PhD Project Proposal

Funder details

Studentship funded by: MRC DTP

Project details

Project title: Investigation of the cancer cell membrane proteome to find new vulnerabilities in Non-small cell lung cancer

Supervisory team

Primary Supervisor: Udai Banerji and Jyoti Choudhary

Associate Supervisor(s):

Secondary Supervisor: George Poulogiannis

Divisional affiliation

Primary Division: Clinical Studies and Division of Cancer Biology

Primary Team: Clinical Pharmacology and Adaptive Therapy and Proteomics and metabolomics

Site: Sutton and Chelsea

Project background

Non-small cell lung cancer (NSCLC) is the leading cause of cancer related deaths across the world. Most patients are diagnosed late and are not curable and finding new treatment is an unmet need. New technologies that bring cancer cells in proximity to cytotoxic agents (e.g. antibody drug conjugates or prodrugs) or immune cells (CAR-T cells or bispecific T cell engagers) have been licensed for the treatment of cancer (1,2). There are now further developments that have led to development of bispecific antibodies that can bind two targets on a cancer cell that have shown benefit e.g. amavantamab (targeting EGFR and MET) (3). This opens out the possibility to further differentiate cancer cells from normal cells by finding unique pairs of proteins on its surface (as opposed to finding single overexpressed or amplified proteins currently used e.g. HER2). We will use a panel of genomically characterized NSCLC organoid panel established in our labs and compare them to a panel of non-cancer ‘normal cells’ using mass spectrometry platforms to find pairs of proteins on the cancer cell membrane. The findings from this project will identify new pairs of proteins on cancer cells which will serve as targets of anticancer drugs in NSCLC.

Project aims

1. To define the membrane proteome of a panel of NSCLC organoids and a panel of 5 non cancer ‘normal’ cell lines.
2. To study characteristics of proteins expressed on the surface and compare them to proteins known to be on membranes of NSLC cells e.g. EGFR or MET. This will be done by proximity labelling with APEX targeting proteins via primary antibody conjugated to an ascorbate peroxidase derivative for catalysing the oxidation of biotin-tyramide for surface labelling.
3. To map the distance between protein pairs on the cell surface using cross-linking mass spectrometry approaches.
4. To study the expression of proteins or pairs of proteins in lung cancer tumour specimens using immunohistochemistry

Research proposal

Hypothesis

NSCLC cells express combinations of proteins on the cell surface that differentiates them from normal tissue and can be used as novel drug targets for therapeutic antibodies, antibody drug conjugates, bispecific T cell engagers, CAR-T cells and prodrugs.

Aim1

Ten NSCLC organoids will be chosen from a panel of 20 genomically characterized NSCLC organoids. We will use established membrane extraction methods in our labs which includes mild detergent-based, selective extraction protocols and quantify the proteome using quantitative mass spectrometry. It is envisaged that this analysis will quantify proteins not only on the outer cell membrane but also intra-membranous proteins and proteins on the cytoplasmic surface of the cell membrane. To specifically target the proteins expressed on the plasma membrane a surface labelling and capture will be used. A generic surface labelling will be achieved by using a lectin conjugated to horse radish peroxidase that will be used to generate biotin-radicals. Peroxidase-based approaches oxidize biotin–phenol into reactive phenoxyl radicals using hydrogen peroxide, which can diffuse and label the proximal surfaces. We have methods to pull down biotin labelled membranes and analyse the proteins by mass spectrometry from cell lines, but the student will develop and validate methods of quantifying pull downs from organoids. This analysis will enrich proteins on the cell surface rather than intramembranous proteins and proteins on the cytoplasmic surface of cancer cells. We will apply a range of bioinformatics approaches to analyse the proteomes and annotate surface proteins in a knowledge graph.

Aim 2

To use protein targeted methods for labelling of proximal surface proteins of selected candidates. We will use the biotinylation by antibody recognition (BAR) method for proximity labelling to study interactions of key membrane proteins (4). Targets will be selected from Aim 1, this will be an orthogonal method to identify proteins on the surface of cancer cells and will serve to validate surface expression dynamics (5). In addition, it will allow to finely map protein:protein relationships and the proximity of the novel proteins to known proteins expressed on NSCLC cells such as EGFR or PDL-1 which will educate future use of these novel combinations of proteins as targets for development of bispecific or trispecific T cell engaging antibodies. For a few candidates we develop isogenic cell Ko and tagged cell lines to study the phenotype and the molecular interactions.

Aim 3

The development of bispecific agents benefits for information on the dynamics and distance between the protein pairs. We have validated methods to link antibodies to biotin using specific linkers, the student will validate and use the methodology before applying it to quantify the samples by mass spectrometry. To study protein pairs where antibodies may not be available, we will use conventional crosslinking methods using bifunctional cross-linkers. By varying the linker length we can map the distance constrains between protein pairs. The approach when conducted

with cell impermeable reagents also enables the mapping of externally exposed surfaces providing key information for developing new protein specific reagents as well as targeting protein pairs.

Aim 4

We will study the expression of proteins identified by our mass spectroscopy experiments in NSCLC tissue, normal lung tissue using immunohistochemistry and immunofluorescence. We will evaluate the use of the BAR approach on patient tissues from fresh and FFPE samples.

Literature references

[1] Li MSC, Mok KKS, Mok TSK. Developments in targeted therapy & immunotherapy-how non-small cell lung cancer management will change in the next decade: a narrative review. Ann Transl Med. 2023;11(10):358.

[2] Drago JZ, Modi S, Chandarlapaty S. Unlocking the potential of antibody-drug conjugates for cancer therapy. Nat Rev Clin Oncol. 2021;18(6):327-44.

[3] Chon K, Larkins E, Chatterjee S, Mishra-Kalyani PS, Aungst S, Wearne E, et al. FDA Approval Summary: Amivantamab for the Treatment of Patients with Non-Small Cell Lung Cancer with EGFR Exon 20 Insertion Mutations. Clin Cancer Res. 2023;29(17):3262-6.

[4] Bar DZ, Atkatsh K, Tavarez U, Erdos MR, Gruenbaum Y, Collins FS. Biotinylation by antibody recognition-a method for proximity labeling. Nat Methods. 2018;15(2):127-33.

[5] Lobingier BT, Huttenhain R, Eichel K, Miller KB, Ting AY, von Zastrow M, et al. An Approach to Spatiotemporally Resolve Protein Interaction Networks in Living Cells. Cell. 2017;169(2):350-60 e12.

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:
Undergraduate Honours degree (First or 2:1) or
Masters degree in
Biology/biochemistry/biotechnology

Intended learning outcomes:

- Quantitative mass spectrometry
- Method development and validation in molecular biology and proteomics
- Analyse large (proteomic) data sets
- Using novel (NSCLC organoid) models
- Basic techniques in pathology (immunohistochemistry)
- Working in a multidisciplinary environment

Advertising details

Project suitable for a student with a background in:

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology

☐ Computer Science