Expression of Somatostatin Receptor (SSTR) Subtypes (SSTR-1, 2A, 3, 4 and 5) in Neuroendocrine Tumors Using Real-time RT-PCR Method and Immunohistochemistry

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Molecule targeting therapy using somatostatin (SS) analogues has become a widely accepted modality to treat neuroendocrine tumors (NETs), particularly gastrointestinal (GI) and pancreatic endocrine tumors. On the other hand, little is known about the expression of somatostatin receptor (SSTR) subtypes in neuroendocrine carcinomas (NECs). We investigated the expression of SSTR subtypes (SSTR-1, 2A, 3, 4 and 5) using real-time reverse transcription polymerase chain reaction (RT-PCR) method and immunohistochemistry in 32 neuroendocrine neoplasms (9 NET G1, 2 NET G2, 18 NECs G3 and 3 mixed NEC G3) of various primary sites. Expression of more than two SSTR subtypes was detected in all neuroendocrine neoplasms examined. Expression of SSTR-2A mRNA was significantly higher than other subtypes. In addition, mRNA expression of SSTR-3 and SSTR-5 was significantly low or below the detection level except for gastroduodenal NET G1. No significant difference of the expression of SSTR subtypes was observed between the NET and NEC groups. The expression of protein and mRNA was generally well correlated. In conclusion, NECs would be a good candidate for molecule targeting therapy using SS analogues, and the expression of SSTR-2A can be useful as a biomarker of neuroendocrine differentiation.

Key words: neuroendocrine tumor (NET), neuroendocrine carcinoma (NEC), somatostatin receptor, real-time RT-PCR, immunohistochemistry

I. Introduction

It is well known that neuroendocrine tumors (NETs) can develop in almost all tissues or organs in the body, and are characterized by a wide range of histological appearances and biological behavior [9, 12, 17]. While the nomenclature of neuroendocrine neoplasms has changed several times in the past [1, 4–6, 9], the recent WHO classification of the neuroendocrine neoplasms of gastro-entrapancreatic (GEP) system puts them into three major categories: neuroendocrine tumor (NET) G1, G2 and neuroendocrine carcinoma (NEC) G3, and others including mixed adenoneuroendocrine carcinoma, some rare tumors of special types and hyperplastic/preneplastic lesions, based on histological differentiation (well differentiated and poorly differentiated), proliferative activity (G1, G2 and G3) and TNM factors (size, infiltration/invasion, metastasis) [2]. NET G1 and NET G2 correspond to what were formerly called well differentiated endocrine tumor (WDET) and well differentiated endocrine carcinoma, respectively. NEC G3 is nearly the same as poorly differentiated endocrine

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carcinoma (PDEC) and highly malignant [2]. Nowadays, this classification seems to be gradually accepted in the neuroendocrine neoplasms of other primary sites besides GEP system.

From the standpoint of molecule targeting therapy for NETs, somatostatin (SS) analogues have been applied to recurrent and/or metastatic diseases of well differentiated NETs and well differentiated NECs, because such tumors express somatostatin receptor (SSTR) subtypes on their cell membrane and/or in cytoplasm, particularly gastrointestinal (GI) tract primary and pancreatic islet cell tumors [7, 11]. In addition, previous clinical studies showed preventive effect on tumor growth and symptoms due to hormone over-secretion even in inoperable cases of NETs and NECs [8, 19, 21]. Furthermore, the potential clinical utility of SS analogue therapy was pointed out in the hepatic metastasis of neuroendocrine tumors with high expression of SSTR subtypes [14].

Up to now, 5 subtypes of SSTR (SSTR-1, 2A, 3, 4, and 5) have been cloned and characterized [18, 22, 23]. SSTR-2A is known to be closely related with regulation of hormone synthesis and secretion, and control of cell cycle including cell proliferation and apoptosis induction [20]. Although the affinity of SS analogues to SSTR subtypes is quite different, the highest affinity to SSTR-2A and the quite low affinity to SSTR-4 have been demonstrated [3]. On the basis of such clinical background, it is important to know the precise expression status of SSTR subtypes in NETs and NECs. Therefore, we investigated the expression status of SSTR subtypes in NET G1, G2, and NEC G3 of various primary sites, using real-time RT-PCR method and immunohistochemistry.

