Cell of origin session

Dr Beatriz Salvador, University of Cardiff

Identifying KRas mutant cell mechanisms to overcome cell competition and promote disease in pancreatic cancer initiation.

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Late diagnosis sets pancreatic cancer among the most aggressive cancers with very poor survival rate. Understanding its development is necessary to achieve new early detection strategies to improve patient outcome.

Activation of oncogenic KRAS is the first genetic event occurring (>90%), and it is associated with the appearance of Pancreatic Intraepithelial Neoplasias (PanINs), driving the development of PDAC. Using the mouse model Pdx-1Cre^{ER};KRas$^{G12D/+}$;Rosa26$^{LSL-RFP}$ and inducing KRasG12D expresion in few cells within pancreatic epithelium, we demonstrated that most KrasG12D-expressing cells are removed from tissues through cell competition. However, when cells are mutant for both KRAS and TP53 oncogenes (low ratio), they are never eliminated and instead are retained in tissues promoting PanINs and, eventually, PDAC. Interestingly, a small population of KRas mutant cells in adult pancreas can overcome tissue homeostasis mechanisms, remain in the tissue, and initiate PanINs. We compared the transcriptome of non-eliminated KRas and/or TP53 mutant cells to identify common signatures. Our data show that non-eliminated cells dysregulate cell adhesion and migration, antigen presentation and differentiation transcriptional signatures; suggesting that mutant cells de-regulate these pathways to avoid cell competition and elimination. Also, KRas mutant cells express gene signatures such as gastric/duodenal stem cell signatures, disruption of adherence junctions and downregulation of NFκB pathway, indicating that overcoming cell elimination also promotes fate changes.

Our objective is to validate these signatures at protein level and study their functional implications in pancreatic cancer disease. Understanding the mechanism underlying early disease will help to develop new and improved methods to detect cancer earlier.

Curtis Rink, CRUK Beatson Institute

Identification of the cell(s) of origin of PDAC is still a matter of some controversy. Pancreatic duct cells were originally presumed to be the cell of origin due to the ductal morphology of the tumours. However, recent studies have shown that acinar cells are highly sensitive to Kras mutation and inflammation and can give rise to PanIN and PDAC through acinar-to-ductal metaplasia. Both ductal and acinar cells therefore have the potential to develop into PDAC depending on genetics and external stimuli, but more work is required in this area. To that end, in our lab we have employed different models targeting mutations specifically to the pancreatic duct cells and will discuss our findings so far and our future plans.

Haonan Xu, University of Oxford

- Haonan is a current PhD student; their presentation will be put together by Prof Eric O'Neill.

Dr Sam Au, Imperial College London

Microfluidic T Cell Selection by Cellular Avidity

We have recently developed a novel microfluidic fluid shear stress-based approach to identify and recover potent T cell clones based on the cellular avidity between living T cells and tumor cells. Our approach is capable of probing approximately up to 10,000 T cell-tumor cell interactions per run and can discriminate and recover highly potent T cells with up to 100% purity from mixed populations of T cells within 30 minutes. Markers of cytotoxicity, activation and binding avidity persisted when recovered high cellular avidity T cells were subsequently exposed to fresh tumor cells. These results demonstrate how microfluidic probing of cellular avidity may fast track the therapeutic T cell selection process for pancreatic and other cancers.

(epi)Genetics and subtypes session

Dr Pinar Uysal-Onganer, University of Westminster

Why would you choose microRNAs as biomarker?

My research focuses on non-coding RNAs and cancer stem cells. I have been working on developing microRNA expression panel, based on our in silico analysis to use as biomarkers to detect and/or predict the PDAC severity. We have been using different samples such as blood, urine, tissues and cell supernatants as well as extracellular vesicles to detect microRNA expressions.

One of our on-going projects is on environmental toxins and their effects on PDAC prognosis. I have started and currently leading an international consortium that focuses on toxins such as Cadmium and Nickel. Our international group consists of clinicians, toxicologists and molecular & cell biologists, so far we published 2 review articles, 2 research papers on this projects.

Another current project is on developing microRNA expression panel to use for early detection of PDAC. We have obtained fresh frozen tissues, plasma and serum samples from the Pancreatic Cancer Biobank. We have been validating our microRNAs by using in vivo samples while studying molecular mechanisms of microRNA triggered metastasis and drug resistance by using genome engineered PDAC cell models. We have published a review article and several research papers from this project.

