

## EXPRESSION AND PROCESSING OF SOMATOSTATIN IN DEVELOPING PANCREAS AND PANCREATIC DUCTAL ADENOCARCINOMA

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**SUMMARY** – Somatostatin is a gastrointestinal peptide hormone that inhibits growth of pancreatic cancer as reported by an increasing body of evidence. Yet this is not always the case. To clarify the controversy we aimed to identify the expression of somatostatin in developing human embryonic pancreatic tissue and pancreatic adenocarcinoma given that somatostatin positive cells were shown either into primitive pancreatic ductal epithelium or into pancreatic carcinoma. Tissue sections representing pancreatic fetal specimens (n=15) and ductal pancreatic adenocarcinoma specimens (n=15) were assessed using immunohistochemical methods for somatostatin expression. Normal primitive exocrine ductal epithelium and endocrine epithelium showed a definite, statistically significant, higher expression of somatostatin over neoplastic pancreatic tissue of mixed (ductal-endocrine) and pure ductal type (p1=0.021, p2=0.001, p3<0.0001, and p4=0.003 respectively) during the 8<sup>th</sup> to the 10<sup>th</sup> week. No statistically significantly different expression of somatostatin in the mantle zone of the islets over neoplastic tissue of mixed (p5=0.16) and pure ductal type (p6=0.65), from the 13<sup>th</sup> to the 24<sup>th</sup> week was demonstrated. Pancreatic cancer cells can express somatostatin in a model that reproduces the normal expression of the peptide by  $\delta$ -cells during embryonal organogenesis. Therapy aimed at pancreatic cancer must be targeted to somatostatin and analogues as a potential adjuvant novel option.

**Key words:** *pancreatic neoplasms – pathology, pancreatic neoplasms – physiopathology, pancreatic neoplasms – therapy; Gastrointestinal hormones – physiology; Somatostatin – physiology; Neoplasms metastasis – preventative control*

### Introduction

The development of the endocrine pancreas is complex and inter-related with the development of the exocrine portion of the organ. It is now clear that both the exocrine and endocrine pancreas are of endodermal origin<sup>1,2</sup>. The evaginations of pancreatic endoderm (fifth week of gestation) into the investing mesenchyma become tubular structures which branch progressively. The prim-

itive duct epithelium provides the stem cell population for all the secretory cells of the pancreas. It gives rise to  $\alpha$  cells which produce glucagon,  $\beta$  cells which produce insulin, and  $\delta$  cells which produce somatostatin during weeks 8-10. Cells containing pancreatic polypeptide (PP) appear somewhat later. All four different endocrine cell types can be distinguished by immunocytochemistry<sup>3,4</sup>. Initially these endocrine cells are located in the duct walls or in buds developing from them. Around the thirteenth week of gestation, formation of the islets of Langerhans commences with the appearance of duct-associated, non-vascularized buds that separate to form the mantle type of vascularized islets characterized by a central mass of insulin-producing cells surrounded by several layers of non- $\beta$  cells<sup>5</sup>. Between

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twenty and twenty-two weeks of gestation, non- $\beta$  cells appear in peripheral and central parts of the islets, and the adult type of islets are formed. Islet formation persists during intrauterine life and can be seen at different phases of the neonatal period. Histologic sections of pancreas examined during this period show both the fetal and the adult types of islets.

Pancreatic carcinoma remains one of the least curable malignant diseases. At present, pancreatic cancer is the fourth leading cause of death in Western countries. It is presently the fifth most common cause of cancer death in the United States, accounting for over 25,000 cancer deaths annually. The only curative treatment for pancreatic tumor is surgical resection. Unfortunately, a surgery for curative purposes is only possible in 10% to 15% of cases, and the overall five-year survival rate of pancreatic cancer is as low as 3.5%. The dismal prognosis of this disease may someday be improved by better understanding of its pathogenesis. Neoplasms of the pancreas arise from ductal, acinar, stromal, or islet cells. The term carcinoma of the pancreas is customarily used only in reference to exocrine tumors and rare mixed endocrine-exocrine carcinomas. Neoplasms including carcinomas composed primarily of endocrine cells are collectively termed islet cell tumors. The precursors of these tumors are presumably developmentally multipotent in terms of their capacity to differentiate into various cell types producing various hormones and regulatory peptides. Whether these cells originate from the ductular epithelium or the islet cells is a matter of debate<sup>7</sup>.

