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Differentiation of Small Bowel and Pancreatic Neuroendocrine Tumors by Gene Expression Profiling

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INTRODUCTION

Neuroendocrine tumors (NETs) are rare neoplasms whose incidence has increased five-fold over the past three decades[1]. These tumors are derived from enterochromaffin and islet cells, and may secrete a variety of polypeptides, which may cause symptoms and can be useful for diagnosis, detection, and treatment of these tumors[2]. Two of the most common sites of these malignancies involving the digestive system are the small bowel (SBNETs) and pancreas (PNETs), and approximately 58% of patients with SBNETs and 72% of those with PNETs present with metastatic disease[3], primarily to the liver. It has been estimated that as many as 10–20% of patients with liver metastases present with an unknown primary site[4], and in these cases, being able to determine the primary site has important implications for surgical approach and therapeutic measures.

The preferred treatment for SBNETs and PNETs is surgical resection, even in the face of metastatic disease[5]. Medical therapy for metastatic NETs includes the use of somatostatin analogs, which helps to control hormonal symptoms as well as improve time to progression[6]. Sandostatin is an 8 amino acid analog of somatostatin which mediates its effects by binding to the type 2 somatostatin receptor (SSTR2, a G-protein coupled receptor [GPCR]) on the cell surface of NETs. Sandostatin has also been useful in localization of tumors when labeled with Indium, but its success in locating primary GI sites of NETs in patients presenting with liver metastases has been limited[4].

Chemotherapy has also had limited response rates in NETs, with improved survival rates in those with pancreatic tumor sites. Treatment of pancreatic NETs (PNETs) has included combinations of streptozotocin and 5-fluorouracil and/or doxorubicin, with response rates of approximately 40%[7]. However, in non-pancreatic NETs, the response rate is only 25%[7].

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More recently, Sunitinib and Everolimus have been demonstrated to lead to improved disease free survival for patients with advanced PNETs[8, 9].

Even patients with advanced disease may have prolonged survival, with median survivals of 56 and 24 months in metastatic SBNETs and PNETs, respectively[1]. These statistics could potentially be further improved by uncovering new targets, such as additional cell surface receptors (besides SSTR2) which could be exploited for diagnosis, imaging, and therapy. Further understanding of these NETs could come from analysis of their expression patterns, which might then be used to categorize the genetic profiles of different primary sites. This would hold promise for determining the site of origin from biopsies of liver metastases, and to identify targets for future therapy. To date, the limited gene expression studies performed in SBNETs and PNETs have not identified consistent and useful patterns of over and underexpressed genes[10–14], and therefore the objective of this study was to examine matched tumor and normal tissues from these sites, then determine whether these could be used to predict the primary sites from liver metastases.

PATIENTS AND METHODS

Patients and Approach

Patients were recruited from the Neuroendocrine Cancer Clinic at the University of Iowa, a multidisciplinary effort between the Departments of Surgery, Endocrinology, and Medical Oncology. Patients were consented to participate in the NET registry, and for collection of tissue samples for research purposes under a protocol approved by the University of Iowa IRB. At operation, 500–1000 mg of primary tumor, normal tissue from that site, metastatic lymph nodes, and liver metastases were retrieved, where available. These were divided into 3-5 mm pieces, immediately placed in RNAlater solution, then stored at −20°C.

RNA Extraction

One hundred mg of tumor or normal tissue was removed from the RNAlater solution, placed in Trizol reagent (Invitrogen, Grand Island, NY), then homogenized using a Polytron PT 3100 (Kinematica AG, Bohemia, NY). RNA was extracted using the standard Trizol method, with isolation of the aqueous phase separation after tissue homogenization. RNA was precipitated using 100% isopropanol, washed with 75% ethanol, and then resuspended in 40 uL of RNase-free water. RNA quality was assessed spectrophotochemically by $A_{260/280}$ ratios, and RNA integrity number (RIN) using an Agilent 2100 bioanalyzer (Agilent, Santa Clara, CA). RNA was either stored at −80°C, or used immediately for the downstream applications noted below.

