B-4

Single-nucleus transcriptomic analysis of the tumor microenvironment in small intestinal NETs

Netta Mäkinen[1,2], Yosuke Kasai[3], Grace E. Kim[4], Chrissie Thirlwell[5,6], Eric Nakakura[3], Matthew Meyerson[1,2].

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA; [2]Cancer Program, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA; [3]Department of Surgery, University of California, San Francisco, California, USA; [4]Department of Pathology, University of California, San Francisco, California, USA; [5]Bristol Medical School, University of Bristol, Bristol, UK; [6]Department of Oncology, UCL Cancer Institute, London, UK.

BACKGROUND

Small intestinal neuroendocrine tumors (SI-NETs) are one of the major cancer subtypes of the small bowel and are believed to arise from enterochromaffin (EC) cells, a rare type of enteroendocrine cell, which account for less than 1% of the intestinal epithelium. Previous high-throughput sequencing studies have shown that concurrent primary tumors from the same SI-NET patient display distinct somatic mutational profiles (despite few clear driver mutations). The independent clonal nature of these lesions suggests that other, non-genetic mechanisms are likely involved in their growth and development. The goal of this project has been to characterize the tumor microenvironment in SI-NETs and study its potential role in their pathogenesis.

METHODS

Our sample cohort included nine primary tumors, six lymph node metastases, and patient-matched normal ileal mucosa specimens from five multifocal SI-NET patients. To improve the chances of capturing EC cells from normal ileal tissue, we included 18 additional normal ileal tissue specimens in our study. Single-nucleus RNA (snRNA) sequencing was performed using 10x Chromium Single Cell 5' High-Throughput v2 technology. We used Seurat (v5) and harmony for the data analysis and integration of the samples. The identification of enterochromaffin cells in our data was based on four cell markers: SLC18A1, TPH1, CHGA, and LMX1A. Pseudobulk differential expression analysis was performed with DESeq2.

RESULTS

A total of 251,680 high-quality nuclei were available for our analysis. After the integration of snRNA sequencing data from normal ileal mucosa samples, we detected altogether slightly under 300 enterochromaffin cells (0.2%). Additionally, we identified 32,558 tumor cells (40.3%) among the primary tumors and 20,618 (45.6%) among the metastases. Smooth muscle cells and (myo)fibroblasts represented the most common types of stromal cells within the primary tumors. Differential expression analysis between tumor cells and enterochromaffin cells revealed several statistically significant differentially expressed genes that are involved in cell transport and cell cycle regulation.

CONCLUSIONS

For the first time, we have been able to examine expression changes between tumor cells and their putative cells-of-origin in SI-NETs, elucidating mechanisms that are involved in the growth and development of these tumors. A deeper knowledge of the cellular and molecular mechanisms that underlie SI-NET development is essential for the non-invasive management, early detection and prevention of the tumors.

ABSTRACT ID 33446