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

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SUPPORTED BY THE IOWA NEUROENDOCRINE TUMOR SPORE

Introduction

Airing from the enterochromaffin cells of the small bowel, small bowel neuroendocrine tumors (SBNETs) are the most common tumors of the small intestine1. 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 pathways2; however little is known regarding the genetic changes underlying the metastatic potential of these tumors. Improved understanding of these genetic 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 (pSBT) and liver metastases (LiMet) 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.

Results

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.

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 significant differential expression. This included TGFBR2 which has been implicated in SBNETs3 and colon cancer4, and SERPIN A10 which has been previously described as being upregulated in both SBNETs and PNETs5,6. 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.

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.

Acknowledgements

This project was supported through T32 Grant: T32CA148062 and SPORE Grant: P50CA174321.

Figure 1: Serial Over/Underexpression

Figure 2: Selection of Upregulated Genes

Figure 3: Selection of Downregulated Genes

Figure 4: qPCR Validation of Upregulated Genes

Figure 5: qPCR Validation of Downregulated Genes

Figure 6: Gene Expression from qPCR Validation

Figure 7: IPA Gene Network