Unravelling the role of peri-neural invasion in pancreatic cancer using single cell and spatial transcriptomics

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Introduction

The role of tumour innervation with respect cancer progression is an emerging field of study. One of the pervasive mechanisms associated with metastasis and poor prognosis in various cancer types is perineural invasion (PNI), by which tumour cells invade the space surrounding a nerve [1]. It is hypothesized that PNI is a consequence of a complex multi-step process where peripheral nerves, malignant cells, and stromal cells cooperate to drive processes such as neurotropism [2], ECM remodeling and epithelial-mesenchymal transition (EMT) [3].

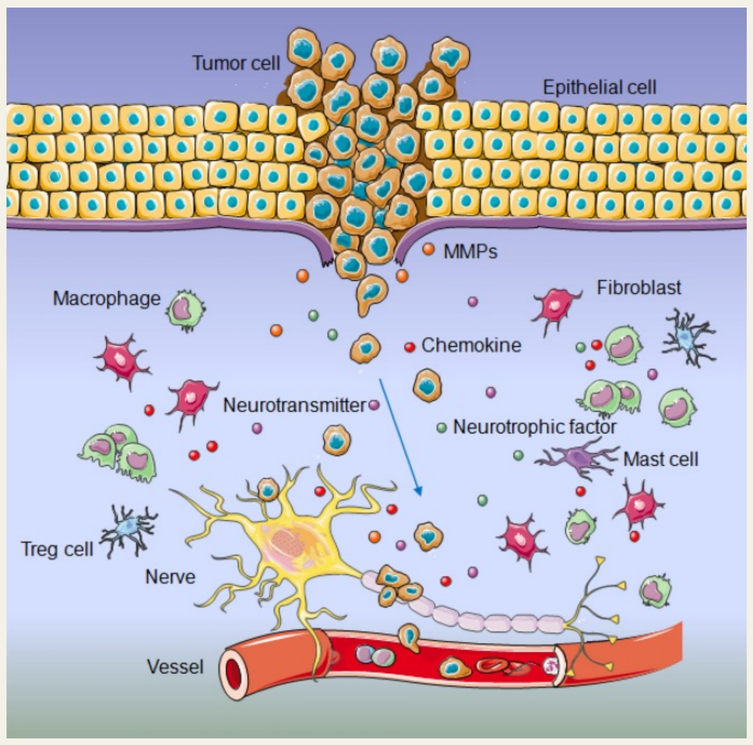

Figure 1. Schematic diagram illustrating key cellular and molecular factors of perineural invasion [4]

Yet, most published transcriptomics studies on PNI were limited by the lack of spatial information [5]. Analysis at bulk and single cell level failed to locate the cell populations in an invaded peri-neural niche thus establish causal relationship between the mentioned transcriptomic response and PNI. Therefore, the major objective of this study is to explore whether characterization of PNI signatures and mechanisms is feasible using published single cell and spatial transcriptomic datasets of pancreatic ductal adenocarcinoma (PDAC) tumour samples.

Materials and Methods

To elucidate transcriptomic signatures around nerve bundles in the PDAC tumour microenvironment (TME), paired single cell and spatial transcriptomic gene expression matrices of 10 Formalin-Fixed Paraffin-Embedded (FFPE) samples sequenced using the 10x Chromium and 10x Visium technology were obtained from the Human Tumour Atlas Network (HTAN) under the WUSTL atlas. [6]

The single cell dataset was first processed using R package Seurat v.5.0.0. [7] Quality control was performed to filter away low-quality cells based on the following criteria: total transcript counts <300; genes expressed <200 or > 10,000; unique molecular identifiers (UMIs) <1,000 or > 10,000; proportion of mitochondrial genes > 10%. Each sample was scaled and normalized using Seurat's SCTransform to correct for batch effects. Cells were then clustered via the FindNeighbors and FindClusters function using the top 30 Principal Components. FindAllMarkers was used to identify the differentially expressed genes of each cluster and guide cell type annotation by comparing results with curated marker gene set.

Ligand-receptor (LR) analysis was performed using CellPhoneDB [8] with default parameters to score average interaction across cell populations and identify originating cells of significant ligand-receptor pairs related to PNI as described from literature [9].

Finally, the cell type annotation was transferred to the normalized spatial transcriptomic dataset using RCTD [10] with multi-mode and a minimum of 25 cells to deconvolve.

