



Annie Toja. *Mediterranean Fishing Boats*. Acrylic.

*In patients with gastroenteropancreatic neuroendocrine neoplasms, SSTR subtyping may help to predict the clinical outcome following somatostatin analog therapy.*

## **Somatostatin Receptor Profiling in Hepatic Metastases From Small Intestinal and Pancreatic Neuroendocrine Neoplasms: Immunohistochemical Approach With Potential Clinical Utility**

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**Background:** *The expression of somatostatin receptors (SSTRs) on endocrine tumor (ET) cells forms the basis for somatostatin analog treatment of patients with SSTR-positive, hormonally active ETs. In patients with SSTR-negative ETs, the clinical response is generally absent or suboptimal, while nonfunctioning ETs with SSTR positivity show a variable response to such therapy.*

**Methods:** *We retrospectively studied SSTR subtype expression in hepatic metastases from 14 adult patients with primary endocrine carcinomas (ECAs) of the small intestine and pancreas and compared SSTR subtype expression among the primary and metastatic ECAs. Polyclonal antibodies against the 5 SSTR subtypes were used on formalin-fixed, paraffin sections from each primary and metastatic ECA. Both qualitative and semiquantitative evaluation of the stained ECA sections was carried out.*

**Results:** *Eleven (61%) of 18 hepatic metastases from small intestinal and pancreatic ECAs were positive for SSTR-1, 15 (83%) for SSTR-2, 13 (72%) for SSTR-3, 10 (56%) for SSTR-4, and 15 (83%) for SSTR-5. Among 11 hepatic ECA metastases from small intestinal ECAs (carcinoids), 7 (63%) expressed SSTR-1, 9 (81%) expressed SSTR-2, 8 (72%) expressed SSTR-3, 6 (54%) expressed SSTR-4, and 10 (91%) expressed SSTR-5. Of 7 hepatic ECA metastases from pancreatic ECAs, 4 expressed SSTR-1 and SSTR-4, 6 expressed SSTR-2, and 5 expressed SSTR-3 and SSTR-5 each. We also observed the immunohistochemical evidence of heterogeneity of expression of various SSTR subtypes in the primary enteropancreatic ECAs and their hepatic metastases.*

**Conclusions:** *SSTR subtype expression needs to be correlated to somatostatin analog therapy. Immunohistochemical profiling of various SSTR subtypes as a part of routine surgical pathologic analysis of enteropancreatic ETs may become a useful predictor of responsiveness of ETs to various SSTR analogs.*

## Introduction

Treatment with radiolabeled somatostatin analogs is effective in the management of patients with inoperable or metastasized neuroendocrine tumors (NETs).<sup>1</sup> Such therapy results in reduced hormonal overproduction and symptomatic relief in most of the NET patients, although it is seldom successful in reducing the tumor size.<sup>2</sup> Specifically, in patients with metastatic carcinoids, octreotide and lanreotide have been shown to result in biochemical response in 40% to 50% of cases, with temporary stabilization of tumor growth in more than 80% and tumor regression in less than 10%.<sup>3,4</sup> The expression of SSTRs on tumor cells forms the basis for somatostatin analog treatment of patients with SSTR-positive NETs.<sup>5</sup> In malignant NETs, the presence of SSTRs has been shown to predict favorable clinical response to octreotide (somatostatin analog) therapy.<sup>6</sup>

SSTRs are divided into five subtypes, all of which have an antiproliferative effect by either inhibiting mitogenesis or stimulating apoptosis.<sup>7</sup> SSTR-1, -2, -4 and -5 induce G<sub>1</sub> cell cycle growth arrest, while SSTR-3 is proapoptotic via the induction of p53 and BAX.<sup>8,9</sup> These subtypes have been identified in human ETs and other tumors and their metastases, using a variety of techniques including autoradiography,<sup>10,11</sup> reverse-transcriptase polymerase chain reaction, and immunohistochemistry (IHC).<sup>12,13</sup> In ETs, the expression of SSTRs correlates with the degree of endocrine differentiation, lower histopathologic tumor grades,<sup>11</sup> and clinical response to somatostatin analog (octreotide) therapy.<sup>6</sup> While the majority of well-differentiated ETs and islet cell carcinomas are SSTR-positive, thus improving their response to somatostatin analog therapy, the poorly differentiated ETs are usually SSTR-negative<sup>11</sup> and rarely respond to somatostatin analog therapy.

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*Abbreviations used in this paper:* SSTR = somatostatin receptor, ET = endocrine tumor, ECA = endocrine carcinoma, NET = neuroendocrine tumor, IHC = immunohistochemistry.

While the demonstration of expression of various SSTR subtypes may be useful in predicting favorable clinical response of NETs to somatostatin analog (octreotide) therapy, some clinical subsets of NETs may specifically benefit from SSTR subtyping in the NET tissues. These include (1) SSTR-negative gastroenteropancreatic (GEP) NETs, in which clinical response to somatostatin analog therapy is generally absent or suboptimal, (2) nonfunctioning GEP NETs, in which role of octreotide therapy is controversial, and (3) OctreoScan plus GEP NETs, which may show a variable clinical response to somatostatin analog treatment. Furthermore, with the availability of newer subtype-specific ligands such as pasireotide (SOM-230) with selective affinity for various SSTR subtypes, compared to the older somatostatin analogs (eg, octreotide and lanreotide), it is becoming increasingly important to establish the pattern of tumor SSTR expression in order to select one or more somatostatin analogs for optimal therapeutic effect in patients with NETs.<sup>14</sup> Such an approach will allow improved patient selection based on expression of various SSTRs in enteropancreatic NET tissues, and it may contribute to higher clinical response rates for various somatostatin analog therapies in such patients. With these clinical rationales in mind, we carried out a retrospective analysis of the IHC expression of the five SSTR subtypes in a series of hepatic metastases from the primary endocrine carcinomas (ECAs) of the small intestine and pancreas.