Dr Rhiannon French, University of Oxford

Diagnosis and treatment of pancreatic ductal adenocarcinoma (PDAC) could be improved by a greater understanding of the highly malignant subset of cancer cells termed Cancer Stem Cells (CSCs). CSCs are considered responsible for tumour initiation and metastasis and exhibit pluripotent gene expression signatures similar to those of embryonic stem cells (ESCs). Ten-Eleven Transcription Factors (TET) 1-3 oxidise 5'mC to 5'hmC (hydroxy-methyl-cytosine) to reverse gene silencing. TET1 is essential both in ESCs as a binding partner of Nanog and in the induction of pluripotency where it can replace OCT4 as an induction factor. As TET1 and 3 are also elevated in PDAC and are associated with poor prognosis, we hypothesised that TET1/3 are important for pancreatic CSC function. We have observed elevated expression of TET1/3 in the tumoursphere-forming (CSC-like) subset of PDAC cell lines, accompanied by increased 5'hmC at key ESC-associated loci. CRISPRi-mediated knockdown of TET1 or 3 reduced colony and tumoursphere formation and expression of pluripotency-related factors including Oct4, Sox2 and Nanog. TET3 knockdown also impaired Activin signalling, an important pathway in PDAC CSCs. ChIP-seq of TET1/3 has identified key target genes including PBX1 and SALL4 which may also mediate CSC properties. We are currently investigating the effect of TET knockdown on tumour initiation in vivo and exploring the possibility of identifying a CSC-associated 5'hmC gene signature in circulating tumour DNA of patient blood samples. Our findings so far suggest that both TET1 and 3 promote CSC properties in PDAC cells and therefore may have potential as therapeutic targets.

Dr Shalini Rao, University of Cambridge

Despite recent advances in the treatment of Pancreatic Adenocarcinoma (PDAC), the median survival remains <12 months. Patients typically present with late stage disease and are often unable to tolerate the drug combination regimens due to the associated toxicity. Therefore, there is an immediate need for the generation of new and innovative therapeutic targets that are subtype specific. Little is known about the role of pioneer factors such as FOXA1 and their interaction partners in a PDAC context. Our project explores whether FOXA1 associated proteins, define subtype specificities and whether these are therapeutically relevant. Inspired by literature reports implicating FOXA1 and GATA5/GATA6 in regulating pancreatic cancer metastasis, we hypothesised that there might be an undiscovered Nuclear Receptor (NR) that works with FOXA1 that could be a potential therapeutic target (similar to estrogen receptor in breast cancer). Using innovative 'omic' approaches developed in our laboratory, we explore the putative role of NR's to delineate mechanisms underlying transcription factor biology 1. Using complex models our findings support a model in which gene transcription in the classical type of pancreatic cancer is regulated by FOXA1/GATA5/6 and a recently discovered (but previously undisclosed) nuclear receptor (NR) complex. Here, we elucidate a detailed molecular investigation of the NR/FOXA1 transcriptional network to validate the role of these NRs and their therapeutic relevance in PDAC.

Immunology Session

Prof Michael Dustin, Kennedy Inst Oxford

My colleagues and I are carrying out a large single cell RNA seq analysis of immune system cells from pancreatic cancer. We have observed two patterns with lymphocytes or myeloid cells dominating the infiltrate. Even when lymphocytes are present they are dominated by senescent features and expression of negative regulatory receptors such as TIGIT. We are also beginning to study the macrosecretome of immune cells and pancreatic cancer cells focusing on proteins released in particles >100 nm in size. This includes some extracellular vesicles and supramolecular attack particles. Immune cells can use such particle to kill cancer cells and we are examining the possibility that pancreatic cancer cells use such particles for defence.

Dr Seth Coffelt, CRUK Beatson Institute

Immune cells play a pivotal role in pancreatic ductal adenocarcimona (PDAC) progression. However, this disease is mostly resistant to currently available immunotherapies, highlighting the urgent need for new and effective therapies that target the immune system. Our lab is focused on gamma delta T cells, a rare T cell receptor-expressing cell type with innate-like qualities. We have found that these cells are absent from normal pancreas tissue but they accumulate in pancreatic tumours. Using the *Kras*$^{G12D/+}$*; Trp53*$^{R172H/+}$*; Pdx1-Cre* (KPC) mouse model, we have found that gamma delta T cells drive metastasis to the liver. Given the importance of pro-metastatic neutrophils in PDAC, we investigated the relationship between neutrophils and gamma delta T cells. Genetic deletion of gamma delta T cells in KPC mice failed to influence neutrophil numbers in the blood, tumour or liver, suggesting a disconnect between neutrophil and gamma delta T cell function. By contast, the absence of gamma delta T cells in KPC mice associated with reduction of tumour-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) within the primary tumour microenvironment. Current efforts are underway to understand how this small population of T cells educates TAMs and CAFs to progress metastatic disease.