Somatostatin is a cyclic polypeptide hormone isolated from the hypothalamus and characterized by its ability to inhibit release of growth hormone from the pituitary gland. It exists in two forms, somatostatin-14, composed of 14 amino acids, and somatostatin-28, a prohormone composed of 28 amino acids. In the digestive system, somatostatin has been immunolocalized in intrinsic nerves of the intestinal wall and in endocrine cells of the digestive mucosa and pancreatic islets. The antrum, duodenum and pancreas contain almost exclusively somatostatin-14, whereas the gastric body and the rest of the intestine contain 40 to 80 per cent of somatostatin-28. NCL-SOMATOp will label cells of the endocrine mammalian pancreas and also cells of the hypothalamic parvicellular region. NCL-SOMATOp may prove useful for the identification of islet cell tumors and hyperplasia of the pancreatic islets.

Somatostatin and its analogs have been included in experimental treatment for advanced pancreatic carcinoma patients, based on their antisecretory and antiproliferative properties, although cases not responding to monotherapy have been reported.

We investigated the immunohistochemical expression of somatostatin in a series of embryonal and neoplastic human pancreatic tissues. We tried to trace the normal expression profile of somatostatin in tissues with different proliferative and differentiating compartments and to investigate whether somatostatin expression in pancreatic carcinoma recapitulates the normal pattern of expression, or may occur as a result of neoplastic deregulation.

## Materials and Methods

### Tissue sampling

The pancreatic tissues were obtained by pancreatoduodenectomy (the Whipple procedure) for carcinoma of the pancreas. Samples from the pancreas of 15 consecutive surgical patients were included in the study. Two tissue samples were taken from each patient: one from the tumor and one from the resection margin. All tumors were verified as pancreatic adenocarcinomas with various degrees of differentiation. The tissues from the resection margins likewise were examined histologically and were found to be free of tumor cells.

Human embryonic (fetal) pancreatic tissue from fifteen fetuses after involuntary abortion (8 to 10 gestational weeks: 8 samples, 13 to 24 weeks: 7 samples) were investigated.

The local hospital ethics committee approved the use of human tissue, and a written informed consent was obtained from all patients.

### Immunohistochemical procedure

Somatostatin immunoreactivity was evaluated using the lyophilized polyclonal antibody (NCL-SOMATOp) on formalin-fixed, paraffin-embedded samples. Continuous sections of the tissue were cut into 3- $\mu$ m thick slices and immunohistochemistry was performed by the avidin-biotin complex (ABC) method, using DAKO kits. Briefly, after the sections had been dewaxed and rehydrated, they were washed in phosphate-buffered saline (PBS) and incubated for 30 min in normal goat serum to inhibit nonspecific binding. The sections were then washed in PBS and incubated with antibody against somatostatin (NCL-SOMATOp) overnight at 4 °C. The primary antibody was used after dilution (1:150).

Somatostatin (NCL-SOMATOp) immunoreactivity was cytoplasmic, with only occasional and faint nuclear immunostaining. For each sample positive cells in the ducts, islets of Langerhans, aggregates or isolated cells in the pancreatic parenchyma were assessed by enumeration of labeled cells in each tissue compartment for a minimum

Table 1. Reactivity of somatostatin (NCL-SOMATOp) in human embryonal and neoplastic pancreatic tissue

Pancreatic tissue	Number of cases	Density of somatostatinpositive cells (average cells/mm <sup>2</sup> of tissue $\pm$ SEM)
<b>Embryonal (8-10 weeks)</b>	8	
Primitive exocrine duct walls		36.4 $\pm$ 2.3
Primitive exocrine ductal buds		20.9 $\pm$ 0.9
<b>Embryonal (13-24 weeks)</b>	7	
Mantle zone of the islets of Langerhans		27.8 $\pm$ 2.1
<b>Neoplastic tissue</b>	10	
Mixed ductal-endocrine carcinoma	6	32.1 $\pm$ 1.7
Pure ductal carcinoma	4	26.6 $\pm$ 1.3

of five random fields *per* section viewed at 40-fold magnification through a grid. Cell number was calculated *per* 1 mm<sup>2</sup> of tissue section. The counted areas were selected from random fetal and neoplastic pancreatic tissue sections, taking into account that the ratio of the exocrine pancreatic area (acinaracemose), according to the endocrine pancreatic area (islets of Langerhans) was entirely representative. Statistical analysis was done by use of t-test.

## Results

Results are presented in Table 1.