## Patients and Methods

### Patient Group

The study comprised 14 randomly selected patients with primary and metastatic ECAs of the small intestine and pancreas. This selection was based on a computer-generated list from NET archives at our institute. Nine men and 5 women were included, with a mean age of 53 years (range 28-73 years). This study was carried out in compliance with the guidelines of the Institutional Review Board at our institute.

### ECA Tissue Specimens

Twenty-two ECA tissues (3 primary and 19 metastatic) were available from the 14 patients for SSTR subtyping. Five (36%) had their primary ECAs of the small intestine, and another 5 patients (36%) had their primary ECAs of the pancreas resected at another institution. For these 10 patients, ECA tissues both from the primary sites and known hepatic ECA metastases were available for this study. Of the remaining 4 patients, 1 was originally diagnosed as primary moderately differentiated ECA of the pancreas (case 11), 1 as primary well-differentiated ECA of the ileum (case 12), and 1 as primary well-differentiated ECA of the duodenum (case 13),

**Table 1. — Dilution and Timing for SSTR Antibodies**

Primary Antibody	Dilution	Incubation Time (minutes)	Incubation Temperature
SSTR-1	1:1200	60	Room temperature
SSTR-2	1:1200	60	Room temperature
SSTR-3	1:1200	60	Room temperature
SSTR-4	1:400	60	Room temperature
SSTR-5	1:600	60	Room temperature

using the WHO 2000 criteria.<sup>15</sup> For each of these 3 patients, ECA tissues both from the primary and 1 or more corresponding hepatic metastases were also available for SSTR subtyping. In addition, metastatic ECA tissue from a mesenteric lymph node was available for case 12, and metastatic ECA tissues from three separate hepatic metastases were available for case 14.

In 12 (85%) of the 14 patients, both primary and metastatic ECAs were well-differentiated. In case 11, the primary pancreatic ECA and both hepatic metastases were moderately differentiated, and in case 6, the metastatic ECA in the liver was not assigned a histologic grade due to marked cytologic atypia of the ECA cells, attributed to extensive preoperative chemotherapy. All 14 patients were undergoing octreotide therapy at the time of resection of their primary and metastatic ECAs.

### Histopathologic Review and Selection of ECA Tissue Blocks

In all selected cases, the original formalin-fixed, paraffin-embedded ECA tissue sections were reviewed by a pathologist with interest in endocrine pathology to verify the originally rendered histopathologic diagnoses and histologic grades, to unify the nomenclature used in the original histopathologic reports, and to select the most appropriate archival ECA tissue blocks for SSTR IHC.

## Somatostatin Receptor Immunohistochemistry

### Technique

Sections of 3 to 4 microns in thickness were cut from the selected formalin-fixed, paraffin-embedded ECA tissue blocks and subjected to SSTR IHC staining protocol as already optimized in the Histopathology Core Research Laboratory at the University of South Florida, using the DakoCytomation Autostainer (DakoCytomation, Carpinteria, Calif). Microwave antigen retrieval with IHC Select EDTA buffer, pH 7.5 (Chemicon International, Temecula, Calif) was utilized. Endogenous peroxidase was blocked by hydrogen peroxide. Blockage of avidin-binding protein was accomplished by using the Avidin-Biotin blocking kit (Vector Laboratories, Burlingame, Calif). The rabbit polyclonal primary antibodies against SSTR-1, -2, -3, -4, and -5, with cross-reactivity against human SSTR subtypes, were provided by the University of Uppsala, Sweden. The dilutions and timing for the 5 SSTR antibodies used are shown in Table 1. Incubation was performed at room temperature for 60 minutes. The EnVision+ HRP-labeled polymer antirabbit detection system was used. This system resulted in cleaner background compared with the DakoCytomation-labeled Streptavidin Biotin+ detection system, which was used during several runs of initial optimization of the above antibodies in our laboratory. With each test run, sections of known SSTR-positive ETs and the nonneoplastic pancreas demonstrating SSTR subtype expression in the various cell types of the islets of Langerhans were also included. Rabbit immunoglobulin G was used for 60 minutes to replace the primary antibodies in the negative control sections.

### Specificity of SSTR Immunostaining

In order to confirm the specificity of the antibodies

**Table 2. — Semiquantitative Assessment of Immunohistochemical Expression of SSTR Subtypes in Hepatic Metastases From Endocrine Carcinomas of the Small Intestine and Pancreas**

