Prevalence of a Variant Gastrin Receptor RNA and Correlating Genomic Polymorphism in Human Pancreatic Cancer

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Abstract
Currently, the five-year survival rate of pancreatic cancer is an abysmal 5%. Our lab has focused on the etiology of CCK2i4svR, a hyperactive splice variant of the gastrin receptor (CCK2R), which has been associated with increased pancreatic tumor aggressiveness. To determine if a correlation exists between a single nucleotide polymorphism (SNP) in the receptor, and expression of the variant RNA in patients, we aim to genotype human pancreatic tumor samples and quantify CCK2R and CCK2i4svR mRNA. Initial analysis of RNA samples by Real Time RT-qPCR has demonstrated successful quantification of CCK2R expression in both tumor and normal tissue samples as well as very low levels of CCK2i4svR transcript. A higher frequency of splice variant receptor was detected in normal pancreatic tissue. Ongoing efforts will bolster sample size and test for the mis-spliced receptor in true normal samples.

Introduction
• Currently, treatments for pancreatic cancer are ineffective due to initial asymptomatic tumor development prior to diagnosis.
• Gastrin, a secreted peptide hormone, can engage in an autocrine signaling loop leading to increased tumor aggressiveness.
• Gastrin signaling is mediated through the cholecystokinin 2 receptor (CCK2R).
• A splice variant of CCK2R, CCK2i4svR, binds gastrin at a higher affinity and further increases tumor aggressiveness.
• The CCK2i4svR variant arises from retention of intron 4, which leads to a 69 amino acid insertion in the third intracellular loop.
• Heretofore, this splice variant has only been detected in colon and pancreatic cancer cells.
• A possible explanation for the intron retention is a single nucleotide polymorphism (SNP) in the 32nd nucleotide of the 4th intron, which switches from a cytosine (C) to an adenine (A)³.

Hypothesis: CCK2i4svR mis-splicing may result from a multi-factor in vivo phenomenon.

Results

Figure 1. Banked patient samples were mostly normal. Paraffin-embedded tissue samples were sectioned and H&E stained for histological analysis. (A) A representative image of normal pancreatic acinar cells and ducts. (B) Carcinous tissue shows increased fibrosis, dysplastic ductal cells (arrowhead), presence of immune infiltrate and a lack of uniformity. (C) 106 samples were graded and the 14% were contained adenocarcinoma (PDAC) or precursor PanIn lesions. Approximately one-third of samples exhibited other pathologies, including neuroendocrine, duodenal, or ampullary tumors.

Figure 2. PDAC samples exhibited a higher frequency of the A allele (A/A or A/C). Genotype distributions of normal pancreatic samples (A) and PDAC/PanIn samples (B) are shown above. This SNP resides within intron 4 of the CCK2R receptor mRNA and was determined utilizing a rhAMP SNP assay.

Figure 3. CCK2i4svR was detected in A allele-containing, normal pancreatic patient samples. Through RT-qPCR, expression levels for CCK2R and CCK2i4svR were quantified using specific primers to discriminate between the splice variants. Receptor expression was calibrated to the housekeeping gene, PPIA. Across patient samples, high variability in total receptor expression was observed. Expression of both receptor variants was significantly higher in normal (A, B) than PDAC (C, D) samples (note axis scaling). CCK2i4svR was more frequently detected in normal (B) than PDAC (D) samples.

Methods
Tissue grading: Paraffin-embedded tissue samples were sectioned and slides were stained with hematoxylin and eosin. Histological determination of tumor vs. normal was provided by a certified anatomic pathologist.

RNA and DNA Extraction and Isolation: Frozen tissue samples were ground into a powder using a mortar and pestle. Guanidinium thiocyanate-phenol-chloroform extraction with needle homogenization was utilized for extraction of RNA and DNA. DNA was isolated from the organic layer by ethanol precipitation and trisodium citrate:10% ethanol and 75% ethanol washes prior to resuspension in DNA hydration solution (GenDia Puregene, Qiagen). RNA was purified with a RNeasy kit (Qiagen) per manufacturer’s protocol.

Genotyping: Genotyping was accomplished with a custom rhAMP assay (IDT) for the specified SNP. RT-qPCR was utilized for the CCK2R mRNA. The CDNA synthesis was conducted with a high-capacity reverse transcription kit (Applied Biosystems) per recommended protocol.

SNP Green Real Time PCR: SYBR green was used as a fluorescent reporter in RT-qPCR experiments using variant-specific primers (Yoon, et al. 2017). The PCR parameters were as follows: 95ºC for 15 seconds, 50 cycles at 95ºC for 3 seconds, and 62ºC annealing and elongation for 15 seconds. Pseudoprolin isomerase (PPIA) was used as the endogenous control.

Conclusions:
• Compared to A-allele frequency in the normal human population (14%):
  • Elevated frequency (47%) in PDAC samples may support a correlation between the SNP and PDAC.
  • Elevated frequency (41%) in our normal samples suggests these may not represent true normal.
• No clear correlation is discernable between the SNP and CCK2i4svR mRNA expression.
• Higher receptor expression in normal samples may reflect differential tissue composition (normal acinar vs. fibrotic ductal/cancer tissue).
• CCK2i4svR was detected in normal tissue, which has not been previously reported.

Acknowledgments
We would like to thank Messiah College Department of Biological Sciences for providing necessary resources. Joseph Yoon for developing the primers used in this study®, Gail Metters and Christopher McGovern for providing the tissues used, and Dr. Fran Ruggiero, Penn State Milton S. Hershey Medical Center, for histological interpretation. The Stinebrecher Research Fellowship provided funding for summer efforts.

References

Notes: