Single-Cell Transcriptome Analysis Reveals Mechanisms of Tumor Immune Escape in NETs

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INTRODUCTION

- Immunotherapy is rapidly becoming a mainstay of cancer treatment.
- Little is known about the role of these therapies in pancreatic neuroendocrine tumors (PNETs).
- We sought to :

(1) Identify the immune cell populations present in the tumor microenvironment (TME)

(2) Explore expression patterns of immunoregulatory genes in immune cells in the TME

(3) Explore immunoregulatory gene expression of tumor cell populations.

MATERIALS & METHODS

Materials

- **Human Tissue Specimens** - 7 PNET tissues [5 non-functional (NF), 2 insulinomas]

Experimental Methods

- **Single cell RNA-sequencing:** Data were acquired using 10X Chromium platform to analyze tumor cells as well as infiltrating immune cells
- **Analysis:** Sequencing results were mapped to UMI count matrix (CellRanger v.6.0.1, built-in library GRCh38-2020-A) and analyzed (R package Seurat v4.3.0.1).
- **IHC:** FFPE tissue sections were stained with H&E as well as CD161 antibody (1:400, Bioss, BS-4682R) and CD8 antibody (1:25, BD Pharmingen, 550372) followed by incubation with an appropriate conjugated secondary antibody.

RESULTS

Specimen ID	Inhibitory T-cell Receptors			Inhibitory NK-cell Receptors				
	PD-1	LAG-3	CTLA4	KIR2DL1	KIR2DL3	KIR2DL4	KIR3DL1	KIR3DL2
62572 (NF)	+	+	+	+	+	+	+	+
62635 (NF)	-	-	-	-	+	+	+	+
64330 (NF)	+	+	-	+	+	+	+	+
64437 (NF)	+	+	-	+	+	+	+	+
69927 (NF)	-	-	-	-	-	-	-	-
61619 (Insulinoma)	+	+	+	+	+	+	+	+
66110 (Insulinoma)	+	-	-	-	-	-	-	-

Table 1. Prescence of T-cell and NK-cell inhibitory receptors for each PNET specimen

Figure 1. Representative image of scRNA sequencing immune checkpoint data analysis, specimen 62572

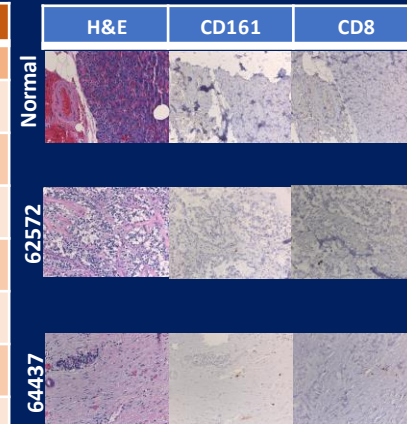**Figure 2.** H&E and IHC staining for Cytotoxic T-cells (CD8) and NK cells (CD161) in FFPE normal and PNET tissue. 20x magnification
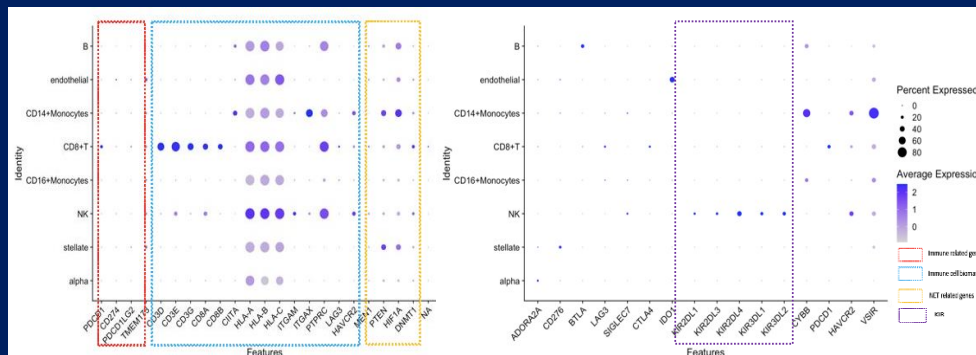
CONCLUSIONS

- scRNA sequencing identified immune cells within tumor samples from human PNETs.
- Data suggest a suppressive immune phenotype.
- Continued analysis is need to explore mechanisms of immune regulation in PNETs.