Exon Arrays

Microarray hybridizations were performed at the University of Iowa Core DNA Facility. Briefly, 50 ng total RNA was converted to Single Primer Isothermal Amplified (SPIA)-cDNA using the Whole Transcript (WT)-Ovation Pico RNA Amplification System (NuGEN Technologies, San Carlos, CA). The amplified SPIA cDNA product was purified through a QIAGEN QIAquick PCR column (QIAGEN, Valencia, CA), then 4 ug of this product were used to generate single transcript (ST)-cDNA using the WT-Ovation Exon Module. Five ug of QIAquick column-purified product was fragmented (average fragment size = 85 bases) and biotin labeled using the NuGEN FL-Ovation cDNA Biotin Module. The resulting biotin-labeled cDNA was mixed with Affymetrix eukaryotic hybridization buffer (Affymetrix, Inc., Santa Clara, CA), and hybridized with the Human Exon 1.0 ST arrays (Affymetrix) at 45° C for 18 hrs. with 60 rpm rotation in an Affymetrix Model 640 Genechip Hybridization Oven. Following hybridization, arrays were washed, stained with streptavidin-phycoerythrin (Molecular Probes, Inc., Eugene, OR), and signal amplified with

antistreptavidin antibody (Vector Laboratories, Inc., Burlingame, CA) using the Affymetrix Model 450 Fluidics Station. Arrays were scanned with the Affymetrix Model 3000 scanner with 7G upgrade and data were collected using the GeneChip operating software (GCOS) v1.4.

GPCR Arrays

One ug of total RNA was converted to cDNA using the ABI high capacity RNA to cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA). 500 ng of cDNA was added to TaqMan Gene Expression Master Mix (Applied Biosystems) to a total volume of 100 uL. Each fill reservoir of the TaqMan Human GPCR array (which contains fluorescent primers for 367 GPCR gene targets and 14 housekeeping genes) was filled with the sample as noted above, then centrifuged to evenly distribute the reaction mix to the wells. Cards were sealed with the TaqMan Array MicroFluidic Card Sealer, and real-time PCR performed using the 7900HT Fast System (Applied Biosystems).

qPCR

Quantitative PCR was performed using probes for the genes of interest, as determined by the GPCR arrays. For these studies, RNA was extracted from liver metastases from 5 SBNETs and 5 PNETs, as described above. 500 ng of total RNA was converted to cDNA as described for GPCR arrays, of which 20 ng was added to the PCR master mix (5 uL of TaqMan Fast Universal PCR Master Mix (Applied Biosystems), 2.5 uL RNAse free water, and 0.5 uL of the gene primers and GAPDH control primers). qPCR was performed in triplicate for each sample using the StepOnePlus Real-Time PCR System (Applied Biosystems).

Data Analysis

For GPCR arrays, data from the 7900 Fast System was exported to the SDS RQ Manager v1.2 (Applied Biosystems). The Ct was set to 0.2. Data analysis was based on the comparative CT method ($2^{-\Delta\Delta CT}$)method[15]. Data were then exported to the RT^2 Profiler PCR Array system (SABiosciences, Frederick, MD). Data were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hypoxanthine guanine phosphoribosyl transferase 1 (HPRT1), and polymerase (RNA) II (DNA directed) polypeptide A (POLR2A). For the GPCR arrays, p-values were generated for pooled samples of both PNETs and SBNETs (tumor compared to normal surrounding tissue), with statistical significance noted at $p<0.05$.

For Exon Arrays, data derived from the exon chip hybridizations were exported from GCOS to the PartekGS software (Partek Inc). Data were normalized, with median polish performed using Robust Multichip Average background correction. The median value of probe-sets for all exons of each gene were calculated. False discovery rate (FDR) correction was applied to the p-value to correct for the potential false positive results due to paired testing of the approximately 30,000 gene targets. Significance was assessed by a combination of maximum FDR q-value and minimum fold-change magnitude. Samples were also compared using ANOVA with tumor and normal linear contrast sub-sets, as well as hierarchical and k-means clustering techniques with Cluster 3.0 followed by Treeview for visualization[16]. In order to develop expression profiles, hierarchical clustering and self-organizing maps (SOM) were used to classify the initial groups of small bowel and pancreatic tumors as described[17,18]

For qPCR, each sample was normalized to GAPDH. The normal tissue was then used as the baseline for comparison, setting this value to 1. The tumor sample output was then analyzed using the internal software, with fold changes calculated using the $2^{-\Delta\Delta CT}$ method.

RESULTS

Exon Arrays

A total of 11 patients with SBNETs and 5 patients with PNETs were evaluated, including corresponding normal and tumor tissues from each site. All genes were assessed and sorted by overall fold change between the tumor and surrounding normal tissue. An initial cutoff value of greater than 5-fold change was used (either up or down regulated in tumor), and in which the expression differences were statistically significant ($p<0.05$). In small bowel samples, 56 genes were significantly upregulated in tumor samples vs. normal tissue which met these criteria, and 117 genes were downregulated. In pancreatic samples, 39 genes met these criteria for upregulation, and 13 for downregulation. Table I displays 10 selected genes that demonstrated significant up or down regulation for pancreatic and small bowel sites.

