Functional Genomics of Pancreatic Neuroendocrine Tumor Malignancy

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Background

Pancreatic neuroendocrine tumors (PanNETs) are among one of the fastest growing cancer diagnoses due to improved detection of asymptomatic malignancies during cross-sectional imaging; however, it is difficult to determine which patients will develop malignant disease. Preliminary genomic analysis of primary PanNETs revealed a complex mutational landscape with four common oncogenic events; yet, critical activation pathways that correlate with tumor aggressiveness have yet to be elucidated. Therefore, pan-genomic analysis of both primary and metastatic PanNETs is necessary to understand which genes/pathways are deregulated in PanNET progression and lead to malignancy.

Methods

We initiated a preliminary genomic sequencing study to evaluate mutations in a set of matched primary and metastatic PanNETs to determine genetic variants involved in metastasis to the liver. De-identified FFPE tumor samples were analyzed from patients who underwent surgical resection. Genomic DNA was isolated and whole exome sequencing was performed using the Nextera Rapid Capture Exome Kit by Illumina on an Illumina HiSeq 2000/2500. The following criteria were used to define genetic variants: bidirectional, clean mapping in IGV, non-synonymous, read depth ≥ 15, and a heterozygous alternate allele frequency of $0.3 \leq x \leq 0.7$. String analysis was performed to determine functional enrichment of pathways and biological processes that are deregulated in the primary tumors and liver metastases.

	Age at Resection	Sex	Tumor Location	Tumor Stage	Functional Status	KI67 Labeling Index	Status
Patient 1	38	F	Head	pT2N0M1	Nonfunctional	5%	Alive
Patient 2	70	M	Body	pT3N1M1	Nonfunctional	60%	Alive
Patient 3	39	F	Head	pT3N1M1	Nonfunctional	15%	Alive
Patient 4	58	F	Body and Tail	pT2N1M1	Nonfunctional	15%	Alive

Table 1. Clinical and pathological features of the primary PanNET tumors sequenced.

