University of Iowa Neuroendocrine Tumor Program

Gene Expression Changes in Small Bowel Neuroendocrine Tumors Associated with Progression to Metastases

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Introduction

Arising from the enterochromaffin cells of the small bowel, small bowel neuroendocrine tumors (SBNETs) are the most common tumors of the small intestine[1]. Although they are generally slow growing, a significant proportion will present with liver metastases. Exome sequencing of SBNETs has revealed non-recurring mutations in a variety of genes, including genes involved in the AKT and SMAD pathways[2], however little is known regarding the genetic changes underlying the the metastatic potential of these tumors. Improved understanding of these changes would aid in the identification of genes and pathways important to the evolution of SBNETs and potentially assist in the development of new diagnostic and therapeutic strategies.

The objective of this study was to compare changes in whole transcriptome expression between normal small bowel, primary SBNETs and synchronous SBNET liver metastases using two complimentary platforms to identify genes associated with this progression.

Methods

Tissue from normal small bowel (NI), primary SBNETs (pSBTs) and liver metastases (LiMets) were collected at the time of surgery from 12 patients and RNA was extracted from each tissue. Whole transcriptome analysis was performed using two complementary methods: RNA-Seq (Illumina TruSeq protocol) and whole transcriptome microarrays (Affymetrix GeneChip® HTA). Ten genes which were serially over or underexpressed (Fig. 1) on RNA-Seq and HTA were selected for qPCR validation in 40 additional SBNET patients based on the magnitude of expression difference or having been previously identified as being involved in tumor formation.

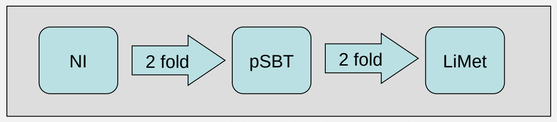

Figure 1: Serial Over/Underexpression

Acknowledgements

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Results

Serial Upregulation

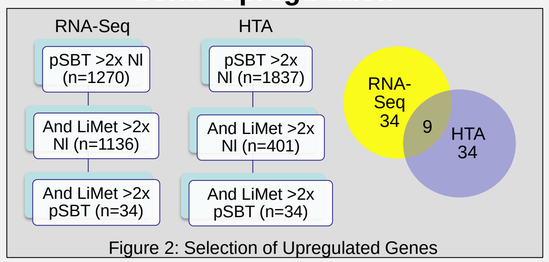


Figure 2: Selection of Upregulated Genes

Gene	Chr	Name	Cellular Location	Function
CAMK1D	10	Calcium/Calmodulin Dependent Protein Kinase 1D	Cytoplasm	Regulates granulocyte function, differentiation and activation of neutrophils and activates CREB-dependent gene transcription,.
ERRFI1	1	ERBB Receptor Feedback Inhibitor 1	Cytoplasm	Upregulated with cell growth. Negative regulator of several EGFR members.
GABRQ	X	GABA Receptor Subunit Theta	Plasma Membrane	Part of a multisubunit chloride channel, mediating inhibitory synaptic transmission.
SERPINA10	14	Serine Protease Inhibitor, A10	Extracellular Space	Primarily expressed in liver and excreted into plasma. Inhibits factors Xa and XIa.
SYT13	11	Synaptotagmin 13	Plasma Membrane	Membrane trafficker. Calcium-dependent neurotransmitter

Table 1: Upregulated Genes for Validation

Serial Downregulation

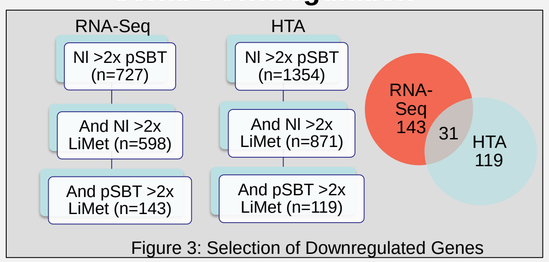
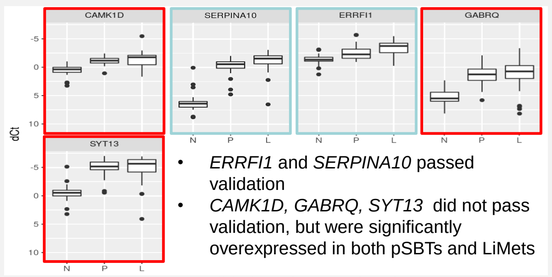


