The application of single cell analysis to understand clear cell Renal Cell Carcinoma (ccRCC)

Hosted by: Antibody and Vaccine Group, University of Southampton

Objectives

1) Isolate primary tissue from 3 ccRCC samples

2) Perform single cell sequencing on isolated tumour and myeloid cells

3) Validate potential targets

Progress

Following a period of initial training in sample preparation and flow cytometry, primary tissue was isolated from six patients with ccRCC. Single cell suspensions and formalin-fixed paraffin-embedded (FFPE) blocks of tumour and matched background tissue were obtained from 6/6 patients. Flow cytometry was performed on 5/6 patients and multiplex immunohistochemistry on 6/6 to aid validation.

Single cells were isolated from a subset of these tumours and evaluated for single cell RNA sequencing (scRNA-seq) alongside paired matched normal tissue from the same samples. Two samples (tumour versus normal) were then progressed through the scRNA-seq pipeline. Briefly, gel bead-in emulsions (GEMs) were generated and barcoded before cDNA amplification and gene expression library construction using specific indexes. A separate cell surface protein library was generated for one of the tumour samples as part of Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq).

Initial results

Flow cytometry data was analysed using FlowJo and highlighted a higher proportion of viable CD45+ cells in the tumour samples than the background samples (65.28 – 91.03% in tumour and 26.90 – 47.60% in background). This information informed the experimental plan, as background samples were subsequently CD45+ enriched prior to input into the scRNA-seq pipeline to maximise immune infiltrate to be sequenced.

Immunohistochemistry was performed on FFPE blocks from all samples. The tumour showed diffuse expression of carbonic anhydrase-IX (CA-IX). There was infiltration of

macrophages within the tumour, evidenced by CD68 and CD163 staining. There was also infiltration of lymphocytes within the tumour, which were predominantly CD3+ although there was a smaller proportion of CD4+ and/or T regulatory lymphocytes expressing FOXP3.

Raw single cell sequencing data was obtained with high quality metrics. Following cellranger count and cellranger mkfastq, count data was loaded into Seurat and a standard pre-processing workflow was followed. Macrophages were identified in the tumour samples with a C1QA+/TREM2+/APOE+ transcriptional profile associated with tumour-associated macrophages, when compared to macrophages in background samples.

Problems

Tissue was isolated from more than three patients to facilitate preliminary data and develop robust experimental pipelines. Further patients were included due to significant inter-tumour variability identified with regard cell counts and viability. It was only possible to undertake scRNA-seq or CITE-seq on 2/6 samples due to issues surrounding the COVID-19 pandemic leading to external NHS operational pressures including critical care capacity and variability in cell counts and/or viability.

Future steps

Single cell data is undergoing further interrogation, including differential gene expression analysis and cell to cell interactions. This will inform future research on primary tissue across a range of tumour types. An abstract will be submitted to the next available Pathological Society meeting and an original research paper will follow.

Acknowledgements

I would like to extend my thanks to the Jean Shanks Foundation and the Pathological Society for the opportunity to undertake this project and learn new skills. Alongside basic training in laboratory skills I have become proficient in use of the R programming language. I am also now familiar with several scientific software packages including FlowJo and GraphPad.

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