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DEEP PHENOTYPING OF SOLID TUMOURS

Group Leader
John Le Quesne FRCPath

Associate Scientist
Ian Powley[1]

Research Scientist
Zhangyi He

Scientific Officers
Fiona Ballantyne
Silvia Martinelli[1]
Rachel Pennie

Histopathology Technology Manager
Leah Officer

Graduate Student
Kai Rakovic[2]

[1]Mazumdar-Shaw Molecular Pathology Chair
[2]Jean Shanks Foundation/ Pathological Society Clinical Fellow

Solid tumours are complex assemblages of malignant cells, lymphocytes, fibroblasts, blood vessels and other tissue types, and are best thought of as complex neo-organs built around a never-ending cycle of injury and frustrated repair. To understand how malignant cells survive and spread in this potentially extremely hostile habitat, we must understand the microscopic environment at a cellular level and visualise the competing cellular strategies of malignant cells and their genomically normal stromal neighbours. We aim to answer a range of key questions in tumour biology by using the latest deep phenotyping technologies to directly observe and quantify cellular behaviours in intact tumour tissue.

We routinely develop highly multiplexed IF/ISH staining assays using Ventana autostainer platforms and collect multiplex images from human and mouse tumour tissues using Akoya Mantra and Polaris imaging platforms, as well as the FUSION ultra-deep imaging system.
In essence, most of the technologies that we apply consist of three steps (Figure 1). First, we detect multiple RNA or protein targets with a range of immunofluorescent antibodies and probes. We then acquire high-resolution images, with separate layers for each marker of interest. These images are subsequently converted into quantitative data, typically single-cell quantitative measures and/or cellular phenotypes, obtained by the application of artificial intelligence image segmentation algorithms which we have created for the task. These spatial and quantitative cell data are used as the substrate for classical or more advanced modelling techniques intended to answer biological questions about tumour function.

Key projects:

1) Translational control in tumour cells

The dysregulation of mRNA translation is emerging as a key hallmark of malignant transformation, as tumour cells radically reprogramme their protein output by implementing translational control mechanisms associated with states such as cellular stress and altered nutrient availability. To what extent is mRNA translation regulation altered in human cells? Which hallmark behaviours are linked to which alterations in translational control? Which elements of the translational control machinery have promise as therapeutic targets?
We are examining numerous measures of translational control at the single-cell level in large collections of several common malignancies, and we are using the resulting images both to generate and to test hypotheses. For example, we have found that switching between expression of different mRNA helicases is associated with tumour cell proliferation and invasion as well as immune system evasion, and that stress signalling through eIF2 is intimately associated with tumour cell proliferation and invasion.

2) Tumour immunophenotyping

The most impactful development in cancer therapy in recent years is the introduction of immunotherapies. These treatments work by reversing the ability of tumour cells to mask themselves from the immune system which would otherwise rapidly destroy them. However, we are at present only partially successful in identifying which patients will benefit from these therapies. We believe that quantifying the degree of immune system engagement within tumour biopsy material is likely to improve our ability to do this; can we, by direct observation of complex cellular phenotypes in tissues, identify tumours which are actively evading immune system detection and/or destruction?
To achieve this, we are applying highly multiplexed panels of markers to identify tumour and immune cell phenotypes, for instance using our FUSION platform we can use upwards of 40 markers to distinguish specific cell phenotypes in the tumour microenvironment. We are then able to link the presence and relative spatial distribution of these cells to patient outcomes. We intend to apply these methods to cohorts of tissues from patients receiving immunotherapies with Glasgow's cancer treatment centre, and to see if we can improve our ability to predict patient response to immunotherapy, compared to current methods.