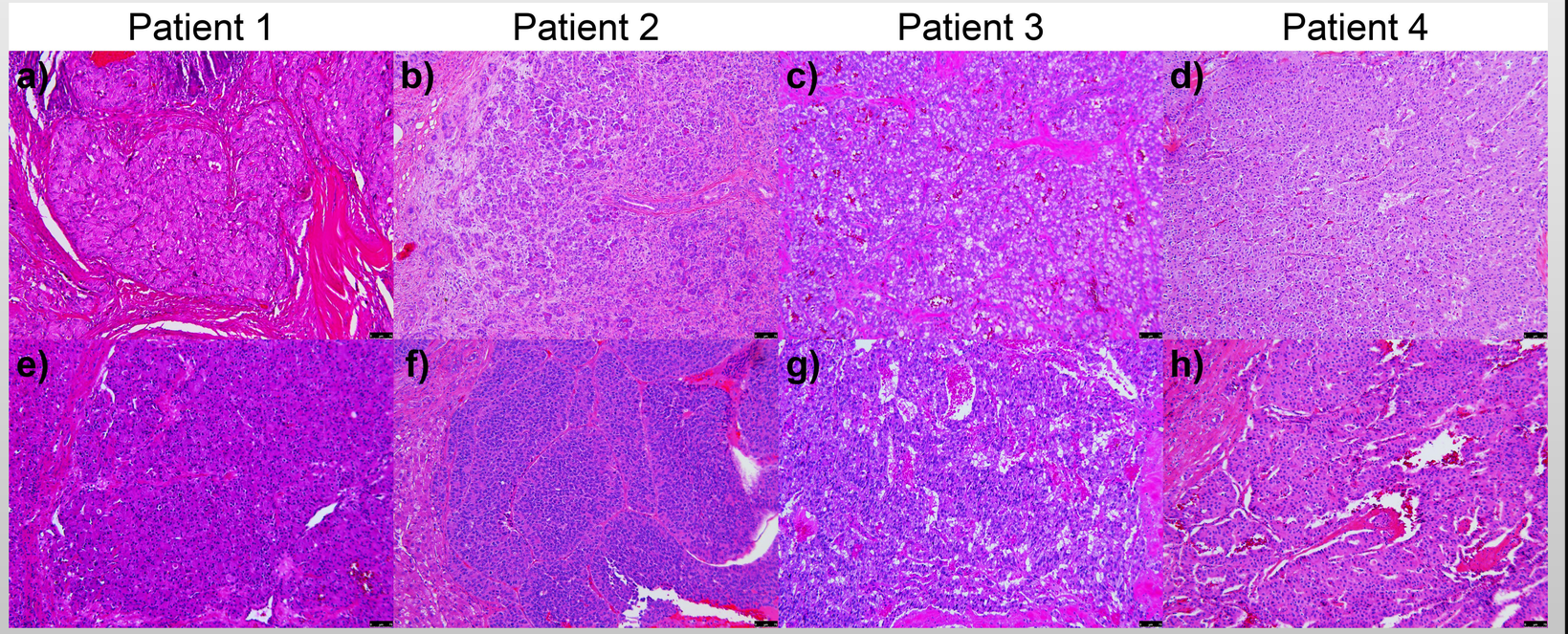

Figure 1. Histology of sequenced primary PanNETs and matched liver metastases. Each column represents one patient. (a-d) primary PanNETs. (e-h) matched liver metastases. Hematoxylin and Eosin stains demonstrate the heterogeneity of primary tumors and liver metastases. Images were taken at 10X magnification.

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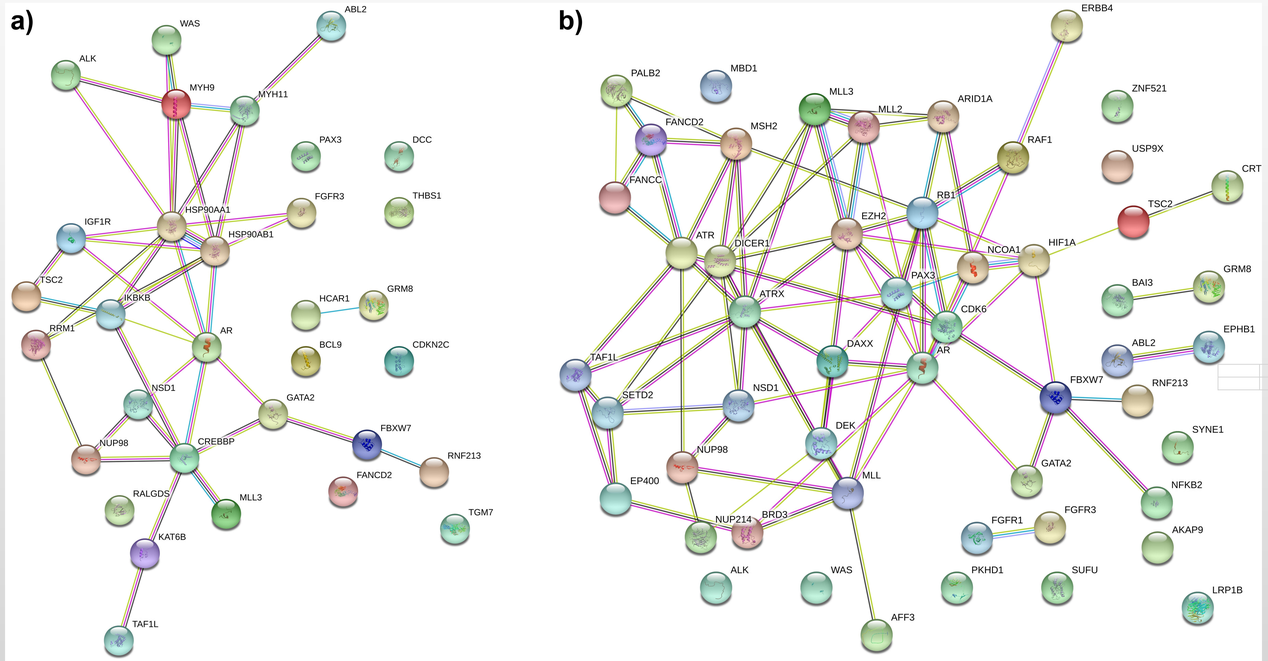

