The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	**An integrative systems-level approach to describe the signalling networks regulating metastatic melanoma cell shape determination**
Short Project Title:	***How physical constraints on lung cancer cells fuel their evolution.***
SUPERVISORY TEAM	
Primary Supervisor(s):	**Dr Chris Bakal**
Other supervisory team members:	**Professor Molly Stevens** **Professor Jessica Downs**
DIVISIONAL AFFILIATION	
Primary Division:	**Cancer Biology**
Primary Team:	**Dynamical Cell Systems**
PROJECT PROPOSAL	

BACKGROUND TO THE PROJECT

Metastatic lung cancer cells have the remarkable ability to change their shape and squeeze through tight constrictions in tissues; which allows them to spread throughout the body. But this ability comes at a cost. As metastatic cells invade tight spaces, their nuclei become deformed which can in some cases damage their DNA. While DNA damage can lead to cell death, it may also generate mutations that drive cancer evolution. DNA damage caused during metastasis may also change how immune cells interact will cancer cells. Thus metastatic cells evolve new abilities that promote their survival as they invade complex 3D tissues. That physical forces encountered during metastasis can damage DNA also presents a therapeutic opportunity, as blocking the ability of cells to repair this DNA damage could be a means to kill metastatic cancer cells.

But how DNA becomes damaged by mechanical forces encountered during metastasis is unknown, the consequences of this damage unclear, and how it might be enhanced therapeutically is poorly understood. Thus gaining new insight regarding the relationship between physical forces and DNA damage/repair is important to both cell and clinical cancer research.

In this project, the student will use sophisticated imaging technologies such as lattice light sheet, total internal reflection fluorescence (TIRF), advanced confocal, and super-resolution microscopy, combined with novel bioengineering approaches, to study DNA damage in single lung cancer cells migrating through confined spaces. The student will also use genetic methods, single cell reporters we have pioneered in the lab (Cooper and Bakal, *Trends in Cell Biology* 2017), and use novel therapeutics developed at the ICR, to describe the mechanisms that link physical forces to DNA damage and repair. Finally, one aim of this project is to establish new models to study the interplay between lung cancer and immune cells in 3D systems.

In addition to gaining training in cutting-edge imaging and bioengineering methods, the student will work in a multi-disciplinary fashion with cancer biologists, chemists, and computational scientists at the ICR, as well as Imperial College, UCL, and the Crick. There are also opportunities for the student to gain further training in microscopy with academic (Janelia Farm - HHMI) and industry (3i, PerkinElmer) partners in the UK and USA.

PROJECT AIMS

Aim 1: Characterise how nuclear deformation during lung cancer cell invasion in 3D generates DNA damage, replication stress, APOPEC3B induction, and affects the DNA damage response.

Aim 2: Test the effects of chemically manipulating the biochemical pathway linking mechanical stress to the DDR, APOPEC3B induction.

Aim 3: Investigate the role of mechanically-induced DNA damage and APOPEC3B induction in dictating the outcome of immune-cancer cell interactions in 2D and 3D models.

RESEARCH PROPOSAL

Keywords: lung cancer, tissue invasion, nuclear deformation, DNA damage, APOBEC3B, ATR and CHK1/2 inhibitors, immunotherapy.

Key methods: 3D models, matrix engineering, light-sheet imaging, single cell image analysis, small-molecule screening.

Key collaborators: Molly Stevens (Imperial College), Louis Chesler (ICR), Charlie Swanton (UCL/Crick), Sergio Quezada (UCL), Caroline Springer (Manchester).

Aim 1: Characterise how nuclear deformation during lung cancer cell invasion in 3D generates DNA damage, replication stress, APOPEC3B induction, and affects the DNA damage response.

In this aim the student will combine bioengineering and advanced imaging approaches, including lattice light sheet and super-resolution imaging, to study how nuclear deformation, which occurs during the invasion of lung cancer cells in 3D, generates DNA damage, promotes replication stress and APOPEC3B induction. Work in this aim has two important goals:

1) To establish a system in which to study how nuclear deformation affects DNA damage, replication stress, and APOPBEC3B levels. This will involve extensive optimisation to choose the right cell lines, markers of DNA damage (antibodies, fluorescently tagged proteins), systems for deformation (collagen gel, grooved surfaces), and imaging solutions (confocal, light sheet).
2) In the optimised system, the student will characterise how nuclear deformation affects genomic integrity in lung cancer cells, and use different analytical tools to describe, and ultimately predict, the relationship between nuclear deformation and genome integrity.