II. Materials and Methods

Neuroendocrine tumors and neuroendocrine carcinomas

Thirty-two neuroendocrine neoplasms were selected from the surgical pathology files of the Pathology Division, Nihon University Itabashi Hospital. All the diagnosis of the selected materials were confirmed by more than two pathologists on the basis of H-E histopathology and positivity for more than two of the following neuroendocrine markers, such as synaptophysin, chromogranin A, CD56, and neuron specific enolase (NSE) on the previously immunostained sections. At the selection of neuroendocrine neoplasms, we used a criterion of more than 50% immunoreactivity of tumor cells in the previously stained slides of at least two markers mentioned above. The selected tumors were reclassified into NET G1, NET G2, NEC G3, and mixed NEC according to the new WHO classification. All the tissue samples were handled according to the Ethical Guidelines for Clinical Studies (July 30, 2003, amended December 28, 2004, Ministry of Health, Labour and Welfare), and the study was approved by the Ethical committee, Nihon University Itabashi Hospital for clinical investigation using human material. Summary of the pathology information of the material is shown in Table 1.

| Table 1. Summary of the pathology profiles of surgical materials |
| --- |
| WHO classification | Original pathological diagnosis |
| --- | --- |
| 1 NET G1 | carcinoid tumor |
| 2 NET G1 | carcinoid tumor |
| 3 NET G1 | carcinoid tumor |
| 4 NET G1 | carcinoid tumor |
| 5 NET G1 | carcinoid tumor |
| 6 NET G1 | carcinoid tumor |
| 7 NET G1 | carcinoid tumor |
| 8 NET G1 | carcinoid tumor |
| 9 NET G1 | carcinoid tumor |
| 10 NET G2 | atypical carcinoid tumor |
| 11 NET G2 | atypical carcinoid tumor |
| 12 NEC G3 | small cell carcinoma |
| 13 NEC G3 | small cell carcinoma |
| 14 NEC G3 | neuroendocrine carcinoma |
| 15 NEC G3 | neuroendocrine carcinoma |
| 16 NEC G3 | small cell carcinoma |
| 17 NEC G3 | small cell carcinoma |
| 18 NEC G3 | large cell neuroendocrine carcinoma |
| 19 NEC G3 | large cell neuroendocrine carcinoma |
| 20 NEC G3 | large cell neuroendocrine carcinoma |
| 21 NEC G3 | large cell neuroendocrine carcinoma |
| 22 NEC G3 | thymic neuroendocrine carcinoma |
| 23 NEC G3 | small cell carcinoma |
| 24 NEC G3 | neuroendocrine carcinoma |
| 25 NEC G3 | carcinoma with NE differentiation |
| 26 NEC G3 | non-invasive neuroendocrine carcinoma |
| 27 NEC G3 | invasive ductal carcinoma with NE differentiation |
| 28 NEC G3 | neuroendocrine carcinoma |
| 29 mixed NEC G3 | invasive ductal carcinoma with NE differentiation |
| 30 mixed NEC G3 | mucinous carcinoma with NE differentiation |
| 31 NEC G3 | carcinoma with NE differentiation |
| 32 mixed NEC G3 | combined squamous cell and small cell carcinoma |

NET: neuroendocrine tumor, NEC: neuroendocrine carcinoma.

Immunohistochemistry for SSTR subtypes

Immunohistochemistry was carried out on 10% formalin fixed paraffin embedded tissue sections using EnVision/HRP-labelled polymer system (DAKO, Tokyo, Japan) and an autostainer (Histostainer, Nichirei Biosciences Inc., Tokyo, Japan). For antigen retrieval, dewaxed 4 µm tissue sections were immersed in citrate buffer pH 6.0 and boiled in water bath for 40 min at 95°C and cooled down at room temperature. After washing several times in
PBS pH 7.2, the sections were processed for quenching the endogenous peroxidase activity with 0.3% hydrogen peroxide and for blocking the non-specific binding with 1% goat serum. Sections were then processed in the usual manner, and incubated with primary antibodies of anti-SSTR-1, 2A, 3 and 5 (Gramsch Laboratories, Germany) at the working dilution of 1:400 to 1:1000 for 30 min at room temperature. For the positive control of SSTR immunohistochemistry, we used normal pancreas islets. For Ki 67 immunohistochemistry, we used anti-human Ki 67 mouse monoclonal antibody (clone MIB-1, DakoCytomation, Denmark) and LSAB method (Histofine SAB-PO (M) kit, Nichirei Biosciences Inc., Japan). Negative controls were done by omitting the specific primary antibodies and processed in the same way. The tissue-bound HRP activity was visualized by immersing the sections in 0.005% 3,3′-diaminobenzidine tetrahydrochloride (DAB) in PBS containing hydrogen peroxide (10 μl/150 ml DAB solution). After the completion of the immunohistochemical process, the sections were stained lightly with hematoxylin, and processed and mounted in the usual manner.