Figure 3: Selection of Downregulated Genes

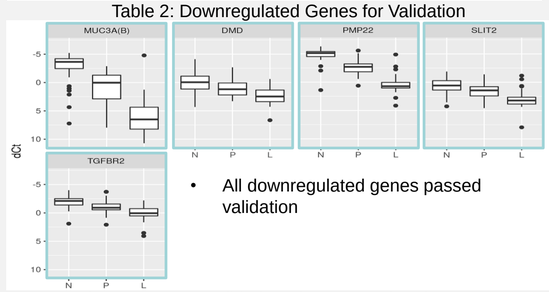
Gene	Chr	Name	Cellular Location	Function
DMD	X	Dystrophin	Plasma Membrane	Part of dystrophin-glycoprotein complex (DGC) bridging the cytoskeleton and extracellular matrix, present at neuron synapses
MUC3A	7	Mucin 3A	Extracellular Space	Epithelial Glycoprotein. May protect mucosal surfaces from foreign particles and infectious agents.
PMP22	17	Peripheral Myelin Protein 22	Plasma Membrane	Integral membrane protein. Involved in demyelinating disease and apoptosis.
SLIT2	4	Slit Guidance Ligand 2	Extracellular Space	Role in migration of neurons and other cells as well as guidance of axons. Decreases growth and migration in cancer cell lines.
TGFBR2	3	Transforming Growth Factor b Receptor 2	Plasma Membrane	Transmembrane binder of TGF-b. Regulates transcription of genes related to cell proliferation. Negative regulator of cellular proliferation.

Table 2: Downregulated Genes for Validation



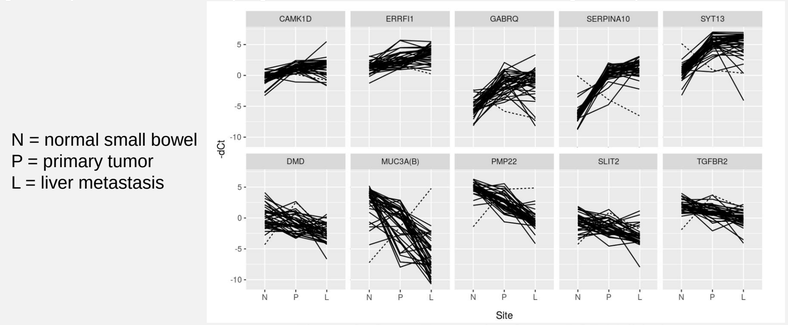
- *ERRFI1* and *SERPINA10* passed validation
- *CAMK1D, GABRQ, SYT13* did not pass validation, but were significantly overexpressed in both pSBTs and LiMets

Figure 4: qPCR Validation of Upregulated Genes



- All downregulated genes passed validation

Figure 5: qPCR Validation of Downregulated Genes



N = normal small bowel
P = primary tumor
L = liver metastasis

Figure 6: Gene Expression from qPCR Validation

Discussion

The use of two complimentary platforms for transcriptome analysis allowed for identification of 40 genes which were serially over (9) or underexpressed (31) in progression from NI to pSBT to LiMet. Of these, 10 genes were validated using qPCR and 7/10 were found to have serial differential expression. This included *TGFBR2* which has been implicated in SBNETs[3] and colon cancer[4], and *SERPINA10* which has been previously described as being upregulated in both SBNETs and PNETs[5,6]. A network was constructed using Ingenuity Pathway Analysis (IPA) using 8 of these genes (Fig. 7), which were found to interact with known cancer pathways *AKT*, *MYC*, and *MAPK3*.

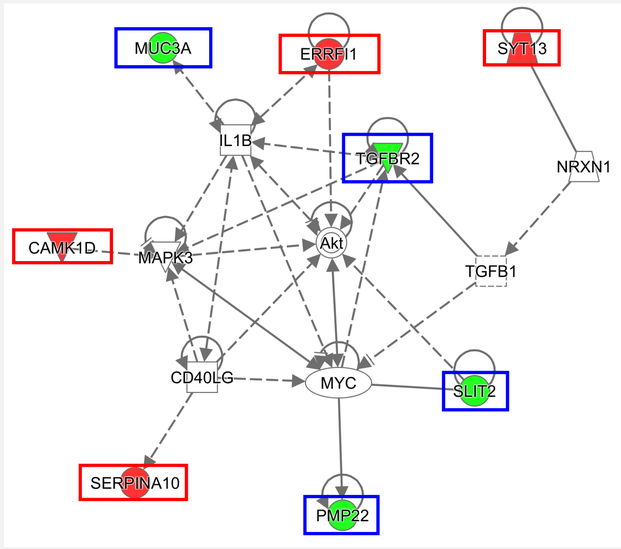


Figure 7: IPA Gene Network

Conclusions

Identification of serially, differentially expressed genes from normal tissues to primary tumors to metastases lends insight into important pathways for SBNET progression. Further study of these genes could help identify additional targets for diagnosis and treatment of SBNETs.

Work Cited

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