Characterizing the tumor microenvironment of multifocal SI-NETs

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Background:

Small intestinal neuroendocrine tumors (SI-NETs) represent the most common neoplasms of the small intestine, accounting for ~25% of all gastrointestinal NETs. Majority of the tumors locate in the terminal ileum with a high incidence of multiple synchronous primary tumors. Although multifocality is a common clinical scenario, there is a lack of evidence for their optimal treatment. Currently, the only essentially curative treatment of SI-NETs is complete surgical resection; however, most SI-NET patients cannot undergo surgery as they typically present with an extensive metastatic disease.

Genomics of SI-NETs

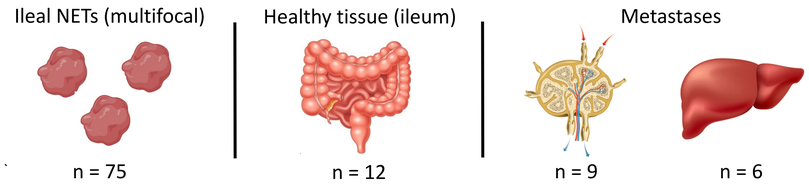
- Low somatic mutation frequency
- Loss of heterozygosity (LOH) at chr18 (~60% tumors)
- Loss-of-function mutations in *CDKN1B* (~8-10% tumors)
- Independent clonal origins of multifocal ileal NETs

Overall aim

The lack of shared somatic variation among the synchronous primary tumors in SI-NET patients suggests that their tumorigenesis is unlikely driven by genomic alterations alone. We hypothesize that the tumor microenvironment plays a key role in the growth and development of these tumors.

Methods:

Sample cohort (n=102).



Whole genome sequencing (WGS). DNA extraction and WGS (Illumina HiSeq X Ten) were performed for all 102 fresh-frozen tissue specimens at the Broad Institute's Genomics Platform (GP). Sequenced reads were aligned to GRCh38 reference assembly.

Microbiome analysis. GATK PathSeq was used to detect microbial organisms in the WGS data. PathSeq subtracts sequence reads derived from the human reference genome and aligns the remaining non-human reads to microbial reference sequences (viral, fungal, bacterial, eukaryotic) to determine their presence and abundance in the data.

Single-nucleus RNA sequencing (snRNA-seq). SnRNA-seq of 17 tumor and 23 normal ileum samples is being performed at the Center for Cancer Genomics (CCG) at Dana-Farber Cancer Institute using 10x Genomics Chromium Single Cell 5' HT v2. We have already received data from 12 normal ileum samples, which are analyzed using Seurat toolkit.

Main Findings

1. SI-NETs have more reads mapped to microbial reference sequences than normal ileum or metastasis specimens.
2. Most microbial reads in multifocal SI-NET patients are derived from bacteria.
3. The most prevalent bacterial species identified in multifocal SI-NET patients belong to known gut microbial phyla, such as Firmicutes and Bacteroidetes.
4. Despite the low frequency of enterochromaffin cells in the ileum, these cells can be detected on single-nucleus level in the normal ileum samples of multifocal SI-NET patients.

Acknowledgements: We thank all the patients who participated in this study. This study is supported by the Neuroendocrine Tumor Research Foundation (NETRF).

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Results:

Microbiome of multifocal SI-NET patients

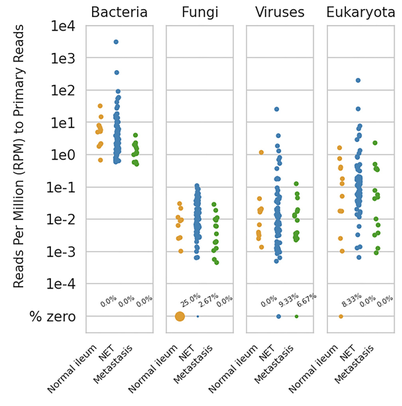

Figure 1. Most microbial reads identified in multifocal SI-NET patients are derived from bacteria. The data are presented on kingdom level, and the read counts have been normalized for sequencing depth.

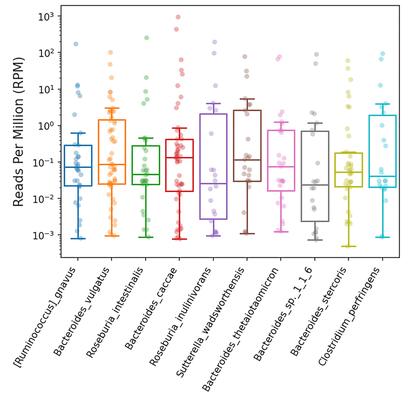

Figure 2. The top 10 most prevalent bacterial species in multifocal SI-NET patients after filtering. This boxplot includes only samples in which these bacterial species are present.

Identification of enterochromaffin (EC) cells on single-nucleus level

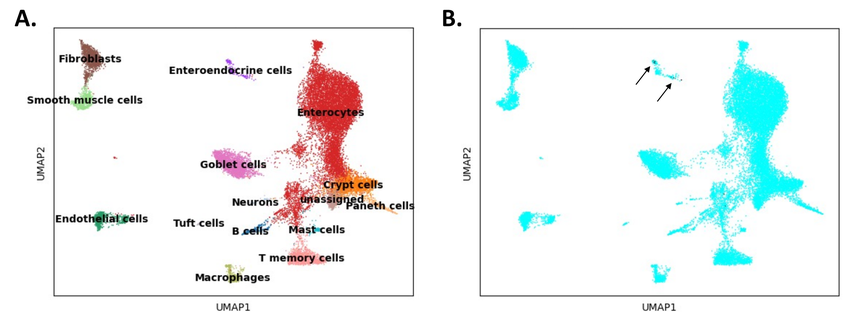

Figure 3. EC cells cluster together with other enteroendocrine cells. A) Example of harmonized cell type clusters in four normal ileum samples. **B)** EC cells are rare neuroendocrine cells in the gut epithelium. We have used four marker genes (CHGA, CHGB, TPH1, NEUROD1) to identify EC cells (marked with arrows) in the snRNA-seq data.

Future Directions for Research:

- Differential gene expression analysis using the transcriptomic profile of enterochromaffin cells as control could provide more robust results.
- Understanding the interactions of cancer cells with their microenvironment will help to illuminate SI-NET biology.