**Laser assisted microdissection for tissue sections**

Eight-μm thick paraffin sections were mounted on the membrane film-coated slide glasses. After dewaxing with xylene, the sections were stained lightly with toluidine blue, then the target tumor areas were microdissected using a laser assisted microdissection system (PALM MBII-N, Zeiss, Germany) by ultraviolet laser beam under a light microscope. The microdissected target tumor cells were retrieved precisely into an Eppendorf lid with mineral oil. Details of the procedure of laser assisted microdissection have been described elsewhere [15].

**Total RNA extraction from the tumor tissue**

Target tumor cell sample was mixed with 200 μl of denaturing buffer containing with 2% SDS, 0.1 mM EDTA, 10 mM Tris-HCl. They were incubated at 55°C with proteinase K until sections were dissolved completely. Total RNA was purified with 20 μl 2M sodium acetate (pH 4.0), 220 μl citrate saturated phenol (pH 4.3), and 60 μl chloroform-isooamyl alcohol, centrifuged for 15 min at 15,000 rpm and the upper aqueous layer transferred into new tubes. Two hundred μl isopropanol and 2 μl glycogen were added as a carrier and stored at −80°C for more than 30 min. The pellets were corrected by centrifugation for 30 min at 14,000 rpm, washed with 70% ethanol and air dried on ice. They were then dissolved with 5–10 μl RNase free water and quantified with a spectrophotometer at the 260-nm optical density using Nanodrop 1000 (Thermo Fisher Scientific, Waltham, MA, U.S.A.).

Quantitative real-time RT-PCR for measurement SSTR subtypes

Quantitative mRNA expression of SSTR subtypes in tumor tissues was measured by SYBR Green PCR Master Mix (Life Technologies Japan). real-time RT-PCR was performed with SYBR Green PCR Master Mix (Life Technologies Japan, Tokyo, Japan) and the primers used in this study are shown in Table 2. The reaction mixture was preheated at 95°C for 10 min, followed by 45 cycles at 95°C for 15 sec and 60°C for 1 min. Each SSTR subtype mRNA relative value was measured by ΔΔCt method with threshold cycle times of each target SSTR and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [13].

### Table 2. Primer sequences for RT-PCR assay

| Target  | Sequence | Products (bp) |
|---------|----------|---------------|
| SSTR 1  | forward  | tgaacctagcgtggtcact | 93 |
|         | reverse  | ggaagaagccccgtggagtt | |
| SSTR 2  | forward  | cttgctggtcgttcacact | 100 |
|         | reverse  | gcagagacattcggagga | |
| SSTR 3  | forward  | ttctctcctcaectcctca | 123 |
|         | reverse  | ctctctcctcaectcctcct | |
| SSTR 4  | forward  | tttgctgctctggtgagttg | 96 |
|         | reverse  | ggtgaaaagccacggtgagt | |
| SSTR 5  | forward  | ccctctcctcgcctca | 102 |
|         | reverse  | gtgctgctgagagagagagag | |
| GAPDH   | forward  | ggaaagtgaaggctggagttca | 101 |
|         | reverse  | gctcattgatgcaaatatccact | |

Statistical analysis

Statistical analysis was performed using SPSS software for Windows version 14, using Mann-Whitney’s U test to assess the significance of the difference between the means±SD of two samples. A p value of <0.05 was considered to be significant.

