B-1

Spatial and Transcriptional Profiling Reveals Immune Remodeling and Microenvironmental Heterogeneity in Benign and Malignant Pancreatic Neuroendocrine Tumors

Gareth Pollin, PhD; Angela J. Mathison, PhD; Raul Urrutia, MD, PhD; Gwen Lomberk, PhD; Mohammed Aldakkak, MD; Y. David Seo, MD; Anai Kothari, MD; Kathleen Christians, MD; Alex Phan, MD; Douglas B. Evans, MD; Callisia N. Clarke, MD, MS.

Division of Surgical Oncology, Medical College of Wisconsin.

BACKGROUND

Pancreatic neuroendocrine tumors (PNETs) are rare, clinically heterogeneous malignancies with limited insight into the molecular and immune mechanisms driving progression from benign to malignant states, especially regarding tumor microenvironment (TME) remodeling.

METHODS

We integrated clinical-pathological genomics to dissect transcriptional and immunologic features across PNET subtypes. We performed bulk RNA sequencing and spatial transcriptomics on 23 clinically annotated PNET samples, including functional (insulinomas, gastrinomas) and non-functional (indolent and aggressive) tumors.

RESULTS

Spatial transcriptomics revealed substantial variation in immune infiltration and inflammatory states. Functional insulinomas showed sparse immune presence with low $CD4^+$ T-cells (9.5–15.1%) and minimal exhausted T-cells (1.9–4.6%). Indolent and aggressive non-functional tumors displayed increased $CD4^+$ infiltration and exhausted T-cells (7.3–15.8%). Macrophage density rose with malignancy, occupying up to 50% of spatial spots in aggressive tumors. The tumor inflammation signature confirmed low inflammation in benign tumors (0.99–2.1%) versus substantial increases in aggressive tumors (up to 27.7%), indicating immune activation.

Bulk RNA-seq gene set enrichment analysis comparing malignant and indolent tumors to benign functional tumors revealed consistent downregulation of pancreatic endocrine pathways (NES −2.23 to −2.64) and biosynthetic programs critical for protein production and proteostasis (NES −2.82 to −1.28) in malignant tumors. Furthermore, Indolent tumors frequently activated extracellular matrix (ECM) pathways (ECM receptor interaction NES 1.59 to 1.82), with some showing moderate MAPK and VEGF signaling (NES 1.36 to 1.90), reflecting stromal and vascular engagement. Immune pathways such as cytokine, JAK-STAT, and complement signaling were moderately enriched (NES 1.55 to 2.17), indicating low-grade immune activation.

Aggressive non-functional tumors showed pronounced heterogeneity. A subset displayed a “hot” phenotype with broad upregulation of inflammatory pathways, including T-cell receptor, antigen processing, JAK-STAT, and cytokine-cytokine receptor signaling (NES 1.55 to 2.61). Others exhibited a “cold” phenotype with downregulation of immune pathways such as leukocyte migration, B-cell

receptor signaling, and T-cell receptor signaling (NES −2.31 to −1.50). Immune deconvolution confirmed immune infiltration correlated with malignancy, with benign tumors showing low immune gene expression (mean 0.19), increasing in indolent tumors (mean 0.34), while aggressive tumors exhibited variable infiltration (0.19 to 0.42), reflecting both “hot” and “cold” immune states.

CONCLUSIONS

We define molecular programs that distinguish benign and malignant PNETs, underscoring a central role of immune remodeling in malignancy. We observed loss of pancreatic identity and diverse tumor microenvironments. The marked heterogeneity within aggressive tumors, comprising “hot” inflammatory and “cold” immunosuppressed phenotypes, highlights the complexity of tumor-immune interactions and offers critical insights for patient stratification and immunotherapy development.

ABSTRACT ID #33416