
6	+	+++	++	+	−	−	−	−
7	−	+++	+	++				

Positive rate of expressed neuro-endocrine tumor cells, − negative, + less than 25%, ++ 25–50%, +++ more than 50%

CGA chromogranin A, *SYN* synaptophysin

classified as ++; and when more than 50% cells were stained positively, they were classified as +++.

Results

Neuroendocrine carcinomas showed immunohistochemical expression of NCAM in five cases, that of chromogranin A in six cases, and that of synaptophysin in all the seven cases. In one of the five cases of small cell carcinoma and both the cases of large cell neuroendocrine carcinomas, positive staining for SSTR2A was observed. SSTR2A-positive cases also frequently expressed chromogranin A. In a case where high SSTR2A expression was noted, other endocrine markers (NCAM, chromogranin A, and synaptophysin) were also frequently expressed. In the two mixed tumors comprising of small cell carcinoma and non-neuroendocrine carcinoma (squamous cell carcinoma or adenosquamous carcinoma), SSTR2A was not expressed in the non-neuroendocrine components. SSTR2A was detected on the cell membrane (Figs. 1 and 2).

Discussion

Uterine cervical neuroendocrine tumors are histopathologically classified into four groups: carcinoid tumors, atypical carcinoid tumors, small-cell carcinomas, and large-cell neuroendocrine carcinomas [9]. These tumors arise in reserve cells of the basal epithelial layer, as well as squamous cell carcinoma and adenocarcinoma. Therefore, mixed tumors of small cell carcinoma and squamous or

Fig. 1 Large-cell neuroendocrine carcinoma (case 3). Tumor cells are frequently positive for SSTR2A (**b**), chromogranin A (**c**) and NCAM (**d**)
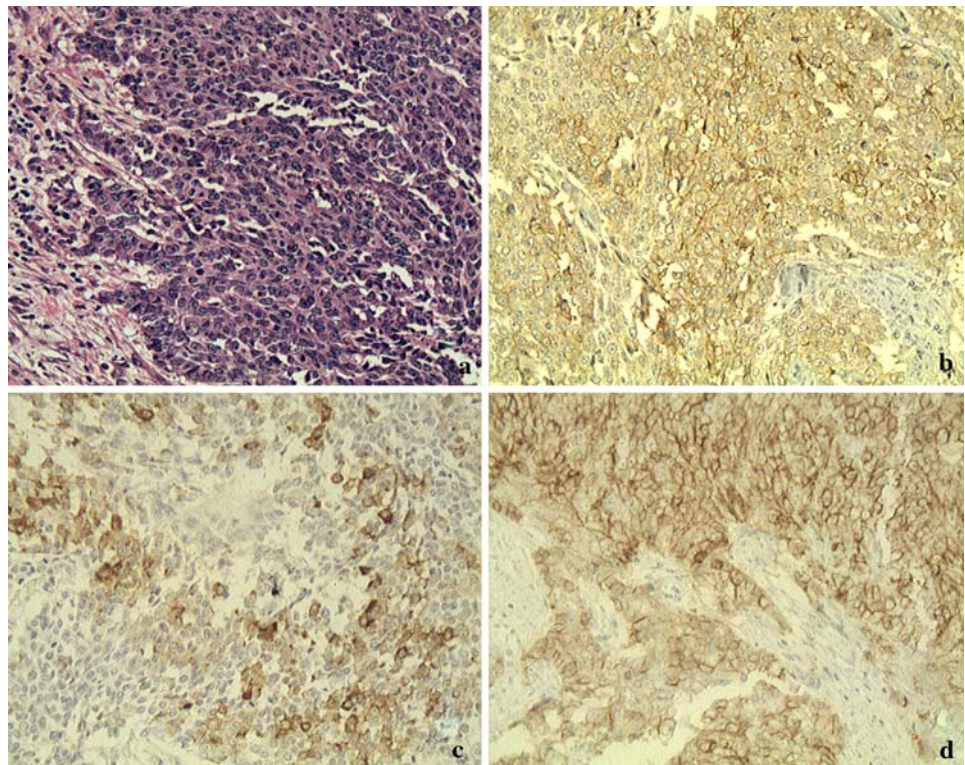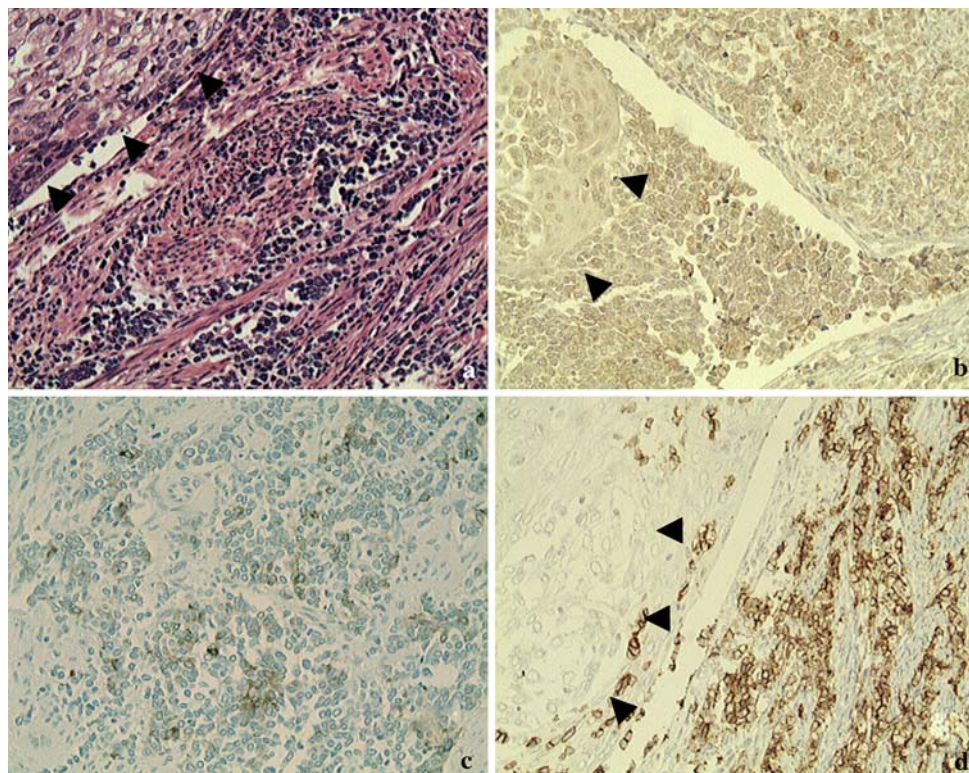
Fig. 2 Small-cell carcinoma and squamous cell carcinoma (case 6). In the small-cell carcinoma component, tumor cells are positive for SSTR2A (**b**), chromogranin A (**c**), and NCAM (**d**). In the squamous cell component (*arrow head*), tumor cells are negative for SSTR2A and NCAM