**Embryonal pancreatic tissue** (8 to 10 weeks, old human embryos). During this period of development, endocrine cells (d cells) demonstrated a strong positive immunoreactivity for somatostatin (NCL-SOMATOp), initially in the primitive exocrine duct epithelium (density of somatostatin positive cells = mean of cells/mm<sup>2</sup> of tissue

$\pm$  SEM = 36.4  $\pm$  2.3) (Fig. 1) or forming small aggregates (buds) in the surrounding ductal structures, loose mesenchymal tissue (density of somatostatin positive cells = mean of cells/mm<sup>2</sup> of tissue  $\pm$  SEM = 20.9  $\pm$  0.9) (Fig. 2). From the 13<sup>th</sup> to the 24<sup>th</sup> week of gestation, a period that coincides with the formation of the islets of Langerhans, a strong positive immunostaining for somatostatin (NCL-SOMATOp) was observed to the endocrine cells (d cells) at the periphery (mantle zone) of the endocrine pancreatic islets (density of somatostatin positive cells = mean of cells/mm<sup>2</sup> of tissue  $\pm$  SEM = 27.8 $\pm$ 2.1) (Fig. 3).

**Neoplastic pancreatic tissue.** Somatostatin was demonstrated in ten out of fifteen pancreatic adenocarcinomas. The five somatostatin negative pancreatic adenocarcinomas were of mucinous type. Somatostatin positive cells constituted the majority of neoplastic cells in the duct-like structures or small cords of the tumor. Especially, in six cases diagnosed as mixed ductal-endocrine carcinoma, the density of somatostatin positive cells was 32.1  $\pm$  1.7 cells/

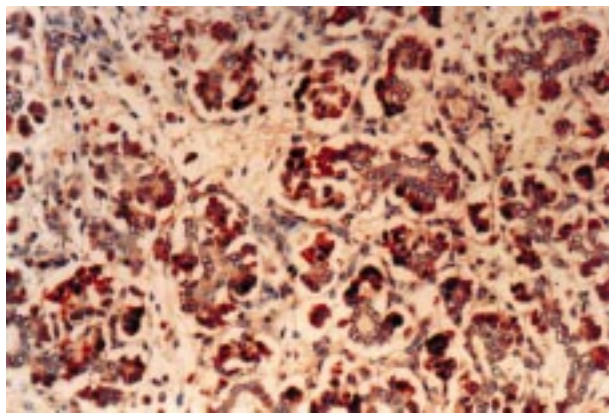


Fig. 1. Somatostatin expression in the primitive exocrine ductal epithelium. NCL-SOMATOp X200.

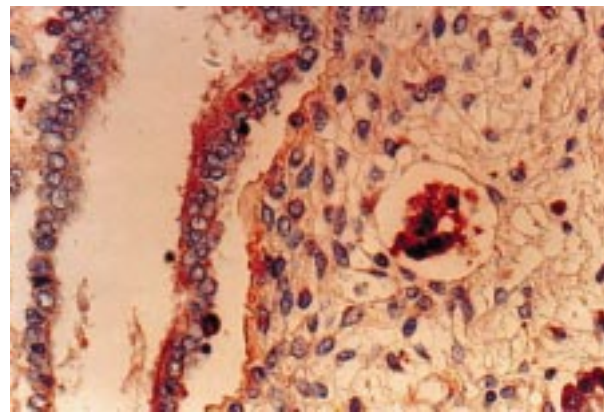


Fig. 2. Somatostatin expression in the primitive exocrine ductal buds. NCL-SOMATOp X400.



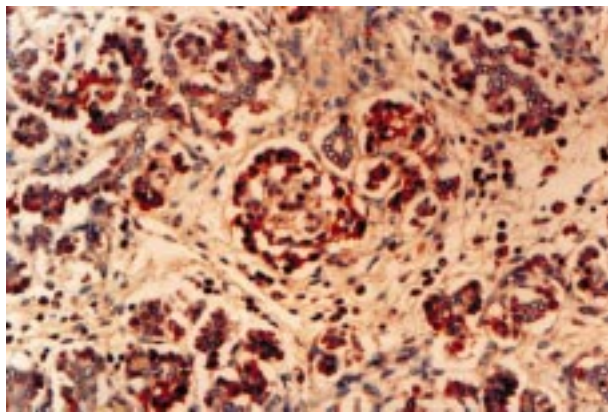


Fig. 3. Somatostatin expression in the primitive endocrine mantle zone of the islet. NCL-SOMATOp X200.

