The Institute of Cancer Research

PHD STUDENTSHP PROJECT PROPOSAL:

PROJECT DETAILS

Project Title: Characterising the immunopeptidome by proteogenomics and elucidating their associated immune response in head and neck cancer

Short Project Title: Elucidating the antigenic drivers that underpin immune response in head and neck cancer

SUPERVISORY TEAM

Primary Supervisor: Dr Jyoti Choudhary

Other members of the supervisory team: Professor Alan Melcher and Professor Kevin Harrington

DIVISIONAL AFFILIATION

Primary Division: Cancer Biology

Primary Team: Functional proteomics

Other Division: Radiotherapy & Imaging

Other Team: Targeted Therapy

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

It has now become possible to comprehensively quantify the proteome of cancer cells. We have recently reported whole proteome analysis to decipher the functional consequences of somatic genomic variants by revealing impact of genomic alterations on protein networks. The robust quantification of over 9,000 proteins and 11,000 phosphopeptides enabled the de novo construction of a functional protein correlation network, which ultimately exposed the collateral effects of mutations on protein complexes. These networks reveal communication within cancer cells and how driver mutations can influence cellular rewiring. These baseline cancer proteomes provide a readout of cell signalling events and highlighted the activity of the immune pathways in some cell lines (ROUMELIOTIS 2017). We have established a proteogenomics pipeline to integrate RNA sequencing and proteomics data, and demonstrated its applicability to detect and quantify neopeptides in cancer cells (Weisser 2016). This project will build on this foundation to characterise the secretory and immunopeptidome by quantitative proteomics.

The immune system has been shown to influence the fate of developing cancers in diverse ways, encompassing tumour promoter roles leading to cellular transformation and tumour growth. The influence of tumour cell immunogenicity has also been associated with cancer-induced immunosuppression that lead to cancer immune evasion in immunocompetent individuals. Tumour suppressor functions that either destroys tumours or restrains their expansion have also been ascribed to the immune system. The signatures that discriminate these opposing outcomes remains unknown.

The comprehensive identification of mutated peptide ligands on the surface of native tumour tissue...
using leading edge mass spectrometry (MS) analysis has recently become possible. Bassani-Sternberg surveyed the melanoma-associated immunopeptidome and revealed close to 100k patient-presented peptides. Importantly, they demonstrate that these include peptide ligands presented on native tumour tissue samples and represent neoepitopes representative of somatic mutations. They show that some of the neoantigens immunogenic and tumour-reactive T cells with specificity for these neoantigens could be detected tumour samples and peripheral blood of patients. This work demonstrates the potential of immunoproteomics for discovery of ligands from primary tumour material and that mutations in cancer driver genes represent attractive targets for immunotherapy. These neoepitopes are actionable as biomarkers and potentially for targeting immunotherapeutics in cancer. (BASSANI-STERNBERG 2016).

This project to discover immunopeptidome will focus on head & neck cancers, where immunotherapy been demonstrated as an effective treatment with great benefits. However, there remains much scope for improvement and biology of this tumour remains mechanistically poorly understood.

**PROJECT AIMS**

- Develop proteogenomics methods for direct identification of clinically relevant epitopes presented on native cancer tissues and cells by mass spectrometry
- To associate signalling pathways with the generation of distinct antigens
- To elucidate the immune responses to distinct epitopes in head and neck cancer
- To apply immunopeptidomics to probe the mechanistic underpinnings of different chemotherapies and/or radiotherapy and discover biomarkers of disease and drug response states.

**RESEARCH PROPOSAL**

The PhD candidate will use and develop experimental and computational proteogenomics methods to understand the antigenic drivers of immune response in head and neck cancer.

A key part of this project will be to develop quantitative mass spectrometry methods to characterise features impacting on the tumour microenvironment, including antigens and secreted proteins. This will involve establishing methods to isolate, sequence and quantify the immunopeptides. Immunoaffinity purification will be performed to capture MHC class I-associated peptides for analysis by liquid chromatography-mass spectrometry (LC-MS). Computational methods will also be developed to generate personalised sequence databases to facilitate the characterisation of neoantigens. This will build on the novel database search pipeline that has been recently established by our group for personal proteogenomics (Weisser and Choudhary, 2017). Data analysis methods will be used to explore the relationship between the immunopeptides and genetic drivers. Correlation analysis will be used to associate pathways with corresponding antigens.

Gene expression (RNAseq) and whole proteomic analysis will be integrated to provide a comprehensive overview of the molecular phenotype. Quantitative multiomics will be used to identify immune signalling pathways of the cancer cells. This analysis will enable the identification of proteins and/or pathways that might be modulated by cancer driver genes. Protein correlation analysis and affinity purification approaches will be used to uncover functional relationships between cellular processes and/or protein complexes affected.

Targeted sequencing will be used to determine the sequence for MHCs in individuals, this will be used for modelling peptide binding that will help refine candidate selection for detailed follow up.

Drug stimulations will be used to modulate the secretory pathway and elucidate the consequences on, for example, immune cell recruitment using co-culture assays. This will enable antigens candidates to be
selected for detailed functional characterisation using dedicated assays.

This highly multidisciplinary PhD will offer a broad range of training in state of the art proteomics mass spectrometry, immunology, cell biology, tissue culture and bioinformatics. There will also be exposure to the protein structure field through interactions with Protein Structure groups.

**LITERATURE REFERENCES**


**CANDIDATE PROFILE**

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

| Pre-requisite qualifications of applicants: | Biological Science or Bioinformatics. |

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**Page 3 of 4**
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