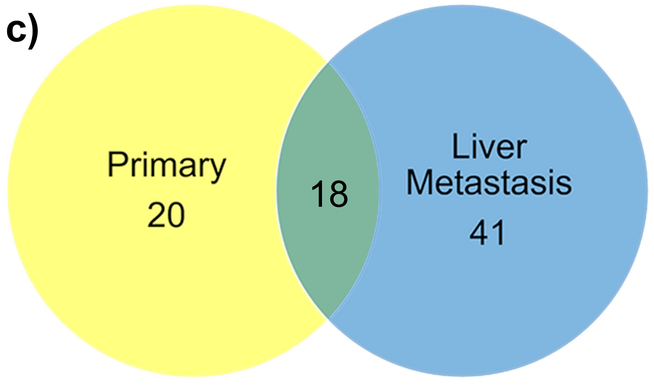

Figure 2. String network analysis for mutations found in the primary tumor and matched liver metastasis in Patient 1. (a) The primary tumor showed enrichment for pathways associated with PI3K/AKT signaling, Ras signaling, and Rap1 signaling. (b) The liver metastasis showed enrichment for pathways associated with MAPK signaling, p53 signaling, and ErbB signaling. (c) Venn diagram showing the number of mutations in the primary tumor (yellow) and matched liver metastasis (blue). The primary tumor contained 20 distinct mutations while the liver metastasis had 41 distinct mutations. The two samples shared 18 common mutations (green).

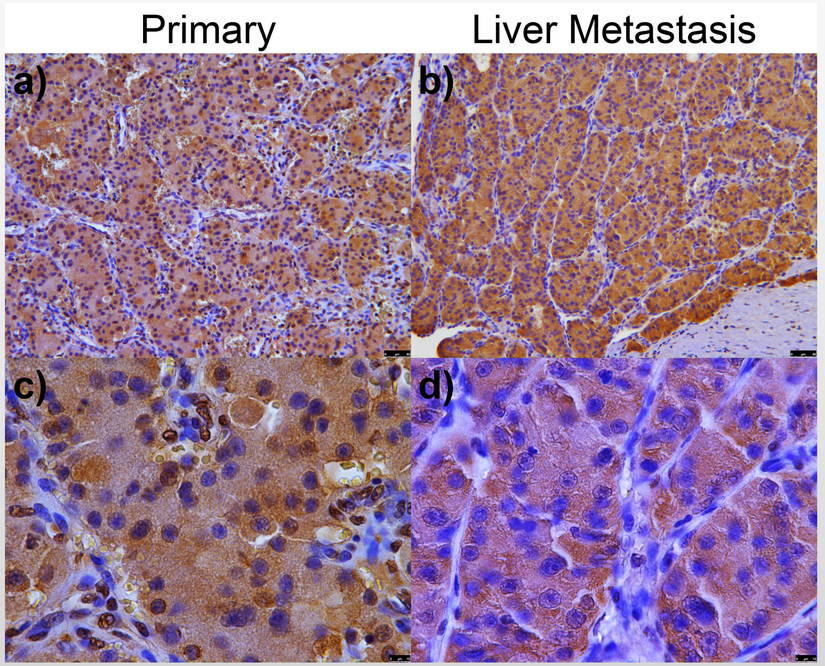

Figure 3. Immunohistochemistry for Synaptophysin (SYP) in Patient 1. (a and c) primary tumor. (b and d) matched liver metastasis. (a-d) both the primary tumor and matched liver metastasis show strong positive staining for SYP in the cytoplasm. (a and b) images were taken at 10X magnification. (c and d) images were taken at 40X magnification.

Sample Name	Gene	Protein	Exon Location
Patient 1 Primary	*MEN1*	p. Arg521Glyfs	10 (non-translated region)
Patient 1 Liver Metastasis	*MEN1*	p. Arg521Glyfs	10 (non-translated region)
Patient 1 Primary	*FGFR3*	N/A	N/A
Patient 1 Liver Metastasis	*FGFR3*	p. Val682Ile	16 (kinase domain)

Table 2. List of *MEN1* and *FGFR3* mutations found in Patient 1.

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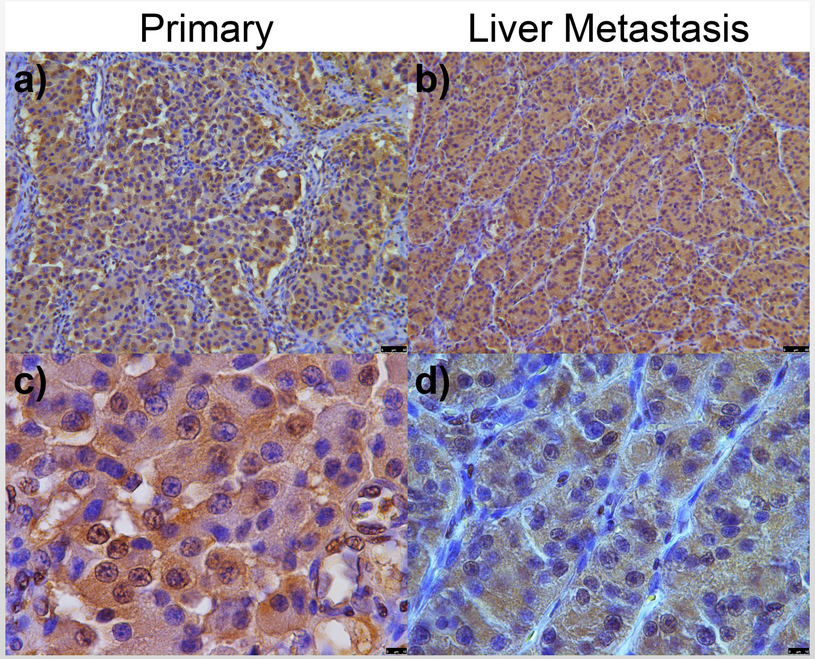