GPCR Arrays

A total of 11 patients with SBNETs and 15 patients with PNETs were evaluated using this method. As in the exon arrays, data resulting from the 367 GPCR gene array was evaluated first by a fold change greater than 5, then by a statistically significant change with a p value <0.05. In small bowel samples, of the 367 genes evaluated, there were 23 which had significant upregulation in tumor samples when compared to their normal tissue counterparts. Only 5 genes were significantly downregulated in small bowel tumors versus normal tissue (Table II). In pancreatic samples, one gene was significantly upregulated, and 17 were found to be significantly downregulated relative to normals (Table III).

Formulation of Expression Profiles

The tumor/normal pairs for each site were then evaluated by constructing a chart noting which receptors were significantly up or downregulated for each sample. After the data were compiled, a receptor expression profile was created for each site using the least number of genes possible. For small bowel samples, three receptors were noted to be upregulated in every tumor: the G-protein coupled receptor 113 (*GPR113*), G-protein coupled receptor 116 (*GPR116*), and the oxytocin receptor (*OXTR*). Each receptor showed a change of >5 fold in the tumor as compared to normal tissue, and each was noted to be statistically significant on initial evaluation for all pooled small bowel samples. This model was further refined to ensure no pancreatic samples would be identified. In order to do so, the *GPR116* receptor was discarded, as it was upregulated in a small number of pancreatic samples. Therefore, *GPR113* and *OXTR* remained the two genes that were both upregulated >5 fold in all small bowel samples; no pancreatic sample shared this expression profile (Table IV).

For pancreatic samples, it was impossible to find a receptor profile that discriminated this site from small bowel primaries in all cases, except for the absence of >5-fold upregulation of *GPR113* and *OXTR*. However, in 11 of 15 pancreatic samples, there were two receptors which showed >5-fold downregulation in the tumor samples when compared to their normal tissue counterparts, the secretin receptor (*SCTR*) and the adenosine A1 receptor (*ADORA1)*. The simplest classification scheme to differentiate primary SBNETs from PNETs was therefore: 1) >5-fold upregulation of both *OXTR* and *GPR113* as compared to normal tissue=SBNET; 2) <5-fold upregulation of both *OXTR* and *GPR113* as compared to normal tissue=PNET; 3) >5-fold downregulation of *ADORA1* and *SCTR* as compared to normal tissue was further supportive evidence for PNETs.

Although this profile correctly identified all of our NETs, it relies on the use of both tumor and normal tissue. However, when one is faced with a neuroendocrine tumor liver metasasis of unknown primary, this will be the only site of tissue available for testing. Therefore, to further refine our model, we reassessed our results using tumor samples only, in order to

exclude the need for normal tissue. After examining several parameters, the best fit was found by simply examining the C_t results of OXTR from SCTR (where $2^{(CT\ SCTR-CT\ OXTR)}$=fold change). *OXTR* expression of >20 fold *SCTR*defined SBNETs, while <5 fold expression change defined PNETs; fold changes between 5 and 20 were deemed indeterminate. Assessing our primary samples, we found that 8 of our 11 SBNETs fit this profile, as did 14 of 15 of our PNETs; 3 SBNETs and 1 PNET were indeterminate.

qPCR in Liver Tumors

Results from the qPCR in the liver metastases and corresponding normal tissue were analyzed according to the original classification. When the criteria of >5-fold upregulation of *OXTR* and *GPR113* to be of SBNET origin were applied in blinded fashion to 10 liver metastases (Table V), 4 of 5 SBNET metastases displayed this profile, which was not observed in any of the 5 PNET metastases. The presence of >5-fold downregulation of *ADORA1* and *SCTR* did not occur in any of the SBNET metastases, and appeared to be less useful for differentiating PNET metastases than seen with the primary tumors. These determinations required comparison of qPCR results from both the metastases and corresponding normal tissues, and therefore were not a good surrogate for testing of liver biopsy samples in isolation. We then applied our more refined profile of $2^{(CT\ SCTR-CT\ OXTR)}$, and found in the qPCR results from 5 small bowel and 5 pancreatic metastases blinded to site, 4 of the 5 small bowel samples fell in the SBNET range, 4 of 5 pancreatic samples were in the PNET range, and the remaining two samples were indeterminate.

DISCUSSION

To date, there have been few publications analyzing gene expression in primary SBNETs. One study by Drozdov et al. examined 9 SBNETs and normal small bowel mucosa samples using Affymetrix U133A arrays, with probesets corresponding to 14,500 genes. They performed a gene network analysis and ultimately reported that of 3470 genes in 10 ontology pathways, 27% were differentially expressed. They then focused upon 2 GPCR and 7 cAMP response-element binding genes, and hypothesized that overexpression of these 2 classes of genes may result in neural activation of secretory genes, providing a possible explanation of the hormonal behavior of these tumors[12]. In our study there was no significant upregulation of these 2 GPCR targets (*ADCY2* and *PRKAR1A*). Four of the postulated CREB targets showed upregulation >5 fold in SBNETs samples; CHGB was upregulated 17.5 fold, BEX1 was upregulated 15.1 fold, and SCG3 was upregulated 7.2 fold. In PNETs, CHGB was also upregulated 7.4 fold. SCG2 and 3 were upregulated in PNETs a well, to 8.0 and 5.8 fold, respectively (Table VI).

