ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	ICR

Project details

Project title:	Targeting cancer cell dependency on RNA-binding proteins

Supervisory team

Primary Supervisor:	Paul Clarke
Associate Supervisor(s):	Marissa Powers Benjamin Bellenie
Secondary Supervisor:	Olivia Rossanese

Divisional affiliation

Primary Division:	Cancer Therapeutics
Primary Team:	RNA Biology and Molecular Therapeutics
Site:	Sutton

Project background

RNA−protein interactions play a key role in the life of an RNA, from transcription to maturation, transport, localization and degradation (reviewed in Hentze et al. 2018; Gebauer et al., 2021). Dynamic interactions between RNA and RNA-binding proteins (RBPs) result in the formation of ribonucleoprotein complexes whose composition constantly changes in a context-dependent manner. RBPs often recognize hundreds of transcripts and form extensive regulatory networks to maintain cellular homeostasis. Some RBPs have restricted expression patterns and/or their activity may be regulated by post-translational modifications, co-factor binding or protein interactions. RBPs are characterized by their RNA-binding domains that are computationally predictable, an approach that allowed curation of 1542 human RBPs (Gerstberger et al., 2014). More recent proteome-wide studies have identified additional RBPs and revealed new paradigms: from structurally disordered RNA-binding regions to connections between intermediary metabolism and RNA-regulation through nucleotide binding domains.

Perturbations in the activity of RBP networks have been causally associated with cancer, albeit with a myriad of complexities that are still being untangled (reviewed in Pereira et al., 2017; Gebauer et al., 2021). A recent screen identified RBPs with distinct roles in supporting MYC-driven tumorigenesis (Einstein et al., 2021). Hits included RBPs that counteracted the global increase of mRNA synthesis in MYC-driven cancers that would otherwise overwhelm the translational machinery and induce proteotoxic stress.

RBPs have traditionally been considered 'undruggable', however, they are now emerging as promising therapeutic targets with several small molecules in early clinical studies. For example, Zotatifin, an inhibitor of eIF4A1, or H3B-8800 a small molecule that binds to splicing factor SF3B. Our group are currently developing small molecule inhibitors of an RBP that protects cells from stress induced by oncogene activation and are also leading a recent Cancer Therapeutics Unit initiative to target a family of RBPs that regulate RNA structure throughout the RNA life cycle.

Project aims

- Identify and confirm active RNA binding proteins in colon cancer organoid cultures compared to normal colorectal organoid cultures using orthogonal organic phase separation
- Rank and select candidate hits for genetic validation by knockout or knockdown approaches
- For one or two candidates build dTAG models for further validation, including genetic rescue to explore the role of potential active sites or binding pockets
- Develop RNA oligo-PROTAC tools for further validation in wider cancer cell line or organoid panels

Research proposal

Hypothesis

Cancer cells have small molecule-targetable dependencies on RBPs that either support oncogene function or overcome the stresses associated with oncogene activation that arise during tumorigenesis

Aim

To use orthogonal experimental methods and public databases to identify candidate RNA binding proteins for further target validation using genetic approaches, protein degradation dTAG-rescue and RNA-oligo PROTAC methods

Available Resources

The Team has considerable experience in small molecule drug discovery and targeting of RBPs, with established methods and analytical pipelines for RNAseq, hi-depth RNAseq for analysing pre-mRNA splicing and RIBOseq footprinting to globally determine ribosome loading and translation of mRNAs. We have recently introduced orthogonal organic phase separation a new highly efficient, low-cost method for the isolation of RNA–protein complexes, enabling the analysis of both the RNA and protein components (Queiroz et al., 2021). This method combines UV cross-linking to stabilize RNA–protein interactions with acidic guanidine phenol chloroform RNA–protein extraction. Cell lysis followed by addition of chloroform produces two distinct phases: an aqueous phase containing RNA, an organic phase containing proteins and an interface between the phases that retains crosslinked RNA:protein adducts. RNA bound proteins can subsequently be identified by purification of the interface followed by mass spectroscopy. The Team has also established dTAG-mediated protein degradation coupled with rescue by re-expression of wild-type or mutant proteins to validate compound-binding pockets and domains required for protein function or activity (Nabet et al., 2018). The Team have access to a small number of colorectal cancer and normal bowel organoid cultures and a panel of 50 plus molecularly characterised colorectal cancer cell lines cell lines. With input from computational biology colleagues the Team has also established an RBP database incorporating public datasets such as DEPMAP (https://depmap.org/portal/), CCLE (https://sites.broadinstitute.org/ccle/) ENCODE (https://www.encodeproject.org/) and other literature published screens for RNA binding proteins.