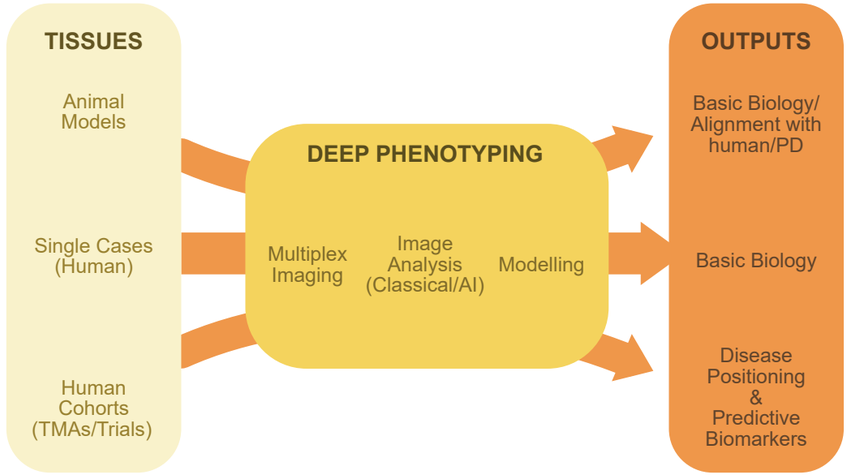

Figure 1
Workflow schematic of deep phenotyping methods. The basic pipeline (centre) is applied to a range of tissue types to achieve answers to diverse scientific questions.

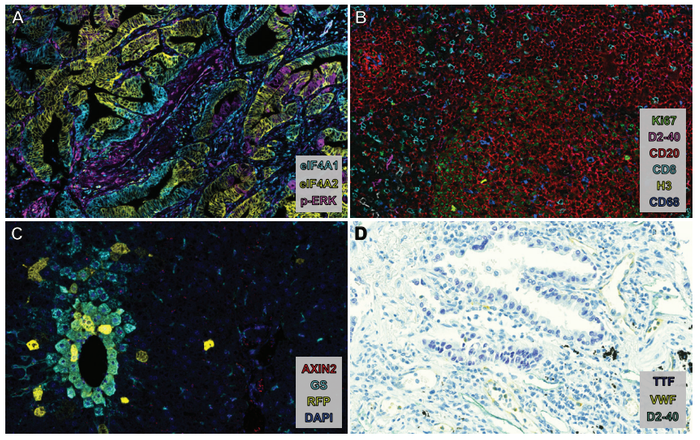

Figure 2
Example multiplex images. **A** Spectrally unmixed multiplex staining of eIF4A1, eIF4A2 and P-ERK in archival human lung adenocarcinoma tissue. **B** FUSION image of indicated protein markers on human tonsil tissue sections; only a small subset of the stained markers are shown **C** Spectrally unmixed co-ISH IHC of AXIN 2 mRNA with IF markers for red fluorescent protein and glutamine synthase in transgenic mouse liver. **D** four-colour chromogenic staining for human lung adenocarcinoma cell nuclei (TTF-1), capillaries (VWF) and lymphatics (D2-40) with haematoxylin counterstain.

3) Application of machine learning to tumour microscopy

Machine learning and artificial intelligence offer us the potential to reach deeply into the information present within microscopy images without necessarily knowing which features of the images are likely to be important *a priori*. These methods are potentially very powerful, and able to answer both clinical and basic scientific questions. Can we train machines to predict patient outcomes, and response to therapies? We have accumulated very large collections of microscopy images from archival lung cancers and mesotheliomas, and, in collaboration with computer scientists, we are using these to train machine algorithms to attempt these tasks. In addition, we aim to use generative methods to identify image features which are particularly strongly associated with key tumour features (e.g. lethality, hallmark behaviours or genomic alterations). Furthermore, we are about to start applying these methods to highly multiplexed tissue images, which holds the potential for even deeper understanding.

4) Deep phenotyping of respiratory malignancies

We have particular interests in non-small cell lung cancer (NSCLC) and malignant mesothelioma. Both have high incidence in Glasgow and are in great need of improved therapies. We are using a combination of classical microscopy methods and multiplex methods to tackle key questions in these disease types. In particular, we are using linked RNASeq and multiplex image data to deconvolute gene expression in very large case cohorts, gaining unique insights across the breadth of human tumour variance.
Malignant mesothelioma is a difficult diagnosis to make in tissue biopsies, and we hope to improve this, as well as our ability to predict progression to invasive malignancy, by discovering novel biomarkers of malignancy, using a combination of classical methods and machine learning algorithms, and building upon Glasgow's flagship PREDICT-Meso physician-led study of early mesothelioma.

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