A Novel Secretin Receptor Splice Variant Potentially Useful for Early Diagnosis of Pancreatic Carcinoma

GREGORY M. HAYES,* PATRICIA E. CARRIGAN,* MAOQING DONG,* JEAN–CLAUDE REUBI,‡ and LAURENCE J. MILLER*

*Mayo Clinic, Cancer Center and Department of Molecular Pharmacology and Experimental Therapeutics, Scottsdale, Arizona, and ‡Institute of Pathology of the University of Bern, Division of Cell Biology and Experimental Cancer Research, Bern, Switzerland

Background & Aims: **Pancreatic and bile duct carcinomas represent highly aggressive malignancies that evolve from secretin receptor-rich ductular cells. With premessenger RNA splicing abnormalities common in cancer, we evaluated whether an abnormal secretin receptor spliceoform were present, characterized it, and developed a serum assay for it.** ***Methods:*** **Cancer cell lines and healthy and neoplastic tissue were studied by nested reverse-transcription polymerase chain reaction and sequencing. A promising spliceoform was isolated and characterized, and monoclonal antibodies were raised to 2 distinct regions. A dual antibody enzyme-linked immunosorbent assay was developed and applied to blinded serum samples from 26 patients with pancreatic carcinoma, 10 patients with chronic pancreatitis, and 14 controls.** ***Results:*** **Each of 9 pancreatic cancer specimens and no normal tissue expressed a secretin receptor variant with exons 3 and 4 deleted. This encoded a 111-residue peptide with its first 43 residues identical to wild-type receptor, but, subsequent to a shift in coding frame and early truncation, the next 68 residues were unique in the transcriptome/proteome. This nonfunctional soluble protein did not bind or signal in response to secretin and was secreted from transfected MiaPaCa-2 cells. Elevated serum levels of this variant were present in 69% of pancreatic cancer patients, 60% of chronic pancreatitis patients, and 1 of 14 controls.** ***Conclusions:*** **We identified a novel abnormal spliceoform of the secretin receptor in pancreatic and bile duct cancers and developed a dual antibody sandwich enzyme-linked immunosorbent assay to measure it in the circulation. Initial application of this assay in patients with pancreatic cancer and chronic pancreatitis was promising, but additional validation will be required to evaluate its clinical utility.**

Pancreatic and bile duct cancers are highly aggressive tumors, having extremely poor prognosis. With no proven curative chemotherapy or radiation therapy, and with the success of surgical resection correlating inversely with the size and invasiveness of these tumors, tests that would facilitate earlier diagnosis would represent a major advance. Pre-messenger RNA (pre-mRNA) splicing has been shown to be altered in the majority of neoplasms, including both pancreatic carcinoma and cholangiocarcinoma.[1–3] In the current report, we focus on a newly identified aberrant spliceoform of the secretin receptor that has characteristics making it a promising candidate to represent an early diagnostic biomarker for these tumors.

Normal pancreatic and biliary ductular epithelial cells uniquely express high concentrations of the wild-type secretin receptor, a molecule that mediates the postcibal stimulation of alkaline secretion from these cells.[4,5] Pancreatic cancer cell lines and pancreatic and biliary ductular carcinomas have also been shown to express the secretin receptor, as well as expressing a misspliced form of the secretin receptor in which exon 3 is inappropriately excised.[2,6] This aberrant spliceoform results in the in-frame deletion of a fragment encoding 36 amino acids within the receptor amino-terminus (residues 44 through 79), a domain known to be critical for the natural agonist peptide binding and biologic activity of this receptor.[6] Although this isoform has been shown to be nonfunctional, with no secretin binding or biologic activity, it has been shown to act as a dominant negative inhibitor of coexpressed wild-type secretin receptor.[6,7] This has important implications for cancer growth because secretin is normally an inhibitor of cell growth, and interference with this growth-inhibitory action can result in enhanced malignant cell growth.[6]

Recent evaluations of secretin receptor expression in normal and pathologic conditions of the human pancreas and liver identified potential splice variants of the secretin receptor mRNA in both types of cancer that were distinct from the abnormal spliceoform reported previously in a patient with gastrin-secreting islet cell tumor who had a false-negative secretin stimulation test[7] and in patients with pancreatic ductular adenocarcinoma.[2,6]

Abbreviations used in this paper: GST, glutathione *S*-transferase; HA, hemaglutinin; hSecR, human secretin receptor.

0016-5085/07/$32.00
doi:10.1053/j.gastro.2007.06.013

Furthermore, the high concentration of secretin receptor expression early in induced biliary ductular proliferation in distinct animal models, such as in the bile duct-ligated mouse,[4] makes variants of this protein even more attractive as a potential early biomarker. It was particularly interesting that, in the current work, the pattern of the pre-mRNA transcripts observed in the primary tumors was different from that observed in the commonly studied cultured tumor cell lines.

