PhD Project Proposal

Funder details

Studentship funded by:	MRC iCASE (AstraZeneca)

Project details

Project title:	Understanding the mechanisms underpinning therapeutic vulnerability of SWI/SNF deficient cancers

Supervisory team

Primary Supervisor:	Jessica Downs
Associate Supervisor(s):	Alan Lau (AstraZeneca)
Secondary Supervisor:	Wojciech Niedzwiedz

Divisional affiliation

Primary Division:	Cancer Biology
Primary Team:	Epigenetics and Genome Stability
Site:	Chelsea

Project background

Genes encoding subunits of SWI/SNF complexes are mutated in a striking number of human cancers, with approximately one in five tumour samples having deleterious changes in at least one SWI/SNF subunit (Harrod et al, 2020). This dysregulation of SWI/SNF contributes to the progression of cancer, but exactly how this happens is still not fully understood. Previously, we and others found that SWI/SNF is important for maintaining genome stability through several cellular activities, including DNA double strand break repair, replication stress responses, and chromosome segregation (e.g. Harrod et al, 2020, Schiavoni et al, 2022, Feng et al, 2022).

The frequent loss of SWI/SNF in cancer cells provides a therapeutic opportunity. By identifying synthetic lethal relationships, or genes required for viability in SWI/SNF deficient cells, we can selectively target the cancer cells using inhibitors to the synthetic lethal targets. Many synthetic lethal relationships have been identified that can be clinically exploited. For example, there is evidence that cells lacking the PBRM1 or ARID1A subunits of SWI/SNF remodelling complexes are selectively sensitive to inhibition of the DNA damage response protein kinase ATR (Williamson et al, 2018, Chabanon et al, 2021). Clinical studies are ongoing testing this hypothesis in the ATARI (NCT0405269; Banerjee et al 2021) and Aggarwal (NCT03682289) trails with limited but early reports of clinical activity in a subset of endometrial cancer patients with ARID1A-loss (Aggarwal et al 2021). However, we find that these relationships are not shared in all cell lines (or patients) and we have yet to identify the genetic or epigenetic factors that would allow us to accurately predict synthetic lethality. Moreover, whether these drugs would provide synergistic effects when used in combination with other treatments has not yet been fully explored.

Project aims

- To assess the cellular responses to ATR inhibitors in SWI/SNF deficient cells. To do this, SWI/SNF deficient cell lines will be characterised following exposure to ATR inhibitors using a panel of isogenic cell lines. This will include mapping of SWI/SNF subunits and DNA breaks combined with transcriptome and proteome analyses.
- To determine the molecular mechanisms of ATR inhibitor sensitivity in different cellular contexts. Assays monitoring DNA repair, DNA damage responses, cell cycle progression and replication stress in SWI/SNF deficient cells treated with ATR inhibitors will be performed.
- To identify synergistic combination treatments in SWI/SNF deficient cells by screening isogenic cell line panels with a bespoke panel of inhibitors, and using the assays above, characterising the cellular response to these.

Research proposal

Chromatin remodelling complexes use the energy derived from ATP hydrolysis to reorganise the structure of chromatin. This is a critical mechanism by which access to DNA is regulated in cells, and consequently, chromatin remodelling activity is important for carrying out activities requiring access to DNA, such as transcription, replication, and DNA repair. The SWI/SNF family of chromatin remodelling complexes can be divided into three categories: BAF, PBAF and GBAF. They share a number of core subunits, and have category-specific subunits. For example, the ARID1A subunit is found exclusively within the BAF complex and PBRM1 is found only within PBAF (Harrod et al, 2020).

Loss of function mutations of genes encoding SWI/SNF subunits is strikingly common in cancer. Notably, the ARID1A and PBRM1 genes are among the most frequently mutated, suggesting that these subunits play key roles in preventing tumourigenesis. In addition, identification of synthetic lethal relationships with ARID1A and PBRM1 provides a therapeutic opportunity. Notably, the DNA damage responsive kinase ATR was identified as a synthetic lethal partner to both ARID1A and PBRM1. Whether this relationship is universal or limited to specific cellular or genetic contexts is still not fully understood, and a deeper mechanistic understanding of the relationship will aid in the application of ATR inhibitors in the clinic.

In addition, other synthetic lethal relationships with ARID1A and PBRM1 have been identified. ARID1A deficient cells are sensitive to inhibitors of the methyltransferase EZH2, the poly ADP ribose polymerase PARP1, histone deacetylases, and the mTOR kinase (Mullen et al, 2022). PBRM1 deficient cells are also sensitive to EZH2 and PARP inhibitors, as well as to inhibitors of the TIP60 histone acetyltransferase (Hopkins et al, 2016). Notably, PARP inhibitors have been shown to work well in combination with ATR inhibitors (Wilson et al, 2022). Whether and how these pathways relate to each other in SWI/SNF deficient cells has not yet been explored.

