B-20

Spatial analysis of the tumor microenvironment in pancreatic neuroendocrine tumors

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BACKGROUND

Pancreatic neuroendocrine tumors (PanNETs) represent a heterogeneous group of neoplasms with an increasing incidence, posing a significant clinical challenge. Their clinical presentation, natural history, and prognosis vary widely, underscoring the critical need for precise prognostic biomarkers and effective treatment strategies, including systemic and targeted therapies. Recent advances in genetic and epigenetic research have identified novel PanNET subtypes and validated several prognostic biomarkers, such as the detection of alternative lengthening of telomeres (ALT) and ATRX/DAXX protein loss through immunohistochemistry. However, our understanding of tumor microenvironment (TME) dynamics, particularly the role of tumor architecture and spatially organized immunological processes, remains limited. We utilized advanced technologies to explore the spatial relationships between PanNET subtypes and systematically analyze the information encoded within both the tumor and the intact TME.

METHODS

Tissue microarrays (TMAs) comprising 62 non-functional PanNETs were constructed, with each case sampled from intratumoral and peritumoral regions. Detection of ALT was performed using both a telomere-specific FISH assay and a novel chromogenic in situ assay developed by our team. Protein expression levels of ATRX, DAXX, ARX, and PDX1 were evaluated through immunohistochemistry. To maintain spatial context, unbiased whole transcriptome profiling was conducted on 38 cases using the Visium platform by 10X Genomics. Additionally, a multiplex immunofluorescent assay was optimized on the Lunaphore COMET platform for comprehensive immune cell analysis. Data integration and multi-modal, multi-scale analyses were facilitated using Giotto Suite, a technology-agnostic spatial multi-omics analysis platform.

RESULTS

Overall, ALT was detected in 40% of the cases. Multiplex immunofluorescence identified a variety of immune cell populations, while Visium spatial transcriptomics provided comprehensive profiles for 38 cases (19 ALT-positive and 19 ALT-negative). On average, 400 Visium spots were captured per TMA core, with 83% of the cores achieving a median of over 1,500 genes per spot. The dataset included expression of canonical epithelial, immune, and stromal marker genes, and deconvolution of spots is underway using a single-cell RNA sequencing reference dataset. Z-stack batch effects were minimal across experiments. Image registration was also performed for integration between these spatial

datasets and immunohistochemical and histological markers. These integrated analyses aim to reveal spatial architectural differences between ALT-positive and ALT-negative cases, potentially uncovering key biological pathways.

CONCLUSIONS

This study aims to elucidate specific tumor and TME spatial characteristics, correlating with biomarkers to enhance prognosis and identify therapeutic targets. Integration of spatial data with molecular insights promises to advance personalized medicine for patients.

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