Results

Due to variation in anatomical site and degree of innervation, presence of infiltrating nerves was only evident in 1 sample as indicated by a distinct cluster of peri-islet Schwann cells from the single cell gene expression profile. Apart from Schwann cells, phenotyping analysis identified tumour cells, endocrine cells, ductal cells, immune cells, pancreatic stellate cells and endothelial cells within the TME.

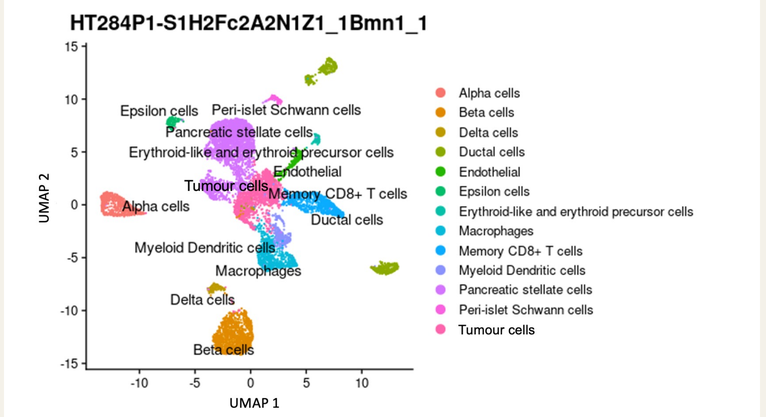

Figure 2. Major cell types identified from single cell gene expression profile in dimensionally reduced UMAP space

Subsequent ligand-receptor analysis on the annotated gene expression matrix did not reveal significant interaction between tumour cells and Schwann cells but indicated substantial interaction between Schwann cells and pancreatic stellate cells, which is a class of quiescent fibroblast-like cells that facilitates process favouring cancer progression such as excess fibrosis, metastasis and induction of resistance to chemotherapy or radiotherapy. [11]

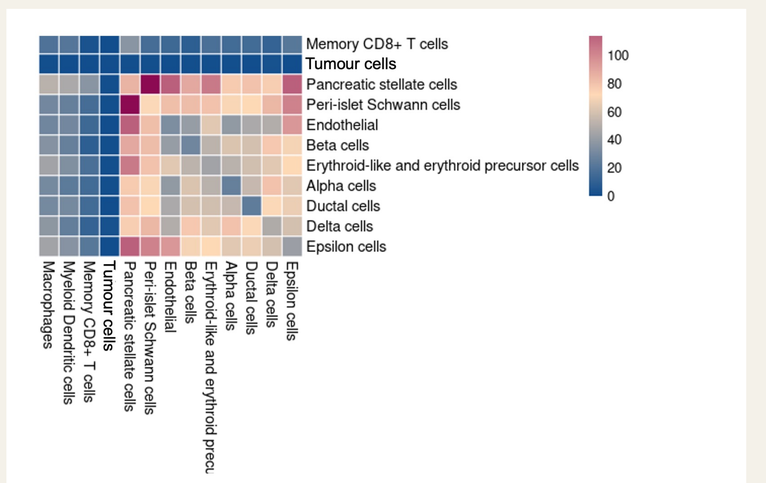

Figure 3. Means of ligand-receptor interactions across all cell populations within the TME.

When investigating specific LR pairs related to PNI signatures identified from literature, only 2 interactions involving neurotrophic factors were revealed between Schwann cells and other cells populations such as endocrine cells, tumour cells and pancreatic stellate cells. Specifically, significant interaction was only identified in the NGF/SORT1 pair, (associated with increased cancer aggressiveness [12]) and the NGF/NGFR pair (associated with nerve growth and regeneration [13]) directed from both epsilon and pancreatic stellate cells to Schwann cells.