The largest study of gene expression profiling in PNETs was by Missiaglia et al., who evaluated 72 PNETs, 7 metastases, and 10 normal pancreatic controls (5 whole pancreas, 5 islet cell preparations) using custom 18.5K arrays[11]. Four categories of tissues emerged from unsupervised cluster analysis: normal pancreas, normal islets, insulinomas, and non-functional PNETs. They found that there were 113 upregulated and 25 downregulated genes in insulinomas compared to normal samples, and 198 upregulated and 55 downregulated genes in non-functional PNETs. The tuberous sclerosis 2 gene (*TSC2*), an inhibitor of the Akt-mTOR pathway, was downregulated in both of these tumor sub-types, and patients with low *TSC2* expression had decreased survival. They also determined that *SSTR2* expression was significantly upregulated in 25 well-differentiated, non-functional PNETs as compared to 14 insulinomas. Fibroblast growth factor 13 (*FGF13*) was found to be overexpressed in metastases versus their primary tumors, and was significantly associated with liver metastasis at diagnosis, as well as decreased survival in well-differentiated PNETs. The clinical implications of this study were validation of a mechanism for mTOR inhibitors'

efficacy in treatment of PNETs, a mechanism supporting the use of somatostatin analogues stabilizing disease progression in non-functional PNETs, and that *FGF13* may be a useful marker for progression. From our ST array data, we found that *TSC2*, *SSTR2*, and *FGF13* showed no significant change in expression between tumor and normal tissue in both SBNETs and PNETs (Table VI).

Previous work by Duerr et al.[10] analyzed 24 PNETs (16 well-differentiated endocrine tumors [WDET WHO classification] and 8 well-differentiated endocrine carcinomas [WDEC]) and 6 malignant GI-NETs (3 primary ileal, 1 colon, 2 liver metastases) using Affymetrix U133A arrays. When examining PNETs, they found that the genes *FEV*, *ADCY2*, *NR4A2*, and *GADD45*β were significantly overexpressed in WDECs as compared to WDETs; they also found that microarrays underestimated the degree of upregulation as compared to qPCR. They reported that previous genes of interest in PNETs (*MEN1*, *hMLH1*, *RASSF1*, *Her2/neu*, cyclin D1, retinoic acid receptor β, $p16^{INK4A}/p14^{ARD}$, $p18^{INK4c}$, and $p27^{Kip1}$) were not significantly different between these WHO subtypes. Comparison of 19 PNETs to 6 GI-NETs revealed 385 differentially-expressed genes with at least 1.5 fold change and p-values of <0.05; 157 were upregulated and 228 downregulated in GI-NETs. The most differentially overexpressed genes in GI-NETS included *ECM1* (28-fold by microarray, 39-fold by qPCR), *VMAT1* (25-fold, 523-fold by qPCR), *LGALS4* (24-fold, 43-fold by qPCR). The implications from this study were that 4 genes were found which could help distinguish between PNETs of different WHO classes, and between GI-NETs and PNETs. The shortcoming of this study was that it only looked at a small number of GI-NETs, and only 3 of these were small bowel primaries. In our evaluation by ST arrays, we found that for these 3 genes, there was statistically significant upregulation of *VMAT1* (22 fold) in SBNETs. *LGALS4* and *ECM1* showed no change between tumor and normal tissue (Table VI).

Couvelard et al.[19] examined 12 benign PNETs (WHO-1 and WH0-2 categories) and 12 malignant PNETs (WHO-3) using custom microarrays representing 9932 transcripts, and found that a cluster of 123 genes could differentiate between these 2 groups. The fold changes observed were relatively modest, ranging from 0.47 (downregulated in WHO-3) to 2.26 (upregulated in WHO-3 group), and did not overlap significantly with genes discussed above by Missiaglia or Duerr et al. Since these studies and others[20, 21, 22] using varying genomic expression array platforms have identified different, non-overlapping genes thought to be of importance, we decided to focus our efforts on one important class of genes known to play a role in these tumors using a qPCR based strategy in order to develop a reliable and predictive model. Other reasons for choosing this strategy include the fact that studies comparing qPCR to microarray results have shown and increased sensitivity of the former, that members of the GPCR group emerged as candidates with differential expression in our exon arrays and in Drozdov's study, and this class of genes is deemed as one of the most promising for development of new therapeutic agents by the pharmaceutical industry[23].

