ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	ICR

Project details

Project title:	Integrative spatial proteomic analysis of tumour microenvironment response to therapy in sarcomas

Supervisory team

Primary Supervisor:	Paul Huang
Associate Supervisor(s):	Andrew Jenks
Secondary Supervisor:	Robin Jones

Divisional affiliation

Primary Division:	Molecular Pathology
Primary Team:	Molecular & Systems Oncology
Site:	Sutton

Project background

Spatial molecular analysis has transformed our understanding of tumour heterogeneity, therapy response and resistance as well as tumour-stromal interactions. While spatial technologies based on genomic and transcriptomic strategies have flourished over the past 5 years, spatial proteomic analysis of tumour specimens has largely been limited to the use of low-resolution antibody panels. Comprehensive spatial proteomic analysis of tumour specimens by mass spectrometry (MS) has been hampered by technical challenges such as limits in analytical sensitivity and comparatively high clinical sample requirements for proteomic workflows. Proteins represent the largest class of druggable targets and directly reflects the functional state of biological pathways and thus provide complementary information for applications in routine care such as guiding treatment choice and in clinical trials for biomarker stratification. In particular, the application of proteomics to formalin-fixed paraffin-embedded (FFPE) tissue has been constrained due to challenges associated with formalin-induced crosslinks and modifications which complicate conventional proteomic workflows. Leveraging on a technology for highly sensitive and quantitative digital proteome mapping of FFPE tissue developed by the Huang Lab, this project seeks to build on this technological advance by extending its application to spatial analysis of tumour specimens.

Through in-depth profiling of tumour specimens from sarcoma patients, this project will employ next generation proteomics based on data-independent acquisition MS as well as classical data-dependent acquisition MS approaches to develop sensitive and highly reproducible assays for measuring the proteome with spatial resolution. These optimised proteomic protocols will subsequently be integrated with orthogonal antibody-based proteomic and transcriptomic spatial analysis to understand the tumour microenvironmental changes associated with immunotherapy and chemotherapy in sarcoma patients that have been treated at the Royal Marsden Hospital.

This translational project will allow us to better understand the molecular alterations associated with immunotherapy and chemotherapy response in sarcoma patients, accelerate the application of proteomics into routine clinical care with potential for future utility in biomarker stratification in clinical trials.

Project aims

- Develop applications in comprehensive spatial proteome mapping for FFPE tumour specimens.
- Compare spatial proteomic readouts with bulk proteomic measurements from whole tumour sections.
- Application of spatial proteomic analysis by mass spectrometry to tissue samples collected from immunotherapy and chemotherapy clinical trials.
- Integration of mass spectrometry-based proteomic data with spatial transcriptomic and antibody-based data.
- Characterise tumour microenvironmental alterations associated with treatment response and resistance.

Research proposal

Intra-tumoural heterogeneity has been shown to be a key contributor to therapy response and resistance. Selective pressures exerted by the administration of therapy fuels tumour evolution and the selection of pre-existing drug resistant clones which ultimately leads to relapse. Furthermore, alterations in the tumour microenvironment including the stromal and immune compartments contribute to drug resistance through a range of different mechanisms. The recent development of spatial molecular profiling approaches, most notably transcriptomic and antibody-based proteomic strategies have substantially improved our ability to study intra-tumour heterogeneity in tissue specimens and develop a deep mechanistic understanding of the molecular drivers of therapy response in patients. However, unlike whole transcriptomic analysis, antibody-based spatial analysis is low-resolution and limited by the availability and quality of antibodies that are compatible with tissue staining (typically up to 60 antibodies). There is a therefore a need for more comprehensive spatial proteomic analysis methodologies to capture the broad diversity of proteins within a tumour specimen.

Digital proteome mapping of tumours by mass spectrometry (MS) employs data independent acquisition (DIA-MS) to convert tissue into a single, permanent digital file comprising the proteome of the sample. The advantage of such digital proteome maps is the ability to conduct retrospective in silico interrogation of proteins using spectral libraries, resulting in highly sensitive and reproducible label-free quantification of proteome. However, the majority of published studies using this next generation proteomics strategy has so far been limited to fresh or flash-frozen tissue. Formalin-fixation and paraffin-embedding of tissue remains the gold-standard for preserving tissue in surgical pathology and presents a rich resource for retrospective studies as well as prospective studies such as clinical trials. Proteomic analysis of FFPE tissue is challenging due to the presence of formalin-induced crosslinks and modifications. The Huang lab has pioneered the use of digital proteomic mapping of FFPE tissue and in the project, the successful candidate will optimise this technology for comprehensive spatial proteomic analysis with application in sarcoma patient specimens collected from immunotherapy and chemotherapy trials.

