Discovery of G Protein Coupled Receptor Tumor Signatures and Identification of Positron Emission Tomography Imaging Targets in Ileal and Pancreatic Neuroendocrine Tumors

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Introduction
Neuroendocrine tumors (NETs), comprised of carcinoids and pancreatic endocrine tumors, are a rare form of cancer that occur in approximately 1 in 100,000 people per year.1 Early detection of NETs can be difficult due to their vague symptoms. This delay in diagnosis can give tumors the opportunity to metastasize, making surgical resection of the tumor impossible.2 G protein coupled receptors (GPCRs) have emerged as candidates for molecular targeting in NETs, with potential uses in both tumor imaging and therapy. GPCR characterization that accounts for their success as drug targets include cell surface overexpression, exquisite specificity of ligand-receptor interactions, and key roles in NET signal transduction pathways. We hypothesize that pancreatic and ileal NETs have distinct GPCR signatures that can be exploited to develop tumor specific Positron Emission Tomography (PET) imaging agents. To test the hypothesis, we isolated RNA from specimens of ileal and pancreatic NETs and compared GPCR expression to adjacent normal tissue. Based on these findings we synthesized multiple peptide ligands targeting the upregulated GPCRs specific to the ileal and pancreatic NETs. Following conjugation of the metal chelator DOTA, the peptides were radiolabeled with 68Ga for in vitro and in vivo studies.

Methods
RNA Isolation and GPCR expression

Solid phase peptide synthesis and DOTA conjugation Peptides were synthesized by standard Fmoc solid phase synthesis at a 0.1 mmol scale on an ABI PRIME A. The peptide sequence was designed to avoid post translation modification and to ensure good cleavage from the resin. After synthesis the peptides were purified by reverse phase HPLC and characterized by LC-MS. The purified peptides were conjugated with DOTA, and radiolabeled with high specific activity [68Ga]-DOTATOC (DOTA-Tyr3-octreotide) or [111In]-DTPA-Octreotide. All scans were performed on the GE Healthcare instrument.

Conclusions
• Tumor specific GPCR signatures were identified in ileal and pancreatic NETs
  − Ileal: OPRK1, GHSR, CALCR, NPY1R, OXTR
  − Pancreatic: SST2, SSTR5, VIPR1, HTR1D
• Both: MCH1R

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Results

Figure 1. GPCR expression in ileal NET compared to normal adjacent tissue. Red color indicates low CT or CTG, corresponding to low expression. Blue is intermediate CT and intermediate expression; green is high CT and low expression. Each gene is compared against ileal NET tumor and normal tissue and significance with p<0.01. Arrows point to the candidate genes significantly p<0.01 upregulated in ileal NET.

Figure 2. GPCR expression in pancreatic NET compared to normal adjacent tissue. Red color indicates low CT or CTG, corresponding to low expression. Blue is intermediate CT and intermediate expression; green is high CT and low expression. Each gene is compared against ileal NET tumor and normal tissue and significance with p<0.01. Arrows point to the candidate genes significantly p<0.01 upregulated in ileal NET.

Results Continued

Table 1. Gene expression fold changes between ileal and pancreatic neuroendocrine tumor and normal tissue for selected candidate GPCRs. GPCRs highlighted in yellow indicate good candidate receptors for PET imaging agents of both NETs. Solid black arrows indicate upregulated candidate receptors for PET imaging agents of ileal NETs. The fold change and p-values were calculated from the statistical analysis program, Stattelor.

Table 2. Patient data for neuroendocrine tumor samples. Note that NET was not visible in 2 of 5 ileal primary lesions while 4 of 5 pancreatic primary lesions were visualized by Octreoscan. Metastatic lesions were positive in both ileal and pancreatic tumors.