Case #	Age (yrs)	Sex	Site of Metastatic Neuroendocrine Carcinoma	Pathologic Diagnosis	Site of Primary EN/ECA	Histologic Grade	SSTR Subtype					
							-1	-2	-3	-4	-5	
1	70	M	Liver	ECA (carcinoid tumor)	Small intestine	WD	2+	1+	1+	1+	1+	1+
2	43	F	Liver	ECA (carcinoid tumor)	Small intestine	WD	1+	1+	1+	0	1+	1+
3	66	M	Liver	ECA (carcinoid tumor)	Small intestine	WD	0	0	0	0	0	0
4	72	F	Liver	ECA (carcinoid tumor)	Small intestine	WD	0	1+	0	1+	1+	1+
5	40	F	Liver	ECA (carcinoid tumor)	Small intestine	WD	0	1+	1+	1+	1+	1+
6	52	M	Liver	ECA	Pancreas	*	2+	1+	2+	0	1+	1+
7	54	M	Liver	ECA	Pancreas	WD	1+	1+	1+	1+	2+	2+
8	43	M	Liver	ECA	Pancreas	WD	0	1+	0	1+	1+	1+
9	40	M	Liver	ECA	Pancreas	WD	0	1+	1+	1+	0	0
10	55	M	Liver	ECA	Pancreas	WD	0	0	0	0	0	0

0 = no expression  
 1+ = mild expression  
 2+ = moderate expression  
 \* Histologic grading not done (cytologic atypia-preoperative chemotherapy)  
 WD = well-differentiated

used in this study, a preincubation of the primary antibody solution and the respective somatostatin antigens (synthetic somatostatin peptides) was performed for 24 hours prior to application to the tissue sections, as described in an earlier study from the University of Uppsala in Sweden.<sup>16</sup>

### IHC Findings

The immunostained ECA sections were examined under a light microscope. Using a semiquantitative scoring system,<sup>16</sup> the intensity of IHC staining for various SSTR subtypes was scored 0 (negative), 1+ (mild positive staining), 2+ (moderate positive), and 3+ (strong positive staining) (Table 2). For the purpose of qualitative analysis, sections showing 0 intensity for SSTR immunostaining were designated SSTR-negative, while those showing 1+ or more intense (2+ or 3+)

SSTR immunostaining in 50% or more of the tumor cells were designated SSTR-positive (Table 3).<sup>17</sup>

## Results

### IHC Expression of SSTR Subtypes in Hepatic Metastases From Small Intestinal and Pancreatic ECAs

Overall, 11 (61%) of 18 hepatic metastases from small intestinal and pancreatic ECAs were positive for SSTR-1, 15 (83%) for SSTR-2, 13 (72%) for SSTR-3, 10 (56%) for SSTR-4, and 15 (83%) for SSTR-5. Among 11 hepatic ECA metastases from small intestinal ECAs (carcinoids), 7 (63%) expressed SSTR-1, 9 (81%) expressed SSTR-2, 8 (72%) expressed SSTR-3, 6 (54%) expressed SSTR-4, and 10 (91%) expressed SSTR-5. Of 7 hepatic ECA metastases from pan-

**Table 3. — Immunohistochemical Expression of SSTR Subtype in Hepatic Metastases From Endocrine Carcinomas of the Small Intestine and Pancreas**

Case #	Age (yrs)	Sex	Site of Metastatic Endocrine Carcinoma	Pathologic Diagnosis	Site of Primary EN/ECA	Grade	SSTR Subtype				
							-1	-2	-3	-4	-5
1	70	M	Liver	ECA (carcinoid tumor)	Small intestine	WD	P	P	P	P	P
2	43	F	Liver	ECA (carcinoid tumor)	Small intestine	WD	P	P	P	N	P
3	66	M	Liver	ECA (carcinoid tumor)	Small intestine	WD	N	N	N	N	N
4	72	F	Liver	ECA (carcinoid tumor)	Small intestine	WD	N	P	N	P	P
5	40	F	Liver	ECA (carcinoid tumor)	Small intestine	WD	N	P	P	P	P
6	52	M	Liver	ECA	Pancreas	*	P	P	P	N	P
7	54	M	Liver	ECA	Pancreas	WD	P	P	P	P	P
8	43	M	Liver	ECA	Pancreas	WD	N	P	N	P	P
9	40	M	Liver	ECA	Pancreas	WD	N	P	P	P	N
10	55	M	Liver	ECA	Pancreas	WD	N	N	N	N	N

P = positive SSTR immunostaining  
N = negative SSTR immunostaining  
WD = well-differentiated  
\* Histologic grading not done (cytologic atypia-preoperative chemotherapy)

**Table 4. — Heterogeneity of SSTR Subtype Expression Among Primary and Metastatic ECAs of the Pancreas and Small Intestine**

Case #	Age (yrs)	Sex	Tumor Site	Primary vs Metastasis	Pathologic Diagnosis	Grade	SSTR Subtype					Fig #s
							-1	-2	-3	-4	-5	
11	28	M	Pancreas	Primary	ECA	MD	2+	1+	2+	0	2+	2A-F
			Liver, met #1	Metastatic	ECA	MD	2+	1+	2+	0	1+	3A-F
			Liver, met #2	Metastatic	ECA	MD	1+	1+	2+	1+	1+	
12	61	M	Ileum	Primary	ECA (carcinoid)	WD	1+	1+	1+	0	1+	4A-F
			Mesenteric lymph node, met	Metastatic	ECA (carcinoid)	WD	3+	2+	1+	2	2+	5A-F
			Liver, met #1	Metastatic	ECA (carcinoid)	WD	1+	1+	2+	0	1+	6A-F
			Liver, met #2	Metastatic	ECA (carcinoid)	WD	1+	1+	1+	1+	1+	
13	55	F	Duodenum	Primary	ECA (carcinoid)	WD	0	1+	2+	1+	1+	
			Liver met	Metastatic	ECA (carcinoid)	WD	1+	1+	0	1+	1+	
14	73	F	Liver, met #1	Metastatic	ECA (carcinoid)	WD	1+	1+	1+	0	1+	
			Liver, met #2	Metastatic	ECA (carcinoid)	WD	2+	1+	1+	0	1+	
			Liver, met #3	Metastatic	ECA (carcinoid)	WD	0	0	1+	1+	1+	

0 = no expression  
1+ = mild expression  
2+ = moderate expression  
met = metastasis  
MD = moderately differentiated  
WD = well-differentiated

creatic ECAs, 4 expressed SSTR-1 and SSTR-4, 6 expressed SSTR-2, and 5 expressed SSTR-3 and SSTR-5 each.