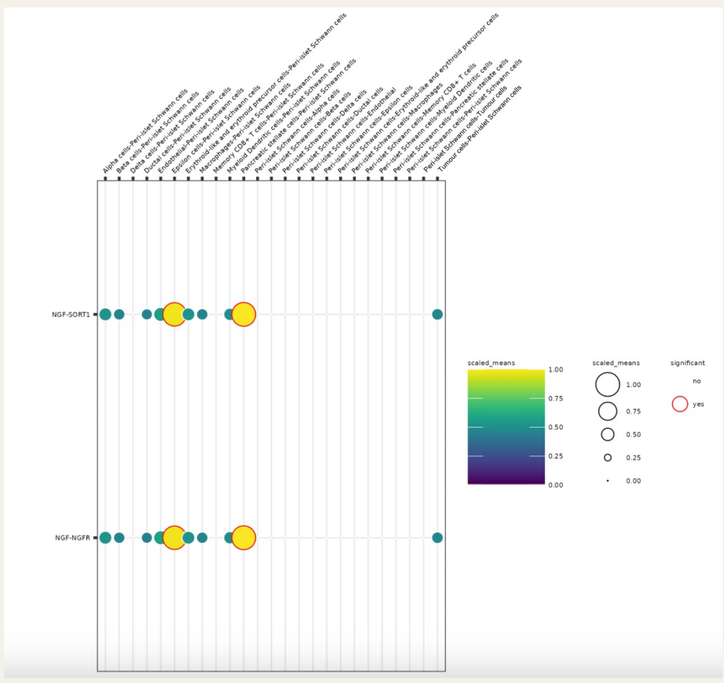

Figure 4. Scaled means of specific ligand-receptor interactions related to PNI signatures between Schwann cells and other cell populations within the TME.

To examine whether the LR interactions between Schwann cells and other cell populations are likely in terms of physical distance, the relative composition of each cell type identified from the single cell expression profile was deconvolved for each grid in the paired spatial transcriptomic data. Results revealed co-localization of Schwann cells with endocrine cells, tumour cells and proximity to pancreatic stellate cells, thus increasing the validity of interactions identified at single cell level with these cell populations. However, further attempt to characterize interaction at spatial level is limited as the relative abundance of Schwann cells in each grid is low (~10%-20%).

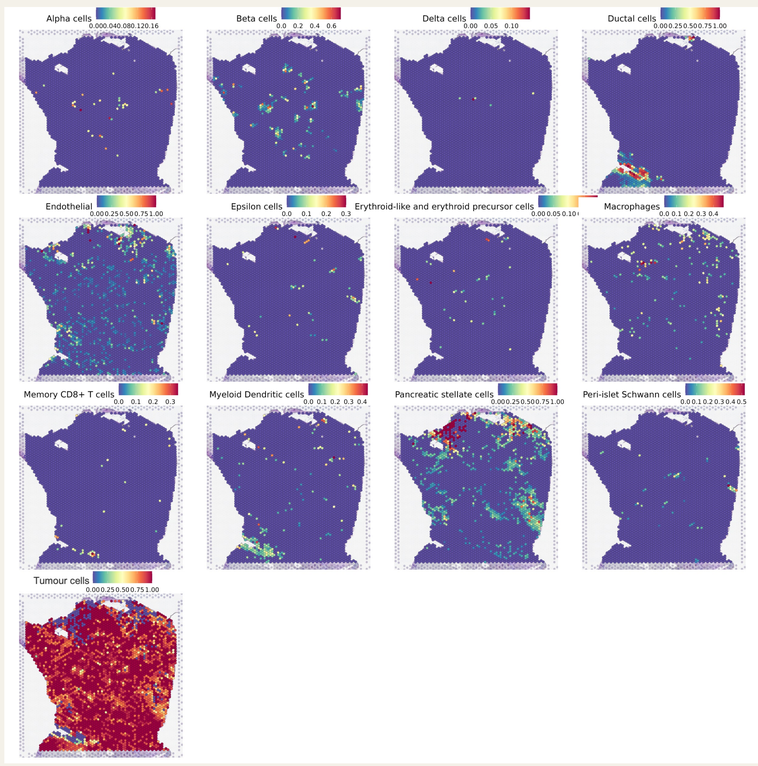

Figure 5. Relative abundance of each cell type in each sequenced grid of the FFPE slide

Conclusion

This study demonstrated the usage of transcriptomic analysis at single cell and spatial level to reveal the interaction between nerves and various cellular components within the TME. However, several challenges hindering further analysis and characterization of PNI mechanisms were highlighted such as high variability in innervation pattern leading to difficulty in obtaining informative samples; low abundance of Schwann cells limits the power of LR analysis and effective usage of spatial transcriptomic dataset. Therefore, future directions include purposive sampling of tumour regions with high degree of innervation, developing alternative methods to integrate spatial information or shifting focus to gene expression program decomposition instead of specific LR analysis on spatial transcriptomic dataset.

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