Figure 4. Immunohistochemistry for Menin in Patient 1. (a and c) primary tumor. (b and d) matched liver metastasis. (a-d) both the primary tumor and matched liver metastasis show strong positive staining for Menin in the cytoplasm and nucleus. No difference in the amount of nuclear staining was seen in the liver metastasis compared to the primary tumor. (a and b) images were taken at 10X magnification. (c and d) images were taken at 40X magnification.

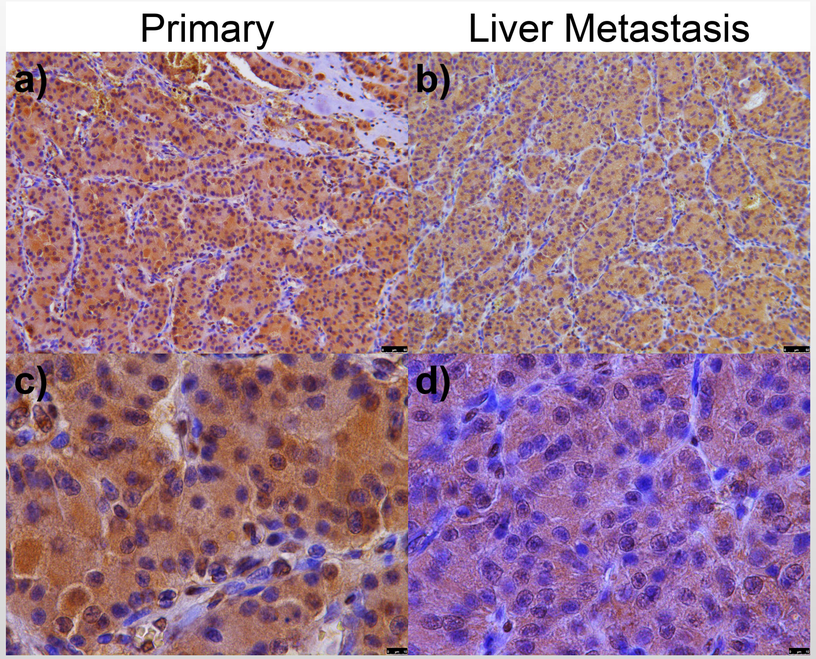

Figure 5. Immunohistochemistry for FGFR3 in Patient 1. (a and c) primary tumor. (b and d) matched liver metastasis. (a-d) both the primary tumor and matched liver metastasis show strong positive staining for FGFR3 in the cytoplasm and nucleus. No difference in the amount of nuclear staining was seen in the liver metastasis compared to the primary tumor. (a and b) images were taken at 10X magnification. (c and d) images were taken at 40X magnification.

Results and Conclusions

There was no difference in tumor stage between PanNETs with liver metastases; however, all PanNETs with liver metastases were categorized as G2/G3 based on their KI-67 labeling index. The primary tumor of Patient 1 showed enrichment for mutations involved in the PI3K and Ras signaling pathways while its matched liver metastasis showed enrichment for mutations involved in the MAPK and ErbB signaling pathways. Immunohistochemistry showed that the both the primary tumor and matched liver metastasis for Patient 1 showed mainly cytoplasmic staining of Menin indicating deregulation of global chromatin modification. Additionally, Patient 1 showed cytoplasmic and nuclear staining for FGFR3 suggesting activation of this pathway in both the primary tumor and matched liver metastasis. In conclusion, we have discovered mutations that may act as a potential driver of PanNET liver metastasis along with an innovative signaling pathway that may sustain liver metastasis growth and survival. In addition, the discovery of activated FGF signaling may provide a novel target for treating patients with non-resectable metastatic disease.

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