There have been just a few studies that have attempted to use gene expression profiling to determine the site of origin from tissue derived from liver metastases. Posorski et al. evaluated 17 NET metastases and 6 primary tumors from 17 patients by both comparative genomic hybridization (CGH) using Agilent 105K CGH microarrays, and genome-wide expression using Agilent 44K expression microarrays. Multiple techniques were used to analyze this data set, including hierarchical clustering of 41,000 genes, which revealed 1,760 differentially expressed genes segregating into 3 clusters (primaries in ileum, pancreas, and stomach). They then attempted to formulate the simplest expression profile they could find that would discriminate between these primary sites. This began by determining whether there was upregulation in the metastasis of *CD302* >13 times that of the primary site, which

indicated that the tumor was ileal in origin. If this was not the case, if *PPWD1* was downregulated >3-fold, the tumors were of pancreatic origin, and if not this, but >4-fold upregulated for *ABHD14B*, then the stomach was the site of the primary tumor[14]. One thing that is not clear from this study is how the fold changes for each were calculated, but it appeared to require using results from the primary as well, which would not make diagnosis from only liver biopsy tissue possible. Furthermore, the overall number of tumors examined here was small. It is difficult to assess whether this profile would be validated in our pancreatic and small bowel samples, as *CD302* was not assessed in our ST arrays. However, our pancreatic samples did not show the same >3-fold downregulation in *PPWD1* as seen in Posorski's study (Table VI).

Another study by Edfeldt et al.[13] analyzed 18 primary tumors, 17 lymph node metastases, and 7 liver metastases from 19 patients with SBNETs. They hybridized RNA to QArray2 microarrays (containing 24,650 genes), then performed cluster analysis, which separated the tumors into 3 groups: 11 primaries, 5 nodes, and 7 liver metastases formed one group; 7 nodes another; and the final group consisted of 5 other primaries. They concluded that the expression clusters did predict clinical outcomes, and that expression patterns were different between primary tumors and their lymph nodes. They reported 8 genes that were differentially expressed between clusters, which included *ACTG2*, *GREM2*, *REG3A*, *TUSC2*, *RUNX1*, *TPH1*, *TGFBR2*, and *CDH6*. Although this study did suggest several gene targets, we only found 3 of these genes (*TPH1, REG3A,* and *CDH6*) to be differentially expressed between primary tumor and normal samples in our study, and only in SBNETs (Table VI).

In this study, RNA was extracted from whole tumor tissues, which often contain a fibrous reaction surrounding the primary tumors. We noted a statistically significant change in expression profile of many genes with this technique, which might have been slightly different if we had extracted from a purer tumor population. Posorski et al. used laser capture microdissection to enrich for the tumor cell population, which they felt was important to obtain useful results[14]. One advantage we did have in this study however, was having normal tissue, primary tumor, and metastases from the same patients. We also did not make full use of the genes identified as being significantly differentially expressed from our ST exon arrays, and instead chose to focus on genes in the GPCR pathway. The higher fold-changes seen with some of these GPCR genes was due in part to the more sensitive qPCR technique used, in contrast to microarray hybridization.

Comparison of the gene expression patterns between normal and tumor tissues allowed us to establish profiles to test in metastases. Of these GPCR genes, *OXTR* and *GPR113* were both >5-fold upregulated in all 11 SBNETs versus normal tissues, and both were not upregulated in all 15 PNETs. Furthermore, 11 of 15 PNETs also had >5-fold downregulation of both *ADORA1* and *SCTR*, which was only seen in 1 poorly-differentiated SBNET (Table IV). When the criteria of upregulation of both *OXTR* and *GPR113* (relative to normal) were used for distinguishing the site of origin in 10 liver metastases, it correctly predicted 4 of 5 SBNETs and 5 of 5 PNETs. However, the practicality of these results are limited in that they were compared to normal tissues, which would not be relevant to a core biopsy sample of a liver metastasis. We reevaluated the expression profiles of *OXTR*, *GPR113*, *ADORA1*, and *SCTR* between primary SBNETs and PNETs relative to our control gene *GAPDH* without normalization to corresponding normal tissues, and found that *OXTR* and *SCTR* expression to be the most useful for distinguishing between the two tumors. *OXTR* expression was >20-fold greater than *SCTR* in 8 primary SBNETs (3 were indeterminate), while there was <5-fold expression difference in 14 PNETs (1 was indeterminate). When blinded to 10 liver metastases' primary site, these differences in *OXTR* and *SCTR* expression correctly identified 4 of the 5 small bowel metastases, 4 of 5 pancreatic metastases, and 1 metastasis

from each site was indeterminate. Since this model correctly predicted the primary site in 80% of these samples, with no incorrect predictions (and 20% indeterminate), this test could have practical clinic value in the evaluation of core biopsy specimens of liver metastases. Validation with a larger number of samples will be needed to confirm the value of this profile, as will evaluation of metastases from other GI sites, such as the stomach, duodenum, and colorectum. Systematic examination of a larger subset of differentially expressed genes from both the exon and GPCR arrays may also prove useful for making these finer discriminations and to improve diagnostic accuracy.

