**PRECLINICAL PRECISION PANCREAS**

Pancreatic Cancer Modelling

Pancreatic cancer is a genetically complex disease, with many genes mutated at low frequency. Whilst mutations in KRAS, TP53, SMAD4 and CDKN2A are common, these are difficult to target therapeutically. Conversely, many of the genes mutated or pathways deregulated less frequently may be clinically relevant targets in subsets of patients. We use genetically engineered mice to model these subsets of patients who may ultimately benefit from more personalised approaches to treatment based on their mutations. KPC mice, which express endogenous mutant KrasG12D and p53R172H targeted to the pancreas (using Cre-Lox technology), develop pancreatic tumours reminiscent of the human disease, whilst novel models also allow us to manipulate genes in different cellular compartments within the tumour, so as to better understand the complex signalling network that exists within the tumour microenvironment. By layering further genetic aberrations onto this model we can study the importance of various signalling pathways in tumourigenesis, and use these mice to test new therapies and combinations. For example, mutations in PTEN/PI3K/mTOR pathway components are found in up to 10% of patients. We previously showed that these tumours might be exquisitely sensitive to mTORC1 inhibition, while tumours lacking these aberrations were sensitive to mTORC2 inhibition (Driscoll et al., Cancer Res. 2016; 76: 6911–23). Thus, we are currently investigating which therapeutic combinations are most effective in targeting signalling pathways downstream of Kras, and in which genetic setting.

Recent studies have also suggested that depletion of B cells, or inhibition of B cell signalling, can reduce tumour growth in xenograft models of pancreatic cancer. However, clinical trials targeting B cells in pancreatic cancer have been conducted recently and the results have been very disappointing. We have now found in our lab, using a more clinically relevant autologous model, that B cells are more likely tumour suppressive. We are now investigating the mechanisms behind this, and the microenvironmental differences that are observed in spontaneous versus allograft models.

Modelling Genomic Instability

Recent data have suggested that genomic instability and increased mutation burden may render tumours sensitive to DNA-damaging therapeutic agents, but also predict the efficacy of immune checkpoint inhibitors. To investigate this, and to create models in which to test response to therapies, we have modelled the effects of deletion of DNA-damage repair genes that are mutated in pancreatic cancer, e.g. ATM and BRCA1–2. We find that tumorigenesis is accelerated in, for example, ATM-deficient mice, suggesting at least some degree of genomic instability. Pharmacological inhibition of the DNA damage repair (DDR) pathway using ATR and PARP inhibitors showed some efficacy in these models; however, there was still a lack of infiltrating T cells. Thus we are examining mutational burden, and investigating whether antigen presentation can be enhanced to render tumours sensitive to immunotherapy in combination with DDR-inhibiting agents.

We have also generated a model of APOBEC overexpression in pancreatic cancer. The APOBEC family of cytidine deaminase enzymes, whose normal function occurs during viral infections, have been revealed as drivers of mutation in a variety of human tumours, including pancreatic cancer. We have engineered mice to express Cre-inducible APOBEC3B and crossed these with the KPC genetically engineered mouse model of pancreatic cancer (Fig. 2). We find that overexpressing APOBEC3B results in a poorer prognosis in these animals, and some changes in the immune microenvironment; however, there is still a lack of T cell infiltration into these tumours. We are currently performing genomic and transcriptomic analyses to determine mutational burden and tumour subtype in an effort to identify therapeutic opportunities in patients bearing this signature.

**Figure 1**

The role of the immune system in pancreatic cancer progression

Crosstalk between tumour cells and the microenvironment can affect cell proliferation, survival, metastasis, immune response and response to different therapeutic agents, while therapeutic agents in turn alter these signals and change the tumour subtype.

**Figure 2**

The KPC APOBEC mouse model

**a** Generation of an APOBEC3B-expressing KPC (Pan-cre, Cre, KrasG12D/+; p53R172H/+) based mouse model of pancreatic cancer

**b** RAQscope shows expression of APOBEC3B in APOBEC3B-expressing KPC tumours. Scale bar = 500μM

**c** Kaplan–Meier survival analysis shows significant acceleration of pancreatic cancer in APOBEC3B-overexpressing KPC mice.

The pancreatic cancer immune microenvironment

A prominent feature of pancreatic cancer is the dense desmoplastic stroma, which can account for up to 90% of the tumour volume. This microenvironment includes fibroblasts, stellate cells, immune cells, blood vessels and extracellular matrix proteins, and is characterised by significant infiltration of immune cells, but a distinct lack of CD4+ and CD8+ T cells (Fig. 1c). Thus, immunotherapies designed to activate effector T cells by blocking IgG function by immune checkpoint ligands such as PD-1, PD-L1, and CTLA-4 have had little efficacy in PC despite promise in other cancers. We previously found that ablating neutrophils homing to pancreatic tumours could enhance T cell infiltration, providing a therapeutic opportunity for PDL-1 blocking immunotherapy (Steel et al., Cancer Cell; 29: 832–45). We have also found that inhibiting macrophages, via CSF-1 antagonists or statins, can enhance anti-tumour activity.

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**Head**

Jennifer Morton

**Research Scientists**

Arantza Perez

Karen Pickering

Mathias Tesson

Viola Paulus-Hock

**Graduate Students**

Laura Lapienyte

Karen Pickering

Mathias Tesson

Peter Repiscak

Arantxa Perez

Saadia Karim

**Scientific Officers**

Saadia Karim

Viola Paulus-Hock

**Bionformatician**

Peter Ruppa

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