### III. Results

**Expression of SSTR subtypes in neuroendocrine neoplasms**

Prior to the quantitative analysis of mRNA expression of SSTR subtypes, the expression of SSTR-1, 2A, 3, 4 and 5 was confirmed in representative cases of NET G1, G2 and NEC G3 by RT-PCR method. Representative expression profiles of SSTR subtypes in NET G1 (SSTR-1=0.08, SSTR-2A=0.55, SSTR-3=0.03, SSTR-4=0.02, SSTR-5=0.28), G2 (SSTR-1=0.88, SSTR-2A=14.42, SSTR-4=0.18, SSTR-5=10.63) and NEC G3 (SSTR-2A=4.00, SSTR-4=2.79, SSTR-5=0.51) are shown in Figure 1.
Expression of SSTR subtypes in NET G1 and G2

All NETs (G1 and G2) expressed more than two SSTR subtypes, and the expression of SSTR-1 and SSTR-2A was 100%, respectively. SSTR-4 expression was also frequently observed. The expression of SSTR-3 was below the detection level in all NETs examined in this study. The expression of SSTR-5 was also quite low or below the detection level in NETs of large intestine, appendix vermiformis and lung primaries, though it was high in the gastroduodenal primary (not significant). The expression of SSTR-2A in gastrointestinal primaries was significantly higher than lung primary (p=–0.02). No expression of SSTR-3 and SSTR-5 was observed in lung primaries (Fig. 2).

Expression of SSTR subtypes in NEC G3 and mixed NEC

In G3 (NEC and mixed NEC) group, more than two SSTR subtype expression was observed in all the primary sites, though the quantity of mRNA of each SSTR subtype was quite varied. However, the majority of NEC G3 constantly expressed SSTR-1 and SSTR-2A (85.7% and 95.2%, respectively). A statistical difference was obtained between the expression of SSTR-2A and the expression of other subtypes in the lung and breast primaries (p<0.05), Table 3. Except for stomach and duodenal primary, the expression of SSTR-3 was very low or below the detection level in other primary sites. Gastrointestinal tumors showed relatively high expression of SSTR subtypes except for SSTR-4.

Table 3. mRNA expression of SSTR subtypes in neuroendocrine carcinoma G3

| Primary sites       | Number | mRNA relative quantities (mean±SD) |
|---------------------|--------|------------------------------------|
|                     |        | SSTR1    | SSTR2    | SSTR3    | SSTR4    | SSTR5    |
| Colon               | 1      | 0.74      | 0.06      | 0         | 0         | 1.80      |
| Stomach, Duodenum   | 2      | 0.41±0.19 | 1.02±0.86 | 1.71±2.41 | 0.02±0.02 | 0.29±0.13 |
| Esophagus           | 2      | 0.005±0.007| 0.04±0.04 | 0.002±0.003| 0         | 0.02±0.02 |
| Thymus              | 1      | 0.79      | 0.54      | 0         | 0         | 0.70      |
| Mediastinum         | 1      | 0.06      | 0.18      | 0.02      | 0.01      | 0.28      |
| Lung                | 5      | 0.31±0.30 | 1.01±2.98 | 0.00±0.01 | 0.46±1.06 | 0.00±0.23 |
| Prostate            | 2      | 0.13±0.13 | 0.21±0.11 | 0         | 0.41±0.28 | 0.00±0.00 |
| Breast              | 5      | 0.11±0.36 | 0.23±1.67 | 0.00±0.01 | 0.00±0.23 | 0.00±0.43 |
| Uterine cervix      | 2      | 0.05±0.08 | 0.15±0.12 | 0         | 0.22±0.31 | 0.11±0.15 |

* p<0.05: Mann-Whitney’s U-test between SSTR2 and other subtypes.
Expression status of SSTR subtypes in NET (G1, G2) and NEC (G3 NEC and mixed NEC)

The expression status of SSTR subtypes was compared between the NET (G1, G2) group and NEC (G3 NEC and mixed NEC) group. In both groups, SSTR-2A expression was significantly higher than other subtypes (Fig. 3). The expression of SSTR-3 was very low or below the detection level in both groups. In addition, SSTR-5 expression was also quite low in the NET group.

Expression status of SSTR subtypes in NEC G3 small cell type and NEC G3 non-small cell type

The expression status of SSTR subtypes was compared between the NEC G3 small cell type and NEC G3 non-small cell type. The expression of SSTR1 and 2A subtypes was significantly high in NEC G3 non-small cell type compared with NEC G3 small cell type.