### Cellular Localization of SSTR Immunostaining

In the vast majority of cases studied, immunostaining for various SSTR subtypes was localized to the ECA cell cytoplasm. In a minority of cases, scattered tumor foci featuring expression of various SSTR subtypes on the ECA cell membranes were noted (Fig 1A). The SSTR subtype immunostaining was also localized to the cell cytoplasm, with some membrane positivity in the islets in sections from nonneoplastic pancreas (Figs 1B-C).

### Heterogeneity of Expression of SSTR Subtypes Among Primary ECAs of the Small Intestine and Pancreas and Their Hepatic Metastases

The intensity of IHC expression of various SSTR subtypes in hepatic metastatic ECA tissues varied from 0 (negative) to 1+ (mild) or 2+ (moderate) (Tables 2 and 4). Two of the metastatic ECAs (1 from the small intestine and 1 from the pancreas) did not express any of the SSTR subtypes (case 3 and case 10, respectively).

**Case 11:** This case is an example of moderately differentiated ECA of the pancreas with 2 separate hepatic metastases (#1, #2). Overall, the intensity of immunostaining for SSTR-1, SSTR-2, SSTR-3, and SSTR-4 was identical among the primary pancreatic (Figs 2A-F) and hepatic metastatic ECA tissue #1 (Figs 3A-F). The intensity of expression of SSTR-5, however, was moderate (2+) in the primary (Fig 2F) and mild (1+) in the hepatic metastasis #1 (Fig 3F). Compared with the primary pancreatic ECA tissue, the intensity of immunostaining for SSTR-1, SSTR-4, and SSTR-5 was also different in the hepatic metastatic ECA tissue #2.

**Case 12:** This case represents a well-differentiated ECA (carcinoid tumor) of the ileum with metastatic ECA in a mesenteric lymph node and 2 separate foci of metastatic ECA in the liver. A comparison of the intensity of expression of various SSTR subtypes in the primary ileal ECA (Figs 4A-F), metastatic ECA in a mesenteric lymph node (Figs 5A-F) and hepatic ECA metastasis #1 (Figs 6A-F) is shown in Table 4. The primary ileal ECA showed 1+ (mild) expression of SSTR-1, -2, and -3, 2+ (moderate) expression of SSTR-5, but no expression of SSTR-4. Compared with the primary ileal ECA, the

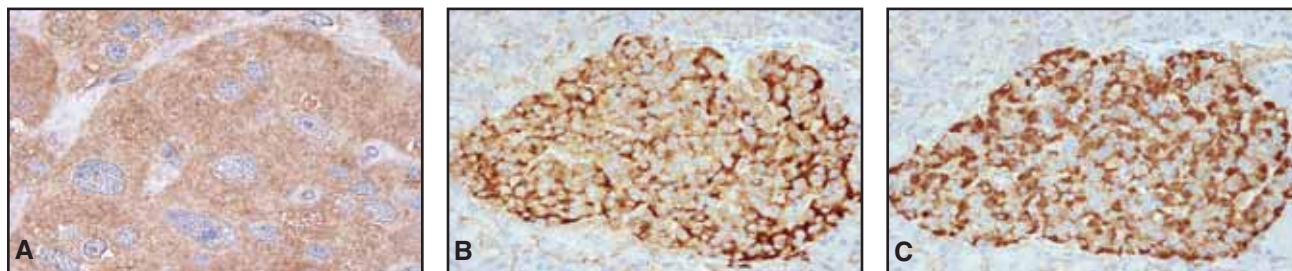
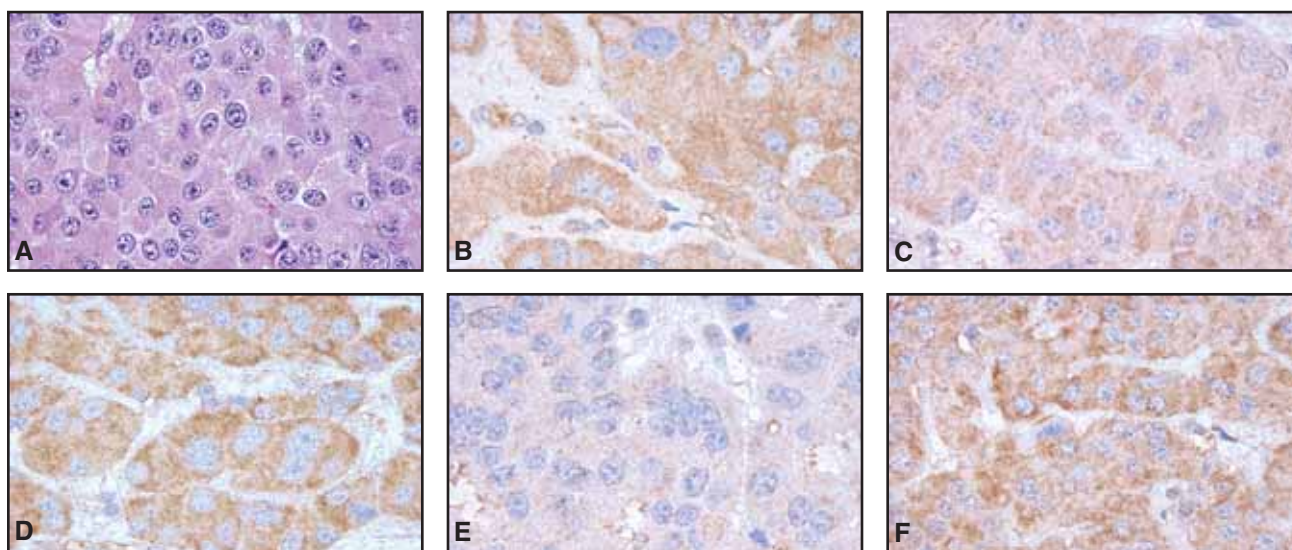