Knowledge that the expression pattern of a metastasis is consistent with a specific primary site could lead to improved surgical exploration. In a study of 123 patients with metastatic NETs, Wang et al. found that only 35% of GI-NETs (small bowel, colorectal, and stomach) were seen on CT scan (and even less on Octreoscan), whereas all PNETs had a pancreatic mass visible on CT[4]. It is our experience that many small bowel tumors are too small to be seen on conventional imaging, and knowledge that a liver metastasis was likely of small bowel origin by virtue of its gene expression pattern would make the decision to explore that patient easier. At exploration, careful palpation of the entire small bowel will uncover primary tumors even a few mm in size, and patients with SBNETs can have relatively small primaries in the face of bulky metastatic disease. In these patients, an aggressive surgical approach is warranted[5], since resection of the primary site in patients with liver metastases leads to both improved progression free survival as well as overall survival[24].

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Table I

Selected Up or DownRegulated Genes in SBNETs and PNETs Relative to Normal Tissue by Exon Arrays

		SBNET vs. Normal		PNET vs. Normal	
Gene	**Gene Name**	**Fold-Change**	**p-value**	**Fold-change**	**p-value**
TTR	Transthyretin	+28.61	2.20E-06	+1.51	5.70E-03
SLC18A1	Solute Carrier Family 18 (Vesicular Monoamine), Member 1	+22.06	3.40E-06	−1.30	1.68E-01
TAC1	Tachykinin, Precursor 1	+18.74	1.26E-06	−1.35	6.28E-02
TPH1	Tryptophan Hydroxylase 1	+17.71	7.82E-05	+2.04	1.13E-01
CHGB	Chromogranin B (Secretogranin 1)	+17.45	4.71E-05	+7.38	8.00E-04
OR51E1*	Olfactory Receptor, Family 51, Subfamily E, Member 1	+5.27	9.84E-04	−1.21	1.86E-02
GPR112*	G-Protein Coupled Receptor 112	+5.05	1.10E-03	−1.13	3.20E-03
DEFA6	Defensin, Alpha 6, Paneth Cell-Specific	−28.16	1.98E-06	+1.05	4.94E-01
SI	Sucrase-Isomaltase (Alpha-Glucosidase)	−24.31	1.21E-05	+1.89	2.95E-01
MEP1B	Meprin A, Beta	−23.71	4.97E-07	+10.04	1.63E-02
FGB	Fibrinogen Beta Chain	+1.17	3.02E-01	+26.63	4.93E-06
IL13RA2	Interleukin 13 Receptor, Alpha 2	+1.10	4.65E-01	+18.15	4.79E-04
MUC13	Mucin 13, Cell Surface Associated	−4.65	8.00E-04	+13.61	4.79E-04
POSTN	Periostin, Osteoblast Specific Factor	+1.23	6.08E-01	+13.17	1.38E-02
KBTBD10	Kelch Repeat and BTB (POZ) Domain Containing 10	+1.18	1.65E-01	+12.86	1.30E-04
ANPEP	Alanyl (Membrane) Aminopeptidase	−13.07	7.75E-06	−10.57	1.54E-02
PDIA2	Protein Disulfide Isomerase Family A, Member 2	−1.19	8.65E-02	−9.61	1.98E-02
LGALS2	Lectin, Galactoside-Binding, Soluble, 2	−4.68	9.79E-05	−8.01	2.36E-02
F11	Coagulation Factor XI	−1.62	9.50E-03	−7.56	1.37E-02
TRHDE	Thyrotropin-Releasing Hormone Degrading Enzyme	−2.13	3.85E-04	−7.36	1.19E-02

*members of GPCR gene family; Gray coloration indicates the primary site of interest with its significantly up and downregulated genes using the Exon Arrays. Non-shaded areas are from the alternate site for comparison

Table II

Small Bowel GPCR Array Data (fold change in tumors relative to normals)

