The Institute of Cancer Research
PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS

<table>
<thead>
<tr>
<th>Project Title:</th>
<th>The role of cancer associated fibroblasts in altering signal transduction and drug resistance in KRAS mutated cancers</th>
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<tr>
<td>Primary Supervisor(s):</td>
<td>Dr Udai Banerji</td>
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<td>Other supervisory team members:</td>
<td>Professor Raj Chopra, Dr Jyoti Choudhary</td>
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DIVISIONAL AFFILIATION

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<tr>
<th>Primary Division:</th>
<th>Cancer Therapeutics</th>
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<td>Primary Team:</td>
<td>Banerji lab</td>
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<tr>
<td>Other Division (if applicable):</td>
<td>Cancer Biology</td>
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<td>Other Team (if applicable):</td>
<td>Functional Proteomics</td>
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BACKGROUND TO THE PROJECT

Introduction
Once cancer has spread from its organ of origin it is called metastatic cancer. At this stage, the majority of cancers are not curable. Treatment includes the use of anticancer drugs but resistance to these anticancer drugs develops, allowing the cancer to progress and eventually leading to death.

Resistance to anticancer drugs is multifactorial. Permanent genetic or long lasting epigenetic changes within the cancer cell can cause resistance and these have been extensively studied. Re-wiring of signal transduction (the flow of information within the cancer cell) has been shown to cause early drug resistance (Al-Lazikani B, 1012). The ability of cancer cells to re-wire signal transduction within themselves can be influenced by the non-cancerous stromal cells that form part of a patient’s tumour. One such subset of stromal cells is called cancer-associated fibroblasts (Kalluri R, 2016), which are derived from multiple sources including endothelium, epithelium or pre-existing fibroblasts. These cells could cause drug resistance by excreting ligands and cytokines that activate cancer cells, in addition to attracting other cells that reduce immune surveillance of the cancer or form a mechanical barrier, reducing drug concentrations within tumours.

The understanding of changes in signalling within a cancer cell driven by proteins secreted by its neighbouring cells with the tumour stroma (Tape CJ, 2016) can help us to find new mechanisms of drug resistance. Importantly, it can bring to light new drug targets for drug discovery efforts to help overcome resistance to current anticancer drugs. Further understanding of re-wiring of signal transduction as a mechanism of drug resistance can lead to the design of clinical trials of combinations of anticancer drugs to overcome this resistance (Lopez JS, 2017).

This studentship broadly aims to study the effects of cancer-associated fibroblasts on drug resistance in a group of colon and lung cancers that harbour a KRAS mutation.
PROJECT AIMS

- Establishing an assay to determine drug resistance associated with co-culture of cancer cells with cancer-associated fibroblasts
- Determine the differences in signalling patterns within cancer cells caused by co-culture with cancer-associated fibroblasts using multiplex antibody-based proteomic platforms
- Identify factors secreted by cancer-associated fibroblasts that influence signal transduction and drug resistance within cancer cells using mass spectroscopic methods
- Validate findings from the mass spectrometry using cell line models and patients’ tumour tissue

RESEARCH PROPOSAL

Hypothesis:
Cancer associated fibroblasts cause drug resistance in mutant KRAS-driven colon and lung cancers by altering signal transduction in cancer cells.

Establishing an assay to determine drug resistance associated with co-culture of cancer cells with cancer-associated fibroblasts
A set of 6 - 10 KRAS mutant cell lines (3 - 5 colon and lung cancer cell lines) will be cultured with and without appropriate cancer associated fibroblasts against a panel of 450 anticancer drugs. The cells will be cultured in 3D, high-throughput 384 or 1536 format and studied using proliferation or cell viability assays over a 7 – 14-day period. The expected outcomes of these experiments are to find differences in sensitivities of anticancer drugs in colon and lung cancer cell lines when co-cultured with appropriate fibroblasts. Expected outcomes: finding 1 - 10 drugs in colon and lung cancer where there are significant differences in sensitivity when cancer cells are co-cultured with cancer-associated fibroblasts.

Determine the differences in signalling patterns within cancer cells caused by co-culture with cancer-associated fibroblasts using cancer multiplex antibody-based proteomic platforms
Cancer cells will be exposed to 10-15 anticancer drugs with known mechanisms of action for 6 and 24 hrs. The experiments will be conducted in 3D cultures in media that have previously been exposed to cancer-associated fibroblasts and those that have not. The differences in re-wiring of signal transduction within the cancer cells will be studied by antibody-based proteomic approaches using the Luminex or Nanostring platforms studying differences in phosphorylation of 50 - 100 key proteins. Existing collaborations with the ICR Bioinformatics teams will be used to model re-wiring of signal transduction caused by different drugs. Expected outcomes: Understanding differences in signal transduction within cancer cells grown in culture medium conditioned by cancer-associated fibroblasts.

Identify factors secreted by cancer associated fibroblasts that influence signal transduction and drug resistance within cancer cells using proteomics
Cancer-associated fibroblasts will be grown in medium containing labelled amino acids which are incorporated within the cells (Eichelbaum, K 2012). Mass spectrometry methods will be used to identify and quantify proteins secreted by the cancer-associated fibroblasts that will be enriched based on selection of the incorporated amino acids.

Validate findings from the mass spectrometry using cell line models and patients’ tumour tissue
A list of proteins will be prioritized and their functional relevance characterized by multiple orthogonal techniques, involving gene editing methods such as ShRNA/ShRNA/CRISPR or pharmacological inhibition with small molecules in cancer cell line models. In addition, expression of proteins secreted by fibroblasts within the stroma of cancer cells will be quantified by immunohistochemistry/immunofluorescence in
tumour samples of patients who have received cancer treatment. Expected outcomes: validation of experimental data from the laboratory in samples from patients' tumours.

The project will expose the candidate to the multidisciplinary approach of cancer research: starting with the clinical problem of drug resistance and then interrogating it using state-of-the-art biological models and technology platforms. This translational research project will allow the candidate to interact with multiple laboratories, including teams specialising in translational biology, proteomics, bioinformatics and drug discovery. This broad training will nurture future leaders in translational medicine to thrive in multidisciplinary scientific research environments.

**LITERATURE REFERENCES**


**CANDIDATE PROFILE**

Note: the ICR’s standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

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<th>Pre-requisite qualifications of applicants:</th>
<th>Biology, molecular biology, biotechnology</th>
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<td>e.g. BSc or equivalent in specific subject area(s)</td>
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**Intended learning outcomes:**

- Broad training within different translational research in cancer medicine which includes screening, discovering and validating markers of drug resistance
- Broad training in molecular biology laboratory techniques, including cell culture, cell viability assays, antibody-based protein detection techniques, mass spectrometry, Si/RNA, ShRNA and immunofluorescence/ immunohistochemistry
- Broad training in basic bioinformatic approaches dealing with phosphoproteomic and proteomic analysis.