Fig 1A-C. — Pancreatic NET. (A) Section from liver metastasis under high magnification, showing mainly diffuse cytoplasmic expression of SSTR-3, with membrane accentuation (immunoperoxidase staining for SSTR-3,  $\times 630$ ). Sections from nonneoplastic pancreas featuring expression of SSTR-4 (B) and SSTR-5 (C) (immunoperoxidase staining for SSTR-4 and -5,  $\times 400$ ).



Figs 2A-F. — (A) Moderately differentiated ECA of the pancreas; paraffin section from the primary pancreatic ECA (hematoxylin-eosin,  $\times 630$ ). (B-F) Paraffin sections from the primary pancreatic ECA featuring 2+, 1+, 2+, negative, and 2+ expression of SSTR-1, -2, -3, -4 and -5, respectively (immunoperoxidase staining for SSTR subtypes 1-5,  $\times 630$ ).

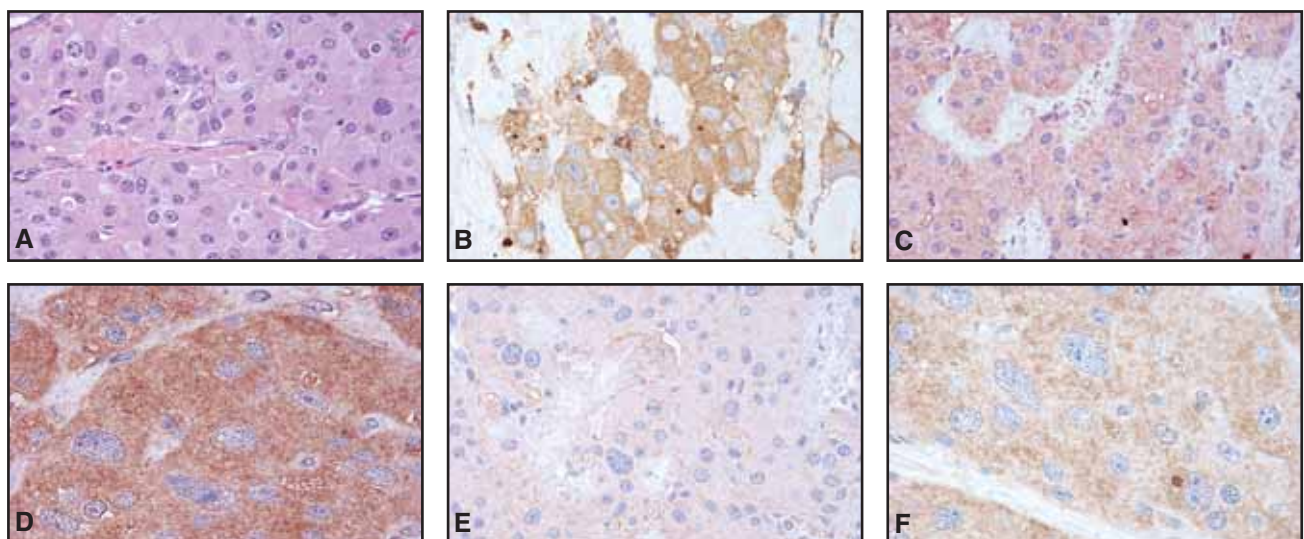
metastatic ECA tissue from the mesenteric lymph node showed higher (3+) expression of SSTR-1 (Fig 5B) and 2+ expression of SSTR-2 (Fig 5C) and SSTR-5 (Fig 5F). The hepatic metastatic ECA tissue #1 showed higher (2+) expression of SSTR-3 (Fig 6D), lower (1+) expression of SSTR-5 (Fig 6F), while expression of SSTR-1, SSTR-2, and SSTR-4 was similar to the primary ileal ECA. These findings represent IHC evidence of heterogeneity of the expression of various SSTR subtypes in synchronously sampled primary ileal ECA and the metastatic ECA tissues from the mesenteric lymph node and liver in the same patient.