The secretin receptor isoform chosen for further characterization and for potential diagnostic assay development in this report represents a splice variant that has not been described previously, which is present in the majority of these tumors that includes a frameshift and early truncation. The translation of this variant mRNA is predicted to result in the production of a secreted soluble 111-residue peptide, with the sequence of its first 43 residues being identical to the amino-terminal end of the secretin receptor, followed by a 68-residue sequence at its carboxyl terminus that is not present in the normal transcriptome/proteome. Indeed, we have demonstrated that this mRNA is stable in a model cellular system and can be translated to produce a novel peptide with the predicted characteristics. Two unique antigenic epitopes within this peptide were used to raise high affinity and specific monoclonal antibodies (mAb). These were shown to recognize a peptide that can be secreted from tumor cell lines. Additionally, this immunoreactive peptide was also demonstrated to be present in the serum of the majority of the patients with pancreatic cancer and of those with chronic pancreatitis and being present in only 1 of 14 controls without clinical evidence of pancreatic disease or cancer. Further clinical characterization and validation of the usefulness of this assay will be essential.

Materials and Methods

Cell Culture

The pancreatic ductal adenocarcinoma cell lines Capan-1, Capan-2, MiaPaCa-2, Panc1, BxPC3, and Su86.86 were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and cultured according to ATCC specifications. The pancreatic cancer cell line L3.6pl was provided by Dr. Isaiah Fidler (MD Anderson Cancer Center) and cultured in modified Eagle medium supplemented with 10% fetal bovine serum, nonessential amino acids, sodium pyruvate, L-glutamine, and vitamin solution (all from Invitrogen, Carlsbad, CA). The near normal human pancreatic ductular cell line HPDE6 was provided by Dr. M. S. Tsao (Ontario Cancer Institute, Canada) and grown in Keratinocyte-serum-free medium (SFM) supplemented with bovine pituitary extract and epidermal growth factor (Invitrogen), as previously described.[8,9] All cells were allowed to grow to a maximum of ~80% confluency before being trypsinized and harvested, washed once in phosphate-buffered saline (PBS), and resuspended at 5×10^7 cells per milliliter Trizol (Invitrogen) for extraction of RNA.

Reverse-Transcription Polymerase Chain Reaction Analysis of Secretin Receptor Expression in Cell Lines and Human Tissues

RNA was isolated from Trizol extracts of cultured pancreatic cell lines (see above) or from macroscopically dissected human pancreatic and hepatic tissues. The protocol for this use of the pancreatic tissue was reviewed and approved by the Mayo Clinic Institutional Review Board. Matched samples of benign and malignant pancreatic tissue were acquired from multiple patients after they had provided informed consent. The hepatic tissues were acquired from the University Hospital of Bern and the Charite Medical Center-Virchow Hospital Berlin, with the approval of the respective institutional review boards, having been utilized previously for secretin receptor expression studies.[3] Tissues were ground in liquid nitrogen and resuspended in Trizol at a concentration of approximately 10 mg per milliliter Trizol. Total RNA was purified from 100-μL aliquots of each Trizol suspension using the Qiagen RNEasy-Isolation Kit (Qiagen Inc, Valencia, CA) and treated with DNAse I (Invitrogen) to remove any possible contamination with genomic DNA. The integrity and concentration of the RNA were established by gel electrophoresis and spectrophotometry. Aliquots of 0.5 μg total RNA were used to produce complementary DNA (cDNA) using Promega's First Strand cDNA synthesis kit (Madison, WI).

Approximately 25 ng cDNA was used as template for standard polymerase chain reaction (PCR) using Platinum Taq polymerase, dNTPs, and primers per manufacturer's protocol (Invitrogen). Nested reverse transcription (RT)-PCR amplification of human secretin receptor (hSecR) transcripts was performed using primers designed against the 5′ and 3′ regions of the cDNA for the first reaction (5′-GCA GCA GCT ACT ACT GCC GGT GC-3′ and 5′-AGC CTT CGC AGG ACC TCT CTT GG-3′, respectively). One microliter of the first PCR reaction was used as template for the nested reaction using primers designed against exons 2 and 5 of the secretin gene (5′-AGA GCA AGA CCA GTG CCT GCA GG-3′ and 5′-AGA GGA TGC CAA GGG CGA CCA G-3′, respectively). Actin control reactions were run utilizing the primers 5′-CCA GCT CAC CAT GGA TGA TGA TAT CG-3′ and 5′-GGA GTT GAA GGT AGT TTC GTG GAT GC-3′. Amplification products were resolved on 2% agarose gels, and representative bands were excised and sequenced to confirm their identities.

Generation of Anti-hSecR(Δexon 3,4) Monoclonal Antibodies

Two potentially unique antigenic determinants were identified via in silico examination of the amino acid sequence encoded by the 111-residue hSecR(Δexon 3,4) splice variant. The first 43 residues of this protein