Dr Tim Halim, University of Cambridge

We are interested in tissue-resident immune cells in the pancreas, and how these cells coordinate tissue homeostasis and inflammation at different stages of tumourigenesis. We focus primarily on a novel immune cell-type, called the Group 2 Innate Lymphoid Cell (ILC2), which has critical roles in directing innate and adaptive immunity. We show that ILC2 are able to directly communicate with other immune and non-immune cells in pancreas, and are localised within a specific niche. This niche expands dramatically during tumourigenesis, and using different reagents, we show that ILC2 are important for suppressing adaptive anti-tumour immunity in models of pancreatic ductal adenocarcinoma. Hence, these tissue-resident ILC2 likely play a critical role in establishing immune-tolerance, and targeting this mechanism has potential to locally reinforce immune surveillance.

Dr Leo Carlin, CRUK Beatson Institute

Neutrophil dynamics in the pancreatic cancer pre-metastatic lung.

The immune system has been implicated in almost every stage of cancer development, from initiation and growth, to dormancy, invasion, and metastasis. As the immune system primarily co-evolved with microbes to protect against infection with pathogens and as cancer cells are mutated host cells, the role of immunity in cancer is complicated. Neutrophils are crucial to many anti-microbial and tissue damage reactions and play a key role in initiating the host immune response to infection. The work of several groups has suggested that neutrophils are important in onco-immunology, and a high neutrophil-to-lymphocyte ratio is associated with poorer prognosis in many advanced cancers. Emerging data suggest neutrophils are exquisitely sensitive to their microenvironment, a feature previously thought to only apply to other myeloid cells. Importantly, studies directly implicate neutrophils in pancreatic cancer metastasis.

Advanced imaging of the lungs of genetically engineered mouse models of pancreatic cancer revealed markedly different neutrophil behaviour. Highly-metastatic (KPC) and poorly-metastatic ($KP^{fl}C$) mice were compared when they had small palpable pancreatic tumours but before overt metastasis was observed. We found that neutrophil numbers were increased in KPC lungs compared with $KP^{fl}C$ lungs and other controls. Bulk RNAseq of sorted neutrophils from the two models revealed transcriptional differences consistent with different behaviour. Although fixed imaging mirrored the increased abundance it did not reveal differences in localisation. However, live ex vivo lung imaging tracking hundreds of neutrophils in situ specifically revealed an enriched static/slow moving neutrophil population in KPC lungs. This exemplifies the power of spatiotemporal information to reveal features of the pre-metastatic tumour immune microenvironment for further mechanistic study.

Stroma Session

John Moir, Freeman Hospital

I have a strong research interest in targeting the stroma in pancreatic cancer, and in particular the cancer-associated fibroblasts, also known as PSCs (Pancreatic stellate cells), which have a key role in the tumour microenvironment.

I am keen to share my CRUK-funded PhD work carried out at the internationally reputed Fibrosis Lab at Newcastle University, which revealed a novel metabolic relationship between PSCs and pancreatic cancer cells which centred upon lactate metabolism and shuttling within the stroma. Notably I demonstrated how the expression of a specific lactate transporter (MCT1) within the stroma is linked to poor prognosis, and thereafter revealed how inhibiting MCT1 with the Astra-Zeneca-produced (and now CRUK-owned) compound AZD3965 had a detrimental effect on both the cancer and stellate cells in co-culture experimentation, which has great potential to translate into prognostic benefit in the treatment of pancreatic cancer patients. The question remains as to which patients may benefit from this treatment, as my experiments revealed that certain tumour phenotypes may respond more favourably; I am keen to discuss this further with experts in the field, particularly those with a metabolomic research interest.

Furthermore, having had some fruitful preliminary discussions recently with CRUK, I am enthusiastic to share my plans to set up a phase 2 RCT utilising AZD3965 in combination with chemotherapy in patients with metastatic pancreatic cancer, in the hope this may improve the outcomes of patients with this devastating disease, and would relish the opportunity to collaborate and work together with attendees to discuss and optimise trial design, as well as garner interest in centres potentially signing up to the project team.

Dr Andrea Mohr, University of Essex

Fas-threshold Signalling in MSCs Promotes Pancreatic Cancer Progression and Metastasis

Mesenchymal stem cells (MSCs) belong to the tumour microenvironment and have been implicated in tumour progression. We found that the number of MSCs significantly increased in tumour-burdened mice driven by Fas-threshold signalling. Consequently, MSCs lacking Fas lost their ability to induce metastasis development in a pancreatic cancer model. Mixing of MSCs with pancreatic cancer cells led to sustained production of the pro-metastatic cytokines CCL2 and IL6 by the stem cells. The levels of these cytokines were dependent on the number of MSCs, linking Fas-mediated MSC-proliferation to their capacity to promote tumour progression. Furthermore, we discovered that CCL2 and IL6 were induced by pancreatic cancer cell-derived IL1. Evidently, analysis of patient transcriptomic data revealed that high FasL expression correlates with high levels of MSC markers as well as increased IL6 and CCL2 levels in pancreatic tumours. Moreover, both FasL and CCL2 are linked to elevated levels of markers specific for monocytes known to possess further pro-metastatic activities. These results confirm our experimental findings of a FasL-MSC-IL1-CCL2/IL6 axis in pancreatic cancer and highlights the role of MSCs in tumour progression.