**Case 13:** This case illustrates a well-differentiated ECA (carcinoid tumor) of the duodenum with hepatic metastasis. The expression of SSTR-2, -4, and -5 was iden-

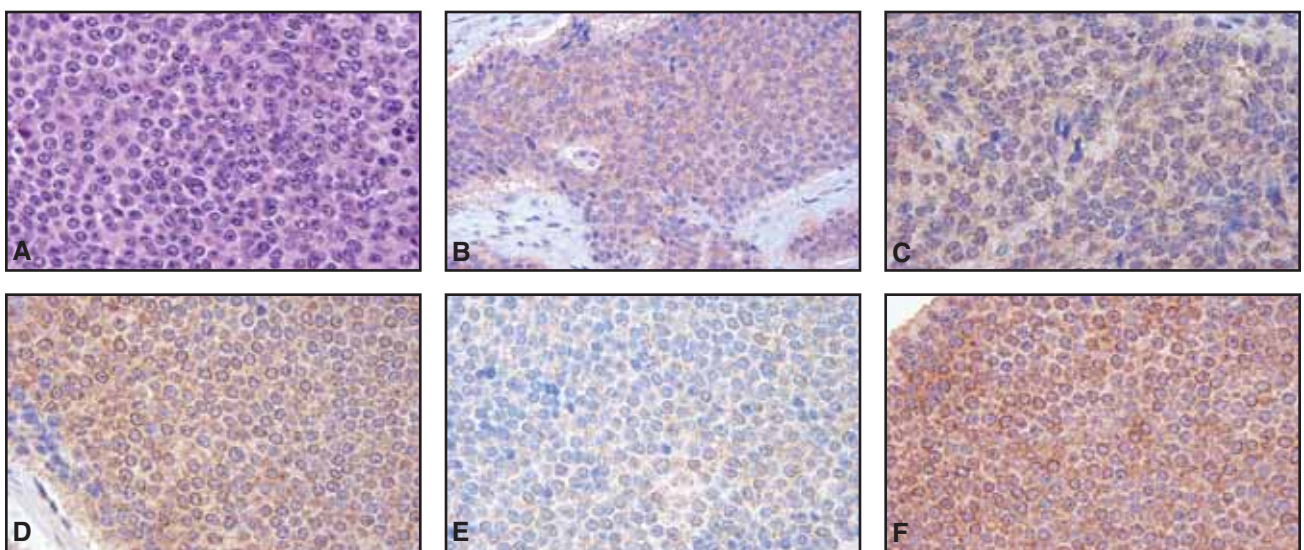
tical in the primary duodenal ECA and the hepatic metastatic ECA tissues in this case (Table 4). However, while the primary duodenal ECA tissue was negative for SSTR-1, the metastatic ECA in the liver showed 1+ (mild) expression. Conversely, moderate (2+) IHC expression of SSTR-3 in the primary duodenal ECA was found to be lost in the hepatic metastatic ECA tissue.

## Discussion

Overall, the most sensitive imaging modality for the detection of metastatic disease in NETs is SSTR scintigraphy (OctreoScan).<sup>18</sup> This imaging technique allows for the noninvasive determination of the presence of SSTRs



Figs 3A-F. — (A) Moderately differentiated ECA of the pancreas (same case shown in Figs 2A-F); paraffin tumor section from the metastatic ECA in the liver (hematoxylin-eosin,  $\times 630$ ). (B-F) Paraffin sections from the metastatic pancreatic ECA in the liver featuring 2+, 1+, 2+, negative, and 1+ expression of SSTR-1, -2, -3, -4, and -5, respectively (immunoperoxidase staining for SSTR subtypes 1-5: 3A-C,  $3E \times 400$ ; 3D and 3F,  $\times 630$ ).

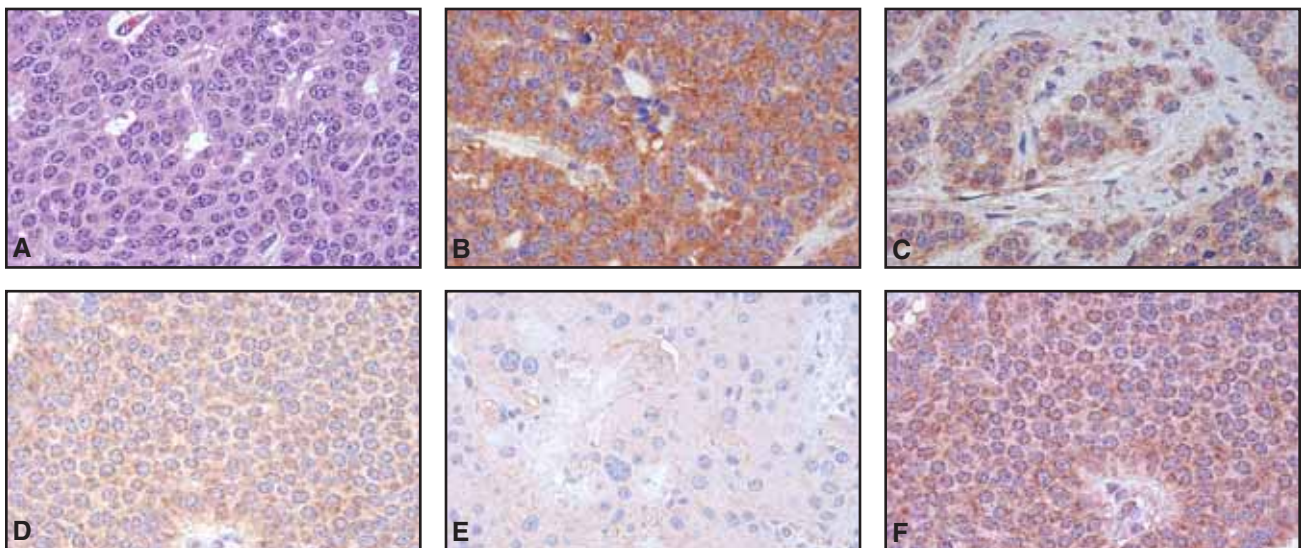


Figs 4A-F. — (A) Well-differentiated ECA of the ileum (carcinoid tumor); paraffin section from the primary ileal neoplasm (hematoxylin-eosin,  $\times 630$ ). (B-F) Paraffin sections from the primary carcinoid of the ileum featuring 1+, 1+, 1+, negative, and 2+ cytoplasmic expression of SSTR-1, -2, -3, -4, and -5, respectively (immunoperoxidase staining for SSTR subtypes 1-5,  $\times 630$ ).

