ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	ICR

Project details

Project title:	Identifying novel therapeutic approaches targeting DNA damage repair pathways for childhood cancers with *ATRX* mutations

Supervisory team

Primary Supervisor:	Dr Sally George
Associate Supervisor(s):	Professor Jyoti Choudhary
Secondary Supervisor:	Professor Louis Chesler

Divisional affiliation

Primary Division:	Clinical Studies
Primary Team:	Developmental Oncology
Site:	Sutton

Project background

Pan-cancer analyses identify an enrichment of mutations in epigenetic genes in tumours of childhood, with *ATRX* being the third most frequently mutated gene across all paediatric cancers [1]. *ATRX* alterations are prevalent in numerous poor outcome tumours of childhood including glioma, osteosarcoma and neuroblastoma. This project primarily focuses on *ATRX* mutant neuroblastoma, a subgroup associated with particularly poor outcomes, with the potential to broaden findings to other malignancies. We have previously shown homologous recombination repair (HRR) deficiency and specific therapeutic vulnerabilities to DNA damage repair inhibitors in *ATRX* mutant neuroblastoma [2]. As a result of this work, the combination of the PARP inhibitor talazoparib and MIBG therapy (a type of molecular radiotherapy) are now being evaluated in a clinical trial for children with relapsed/refractory neuroblastoma.

ATRX is responsible for the maintenance of genomic stability by multiple mechanisms: it is responsible for the mediation of specific HRR sub-pathways [3], in addition to playing a pivotal role in the protection of stalled replication forks [2]. ATRX deficient cells have also been shown to have higher levels of trapped DNA damage response associated proteins on chromatin [4]. Different types of *ATRX* mutations are seen in paediatric cancers including complete loss of function mutations, in-frame focal deletions and missense mutations, each of which may be associated with slightly different phenotypes [5]. How these different types of *ATRX* alteration affect the capacity for DNA damage repair, or their reliance on specific pathways to repair DNA is completely unknown.

Here, we propose an unbiased analysis of the chromatin response to DNA damage to identify candidate proteins that are activated at sites of DNA damage in *ATRX* mutant cells. The effects of genetic and/or therapeutic knockdown of these proteins in the context of DNA damage will also be evaluated to identify specific dependencies in *ATRX* altered cancers.

Project aims

- Quantification of temporal response to ionising radiation in *ATRX* wild type and mutant neuroblastoma models.
- Characterisation of the chromatin bound phospho-proteome in response to ionising radiation in ATRX wild type and mutant neuroblastoma models.
- Evaluation of the effects of knockdown of proteins enriched in *ATRX* mutant models following ionising radiation
- Pre-clinical evaluation of candidate combination therapies in *ATRX* mutant models.

Research proposal

The overall goal of this project is to identify how dynamic changes in the chromatin landscape differ between *ATRX* wild type and mutant cells following the induction of DNA damage, and to use this information to identify specific dependencies on DNA damage repair pathway proteins.

Aim 1: Quantification of temporal response to ionising radiation in ATRX wild type and altered neuroblastoma models

We will use a panel of already available cell lines including:

-A panel of cell lines derived from SKNSH that are isogenic for *ATRX* (wild-type versus complete loss of function [LoF]) [2].

-Neuroblastoma cell lines with focal in-frame deletions of the proximal portion of *ATRX* (but intact helicase domain function), with matched *ATRX*-wild type controls.

We will use external beam irradiation (IR) to induce DNA damage in these cell lines which is a validated methodology to induce double stranded DNA breaks for mechanistic studies. Dose finding experiments will be performed to evaluate the sensitivity to IR alongside identifying the optimal IR dose to induce DNA damage which can then subsequently be repaired (as quantified by the induction, then resolution of γH2AX foci by immunofluorescence). The timing of the formation of γH2AX foci and subsequent recovery will also be evaluated to identify the best experimental conditions for aim 2. Overall expression of known regulators of the DNA damage response (including p-ATM, p-ATR and p-CHK1) at baseline, and after different doses of, and times post IR will also be evaluated by immunofluorescence and/or western blot and results will be correlated with results in aim 2.

