Studying field cancerization as a cause for multifocal ileal NETs

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MULTIFOCAL ILEAL NEUROENDOCRINE TUMORS

Small intestinal neuroendocrine tumors (SI-NETs) represent the most common neoplasms of the small intestine, accounting for ~25% of all gastrointestinal NETs (1). Majority of the tumors locate in the terminal ileum with a high incidence of multiple synchronous primary tumors (2,3). Although multifocality is a common clinical scenario, there is a lack of high-level evidence for their optimal treatment.

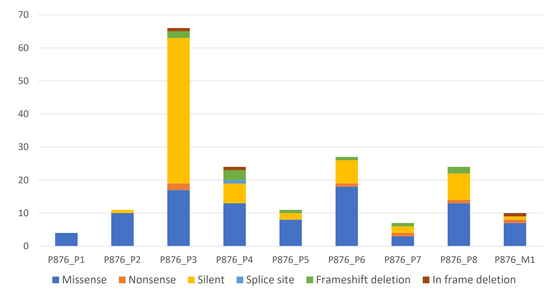
Genomics of SI-NETs (4,5)

- Low somatic mutation frequency
- Loss of heterozygosity (LOH) at chr18 (~70% tumors) as the most frequent genomic alteration
- Loss-of-function mutations in *CDKN1B* (~8-10% tumors) the only reported recurrent mutations

Preliminary data on multifocal ileal NETs (Fig. 1)

- Distinct mutation profiles between primary tumors
- No allele-specific LOH at chr18 (6)
- No obvious alterations in the microbiome
- No distinct transcriptional subtypes

A.



B.

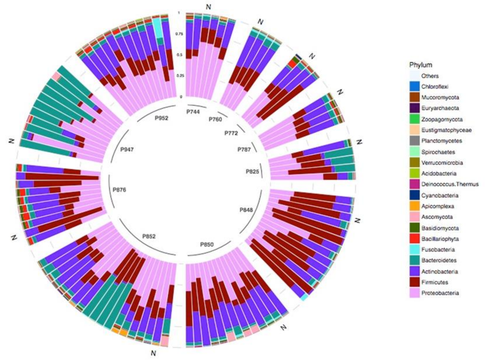


Fig. 1. Preliminary data on molecular genetic characterization of multifocal ileal NETs. A. Whole-genome sequencing data reveal independent clonal origins of multifocal ileal NETs from a single patient. P: primary tumor; M: metastasis. **B.** RNA-sequencing data of 11 patients with multiple synchronous primary ileal NETs show variation in bacterial composition among patients and samples. No bacteria are enriched in tumors compared to the matched normal and vice versa. N: tumor adjacent normal ileum sample.

OVERALL AIM

To examine if multiple synchronous primary ileal NETs arise as the result of field cancerization (Fig. 2), a biological process in which large areas of cells at a tissue surface or within an organ form the substrate for a tumor to develop due to a genetic and/or epigenetic alteration(s) (7).

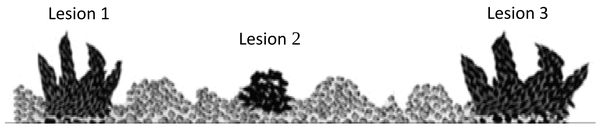


Fig. 2 Field cancerization. The entire epithelial surface has an increased risk for the development of malignant lesions because of multiple genetic and/or epigenetic abnormalities in the tissue region. Modified from Fig. 1 in van Oijen MG & Slootweg PJ. *Cancer Epidemiol Biomarkers Prev*. 2000, 9:249-56.

MATERIALS AND METHODS

Sample material

Our sample material consisted of 72 well-annotated fresh-frozen tissue specimens from 15 de-identified ileal NET patients, including both multi- and unifocal NET patients. At least one primary ileal NET, tumor adjacent normal ileum and whole blood specimen were available from each patient. All tumor and normal ileum specimens were stained with hematoxylin and eosin (H&E) to estimate their tumor cell percentage. Only tumors with ≥ 20% tumor purity were included in the study. DNA extraction of primary ileal NET and tumor adjacent normal ileum specimens was performed at the Broad Genomics Platform (GP), while we carried out DNA extraction of whole blood specimens using QIAamp® DNA Blood kit (Qiagen, Germantown, MD, USA).

Whole genome sequencing (WGS)

WGS was performed for all primary ileal NET, normal ileum and whole blood specimens using the Illumina HiSeq X Ten at the Broad GP.

Table 1. WGS data information.