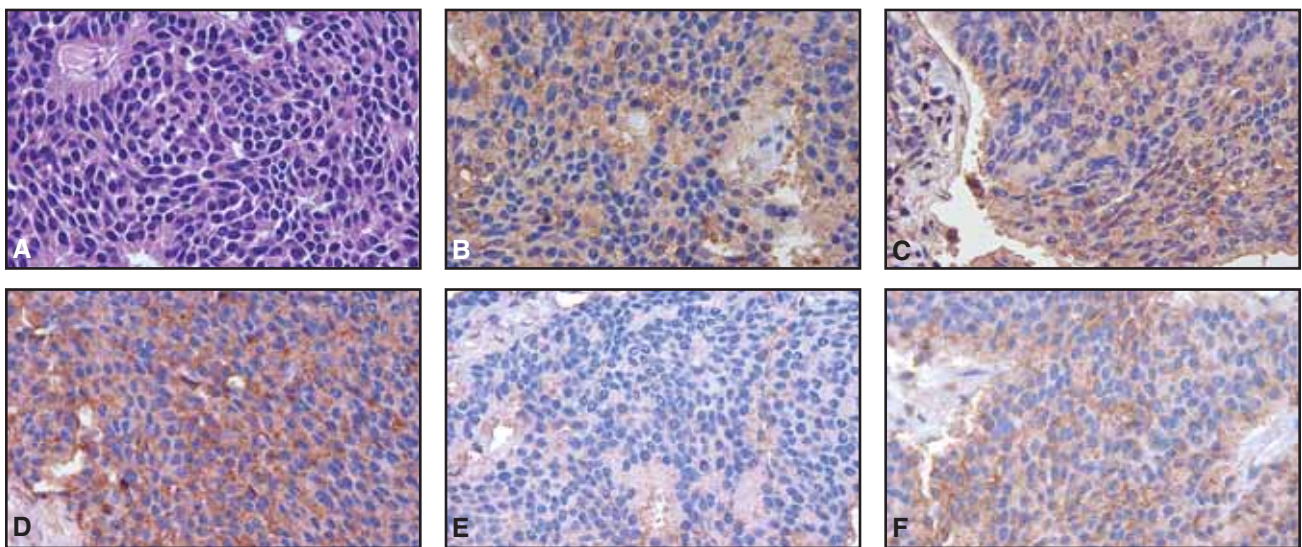
using radiolabeled octreotide (pentetreotide).<sup>19</sup> Therefore, it is widely used for predicting the response of SSTR-positive NETs to somatostatin analogs. However, it does not identify expression of various SSTR subtypes in a given case of NET. With the availability of newer somatostatin analogs, such as pasireotide (SOM230), with specific binding affinities for various SSTR subtypes, it is becoming increasingly important to determine the SSTR profile of NETs in the clinical setting. SOM230 is a novel multiligand somatostatin analog that exhibits high binding affinity to SSTR-1, -2, -3, and -5. Compared with octreotide, SOM230 has 30-, 5- and 40-times greater affinity for SSTR-1, SSTR-3, and SSTR-5, respectively, and a comparable affinity for SSTR-2.<sup>20</sup> IHC technique, although still in its early stages for routine

assessment of SSTR subtyping in NETs, has the potential to allow retrospective assessment of SSTR subtypes in formalin-fixed sections of the surgically resected NETs.

In this IHC study, we determined the frequency of expression of various subtypes of SSTRs in archival tumor sections of hepatic metastases from ECAs of the small intestine and pancreas. SSTR immunostaining in the cases studied was predominantly localized to the cytoplasm of the ECA cells. Some studies have shown dominant localization of various SSTRs to the plasma membrane of the tumor cells,<sup>21,22</sup> while other groups have reported both cytoplasmic and membranous localization.<sup>12,23-26</sup> Our finding of predominant cytoplasmic staining may be best explained by the fact that all of our patients were on octreotide at the time



Figs 5A-F. — (A) Well-differentiated ECA (carcinoid tumor) of the ileum; paraffin section from the mesenteric lymph node metastasis (hematoxylin-eosin,  $\times 630$ ). (B-F) Paraffin tumor sections from the lymph node metastasis featuring 3+, 2+, 1+, negative, and 2+ cytoplasmic expression of SSTR-1, -2, -3, -4, and -5, respectively (immunoperoxidase staining for SSTR subtypes 1-5,  $\times 630$ ).