Aim 2: Characterisation of the chromatin bound phospho-proteome in response to ionising radiation in ATRX wild type and altered neuroblastoma models

Here, in collaboration with Prof Jyoti Choudhary (Functional Proteomics Lab, ICR), we will use a quantitative mass spectrometry analysis to characterise the chromatin bound proteome and phospho-proteome, developed by the Choudhary team. In brief we will apply an established chromatin isolation method +/- phospho-peptide enrichment to reveal changes associated with ATRX status. Quantification of the chromatin associated proteome and phospho-proteome in response to IR treatment in a time course, will be performed and comparison made between *ATRX* wild-type and mutant models. Differential proteins and phsosphorylation will be assigned using bioinformatics to protein complexes and signalling processes. The identified proteins will be validated by western blotting and followed up in Aim 3. Phospho-proteins will also be identified that are specifically enriched at sites where γH2AX (139 serine phosphorylated H2AX) is bound to chromatin, as a biomarker of sites of DNA damage. Comparison will also be made between models with complete *ATRX* LoF and in-frame focal *ATRX* deletions to identify if phenotypic changes are consistent across different types of *ATRX* alteration.

Aim 3: Evaluation of the effects of knockdown of proteins enriched in ATRX mutant models following ionising radiation. Candidate proteins identified in aim 2 will be prioritised for further evaluation, focusing on proteins that are significantly enriched in *ATRX* mutant neuroblastoma models, with potential for therapeutic translation and relevance to other paediatric cancers. The effects of genetic/chemical knockdown of these proteins alone, and in combination with IR will be evaluated. Analysis in the first instance will include quantification of γH2AX foci, alongside apoptosis (cleaved caspase-3, annexin) and cell viability (Cell-titre Glo) assays. Depending on the proteins identified, further mechanistic studies will be performed to evaluate the effects of knockdown of these proteins in the context of DNA damage. Analysis will be broadened to evaluate if genetic/chemical knockdown of key

proteins +/- IR has the same effect in both neuroblastoma with different types of ATRX mutations, and in neuroblastoma compared with other ATRX mutant paediatric cancers.

Aim 4: Pre-clinical evaluation of candidate combination therapies in ATRX mutant models
Here the scope of combination therapies to be evaluated will be broadened to include: rational novel-novel combinations and/or chemotherapy-novel combinations therapies, targeting specific DNA damage repair vulnerabilities in *ATRX* mutant cells based on findings from aims 1-3. This may include chemotherapy - novel agent combinations, focusing on chemotherapy agents that induce double stranded DNA breaks and are commonly used in paediatric malignancies, and/or PARP inhibitor - novel agent combinations based on findings from both this study and our previously published work [2]. In addition, where based on phospho- proteomic findings, two of more proteins are identified to be enriches at sites of DNA damage, the effects of knock-down of both targets versus each target alone will also be evaluated. If clinical compounds are not available for the proteins identified for the phospho-proteomic screen, then genetic knock down will be used as proof of principle for targets that are potentially druggable. The most promising *in-vitro* combination will also be evaluated in *in-vivo* models.

Literature references

1. Grobner, S.N., et al., The landscape of genomic alterations across childhood cancers. Nature, 2018. **555**(7696): p. 321-327.
2. George, S.L., et al., Therapeutic vulnerabilities in the DNA damage response for the treatment of ATRX mutant neuroblastoma. EBioMedicine, 2020. **59**: p. 102971.
3. Elbakry, A., et al., ATRX and RECQ5 define distinct homologous recombination subpathways. Proc Natl Acad Sci U S A, 2021. **118**(3).
4. Rose, A.M., et al., Induction of the alternative lengthening of telomeres pathway by trapping of proteins on DNA. Nucleic Acids Res, 2023. **51**(13): p. 6509-6527.
5. van Gerven, M.R., et al., Mutational spectrum of ATRX aberrations in neuroblastoma and associated patient and tumor characteristics. Cancer Sci, 2022. **113**(6): p. 2167-2178.

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: B.Sc Biological Sciences

Intended learning outcomes:

- Master a range of wet lab experimental techniques
  Design and execution of experiments to address scientific hypotheses
- Develop an understanding of chromatin biology and its relationship with DNA damage response
- Experience of paediatric cancer research spanning from basic biology through to translational research
- Opportunity to learn bioinformatic analysis of proteomics data
- Acquisition of excellent oral and written scientific communication skills.

Advertising details

Project suitable for a student with a background in: ☒ Biological Sciences

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