Project plan

1) Orthogonal organic phase separation will be run in the 7 KRAS mutant CRC organoid cultures the Team has access to, and organoid cultures from normal bowel to identify RBPS that exhibit increased RNA-binding in the cancer cultures. RBPs showing increased binding in the cancer organoids will be confirmed in the organoid models using ELISA or immunoblotting endpoints where possible, and in additional colorectal cancer cell lines to determine if increased RNA-binding in organoids can be reproduced in wider set of adherent tissue culture models.

2) With input from Cancer Therapeutic Unit (CTU) computational biology colleagues the student will use the RBP database established by RBMT to identify and rank candidates identified from their orthogonal organic phase separation data for further genetic validation. siRNA or CRISPRi will be used to initially validate the top (≤10) candidate hits for follow-up in organoid models, and if possible additional tissue culture lines, using proliferative/growth and cell death endpoints. Molecular endpoints will also be assessed where possible and these will be tailored to the candidate hit in question.

3) The top one or two candidate hits that are genetically validated will be followed up using a dTAG-degradation model. The impact of target degradation will be confirmed using molecular and cellular endpoints determined above. Rescue experiments re-expressing wild-type or binding domain/active site mutants will be run to determine the importance of conserved domains or active sites, e.g. ATPase, for cellular and molecular functions. In addition, a computational analysis of target protein druggability will be run with help of CTU computational biologists and dTAG-rescue experiments will run with mutants of pockets that are identified as potential small-molecule binding pockets.

4) Finally, RNA oligonucleotide-PROTACs will be designed either using known RNA binding motifs determined from the ENCODE database or experimentally using SELEX approaches (Ghidini et al., 2021). The RNA oligo-

PROTACs will be transfected into cells and their ability to degrade the target RBP-of-interest will be determined along with the subsequent impact on cancer cell growth and survival. Selectivity of RNA-oligo PROTACs will be determined using global proteomics to determine the profile of proteins showing degradation following treatment. Building dTAG-degradation models is time consuming and labour intensive, and thus only practical for one or two cell lines. In contrast the identification of a selective RNA-oligo PROTAC tool will allow assessment of the impact of target protein loss in multiple cell lines.

Outcome

We anticipate that the orthogonal organic phase separation analysis and follow-up validation by genetic, dTAG and RNA oligo-PROTAC methods will identify and validate a candidate RBP for future follow-up as a therapeutic target in colorectal cancer.

Literature references

[1] Einstein, JM, Perelis W, Chaim IA et al. (2021) Inhibition of YTHDF2 triggers proteotoxic cell death in MYC-driven breast cancer. Mol. Cell., 81:3048-3064.

[2] Gebauer F, Schwarzl T, Valcárcel. J and Hentze WM. (2021) RNA-binding proteins in human genetic disease. Nat. Rev. Gen., 22:185–198.

[3] Gerstberger S, Hafner M, and Tusch T. (2014) A census of human RNA-binding proteins. Nature Rev. Gen., 15:829-845.

[4] Ghidini A, Cléry A, Halloy F et al. (2021) RNA-PROTACs: Degraders of RNA-Binding Proteins. Angew. Chem. Int Ed., 60:3163-3169

[5] Hentze MW, Castello A, Schwarzl T and Preiss T. (2018) A brave new world of RNA-binding proteins. Nature Rev. Mol. Cell. Biol., 19:327–341.

[6] Nabet B, Roberts JM, Buckley DL et al. (2018) The dTAG system for immediate and target-specific protein degradation. Nat. Chem. Biol., 14:431–441.

[7] Pereira B, Billaud M and Almeida R. (2017) RNA-Binding Proteins in Cancer: Old Players and New Actors. Trends Cancer, 3:506-528.

[8] Queiroz RML, Smith T, Eneko Villanueva E et al (2021) Comprehensive identification of RNA–protein interactions in any organism using orthogonal organic phase separation (OOPS). Nature Biotechnology, 37:169-178.

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: Relevant undergraduate Honours degree (First or 2:1)

Intended learning outcomes:

- Knowledge and skills required to run and analyse protein mass spec. data from orthogonal organic phase separation analysis.
- Know-how and skills associated with drug target validation, including genetic approaches and dTAG-rescue strategies.
- Experience in the development of RNA-oligo PROTACS as validation tools.
- Exposure to state-of-the-art drug discovery within the Cancer Therapeutics Unit.

Advertising details

Project suitable for a student with a background in:

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry

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