Figs 6A-F. — (A) Well-differentiated ECA (carcinoid tumor) of the ileum; paraffin section from the hepatic metastasis (hematoxylin-eosin,  $\times 630$ ). (B-F) Paraffin tumor sections from the hepatic metastasis featuring 1+, 1+, 2+, negative, and 1+ cytoplasmic expression of SSTR-1, -2, -3, -4, and -5, respectively (immunoperoxidase staining for SSTR subtypes 1-5,  $\times 630$ ).

of surgery for the NETs, which is known to cause internalization of SSTRs.<sup>19</sup> Such internalization and subsequent nuclear translocation and DNA binding of SSTRs and somatostatin analogs have been shown to increase over time in SSTR-positive cells but not in SSTR-negative cells.<sup>27</sup> Furthermore, Reubi et al<sup>23</sup> have shown that the subcellular (membranous, cytoplasmic, nuclear) distribution of SSTRs may be dependent on the surrounding somatostatin concentration, which is consistent with both the known effect of somatostatin to cause SSTR-2 internalization and an autocrine regulation of tumors by the peptide they produce.

A recent IHC study<sup>17</sup> reported that 90% of the malignant pancreatic NETs expressed SSTR-2 and SSTR-4 and 70% expressed SSTR-1, while only 50% stained positive for SSTR-3 and SSTR-5. In another series,<sup>28</sup> SSTR-2 and -5 were found to be the most frequent SSTR subtypes. In an IHC study of SSTR subtypes, more than 85% of the pancreatic ETs expressed SSTR-1, -2, and -3.<sup>13</sup> In another study, the vast majority of the gastroenteropancreatic ETs expressed SSTR-1, -2, -3, and -5, while SSTR-4 was detected in a small minority.<sup>12</sup> Overall, similar to some earlier studies,<sup>13,17,28-30</sup> we found that SSTR-2 and SSTR-5 were the most frequently expressed subtypes (83%) in the metastatic small intestinal and pancreatic ECAs studied. Such high frequency of SSTR-2 expression in our study suggests that these metastases would likely respond well to octreotide therapy, since clinical efficacy of octreotide therapy has been associated with high expression of SSTR-2 in pancreatic NETs.<sup>13,21</sup> However, SSTR-4 was the least frequently expressed subtype (56%) in our material.

The observed variation in the degree of IHC expression of various SSTR subtypes among the hepatic metastases from the primary small intestinal and pancreatic ECAs provides IHC evidence of the heterogeneity of expression of SSTR subtypes among hepatic metastases (inter-metastatic heterogeneity of expression of SSTR subtypes) (Table 4). Such variation in the degree of IHC expression of SSTR subtypes was also evident when comparing the primary small intestinal and pancreatic ECAs with their respective hepatic metastases (heterogeneity of expression of SSTR subtypes among primary vs metastatic ECAs). Such observed variations in SSTR subtype expression in our study raise the possibility that multiple hepatic metastases from ECAs of the pancreas and small intestine may comprise different clones of metastatic ECA cells that, due to different SSTR subtype expression, may respond differently to various somatostatin analog therapies. In the preliminary efficacy data presented recently, 7 of 28 patients with metastatic carcinoid tumor patients whose symptoms were inadequately controlled by octreotide LAR showed partial response (at least 50% reduction in the frequency of diarrhea over 15 days at a fixed dose) to SOM230 therapy.<sup>20</sup>

## Conclusions

The immunohistologic observations presented in this report are preliminary. However, with the availability of newer somatostatin analogs such as SOM230, the determination of differential expression of various SSTR subtypes, and an assessment of heterogeneity of such expression in larger series of primary and metastatic ECAs of the small intestine and pancreas is clinically relevant. Furthermore, SSTR subtype expression should be correlated with the pattern of clinical response of such patients to somatostatin analog therapies.

Based on the series of optimization experiments carried out in our research laboratory using polyclonal SSTR subtype-specific polyclonal antibodies, we believe that SSTR-IHC is feasible on formalin-fixed ECA tissues. Overall, SSTR-2 was the most frequently expressed subtype in the hepatic metastases of ECAs of the small intestine and pancreas, while SSTR-1 was the least commonly expressed. These findings merit additional SSTR subtype analyses on larger series of patients with endocrine neoplasms. The predominance of cytoplasmic expression of various SSTR subtypes is best explained by prior Sandostatin therapy in our patients. Our observations regarding the immunohistologic evidence of heterogeneity of expression of various SSTR subtypes in primary ECAs of the pancreas and small intestine and their hepatic metastases may have potential relevance in the management of ECA patients in the clinic and should be further investigated.

The new analog of somatostatin, SOM230, binds to 4 of the 5 SSTR subtypes (SSTR-1, -2, -3, and -5). Since our institute was involved in the initial clinical trials of this agent<sup>20</sup> and will be involved in future trials of this agent, we recommend that SSTR subtyping be included in routine histopathologic analysis of enteropancreatic endocrine neoplasms. This recommendation is further supported by our observations that since the expression of various SSTR subtypes in profiles of primary and metastatic enteropancreatic ECAs in a given patient may or may not be the same, we believe that SSTR-subtyping should be included in routine pathologic analysis of these neoplasms. Furthermore, an assessment of heterogeneity of IHC expression of SSTR subtypes among primary and metastatic enteropancreatic ECAs may have potential clinical implications in interpreting the clinical response and the extent of objective tumor regression in response to various somatostatin analog therapies.

As part of our ongoing work in this field, we intend to expand this preliminary study to larger series of neuroendocrine neoplastic tissues, especially with reference to correlation of various pathologic parameters to SSTR subtype expression in these neoplasms. In addition, we are in the process of investigating genomic profiles of these neoplasms to further improve selec-

tion of neuroendocrine cancer patients for various somatostatin analog therapies.

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