B-2

Spatial Transcriptomics of Multifocal Ileal Neuroendocrine Tumors Reveals Tumor Heterogeneity based on Tumor Microenvironment and New Biomarkers

Akitada Yogo[1,2], Naoki Akanuma[3], Grace E. Kim[3], Chrissie Thirwell[4], Netta Mäkinen[5,6], Matthew Meyerson[5,6,7], Eric K. Nakakura[1,2].

[1]Division of Surgical Oncology, Section of Hepatopancreaticobiliary Surgery, Department of Surgery, University of California, San Francisco, San Francisco, CA; [2]Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; [3]Department of Pathology, University of California, San Francisco, CA; [4]Bristol Medical School, University of Bristol, Bristol, UK; [5]Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA; [6]Cancer Program, Broad Institute of Harvard and MIT, Cambridge, MA; [7]Departments of Genetics and Medicine, Harvard Medical School, Boston, MA.

BACKGROUND

Ileal neuroendocrine tumors (i-NETs) are characterized by a high incidence of multiple primary tumors (>30-40%) and production of serotonin/other hormones. Recent whole genome sequencing analyses revealed an absence of shared somatic variations among synchronous primary tumors, so the mechanisms underlying multifocal tumor development are not known. In this study, we evaluated gene expression patterns of multifocal i-NETs with spatial resolution in order to develop new hypotheses about tumorigenesis focusing on the tumor microenvironment.

METHODS

FFPE blocks of surgically resected specimens from 4 patients with multifocal i-NETs were used. Tissue microarrays were constructed from 72 cores (18 one-mm cores per patient). Spatial gene expression libraries were constructed using Visium v1 (10x Genomics). A total of 8,295 spots were analyzed: a median of 3,102 genes, 5,944 UMI counts per spot, and a total of 16,778 genes were detected in each capture area. R packages including Seurat, clusterProfiler, and monocle3 were used for data analysis.

RESULTS

Spatial transcriptomics analysis reliably captured spatial information of malignant and non-malignant cells in distinct tissue compartments within the ileum and regional lymph node/mesenteric masses. Unsupervised clustering demonstrated differences of the i-NET in various microenvironments--mucosa, submucosa, muscularis propria, and intranodal/perinodal regions of the lymph node/mesenteric masses. In all 4 patients, gene expressions of tumor cells in the mucosa were similar among multifocal tumors while tumor cells in other microenvironments clustered separately. Trajectory analysis was consistent with the supposition that tumors in the mucosa are likely the origin of i-NETs found in the other microenvironments. Tumor cells in all microenvironments exhibited characteristic gene expression patterns of serotonin receptors (HTR1B, HTR1D, and HTR7), ghrelin receptor (GHSR), GIP receptor (GIPR). Over-representation or enrichment analysis found that tumor cells in the mucosa or those with potential for metastasis exhibited overexpression of specific gene sets, including genes related to lipid metabolism.

CONCLUSIONS

This is the first spatial transriptomics analysis of multifocal i-NETs revealing similarities among tumors located in the mucosa and distinct clustering of tumors situated in other microenvironments. The finding that tumor heterogeneity in multifocal i-NETs varies depending on the microenvironment has not been previously described. The spatial data also reveal known, as well as, new biomarkers of i-NETs. In particular, we identified serotonin and other hormone receptors—some of which may be tumor specific–suggesting possible endocrine/paracrine signaling in i-NETs.

ABSTRACT ID 28490