Immunohistochemistry for SSTR subtypes

Overall protein expression of SSTR-1, 2A, 3 and 5 was 93.8%, 65.6%, 53.1% and for 6.2%, respectively.

SSTR-1

Immunohistochemical expression of SSTR-1 was cytoplasmic and/or membranous localization along the plasma membrane, though the cytoplasmic localization was predominant. SSTR-1 expression was the most constant in NET G1, G2 group (100%). On the other hand, in NEC G3 and mixed NEC group it was 85.7%. Immunohistochemical intensity varied greatly from case to case, and there was heterogeneous distribution of SSTR-1 positive cells even in the same tumor tissue (Figs. 5–10).

SSTR-2A

The expression of SSTR-2A was the second most common in NET G1, G2 group (81.8%) and in NEC G3 and mixed NEC group (61.9%). Immunohistochemical localization of SSTR-2 was membranous in most cases. However, cytoplasmic localization was also seen in some cases. As with SSTR-1, heterogeneous distribution was also frequently observed (Figs. 5, 6, 7, 8 and 10).

SSTR-3

Approximately 50% of neuroendocrine neoplasms examined in this study expressed SSTR-3 in both NET G1, G2 group (54.5%) and NEC G3 group (52.4%). The immunohistochemical expression of SSTR-3 was usually located in the cytoplasm (Figs. 5, 6 and 10).

SSTR-5

The immunohistochemical expression of SSTR-5 was quite low in both NET G1, G2 group (9.0%) and NEC G3 (4.8%). Very weak membranous staining along the plasma membrane was observed (Fig. 5).

IV. Discussion

In this study, we investigated the expression of SSTR in neuroendocrine neoplasms of various primary sites with different biological behavior, particularly focused on its expression profiles of SSTR subtypes, because the expression pattern of SSTR subtypes is vary greatly even in the same tumor types or same primary sites. Particularly in high grade neuroendocrine neoplasms (NEC G3 and mixed NEC), little is known about the expression profiles of SSTR subtypes. Furthermore, the expression patterns of SSTR subtypes are directly related to the choice of molecule targeting therapy using SS analogues.

Today molecule targeting therapy using SS analogues to low grade NETs (G1 and G2) of GEP system, particularly in cases of recurrent or unresectable tumors, has come to be widely accepted as standard therapy [14, 19,
Fig. 5. Expression of SSTR-1, 2, 3 and 5 in NET G1 (carcinoid tumor) of the appendix vermiformis. a) SSTR-1; Diffuse and intense positive staining identified mostly in cytoplasm of the tumor cells. b) SSTR-2; Weak but positive staining seen along the plasma membrane. c) SSTR-3; Diffuse cytoplasmic staining present. d) SSTR-5; very weak, but membranous staining seen along the plasma membrane. Original magnification ×400 (a–d).

Fig. 6. Expression of SSTR-1, 2 and 3 in NET G1 (carcinoid tumor) of rectum. a) H-E; Ribbon-like arrangement of relatively uniform tumor cells. b) SSTR-1; Diffuse cytoplasmic positive staining in tumor cells. c) SSTR-2; Membranous and/or cytoplasmic localization seen. d) SSTR-3; Cytoplasmic staining identified in tumor cells. Positive staining present in some interstitial cells. Original magnification ×200 (a), ×400 (b–d).
Fig. 7. Expression of SSTR-1 and 2 in NET G2 (atypical carcinoid) of stomach. a) Ribbon-like arrangement of tumor cells of nuclear atypia. b) CD56; Intense membranous staining seen along the plasma membrane. c) SSTR-1; Mostly cytoplasmic positivity in tumor cells. d) SSTR-2; Intense membranous staining along the plasma membrane of tumor cells. Original magnification ×400 (a–d).