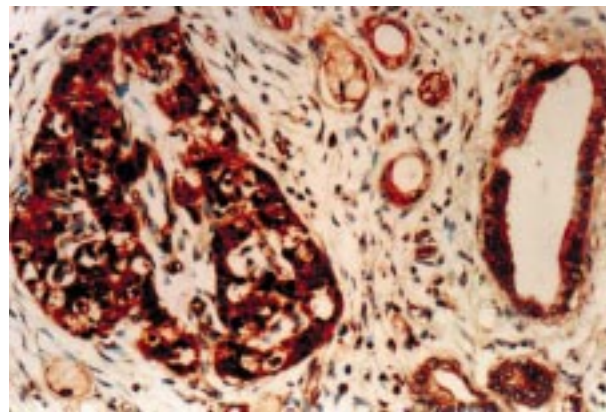


Fig. 4. Somatostatin expression in neoplastic pancreatic tissue with recapitulation of the relevant expression of the antigen in the primitive embryonal pancreatic anlage. NCL-SOMATOp X200.

mm<sup>2</sup> (Fig. 4); in the remaining four cases diagnosed as pure ductal adenocarcinoma the density of somatostatin positive cells was  $26.6 \pm 1.3$  cells/mm<sup>2</sup> (Fig. 5).

There was a statistically significant difference in the expression of somatostatin in the duct-like structures between the primitive exocrine embryonal pancreatic tissue from the 8<sup>th</sup> to the 10<sup>th</sup> gestational week, and the neoplastic pancreatic tissue of mixed type ( $p1=0.021$ ) and pure ductal type ( $p2=0.001$ ).

There was also a statistically significant difference in the expression of somatostatin in the buds surrounding the ductal structures between the primitive exocrine embryonal pancreas from the 8<sup>th</sup> to the 10<sup>th</sup> week, and the neoplastic pancreatic tissue of mixed type ( $p3<0.0001$ ) and pure ductal type ( $p4=0.003$ ).

No statistically significant difference was observed in the expression of somatostatin in the mantle zone between the endocrine embryonal pancreatic tissue from the 13<sup>th</sup> to the 24<sup>th</sup> week, and the neoplastic tissue of mixed type ( $p5=0.16$ ) and pure ductal type ( $p6=0.65$ ).

## Discussion

The prognosis of patients with exocrine pancreatic cancers remains very poor. Only 36.1% of patients are surgically treated, however, with a 5-year postoperative survival rate of less than 20%<sup>8</sup>. Therefore, new therapeutic approaches for the treatment of exocrine pancreatic cancers must be developed. In the past two decades, the employment of certain gastrointestinal hormones, growth factors, and steroids has been reported in new approaches to control exocrine pancreatic cancers<sup>9</sup>.

Somatostatin is a tetradecapeptide that is widely distributed in the body and inhibits hormonal secretion, cell proliferation, and other cellular processes<sup>10</sup>. These inhibitory effects of somatostatin are mediated by cell-surface somatostatin receptors (sstr), which consist of five subtypes and form the sstr family<sup>11-13</sup>. All five sstr subtypes (sstr-1 to -5) differ in their tissue distribution<sup>11</sup>, pharmacological properties<sup>14</sup>, or affinity to somatostatin analogs<sup>15,16</sup>. Many kinds of somatostatin analogs bind selectively and more potently to sstr-2, -3, and -5 than endogenous ligands, SS-14/SS-28, but these analogs lose potency for sstr-1. Sstr-1 and -4 show strikingly low affinities for the octapeptide analogs, SMS 201-995 (octreotide), RC-160 (vapreotide) and BIM 23014 (lanreotide), which are already in clinical use as long-acting somatostatin analogs for the diagnosis and treatment of a variety of neuroendocrine tumors and

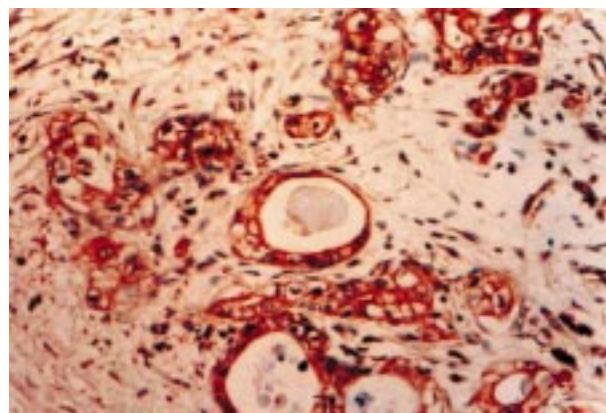


Fig. 5. Somatostatin expression in pancreatic adenocarcinoma of pure ductal type. NCL-SOMATOp X200.