Gene; Gene Name	Fold Change	p-value
GHSR; Growth Hormone Secretagogue Receptor	+97.5	0.0426
OPRK1; Opioid Receptor, Kappa 1	+85.5	0.0021
GPR148; G-Protein Coupled Receptor 148	+71.4	0.0019
MASS1; Monogenic Audiogenic Seizure Susceptibility 1 Homologue	+74.2	0.0051
CALCR; Calcitonin Receptor	+59.8	0.0024
GPR51; G-Protein Coupled Receptor 51	+52.8	0.0056
GPR75; G-Protein Coupled Receptor 75	+33.5	0.0012
GIPR; Gastric Inhibitory Polypeptide Receptor	+27.6	0.0007
OXTR; Oxytocin Receptor	+25.7	0.0088
DRD5; Dopamine Receptor, D5	+21.9	0.0127
BAI3; Brain Specific Angiogenesis Inhibitor 3	+18.6	0.0019
GPR116; G-Protein Coupled Receptor 116	+18.5	0.0239
CELSR3; Cadherin, EGF LAG Seven-Pass G-Type Receptor 3	+18.3	0.0016
CASR; Calcium Sensing Receptor	+17.2	0.0229
GPR113; G-Protein Coupled Receptor 113	+16.7	0.0016
GPR110; G-Protein Coupled Receptor 110	+16.4	0.0149
SSTR2; Somatostatin Receptor 2	+15.2	0.0012
GPBAR1; G-Protein Coupled Bile Acid Receptor 1	+15.0	0.0013
GPR173; G-Protein Coupled Receptor 173	+14.7	0.0014
FZD3; Frizzled Family Receptor 3	+8.4	0.0014
MC1R; Melanocortin Receptor	+7.5	0.0031
GABBR1; Gamma-Aminobutyric Acid (GABA) B Receptor, 1	+6.7	0.000002
ADORA2A; Adenosine A2A Receptor	+5.7	0.0022
GPR128; G-Protein Coupled Receptor 128	−27.2	0.000007
MELL1; Metalloprotease 1	−10.9	0.0408
P2RY2; Purinergic Receptor, G-Protein Coupled, 2	−8.4	0.0047
CCR9; Chemokine Receptor, Type 9	−7.1	0.0200
GPR15; G-Protein Coupled Receptor 15	−5.0	0.0270

Table III

Pancreatic GPCR Array Data (fold change in tumors relative to normals)

Gene; Gene Name	Fold Change	p-value
SSTR2; Somatostatin Receptor 2	+5.6	0.0124
GRPR; Gastrin Releasing Peptide Receptor	−31.0	0.00001
VIPR2; Vasoactive Intestinal Peptide Receptor 2	−25.2	0.00002
SCTR; Secretin Receptor	−25.1	0.07519
ADORA1; Adenosine A1 Receptor	−21.7	0.00007
EDG7; Endothelial Differentiation Gene 7	−21.5	0.0006
PHGDH; Phosphoglycerate Dehydrogenase	−15.3	0.0013
NMUR1; Neuromedin U Receptor 1	−10.3	0.0072
GPR120; G-Protein Coupled Receptor 120	−8.2	0.0186
LGR4; Leucine-Rich Repeat Containing G-Protein Coupled Receptor 4	−7.6	0.00003
GPR161; G-Protein Coupled Receptor 161	−7.3	0.00018
PTGER4; Prostaglandin E Receptor 4, Subtype EP4	−7.3	0.0015
BDKRB1; Bradykinin Receptor B1	−6.6	0.0003
CHRM3 ; Cholinergic Receptor, Muscarinic 3	−6.4	0.0002
CCKBR; Cholecystokinin B Receptor	−6.2	0.0094
P2RY1; Purinergic Receptor P2Y, G-Protein Coupled, 1	−5.6	0.0186
SMO; Smoothened, Frizzled Family Receptor	−5.4	0.0183
IL8RB; Interleukin 8, Receptor Beta	−5.0	0.0193
GPRC5B; G-Protein Coupled Receptor, Family C, Group 5, Member B	−5.0	0.00007

Table IV

Profiles for Select Receptors in SBNETs and PNETs

Tissue Type-Sample #	GPR 113	OXTR	SCTR	ADORA1
SBNET-209	↑	↑		
SBNET-186	↑	↑		
SBNET-160	↑	↑		
SBNET-150	↑	↑		
SBNET-145	↑	↑	↓	↓
SBNET-144	↑	↑		
SBNET-141	↑	↑		
SBNET-134	↑	↑		
SBNET-133	↑	↑		
SBNET-130	↑	↑		
SBNET-119	↑	↑	↓	
PNET-104			↓	↓
PNET-206	↑		↓	↓
PNET-202				
PNET-198			↓	↓
PNET-195				
PNET-184				
PNET-183				
PNET-179	↑		↓	↓
PNET-177	↑		↓	↓
PNET-171	↑		↓	↓
PNET-165		↑	↓	↓
PNET-158	↑		↓	↓
PNET-151		↑	↓	↓
PNET-127	↑		↓	↓
PNET-110			↓	↓