Fig. 8. Expression of SSTR1 and 2 in NEC G3 (neuroendocrine carcinoma) of stomach. a) H-E; Rather solid growth of poorly differentiated tumor cells. b) Synaptophysin; Diffuse and intense cytoplasmic positive staining seen in tumor cells. c) SSTR-1; Diffuse cytoplasmic positivity seen in tumor cells. d) SSTR-2; Intense membranous staining along the plasma membrane. Original magnification ×400 (a–d).
Fig. 9. Expression of SSTR-1 in two different NEC G3 (large cell neuroendocrine carcinoma; LCNEC) of lung. 

a) H-E; Rather solid growth of poorly differentiated tumor cells with incomplete peripheral nuclear palisading.
b) SSTR-1; In this case cytoplasmic positive staining seen.
c) H-E; Another case of LCNEC.
d) Intense membranous and occasional cytoplasmic positivity identified in the tumor cells. Original magnification ×200 (a), ×400 (b–d).

Fig. 10. Expression of SSTR-1, 2 and 3 in NEC G3 (mixed neuroendocrine carcinoma) of breast.

a) H-E; Solid growth of poorly differentiated tumor cells.
b) SSTR-1; Cytoplasmic and/or membranous positivity identified in scattered tumor cells.
c) SSTR-2; Intense membranous and/or cytoplasmic localization present.
d) SSTR-3; Some tumor cells show cytoplasmic positive reaction. Original magnification ×200 (a), ×400 (b–d).
depend greatly on the individual tumor character rather than the same tumor type is not clearly understood, but it seems to be due to the new WHO classification. This classification is much more reliable to evaluate biological behavior, due to the introduction of the Ki-67 labeling system and the subclassification of small cell and large cell types in NEC G3.

In conclusion, high grade neuroendocrine neoplasms (NEC G3 and mixed NEC) would be a good candidate for molecule targeting therapy using SS analogues. We have demonstrated that NEC G3 small cell type show a different expression profile of SSTR subtypes compared with NEC non-small cell type. In addition, the expression of SSTR-2A could serve as a good biomarker for neuroendocrine differentiation.

V. Acknowledgments

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VI. References

1. Arnold, R. (2005) Endocrine tumors of the gastrointestinal tract. Introduction; definition, historical aspects, classification, staging, prognosis and therapeutic options. Best Pract. Res. Clin. Gastroenterol. 19, 491–505.
2. Bosman, F., Carneriro, R., Hruban, R. and Theise, N. (2010) WHO classification of tumors of the stomach. In “WHO Classification of Tumors of the Digestive System”, ed. by F. Bosman, F. Carneriro, R. Hruban and N. Theise, IARC Press, France, pp. 46–47.
3. Burns, C., Lewis, I., Briner, U., Meno-Tetang, G. and Weckbecker, G. (2002) SOM230: a novel somatostatin mimetic with broad somatostatin release inhibiting factor (SRIF) receptor binding and a unique antisecretory profile. Eur. J. Endocrinol. 146; 707–716.
4. Capella, C., Solcia, E., Sobin, L. H. and Arnold, R. (2000) Endocrine tumor of the stomach. In “Pathology and Genetics of Tumors of the Digestive System”, ed. by S. R. Hamilton, L. A. Aaltonen, IARC Press, Lyon, France, pp. 53–57.
5. Capella, C., Solcia, E., Sobin, L. H. and Arnold, R. (2000) Endocrine tumor of the small intestine. In “Pathology and...
6. Capella, C., Solcia, E., Sobin, L. H. and Arnold, R. (2000) Endocrine tumor of the colon and rectum. In “Pathology and Genetics of Tumors of the Digestive System”, ed. by S. R. Hamilton, L. A. Aaltonen, IARC Press, Lyon, France, pp. 137–141.

7. Hofland, L. J. and Lamberts, S. W. (2003) The pathophysiologic consequence of somatostatin receptor internalization and resistance. Endocr. Rev. 24; 28–47.

8. Janson, E. T. and Oberg, K. (1993) Long-term management of the carcinoid syndrome: treatment with octreotide alone and in combination with alpha-interferon. Acta Oncol. 32; 225–229.

9. Jansen, R. (1999) Pancreatic endocrine tumors: Recent advances. Ann. Oncol. 10; S170–176.

10. Kulaksiz, H., Eissele, R., Rossler, D., Schulz, S., Holtt, V., Cetin, Y. and Arnold, R. (2002) Identification of somatostatin receptor subtypes 1, 2A, 3, and 5 in neuroendocrine tumors with subtype specific antibodies. Gut 50; 52–60.

