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PRECISION-PANC PRECLINICAL LAB

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The focus of research in our lab is to better understand how pancreatic cancer develops and progresses and use this knowledge to identify and test new clinically relevant therapies and combinations. To do this, we use mouse models of pancreatic cancer that recapitulate human tumours in terms of both the genetic aberrations and the dense fibrotic and immuno-suppressive stroma. These models, therefore provide a clinically relevant platform, in which to test and validate or de-validate novel tumour cell and microenvironment targeted therapies.

Pancreatic cancer, or pancreatic ductal adenocarcinoma (PDAC), kills over 430,000 people every year. It is one of the deadliest epithelial malignancies, and both incidence and mortality are rising. In the UK alone, there are around 10,500 new cases every year, equivalent to about 30 new cases every day. Less than 8% of those patients will survive their disease for five years, and only 1% are likely to survive beyond ten years. Indeed, despite improvements in surgical management and significant investment in clinical trials, cure rates have only minimally increased over the last 50 years.

Many years of research have improved our understanding of disease evolution, genetic alterations, transcriptional subtypes, and the tumour microenvironment (TME). Activating mutations in KRAS are the most prevalent driver mutations, accompanied by loss of function of tumour suppressor genes. Some mutations found in subsets of patient may confer sensitivity to targeted therapies. For that reason, part of our work involves modelling mutations in the genes that are mutated in smaller subsets of human pancreatic cancer with a view to understanding the biological consequences of those mutations. Another feature characteristic of PDAC is the dense fibrotic stroma that surrounds and supports the tumour cells and can account for up to 90% of the tumour volume in the human disease. This microenvironment consists of fibroblasts and extracellular matrix proteins as well as significant inflammation with prominent myeloid cell infiltration and a dearth of effector T cells. Each component plays an important role in pancreatic cancer progression, able to influence tumour cell proliferation, survival, metabolism, migration, immune surveillance, and response to chemotherapy. Therefore, it is essential to investigate pancreatic tumour biology *in vivo*, in spontaneous tumours with a physiological microenvironment and immune response.

Modelling genetic subsets of patients

With regard to the recurring mutations in patients that may be actionable, we have developed several models to mimic these patients and identify therapeutic targets. Our suite of models covers the majority of genes/pathways identified in the patient tumours. For example, RNF43, the gene encoding ubiquitin E3 ligase ring finger 43, has been shown to be mutated in 10-15% of cases of metastatic pancreatic (10-15%). Using KPC mice as a backbone (*Pdx1-Cre; Kras*$^{G12D/+}$; *Trp53*$^{R172H/+}$), we have now developed a genetically engineered mouse (GEM) model of *Rnf43* deletion and found that *Rnf43* deletion is a strong driver of pancreatic cancer progression, with loss of even a single copy sufficient to significantly accelerate tumour progression. RNF43 inhibits Wnt/β-catenin signalling by reducing membrane Frizzled. Thus, to test whether this subset of patients might be uniquely sensitive to pathway inhibition we are testing a clinically-relevant porcupine inhibitor in these mice.

Mutations in DNA damage repair genes have also been reported in ~15% of pancreatic cancers. We developed models of these patients, by deleting *Atm* or *Brca1* in the KPC mouse model and found differential sensitivities to DNA damaging agents. We are now extending these studies to include radiotherapy, as we predict that these mutations will render tumours more sensitive to radiation. Using our small animal radiotherapy research platform (SARRP) we have developed a protocol for tumour-targeted radiotherapy in GEM models of pancreatic cancer (Figure 1), which we are applying to these models. The use of radiotherapy in pancreatic cancer treatment has been limited thus far, however, this may be due to a lack of understanding of the effect of radiation on the pancreatic TME. Irradiation results in tumour cell death and release of tumour-associated antigens that can elicit a cytotoxic T cell response against the tumour. However, this is impeded by the release of inflammatory cytokines and chemokines which can result in altered fibroblast secretory output, ECM remodelling, macrophage polarisation and an even more immunosuppressive microenvironment. Thus, we are using our models to investigate responses in individual cells in the TME to determine the mechanisms controlling pro-tumourigenic immune and fibrotic responses with the aim of identifying rationale therapeutic combinations to promote anti-tumourigenic immune responses while inhibiting pro-tumourigenic immune and fibrotic responses.