Prof Gerard Evan, University of Cambridge

We have used switchable genetic mouse models of pancreatic adenocarcinoma to map the tumour micro environmental paracrine signals by which oncogenic mutations drive PDAC progression and By which targeted inhibition of driver oncogenes instruct tumour regression.

Progression Session

Dr Pilar Acedo-Nunez, UCL

How can we accelerate the translation of early detection biomarkers into the clinic? Do we have good *in vitro* models mimicking early-stage human disease?

Our multidisciplinary team formed by basic and clinical scientists aims to improve the outcome of patients with pancreatic cancer by **i)** generating clinically relevant *in vitro* models mimicking human disease, **ii)** investigating novel diagnostic markers, and **iii)** developing more effective therapeutic strategies for this cancer. A key issue in pancreatic cancer research is the lack of preclinical models that recapitulate the complexity of human tumours, particularly at early stages, compromising the successful application of research findings to the clinical setting. We have access to surgical and endoscopic ultrasound (EUS) human biopsies, but early-stage cases are difficult to obtain and to maintain in the laboratory. These samples are key to better understand tumour initiation and progression, to discover novel therapeutic targets and early-detection biomarkers, and to develop novel drugs. We have isolated PBMCs from our clinical biobank containing pancreatic cancer cases. We are in the process of isolating CAFs. Our goal is to develop 3D heterotypic culture models to help overcome the current challenges mentioned above. We would be happy to collaborate with other research groups to improve the complexity of our patient-derived models. We have also generated a biobank of blood and urine samples from patients diagnosed with pancreatic cancer and from those at risk of developing the disease. We would highlight this resource during the presentation.

Dr Remi Samain, Barts Cancer Institute

CD73 drives invasion, metastasis and immunosuppression in pancreatic amoeboid cancer cells

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer, mainly because of its high propensity to metastasize and its desmoplastic nature. Using a panel of pancreatic cancer cell lines, 3D invasion systems, microarray gene signatures, mouse models and intra-vital imaging, we demonstrate that ROCK-myosin II activity in PDAC cells regulates actomyosin contractility and confers amoeboid in vivo metastatic abilities. As such, ROCK inhibition blocks cancer cell migration, invasion and in vivo liver metastasis. We further demonstrate that the immune checkpoint CD73 is not only involved in amoeboid cancer cell immune escape and macrophage co-option, but also in regulating Myosin II dependent invasion and metastatic dissemination. CD73 blocking reduces cancer cells invasion abilities in vitro. Tissue microarrays of human PDAC biopsies combined with bioinformatics analysis highlights that the presence of rounded-amoeboid invasive cells with high CD73-ROCK-Myosin II activity and a specific immune infiltrate confers poor prognosis to patients. We therefore propose targeting of immunosuppressive amoeboid cancer cells as a therapeutic strategy in PDAC.

Dr Justin Sturge, University of Hull

Endo180: an emerging therapeutic target and biomarker in pancreatic cancer

The early molecular cellular events that can initiate disease progression in anatomically normal pancreatic epithelia towards pancreatic intraepithelial neoplasia, and its further progression to pancreatic ductal adenocarcinoma (PDAC), represent key therapeutic targets and the basis for development of new diagnostics. In this respect, we have identified a molecular mechanism that can

trigger normal epithelial cell invasiveness. Firstly, we have shown that breakage of an epithelial-to-mesenchymal (EMT)-suppressor complex, which is formed between the fourth C-type lectin domain CTLD4 in the collagen receptor Endo180 (CD280) and the cell adhesion modulator basigin (CD147), is sufficient to initiate the invasion of normal epithelial cells in glandular acini. Secondly, we have shown that CTLD2 in Endo180, which binds to glycosylated collagens, has the capacity to drive invasion of normal acinar epithelial cells when exposed to 'stiff' microenvironments, for example the fibrotic changes that occur during chronic pancreatitis and diabetes, which are both risk factors for metastatic PDAC. Our findings have led to the development of diagnostic tools targeting Endo180 that are currently under evaluation for the detection of the initial stages of metastatic disease in glandular tissues.