11. Kvols, L. K., Reubi, J. C., Horisberger, U., Moertel, C. G., Rubin, J. and Charboneau, J. W. (1992) The presence of somatostatin receptors in malignant neuroendocrine tumor tissue predicts responsiveness to octreotide. Yale J. Biol. Med. 65; 505–536.

12. Macabeo-Ong, M., Ginzinger, D. G., Dekker, N., McMillan, A., Regezi, J. A., Wong, D. T. W. and Jordan, R. C. K. (2002) Effect of duration of fixation on quantitative reverse transcription polymerase chain reaction analysis. Mod. Pathol. 15; 979–987.

13. Modlin, I. M., Oberg, K., Chung, D. C., Jensen, R. T., de Herder, W. W., Thakker, R. V., Caplin, M., Fave, G. D., Kaltas, G. A., Krenning, E. P., Moss, S. F., Nilsson, O., Rindi, G., Salazar, R., Ruszniewski, P. and Sundin, A. (2008) Gastroenteropancreatic neuroendocrine tumors. Lancet Oncol. 9; 61–72.

14. Nasir, A., Stridsberg, M., Strosberg, J., Su, P.-H., Livingston, S., Malik, H. A., Kelly, S. T., Centeno, B. A., Coppola, D., Malafa, M. E., Yeatman, T. J. and Kvols, L. K. (2006) Somatostatin receptor profiling in hepatic metastases from small intestinal and pancreatic neuroendocrine neoplasms: immunohistochemical approach with potential clinical utility. Cancer Control 13; 52–60.

15. Ohno, C., Nakaniishi, Y., Honma, T., Henmi, A., Sugitani, M., Kanaiz, Y. and Nemo, N. (2009) Significance of system L amino acid transporter 1 (LAT-1) and 4F2 heavy chain (4F2hc) expression in human developing intestines. Acta Histochem. Cytochem. 42; 73–81.

16. Osamura, R. Y., Egashira, N., Kajiya, H., Takei, M., Tobita, M., Miyakoshi, T., Inamoto, C., Takekoshi, S. and Teramoto, A. (2009) Pathology, pathogenesis and therapy of growth hormone (GH)-producing pituitary adenomas: technical advances in histology and their contribution. Acta Histochem. Cytochem. 42; 95–104.

17. Pearse, A. G. E. (1977) The diffuse neuroendocrine system and the APUD concept: related ‘endocrine’ peptides in brain, intestine, pituitary, placenta and human cutaneous glands. Med. Biol. 55; 115–125.

18. Petel, Y. C. (1999) Somatostatin and its receptor family. Front. Neuroendocrinol. 20; 157–198.

19. Ruszniewski, P., Ducreux, M., Charyvialle, J. A., Blumberg, J., Cloarec, D., Michel, H., Raymond, J. M., Dupas, J. L., Gouerou, H., Jian, R., Genestin, E., Bemades, P. and Rougier, P. (1996) Treatment of the carcinoid syndrome with the long acting somatostatin analogue lanreotide: a prospective study in 39 patients. Gut 39; 279–283.

20. Sushi, C. and Buscal, L. (2006) Rationale for the use of somatostatin analogs as antitumor agents. Ann. Oncol. 17; 1733–1742.

21. Wymenga, A. N. M., Eriksson, B., Salmela, P. I., Jacobsen, M. B., Van Cutsem, E. J. D. G., Fiasse, R. H., Valimaki, M. J., Renstrup, J., de Vries, E. G. E. and Oberg, K. E. (1999) Efficacy and safety of prolonged-release lanreotide in patients with gastrointestinal neuroendocrine tumors and hormone-related symptoms. J. Clin. Oncol. 17; 1111–1117.

22. Yamada, Y., Post, S. R., Wang, K., Tager, H. S., Bell, G. I. and Seino, S. (1992) Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. Proc. Natl. Acad. Sci. U S A 89; 251–255.

23. Yamada, Y., Reisine, T., Law, S. F., Ihara, Y., Kubota, A., Kagimoto, S., Seino, M., Seino, Y., Bell, G. I. and Seino, S. (1992) Somatostatin receptors, an expanding gene family: Cloning and functional characterization of human SSTR3, a protein coupled to adenylate cyclase. Mol. Endocrinol. 6; 2136–2142.

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