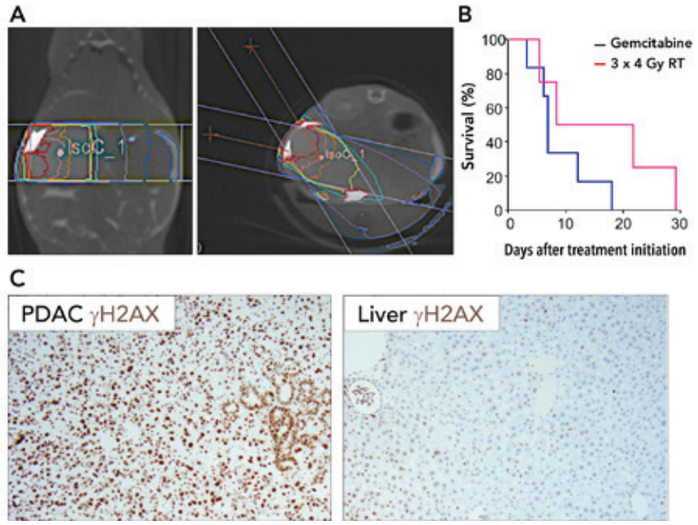

Figure 1
A) Example of CT imaging guided arc radiotherapy plan in a KPC mouse. **B)** Kaplan-Meier survival analysis of KPC mice treated as indicated and aged until clinical endpoint. **C)** Immunohistochemical staining for γH2AX in PDAC and liver from irradiated KPC mice, showing DNA damage in the tumour, but not in the surrounding tissue.

Tumour heterogeneity

Microdissection and single cell sequencing studies in human pancreatic cancer have recently revealed that both the tumour and stromal compartments display significant heterogeneity in terms of gene expression and function. For example, antibody-based single cell analysis (CyTOF) has highlighted two stable populations of cancer-associated fibroblasts with distinct expression profiles and immune cell interactions and defined by differential expression of CD105. CD105+ fibroblasts are tumour-permissive, whilst CD105- fibroblasts exhibit tumour restrictive behaviour which is dependent on the adaptive immune system.

The level of heterogeneity in mouse models has been the subject of some debate, both in terms of inter- and intra-tumour heterogeneity. We have now shown that these models do exhibit significant transcriptional heterogeneity, particularly between animals, despite identical initiating mutations (Figure 2). We now want to investigate the spatially resolved transcriptional landscape of tumours in these models, to monitor and understand this multi-level heterogeneity during tumour progression and in response to therapeutic intervention. This understanding is vital for the development of novel therapeutic strategies to improve the dismal statistics associated with this disease.

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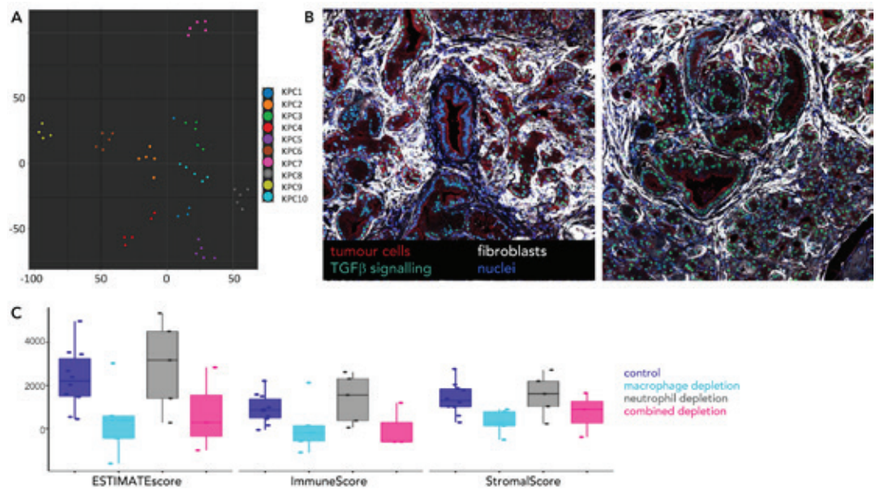

Figure 2
A) t-SNE plot and unsupervised clustering of biopsies taken from KPC autochthonous tumours (each colour represents 1 mouse; each dot represents 1 of 5 biopsies). **B)** co-IF for the markers indicated highlighting significant heterogeneity in the pancreatic cancer microenvironment. **C)** ESTIMATE (Estimation of STromal and Immune cells in MAlignant Tumors using Expression data) evaluation, using gene expression data, of the abundance of immune and stromal components in tumours from KPC mice treated as indicated.