↑ Indicates >5- fold UpRegulation

↓ Indicates >5- fold DownRegulation

Table V

Results of qPCR from Liver Metastases

Metastasis From:	Sample Number	GPR113	OXTR	SCTR	ADORA1
SBNET	141	↑	↑	-	↑
SBNET	144	↑*	↑*	-	-
SBNET	140	↑	↑	↑	↑
SBNET	186	↑	↑	-	-
SBNET	209	↑	↑	-	-
PNET	126	↑	-	↓*	↑
PNET	198	-	↑	-	-
PNET	202	↓	-	↓	↓
PNET	178	↑*	-	↓	-
PNET	173	↓*	↓*	↓*	↓*

*Indicates trend of up or down regulation, which did not reach >5 fold change

-Indicates no change or levels too low to quantify

Table VI

Exon Array Results for Selected Genes of Interest from Other Studies

		SBNET vs. Normal		PNET vs. Normal	
Gene	**Gene Name**	**Fold-Change**	**p-value**	**Fold-change**	**p-value**
ADCY2 *	Adenylate Cyclase (Brain)	+2.21	5.88E-05	+1.28	0.1370
PRKAR1A *	Protein Kinase, cAMP-dependent, Regulatory, Type I, Alpha	+1.84	9.19E-05	+1.69	0.0020
BEX1 *	Brain Expressed, x-Linked 1	+15.11	3.60E-08	+2.75	0.0044
BICD1 *	Bicaudal D Homolog (Drosophila)	+1.93	0.0182	+1.18	0.4131
CHGB *	Chromogranin B (secretogranin 1)	+17.45	4.71E-05	+7.38	0.0008
CPE *	Carboxypeptidase E	+6.27	1.23E-08	+4.59	0.0016
GABRB3 *	Gamma-aminobutyric Acid (GABA) A Receptor, Beta 3	+4.01	6.47E-08	+2.68	0.0031
SCG2 *	Secretogranin II	+2.61	4.52E-05	+7.98	0.0011
SCG3 *	Secretogranin III	+7.20	1.06E-05	+5.80	0.0016
ACTG2 †	Actin, gamma 2, Smooth Muscle, Enteric	−1.55	0.3933	+1.02	0.9551
GREM2 †	Gremlin 2	−1.49	0.1147	−1.27	0.0196
REG3A †	Regenerating Islet-Derived 3 Alpha	−9.18	2.15E-05	−2.82	0.4280
TUSC2 †	Tumor Suppressor Candidate 2	+1.05	0.7133	−1.57	0.0065
RUNX1 †	Runt-Related Transcription Factor 1	−1.03	0.8191	+1.19	0.5596
TPHl †	Tryptophan Hydroxylase 1	+17.71	7.82E-05	+2.04	0.1128
TGFBR2 †	Transforming Growth Factor, Beta Receptor II	−1.45	0.1322	−1.19	0.4957
CDH6 †	Cadherin 6, Type 2, K- Cadherin (Fetal Kidney)	+5.62	1.15E-06	+2.68	0.0068
ECM1 ‡	Extracellular Matrix Protein 1	+2.86	0.0136	−1.02	0.9086
VMAT1 ‡	Solute Carrier Family 18 (Vesicular Monoamine), Member 1	+22.06	3.40E-06	−1.30	0.1677
LGALS4 ‡	Lectin, Galactoside-Binding, Soluble, 4	−3 15	0.0038	−1.99	0.1649
TSC2 §	Tuberous Sclerosis 2	+1.12	0.4872	−1.24	0.0386
SSTR2 §	Somatostatin Receptor 2	+1.49	0.0118	+1.62	0.0680
FGF13 §	Fibroblast Growth Factor 13	+1.41	0.0003	+1.03	0.8247

		SBNET vs. Normal		PNET vs. Normal	
Gene	**Gene Name**	**Fold-Change**	**p-value**	**Fold-change**	**p-value**
FEV[≠]	FEV (ETS Oncogene Family)	+2.15	0.0071	−1.18	0.0263
ADCY2[≠]	Adenylate Cyclase (Brain)	+2.21	5.88E-05	+1.28	0.1370
NR4A2[≠]	Nuclear Receptor Subfamily 4, Group A, Member 2	+1.13	0.6564	−2.17	0.1000
GADD45b[≠]	Growth Arrest and DNA- Damage-Inducible, Beta	−1.08	0.5842	−2.25	0.0016
CD302[//]	Type Lectin Domain Family 13, Member A	NA	NA	NA	NA
PPWD1[//]	Peptidylprolyl Isomerase Domain and WD Repeat Containing 1	+1.14	0.5971	+1.61	0.0030
ABHD14B[//]	Abhydrolase Domain Containing 14B	−1.09	0.3084	−1.35	0.0064

Gray coloration indicates the primary site upon which the study noted focused. Non-shaded areas for comparison purposes from the other primary site.

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