As a starting point the student will make use of novel hydrogels developed in the Stevens laboratory to culture lung cancer cell lines in spatially restricted environments. As the project continues, cells will be cultured on materials and surfaces with defined geometries, matrix composition, and stiffness. Throughout the course of the project, the student will spend extensive time working with members of the Stevens lab to develop novel materials and surfaces.

We have assembled a panel of lung cancer cell lines from Charlie Swanton. These lines can be classified on the basis of their APOBEC3B activity, with some lines having low APOBEC3B activity, and some having high activity.

Using a combination of confocal and lattice light sheet microscopy, the student will quantify the extent of: (i) DNA damage - using readouts such as gH2AX, 53BP1, and phosphoRPA; (ii) the structure of the nuclear membrane - using markers such as LMNA (lamin A), phosphoLMNA, and LMNB; (iii) APOPEC3B upregulation and activation. Initially this will be done using antibodies, but we also aim to establish cell lines where APOPEC3B is endogenously tagged with eGFP. This will allow us to monitor upregulation of APOBEC3B in living single cells; and (iv) cell and nuclear morphology. Imaging will be done on a combination of fixed cells, or on live cells following transfection of fluorescently-tagged proteins.

We will also determine whether nuclear deformation in 3D induces not only DNA damage and APOBEC3B expression – but also whether it affects the DDR and resolution of damage. Thus we will quantify DNA damage, as in (i), in 3D following transient exogenous damage, such as following treatment with DNA damaging agents, or laser-induced damage. It will be important to perform these experiments in live cells when possible, as we predict the kinetics of DDR activation and repair resolution may be particularly affected by DNA damage.

As part of this aim the student will work with partners in industry (3i, PerkinElmer), and academia to develop new 3D imaging methods and analysis tools.

Aim 2: Test the effects of chemically manipulating the biochemical pathway linking mechanical stress to the DDR, APOPEC3B induction.

We propose that nuclear deformation may either induce DNA damage, or lead to impaired mobility of DDR components, that can sensitize cells to either chemotherapeutics, or small-molecule agents; thus a “tipping the balance” of the DDR towards apoptosis/death versus repair. If this model is correct, this could open up therapeutic avenues that aim to either enhance the extent of damage that occurs after nuclear deformation and/or blocks DNA repair pathways that are engaged following deformation.

The student will explore how chemical manipulation of signalling pathways that couple mechanical stress to the DDR affects the levels of DNA damage and APOBEC3B when cells are invading confined spaces. In particular we will work with Louis Chesler to use Chk1 and Chk2 inhibitors developed at the ICR and ATM/ATR inhibitors developed by Astra Zeneca.

Aim 3: Investigate the role of mechanically-induced DNA damage and APOPEC3B induction in dictating the outcome of immune-cancer cell interactions in 2D and 3D models.

Towards examining the interplay between the immune system and tumour cells confined in 3D environments, the student will work with the Stevens laboratory to establish a system in which lung cancer cells and immune cells can be co-cultured and imaged in collagen or hydrogel matrices. This work will be assisted by a collaboration with Sergio Quezada (UCL).

Initially the system will be used purely for characterisation of how mechanically-induced DNA damage and/or changes in APOPEC3B alters the immune response (i.e. macrophage recruitment, dendritic cell activation, T-cell activation and/or repetoire). But manipulation of the components of the system through chemical or genetic means is well beyond the scope of this PhD studentship.

LITERATURE REFERENCES

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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: e.g. BSc or equivalent in specific subject area(s)	BSc in Biology, Biochemistry, Bioengineering, Chemistry, or Physics. Strong background in statistics, programming, or bioinformatics is highly preferred.
Intended learning outcomes:	• Engineering of surfaces and materials for cell culturing • Advanced imaging approaches including light-sheet microscopy • Sophisticated image analysis • Statistical and/or computational modelling of biological pathways • Complex 3D models, including immune-cancer cell models