Aim 1. To assess the cellular responses to ATR inhibitors in SWI/SNF deficient cells. In this aim, we will use panels of isogenic cell lines, in which CRISPR-Cas9 mediated genome editing has been used to inactivate either ARID1A or PBRM1 in a range of cell types. Using these lines, we will explore sensitivity to ATR inhibitor exposure across time and doses. To fully understand the impact of SWI/SNF deficiency on ATR inhibitor sensitivity, we will also map the locations of unrepaired DNA breaks and the genomic locations of SWI/SNF complexes. These datasets will be intersected with unbiased, genome-wide transcriptome and proteome (multi-omic) analyses to provide a comprehensive readout of cellular responses to ATR inhibitors when SWI/SNF subunits are dysfunctional in a variety of genetic contexts and cell types.

Aim 2. To determine the molecular mechanisms of ATR inhibitor sensitivity in different cellular contexts. Using the cell line panels from Aim 1, we will look more specifically at the DNA damage response following ATR inhibitor exposure. SWI/SNF complexes have been implicated in several DNA repair pathways and in preventing replication stress as well as in DNA damage checkpoint responses. We will therefore look at each of these activities in detail to determine which function or functions are consistently impacted by SWI/SNF activity across different genetic contexts and cell types.

Aim 3. To identify synergistic combination treatments in SWI/SNF deficient cells by screening isogenic cell line panels with a bespoke panel of inhibitors, and using the assays above, characterising the cellular response to these. Building on the skills and expertise gained in Aims 1 and 2, cellular responses to inhibitor combinations will be investigated. We will focus on EZH2 and PARP inhibitors because these have been identified as synthetic lethal targets in both PBRM1 and ARID1A deficient cells. In addition, inhibitors of mTOR and the PI3K/AKT pathway will be tested. These inhibitors have been more extensively investigated in ARID1A deficient settings, and a better understanding of their impact on PBRM1 deficient cells will provide important insights. This selection is based on existing evidence, but inhibitor choice for this Aim will also be guided by the outcome of our multiomic profiling analyses in Aim 1.

Consistent findings obtained from these isogenic model systems would then be applied and validated in naturally occurring human cell lines, patient derived organoids/xenografts (PDO/PDX) or clinical tumours (if available) harbouring SWI/SNF alterations.

This work will be carried out between labs in the ICR and AZ, giving the student exposure to both academic and industrial research environments.

Literature references

[1] Aggarwal R, Umetsu S, Dhawan M, Grabowsky J, Carnevale J, Howell M, Wilch L, Chapman J, Alvarez E, Calabrese S, Smith S, Shah N, Dean E, Munster P, and Collisson E. 512O - Interim results from a phase II study of the ATR inhibitor ceralasertib in ARID1A-deficient and ARID1A-intact advanced solid tumor malignancies. Annals of Oncology 2021;32:S583-S620.

[2] Banerjee S, Stewart J, Porta N, Toms C, Leary A, Lheureux S, Khalique S, Tai J, Attygalle A, Vroobel K, Lord CJ, Natrajan R, and Bliss J. ATARI trial: ATR inhibitor in combination with olaparib in gynecological cancers with ARID1A loss or no loss (ENGOT/GYN1/NCRI). Int J Gynecol Cancer 2021;31:1471-75.

[3] Chabanon, R.M. et al (2021) PBRM1 deficiency confers synthetic lethality to DNA repair inhibitors in cancer. Cancer Res, 81(11):2888-2902.

[4] Harrod, A., K.A. Lane, and J.A. Downs (2020) The role of the SWI/SNF chromatin remodelling complex in the response to DNA double strand breaks. DNA Repair, (93):102919.

[5] Feng, H. et al (2022) PBAF loss leads to DNA damage-induced inflammatory signaling through defective G2/M checkpoint maintenance. Genes Dev, doi: 10.1101/gad.349249.121.

[6] Mullen, J. et al (2022) Targeting ARID1A mutations in cancer. Cancer Treat Rev, (100):102287.

[7] Schiavoni, F. et al (2022) Aneuploidy tolerance caused by BRG1 loss allows chromosome gains and recovery of fitness. Nat Commun, 13(1):1731.

[8] Williamson, C.T. et al (2018) ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. Nat Commun, (7):13837.

[9] Wilson, Z. et al (2022) ATR inhibitor AZD6738 (Ceralasertib) exerts antitumor activity as a monotherapy and in combination with chemotherapy and the PARP inhibitor Olaparib. Cancer Res, 82(6):1140-1152.

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:

- Master a range of experimental approaches, including state-of-the-art multi-omic mapping technologies.
- Develop a deep understanding of chromatin biology and its relationship with DNA damage responses and genome stability.
- Learn to work as part of a collaborative team across academic and industrial research environments.
- Procure excellent oral and written scientific communication skills.
- Develop an understanding of the trajectory from discovery science to industrial cancer biology research.

Advertising details

Project suitable for a student with a background in:

☒ Biological Sciences

☐ Physics or Engineering

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