PhD Project Proposal

Funder details

Studentship funded by:	**MRC DTP**

Project details

Project title:	DNA damage deficiency in prostate cancer: functional characterisation and treatment

Supervisory team

Primary Supervisor:	Amanda Swain
Associate Supervisor(s):	
Secondary Supervisor:	Jyoti Choudhary

Divisional affiliation

Primary Division:	Cancer Biology
Primary Team:	Development and Cancer
Site:	CBL

Project background

Genome sequencing studies of prostate tumours have identified common alterations in genes involved in DNA damage response. The Ataxia Telangiectasia Mutated (ATM) gene encodes a PI3K-related protein kinase involved in the maintenance of genome integrity. ATM plays an integral role in the sensing of DNA damage and subsequent cellular response and acts as a signal transducer in the double strand break repair process. ATM mutations are found in multiple cancer types and are associated with poor prognosis. In prostate cancer, up to 10% of patients with aggressive disease show ATM loss and germline ATM variant carriers are associated with higher risk of early onset disease (Neeb et al., 2021, Karlsson et al., 2021). Mutational hotspots within the ATM gene have been observed in prostate cancer, generating missense putative pathogenic variants.

Clinically, tumours with defects in DNA repair genes such as BRCA1/2 show exceptional responses to PARP inhibitors (Mateo et al., 2016). Similar synthetic lethal interactions have been reported for ATM mutants with inhibitors of ATR, PARP and DNA-PK. Although some response to PARP inhibitors for ATM mutants have been seen, these have been modest compared to patients with BRCA1/2 mutations (Carreira et al., 2021). Understanding the effect of ATM mutations on prostate cancer progression and response to treatment is essential to the identification of novel therapies and combinations that would be effective in this group of patients as well as informing ongoing clinical trials with agents such as PARP inhibitors.

In this project the student will use available complementary models from mouse and human prostate including genetically defined organoids and patient derived xenografts (PDXs) to determine the specific contribution of different genomic aberrations and their interaction to tumour progression and response to treatment.

Project aims

- To use human and mouse prostate organoids to investigate the effect of different ATM mutations found in prostate cancer patients on tumour progression and response to treatments that are currently being used in the clinic, including PARP and ATR inhibitors and radiation.
- To understand the effect of co-occurring genetic drivers with ATM mutations in tumour progression and response to therapy.
- To investigate the mechanisms of interaction of prostate cancer driver mutations using proteomics and transcriptomics approaches on pathways such as DNA damage response, genomic instability, and cell cycle to identify more effective therapies for patients.
- To perform in vivo validation and analysis of human prostate cancer samples to generate preclinical data.

Research proposal

Aim 1: To investigate the effect of different ATM mutations on tumour progression and response to clinically relevant treatments using genetically modified organoid and patient derived prostate models.

Recent studies have described the generation of ex vivo 3D organoid cultures from normal and neoplastic mouse and human patient prostate tissue (Karthaus et al., 2014). These have been shown to be robust models to study normal and neoplastic prostate biology and response to therapeutic agents. We have developed workflows to genetically modify prostate organoid cultures using CRISPR based protocols and perform phenotypic and drug treatment studies. Using these workflows, the student will generate prostate mouse and human organoids with ATM mutations in different regions of the protein to mimic those found in patient tumours. These experiments will include using CRISPR-based prime or base editing techniques to generate missense mutations that mimic those found in patients. Phenotypic studies will be performed to investigate the effect of these mutations on growth, stem/precursor function, cell differentiation and dependency on androgens.

To identify the drug sensitivities of different ATM mutations drug and radiation studies will be performed on genetically modified organoids. Therapies currently being used in the clinic to treat prostate cancer patients such as PARP and ATR inhibitors and radiation as well as combinations with androgen deprivation therapies will be chosen. The identified sensitivities will be compared to those of organoids derived from patient ATM mutant tumours. These will be generated from available PDXs, which we have generated in collaboration with Professor Johann de Bono, who leads prostate cancer clinical trials at the Royal Marsden Hospital. In vivo studies with PDXs have shown that they recapitulate tumour histology, genetic background and drug response more faithfully than cell lines.

Aim 2: To understand the effect of co-occurring genetic drivers with ATM mutations in tumour progression and response to therapy.

To investigate the genomic aberrations that could interact with ATM mutants during prostate cancer progression, the student will mine publicly available sequencing datasets including TCGA and Stand up to Cancer (SU2C). This information will be used to model patient tumours through the generation of genetically modified organoids that have mutations co-occurring with mutant ATM. Examples of these will be mutations in the tumour suppressors PTEN, TP53 and RB1, which are commonly found in aggressive disease. These organoids with defined complex genetics will be subjected to phenotypic assays and therapy studies. The aim is to understand the impact of the interaction between genetic drivers on tumour progression and response to therapies.

Aim 3: To investigate the mechanisms of interaction of prostate cancer driver mutations on relevant pathways in an effort to identify more effective therapies for patients.

Mechanistic studies will be performed to investigate the effect of ATM mutants and the interaction with other co-occurring genetic aberrations on different relevant pathways. To identify novel network rewiring unbiased proteomics and transcriptomic studies will be performed on organoids. Quantitative mass spectrometry will be used to map the changes in protein abundance and interactions, as well as phosphorylation to track signalling events. These studies will also be done in the context of damage induction and different therapies, including androgen deprivation. This data will be used to study different processes such as DNA damage response, genomic instability, innate immunity,

cell cycle and signalling pathways such as PI3 Kinase in mutant models. The information generated will be used to identify more effective therapies, including combinations with clinically relevant compounds.

Aim 4: To perform in vivo validation and analysis of human prostate cancer samples to generate preclinical data.

Novel dependencies and phenotypes will be validated in in vivo studies through organoid implantation in the mouse prostate. Syngeneic mice will be used to investigate the role of the microenvironment, including immune cells, in tumour growth and progression. The presence of novel pathways will be confirmed in human prostate cancer samples that carry the combination of mutations being analysed.

Expected outcome

The expected outcome of this project is to generate novel clinically relevant information that can be used to design clinical trials in prostate cancer patients. We aim to inform the process of patient selection, biomarker choice and treatment regimes, particularly for patients with tumours with ATM mutations.

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:

Intended learning outcomes:

- Molecular and cellular biology techniques
- Experience working with 3D and in vivo models

- Genetic modification using CRISPR based techniques
- In depth knowledge of prostate cancer and DNA damage response pathways
- Analysis of proteomic and transcriptomic data
- Knowledge of cancer therapies in a clinical context
- Good writing and presentation skills

Advertising details

Project suitable for a student with a background in:

x Biological Sciences

☐ Physics or Engineering

☐ Chemistry

☐ Maths, Statistics or Epidemiology

☐ Computer Science