gastrointestinal disorders. The antiproliferative effects of somatostatin and its analogs suggest their therapeutic potential for cancer treatment<sup>17</sup>, and these effects are suggested to be mainly mediated by sstr-1, -2, and -5<sup>18</sup>. Sstr-2 mediates the antiproliferative effects of the long-acting somatostatin analogs, SMS201-995 and RC-160, *in vivo* through the stimulation of tyrosine phosphatase activity<sup>19</sup>. Paz-Bouza *et al.*<sup>20</sup> have reported that RC-160 decreased the volume of experimentally induced tumors, and their colleagues also found regressive changes and necrosis of the tumor by histopathologic methods<sup>21</sup>. Although the potential usefulness of somatostatin analogs for the treatment of pancreatic cancers has been discussed previously<sup>22</sup>, the expression of sstr subtypes in human pancreatic cancer tissues has not been fully studied.

The presence of sstr-2 not only in normal surrounding pancreatic tissues but also in pancreatic cancer tissues in the study of Kikutsuji *et al.*<sup>23</sup> is contradictory to observations reported by two groups<sup>24,25</sup>. Buscail *et al.*<sup>24</sup> have reported that sstr-2 was present in the normal human exocrine pancreas as well as in colon tissues, but that sstr-2 was not expressed in transplanted pancreatic and advanced colorectal carcinoma tissues. This discrepancy may be explained by differences in the culture environments, i.e. the monolayer culture in our study and the subcutaneous implant of tumor tissues in nude mice in their study. The expression pattern of the sstr subtype may be affected by the cellular environment, e.g., that of the monolayer culture or in xenografts<sup>26</sup>.

The purpose of this article is to point to somatostatin expression in embryonic and neoplastic pancreata. In the fetus, somatostatin was expressed in selected developmental phases suggesting a differentiation-related role. Our data reveal the dynamic behavior of the glandular epithelium in the neoplastic pancreas as well, thus indicating that the human epithelial cells in the branching ducts of the neoplastic pancreas may serve as stem cells, which if appropriately induced may differentiate into endocrine cells such as the d-cells expressing somatostatin. This finding could be of therapeutic relevance.

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## Sažetak

## EKSPRESIJA I OBRADA SOMATOSTATINA U GUŠTERAČI U RAZVOJU I U DUKTALNOM ADENOKARCINOMU GUŠTERAČE

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Somatostatin je probavni peptidni hormon koji suzbija rast raka gušterače, za što postoji sve više dokaza. No to se ne događa uvijek. Cilj studije bio je utvrditi ekspresiju somatostatina u ljudskom embrijskom tkivu gušterače u razvoju i u adenokarcinomu gušterače, s tim da su na somatostatin pozitivne stanice dokazane ili u primitivnom duktalnom epitelu gušterače ili u karcinomu gušterače. Tkivni isječci koji su predstavljali uzorke fetalne gušterače (n=15) i uzorke adenokarcinoma gušterače (n=15) ispitani su pomoću imunohistokemijskih metoda za ekspresiju somatostatina. Normalan primitivni egzokrini duktalni epitel i endokrini epitel pokazao je konačnu, statistički značajno višu ekspresiju somatostatina iznad neoplastičnog tkiva gušterače miješanog (duktalno-endokrinog) i čistog duktalnog tipa ( $p_1=0,021$ ,  $P_2=0,001$ ,  $p_3<0,0001$  odnosno  $p_4=0,003$ ) tijekom 8. do 10. tjedna. Nije dokazana statistički značajno različita ekspresija somatostatina u ovojnom sloju (mantle zone, mantle layer) otočića iznad neoplastičnog tkiva miješanog ( $p_5=0,16$ ) i čistog duktalnog tipa ( $p_6=0,65$ ) od 13. do 24. tjedna. Dakle, stanice raka gušterače mogu izražavati somatostatin na način koji ponavlja normalnu d-staničnu ekspresiju peptida za vrijeme embrijske organogeneze. Liječenje zbog raka gušterače usmjereno na somatostatin i njegove analoge moglo bi predstavljati novu mogućnost adjuvantne terapije.

Ključne riječi: