Studying field cancerization as a cause for multifocal ileal 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 NETs. Majority of the SI-NETs locate in the distal ileum and have a high incidence of multiple synchronous primary tumors. The mechanisms by which these multifocal lesions arise remain largely unknown. Large-scale next-generation sequencing efforts reveal that SI-NETs have a low somatic mutation rate. Loss of heterozygosity (LOH) at chromosome (chr) 18 is the most frequent genomic event identified, occurring in ~60% of tumors, whereas loss-of-function mutations in cyclin-dependent kinase inhibitor 1B (*CDKN1B*), observed in ~10% of tumors, are the only recurrent somatic mutations reported to date. Based on the multifocal nature of SI-NETs and the lack of recurrent genomic alterations, we hypothesize that multifocal SI-NETs arise from morphologically normal small intestine as a result of an expansion of genetically and/or epigenetically abnormal cell clones, a phenomenon known as field cancerization, and grow multi-clonally in the small intestine.

Materials and Methods: Our sample material consisted of 83 well-annotated fresh-frozen tissue specimens from 16 de-identified ileal NET patients, including both multi- and unifocal ileal NET patients. 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 cells were included in the study. Our aim is to perform both whole genome sequencing (WGS) and genome-wide DNA methylation profiling on sets of patient-matched synchronous primary ileal NETs, adjacent normal ileum and whole blood, to identify genomic and/or epigenomic alterations in the normal ileum likely to represent the earliest events in the tumorigenesis of multifocal ileal NETs. Subsequently, we will assess potential similarities and differences between multi- and unifocal ileal NET patients.

Results: Based on H&E staining, a total of 72 specimens from 15 de-identified ileal NET patients were included in the study (five multi- and 10 unifocal ileal NET patients). We have just received WGS data from all the samples, which are currently being processed. Whole genome sequences of patient-matched normal ileum and whole blood specimens will be compared to identify acquired somatic alterations in the normal ileum suggestive of field cancerization. The acquired alterations in the normal ileum, also present in ileal NETs of the same patient, are likely to represent early events that are important to trigger the tumorigenesis of these lesions. We will also conduct genome-wide DNA methylation profiling of patient-matched tumor adjacent normal ileum and primary ileal NET pairs to uncover epigenomic alterations that have the potential to drive tumorigenesis in the normal small intestine – another sign of field cancerization.

Conclusions: We believe these efforts will provide biological insight into the molecular mechanisms driving the tumorigenesis of multifocal ileal NETs and give clues to their targeted treatment. The identified genomic and/or epigenomic alterations in the normal small intestine may act as potential biomarkers indicative of the genesis of disease, which is essential for early detection and risk assessment, or key cellular factors that function aberrantly and present themselves as potential drug targets.