IMMUNE CELLS AND METASTASIS

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Our lab focuses on the regulation and function of γδ T cells in tumour progression, using genetically engineered mouse models of cancer. γδ T cells are a unique cell population – they are small in number but have major influences on neighboring cells during immunity. We aim to understand how γδ T cells behave in tumour-bearing mice, specifically in pre-metastatic organs, and to uncover regulatory pathways governing these cells that may be manipulated to counteract metastasis formation. Our research is centered on three main cancer types: breast, colon and pancreas.

γδ T cells in breast cancer

A subset of γδ T cells promotes mammary tumour metastasis through expression of the cytokine IL-17 (Coffelt *et al.*, Nature 2015; 522: 345–8). Last year, we found that this subset of IL-17-producing γδ T cells expresses the receptor NKG2D – a molecule more commonly associated with natural killer (NK) cells and cancer cell killing. Our work to understand the function of this killing receptor on pro-metastatic γδ T cells continued into 2018. We found that NKG2D-expressing γδ T cells are enriched in lung tissue, when compared to lymph nodes, spleen, liver or thymus. These data suggest that they may have an important role in metastasis, since the lung is the primary site of cancer cell dissemination in mice. Interestingly, NKG2D-expressing γδ T cells are not present in lungs after birth, but arise sometime after weaning. We are currently exploring the idea that NKG2D is involved in regulation of IL-17 expression in γδ T cells. We have profiled various cell populations in the lung to determine where the ligands for NKG2D are expressed and how mammary tumours affect ligand expression. This analysis uncovered monocytes as the main producers of NKG2D ligands, so we will investigate the relationship between monocytes and γδ T cells over the coming year. We have secured funding from Breast Cancer Now for this project. All our work in breast cancer is done in collaboration with Karen Blyth and her team.

To gain a better understanding of γδ T cell heterogeneity in the lung, we have performed single-cell sequencing on these cells in collaboration with Kristina Kirschner (University of Glasgow) and Nizar Batada (University of Edinburgh). An unbiased transcriptional analysis of 1000s of individual cells revealed two major clusters of γδ T cells in accordance with published literature that largely segregate on cytotoxic molecules (Fig. 1a). Within these two major clusters, we identified populations expressing T cell receptor signalling molecules, co-stimulatory molecules, T cell checkpoint inhibitors, lymph node trafficking molecules, NK cell markers, cytokines (such as IL-17) and cytokine receptors, as well as genes involved in NOTCH signaling. This in-depth analysis of γδ T cells at the single-cell level provides further insight into γδ T cell biology in homeostasis and cancer.

γδ T cells in colorectal cancer

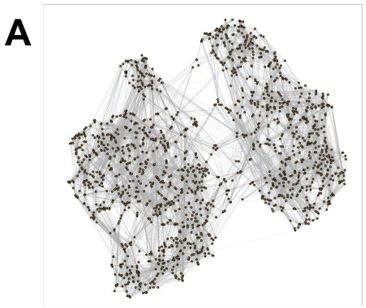
In collaboration with Owen Sansom and his lab, we are investigating the role of γδ T cells in various mouse models that recapitulate distinct molecular subtypes of colon cancer. We have found that the absence of γδ T cells fails to influence tumour growth and survival in models driven by loss of the tumour suppressor Apc. At the same time, we profiled the phenotype of γδ T cells in two other models of colorectal carcinoma, which indicate that γδ T cells may be important. We found that IL-17-producing γδ T cells are increased in the metastatic *Villin-Cre;Kras*^{G12D}*;Trp53*$^{F/F}$*;Nicd1*$^{F/+}$ model and, in a similar fashion to our observations in metastatic mammary tumour models, we find that the increase in IL-17-producing γδ T cells occurs in visceral organs in addition to the primary tumour. Over the next year, we will determine whether IL-17-producing γδ T cells play a role in primary tumour growth or metastasis formation in this model. We have also been using a model of microsatellite instability, which has provided some surprising results. Unlike any other cancer model we have profiled, γδ T cells make up a large proportion of infiltrating T cells in microsatellite unstable tumours (Fig. 1b). We have shown that these γδ T cells produce IL-17 and that their infiltration correlates with neutrophil influx. In the future, we aim to determine whether they are required for tumour growth and the mechanisms by which they are recruited to tumours.

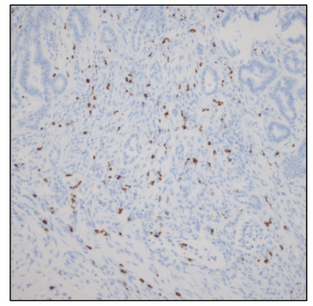
Seth Coffelt giving a tour of the labs to Rebecca Scott, who underwent treatment for ovarian cancer in 2017. Photo credit: Steve Welsh

γδ T cells in pancreatic cancer

Using the *Kras*^{G12D}*;Trp53*^{R172H}*;Pdx1-Cre* (KPC) model, we are exploring the ability of γδ T cells to influence pancreatic ductal adenocarcinoma (PDAC) and liver metastasis. This work is done in collaboration with Jen Morton. We crossed the KPC model with γδ T cell–deficient (*Tcrd*$^{-/-}$) mice and measured neutrophils in the circulation of these mice. In contrast to our previous observations in mammary tumour–bearing mice, we found that neutrophils were unaffected by the absence of γδ T cells (Fig. 1c), indicating that γδ T cells are not required for neutrophil expansion in the presence of PDAC. When we profiled conventional T cells and NK cells in livers of tumour-bearing mice, however, we noticed that NK cell activity was increased in γδ T cell–deficient mice, while T cells remain unchanged. These data suggest that γδ T cells may communicate with NK cells to suppress their function in liver tissue. Over the next year, we will examine the crosstalk between NK cells and γδ T cells and measure any impact on pancreatic cancer metastasis.

Publications listed on page 84





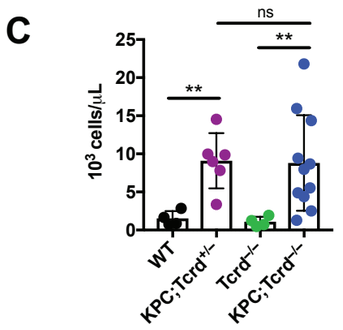


Figure 1
γδ T cells in mammary, pancreatic and colon cancers
(a) γδ T cells sorted from lungs of wild-type mice and analysed by single-cell sequencing. Analysis shows that two main populations of γδ T cells reside in the lungs under homeostatic conditions.
(b) γδ T cell abundance in microsatellite unstable tumours of the gut as determined by RNAscope for the *Trdc* transcript.
(c) Neutrophil counts in blood of wild-type (WT) mice, tumour-bearing KPC mice, γδ T cell–deficient (*Tcrd*$^{-/-}$) mice and tumour-bearing, γδ T cell–deficient KPC mice.
Each dot represents one mouse. P value was determined by one-way ANOVA.