Objective 1: Develop spatial proteome mapping for FFPE specimens using mass spectrometry.

The Huang lab has already developed robust protocols for proteomic profiling of FFPE tissue. The goal of this aim is to extend the capabilities of this approach to spatial analysis which would require highly sensitive detection due to limiting sample amounts. Working with the sarcoma histopathology team at the Royal Marsden, regions of interest (ROIs) will be identified and macro/microdissected from tissue specimens. These samples will then be subjected to dewaxing and reversal of crosslinks prior to MS analysis. The student will further compare the spatial proteomic readouts with bulk proteomic analysis of the entire tumour section to evaluate the information content in the two different approaches. Proteomic data obtained from these studies will be orthogonally validated using immunohistochemistry assessment of selected proteins.

Objective 2: Integration of MS data with complementary spatial transcriptomic and antibody-based approaches.

Prior comparative studies of bulk proteomic and transcriptomic profiling in multiple cancer types have shown relatively poor concordance between abundance levels of genes and proteins indicating that these measurements provide complementary molecular information. In this aim, the student will undertake comparative analysis of protein level measurements by MS with gene expression and antibody-based measurements obtained from the same ROIs in Objective 1 and integrate the different multi-omic datasets. This will be applied to address specific biological questions, for instance, by selecting ROIs that either are rich in immune cell infiltrate or are immune cell excluded, we seek to understand the molecular interplay between the tumour and immune cell interactions in sarcomas.

Objective 3: Deploy spatial proteomics to characterise the tumour microenvironmental changes associated with immunotherapy and chemotherapy.

With the optimised protocols developed in previous aims, here the student will apply spatial proteomics to tissue collected from window-of-opportunity sarcoma studies. In collaboration with Prof Robin Jones, Head of the Royal

Marsden Sarcoma Unit, the goal of this aim is to use spatial MS to compare pre- and on-treatment tissue specimens to characterise the tumour microenvironmental changes associated with therapy. By correlating these changes with patient outcomes, we seek to identify candidate molecular biomarkers for predicting therapy response or resistance.

Training and development

The PhD student will be integrated into the multi-disciplinary Molecular & Systems Oncology Team in the Division of Molecular Pathology. The student will benefit from mentorship and training from other members of the Huang laboratory within a collaborative and supportive environment. There will be close collaborations with other Team Leaders at the ICR and The Royal Marsden, for example Prof Robin Jones. The student will gain hands-on experience in molecular profiling strategies. The student will be exposed to Cancer Biology, Molecular Pathology, Translational Research and Precision Medicine.

Literature references

[1] Guo T, Kouvonen P, Koh CC et al. Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps. Nat Med 2015; 21: 407-413.
[2] Krasny L, and Huang PH. Data-independent acquisition mass spectrometry (DIA-MS) for proteomic applications in oncology. Molecular Omics 2021; 17(1): 29-42.
[3] Burns J, Wilding CP, Jones RL, and Huang PH. Proteomic research in sarcomas-current status and future opportunities. Seminars in Cancer Biology 2020; 61: 56-70.
[4] Milighetti M, et al., Proteomic profiling of soft tissue sarcomas with SWATH mass spectrometry. J Proteomics 2021 doi: j.jprot.2021.104236.
[5] Krasny, L., et al., SWATH mass spectrometry as a tool for quantitative profiling of the matrisome. J Proteomics, 2018 doi: j.jprot.2018.02.026.
[6] Sequencing-based spatial analysis maps the tumour microenvironment. https://www.nature.com/articles/d42473-020-00245-2

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:	Candidates must have a First or 2:1 Honours degree or a Masters in biology/ biochemistry/ cancer biology/ analytical chemistry or a related discipline. Academic knowledge in cancer biology, cell biology, or analytical chemistry. Previous laboratory experience. Good presentation and communication.
Intended learning outcomes:	• Knowledge in proteomics, histology, translational cancer research, molecular diagnostics • Experimental skills in biochemical, protein chemistry, proteomic techniques • Ability to design, manage and progress a defined scientific project • Scientific writing, presenting and communication skills. Ability to read and process relevant literature.

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☐ Physics or Engineering
- ☒ Chemistry

- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science