adenocarcinoma are rarely observed. Neuroendocerine carcinomas of uterine cervix have been known to detect common neuroendocrine markers (NCAM, chromogranin A and synaptophysin) [10, 11].

Neuroendocrine tissues and their tumors have been found to express SSTR by SSTR autoradiography or ISH of SSTR mRNA [5, 6, 12]. Reubi et al. comprehensively investigated the expression of SSTR mRNA in human tumors by using receptor autography. By using an immunohistochemical method, Kimura et al. [2] found that SSTR is expressed in many well-differentiated neuroendocrine carcinomas and some poorly differentiated neuroendocrine carcinomas. The tumors were characterized as having receptors with a high affinity for the synthetic analogue octreotide [13]. Janson et al. [6] reported that the expression of SSTR2 mRNA detected using ISH correlates with therapeutic outcome in patients with carcinoid tumors treated with somatostatin analogs.

However, to our knowledge, there have been no immunohistochemical studies of SSTR2A in poorly differentiated neuroendocrine carcinomas of the uterine cervix. As the numbers of cases studied with the report are rather limited, therefore is should be mentioned that the current study is a pilot study and its results should be confirmed in an extended study containing larger number of cases. Our study showed that SSTR2A is the useful neuroendocrine markers, as well as NCAM, chromogranin A and synaprophysin, in the uterine cervical neuroendocrine carcinomas like other neuroendocrine tumors.

A number of somatostatin analogs have been used for the treatment of acromegaly and endocrine tumors of the gastroenteropancreatic system [14, 15]. Most of these tumors and cell lines express SSTR2 [13, 16], and SSTR5 mRNA is also expressed in gastroenteropancreatic tumors [17]. The activation of a tyrosine phosphatase and the inhibition of calcium mobilization mediated by SSTR2 and SSTR5, respectively, could be important steps in the negative mitogenic signal induced by a somatostatin analog [17]. Janson et al. [6] compared the efficacy of the SSTR2 mRNA expression and the response to treatment with SS analog in patients with carcinoid tumors. They concluded that there was a complete agreement between the presence of SSTR2 mRNA detected using ISH and a positive therapeutic outcome. Recently, they conducted an immunohistochemical study on SSTR2 in the above tumors and obtained the same results, and concluded that immunohistochemical methods are applicable in clinical practice [15]. Although there were no cases of treatment with somatostatin analogs in the present study, we would like to refer to the possible utility of somatostatin analogs for treating not only carcinoid tumors but also malignant neuroendocrine tumors, including metastatic pheochromocytoma, neuroendocrine carcinoma, and infiltrating ganglioneuroma, for which there are no effective treatments at present. Further practical investigations of using somatostatin analogs for treating patients with such tumors should be conducted.

The prognosis of uterine cervical neuroendocrine cancers is poor, and no chemotherapy has been known to be

effective in treating these. One study used a combined chemotherapy, including a somatostatin analog (octreotide), for treating uterine cervical atypical carcinoid tumors [18]. However, the effect has not been confirmed. Somatostatin analogs can be expected effective for SSTR2A-positive uterine cervical neuroendocrine neoplasm in future. In addition, in uterine cervix cancers of mixed histological type comprising of neuroendocrine and non-neuroendocrine components, it seemed that a combination of somatostatin analogs and conventional chemotherapy was necessary.

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