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Single-nucleus transcriptome profiling of enterochromaffin cells in SI-NET patients

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BACKGROUND

Small intestinal neuroendocrine tumors (SI-NETs) are one of the major cancer subtypes of the small bowel. Their putative cells-of-origin are enterochromaffin cells, which account for less than 1% of the intestinal epithelium. Enterochromaffin cells are a specialized type of enteroendocrine cells that synthesize, store and secrete ~90% of the serotonin (5-hydroxytryptamine or 5-HT) in the human body. The low tumor mutational burden and the presence of only few recurrent genomic driver alterations in SI-NETs have motivated the search for other potential causes of SI-NET pathogenesis, including transcriptomic and epigenomic profiling of these lesions. These studies have been limited, however, by the lack of a reference for enterochromaffin cells. The goal of this project has been to characterize the gene expression landscape of enterochromaffin cells in the ileum of SI-NET patients, and to form a reference for cancer-to-normal cell comparisons.

METHODS

Our sample cohort consisted of 21 fresh-frozen normal ileum specimens from 12 multi- and 9 unifocal SI-NET patients. To identify subpopulations of enterochromaffin cells within each sample, single-nucleus RNA (snRNA) sequencing was performed using 10x Chromium Single Cell 5' High-Throughput v2 technology. Seurat v5 and Harmony R packages were used for the data analysis and integration of the samples, respectively. The identification of enterochromaffin cells in our data was based on four cell markers: SLC18A1, TPH1, CHGA and CHGB.

RESULTS

A total of 142,362 high-quality nuclei were available for our analysis. After the integration of snRNA sequencing data from all 21 normal ileum samples, five most variable genes identified were *DEFA5, CNTNAP2, DEFA6, CHGA,* and *CTNNA2*. For example, DEFA5 and DEFA6 are known cell markers for Paneth cells, and *CHGA* for enteroendocrine cells. We successfully detected an enteroendocrine cell cluster in our integrated data set, which included 877 nuclei, and located enterochromaffin cells within this cluster. We are currently calculating the total number of enterochromaffin cells in our data set, and subsequently, we will assess their transcriptomic profile.

CONCLUSIONS

Our results indicate that snRNA sequencing can capture enterochromaffin cells within normal ileal tissue. We will next use the transcriptomic profile of enterochromaffin cells as a reference for cancer-to-normal cell comparisons in a cohort of 10 SI-NETs. A better understanding of the cellular and molecular mechanisms that underlie SI-NETs is essential for the non-invasive management, early detection and prevention of these tumors.

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