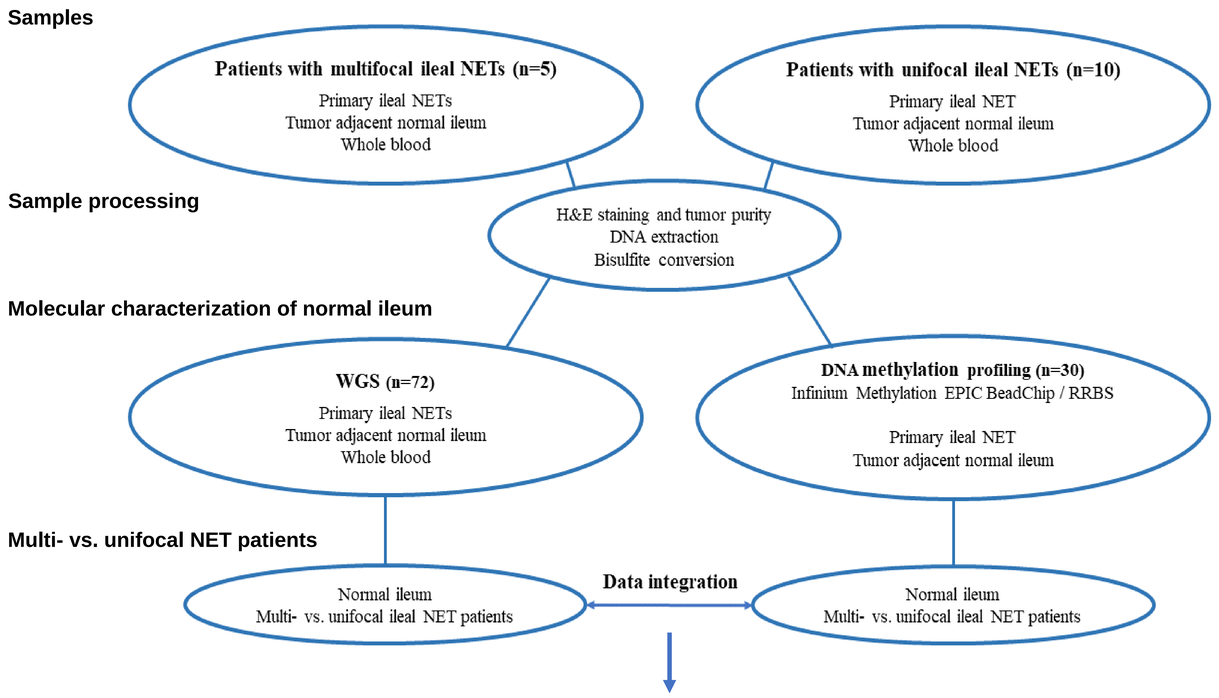
Sample type	Mean coverage [range]	Mean 20x rate [range]	Contamination rate
Whole blood	43.0x [32.3x - 55.3x]	97.9% [95.3% – 99.2%]	0.01% [0% – 0.2%]
Normal ileum	82.7x [67.3x – 103.2x]	99.4% [99.2% - 99.5%]	0.004% [0% - 0.03%]
Tumor	80.1x [62.6x – 108.7x]	99.2% [98.7% - 99.6%]	0.01% [0% - 0.3%]

Our WGS data analysis pipeline utilizes GATK's Mutect2, Strelka, TitanCNA/snakemake (6), and SvABA (7) algorithms to detect somatic single nucleotide variants, indels, copy-number variation, and structural rearrangements.

DNA methylation profiling

We will use Infinium MethylationEPIC BeadChip technology (Illumina Inc.) and reduced representation bisulfite sequencing (RRBS) on patient-matched tumor adjacent normal ileum and primary ileal NET pairs to examine their DNA methylation profiles. Bisulfite conversion and array-based DNA methylation profiling will be conducted at the Broad GP, while RRBS will be conducted in collaboration with Prof. Thirlwell. Data analysis of Infinium MethylationEPIC BeadChips will be undertaken using the ChAMP pipeline (https://bioconductor.org/packages/ release/bioc/vignettes/ChAMP/inst/doc/ChAMP.html), which includes quality control and normalization of the array data followed by analyses for copy-number variation, biological age, cell of origin, differential methylation, and gene set enrichment. RRBS data will be analyzed using an in-house pipeline from Prof. Thirlwell's lab developed at the UCL Cancer Institute and Francis Crick Institute.

STUDY DESIGN



Significance

1) Define key characteristics of normal ileum
2) Provide insight into the molecular mechanisms driving tumorigenesis
3) Give clues to targeted treatment of multifocal ileal NETs

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