PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	**Deciphering cancer cell dependency on eIF4E**

Supervisory team

Primary Supervisor:	Paul Clarke
Associate Supervisor(s):	Marissa Powers
Secondary Supervisor:	Jyoti Choudhary

Divisional affiliation

Primary Division:	Centre for Cancer Drug Discovery
Primary Team:	RNA Biology & Molecular Therapeutics
Site:	Sutton

Project background

Cancer cells rely on enhanced protein synthesis to sustain their rapid growth and adaptability. A key control point is translation initiation, where ribosomes are recruited to mRNA[1]. Central to this step is the eIF4F complex, which assembles at the 5' cap of mRNA. Within this complex, eIF4E binds the cap structure, eIF4A unwinds RNA structures, and eIF4G acts as a scaffold to orchestrate assembly of eIF4F1. Deregulation of these eIF4F components is common in cancer with some acting as oncogenes, highlighting the eIF4F complex as a promising therapeutic target2,3. The potential of eIF4E as a target is underscored by its oncogenic activity in multiple transformation assays and tumorigenesis studies. Importantly, in a haplo-insufficient mouse model loss of one eIF4E allele confers resistance to tumorigenesis, while maintaining normal physiology[2–6]. Despite this promise, eIF4E has proved resistant to traditional drug discovery, with existing tool compounds lacking the characteristics of high-quality chemical probes and subsequently failing to progress to drug-like series[3].

We addressed this challenge in a collaboration with Astex Pharmaceuticals through fragment-based screening and genetic validation[7]. The team at Astex identified a compound that binds eIF4E in a previously uncharacterised pocket adjacent to the eIF4G binding interface[7]. While the compound engaged eIF4E in cells, it did not inhibit protein synthesis. We used genetic rescue experiments in lung cancer cells to confirm that RNA binding is essential for eIF4E function and revealed that eIF4G engages eIF4E through extended interactions with eIF4E that our compound only partially disrupts[7].

Project aims

Hypothesis: Targeting cancer cell dependency on eIF4E, a central component of the eIF4F protein synthesis initiation complex and a regulator of nuclear mRNA export, will suppress tumour cell growth and survival and inform on potential therapeutic strategies.

- **Aim 1 Gain more insight into the contributions of the different eIF4E molecular functions to cancer and normal cell biology.** Investigate the interplay between protein synthesis initiation and other functions of eIF4E, such as nuclear mRNA export, and also the function of potentially druggable sites on eIF4E.

- **Aim 2 Advance eIF4E-targeted drug discovery.** Utilise genetic models and chemical tools to gain a better understanding of features of eIF4E required for its oncogenic activity and their promise as targets for drug development.

- **Aim 3: Determine how tumour cells rely on eIF4E for the pioneer round of protein synthesis and maintain oncogenic-associated protein synthesis.** Investigate the eIF4E dependency of the first pioneer round of protein synthesis, and the extent to which tumour cells can sustain oncogene-associated protein synthesis, including eIF4E-independent mechanisms as a potential source of resistance to eIF4E-targeted therapies.

Overall impact: Identify better therapeutic strategies. This research will clarify the distinct roles of eIF4E and associated translational mechanisms in cancer. Leveraging knowledge gained from **AIMS1-3** will contribute to innovative strategies targeting eIF4E-related vulnerabilities in tumours.

Research proposal

Introduction

The oncogenic activity of eIF4E is largely attributed to its canonical role in cap-dependent mRNA translation, however, recent studies reveal broader functions, including mRNA export[1,3,8,9]. One hypothesis proposes that elevated transcription associated with oncogenic transformation heightens the demand for mRNA export[6], causing eIF4E to shift to facilitating nuclear export and reducing the availability eIF4E for translation. Consequently, the contribution of eIF4E to malignancy may stem more from its nuclear functions than we previously appreciated. The clinical relevance and prevalence of these different eIF4E roles remains unclear, highlighting the need for further investigation and refined tools to dissect and target eIF4E-driven oncogenesis.

We previously established an eIF4E-dTAG protein degradation model to investigate a ligand binding pocket identified by compound screening[7]. To further these studies, we are generating additional eIF4E-dTAG models in lung cancer and immortalised normal airway epithelial cells. These models will be available for rescue experiments with eIF4E mutants, for example, variants that selectively impair translation, but preserve nuclear export, or *vice versa,* to probe function-specific dependencies by characterising eIF4E protein and RNA interactomes.

AIM 1. Saturation mutagenesis to identify domains essential for eIF4E function

To expand on our understanding of domains of eIF4E required for different functions we will use a genetic rescue strategy in our eIF4E-dTAG models, however, we have previously found that several literature-reported eIF4E mutants did not perform as expected in rescue assays[7]. Therefore to gain a wider unbiased understanding of domains important for eIF4E function in cancer and normal cells we constructed a pooled saturation mutagenesis library of eIF4E mutants to enable a systemic and comprehensive rescue strategy[7]. The eIF4E mutant library will be transduced into eIF4E-dTAG cells. Samples will be collected following treatment with $dTAG^V$-1, a compound that induces eIF4E-dTAG degradation, or $dTAG^V$-1-neg, an inactive control. Mutant abundance will be determined by PacBio sequencing. Based on prior experience[7] non-functional eIF4E mutants will be depleted from the cell population as they will not rescue cells from cell death induced by eIF4E-dTAG degradation, conversely mutants that retain eIF4E activity will rescue the cells from death and will be retained in the population. Followup rescue screens will use cellular readouts for mRNA export or translation coupled with FACs-based selection to separate affected and unaffected populations for sequencing. **OUTPUT:** Mapping results onto our existing eIF4E structural data[7] will identify and refine our current knowledge of residues or domains critical for the different functions of eIF4E.

AIM 2. Selective targeting of eIF4E by mutation or compound treatment.

We are developing eIF4E-targeted compounds. Mutants currently available within the team, supplemented with mutants identified from **AIM1,** that interfere with different eIF4E domains, will be compared with compound activity. Endpoints will include proliferation, survival, cap-dependent translation, and RNA export. Binding to protein partners or mRNA subsets will be assessed through co-immunoprecipitation and mass spectrometry or RNAseq to define how mutations

alter complex formation and RNA binding. **OUTPUT:** These studies will help us understand the role and dependency of cancer cells on different eIF4E functions. They will also define the selectivity of compounds across eIF4E functional complexes and help guide rational eIF4E-degrader development. The obtained results could also lead to the identification of molecular glues that selectively bind eIF4E, enabling the development of either an alternative single-agent therapy or a multi-target therapy involving other key components in cancer development.

AIM 3. Assessing the role of eIF4E in the "pioneer" round of translation or maintenance of established translation.
Oncogenic transformation places a heavy transcriptional burden on cancer cells, potentially sequestering eIF4E for mRNA nuclear export and limiting its role in translation[6]. Using transfection of a synthetic cap-dependent translation reporter mRNA before or after loss of eIF4E-dTAG protein we observed that eIF4E is essential for the first or "pioneer" round of translation, but not for subsequent rounds once translation of the reporter mRNA was established. As the pioneer round of translation is also linked to mRNA nuclear export, this may reflect the elevated dependency of cancer cells on eIF4E during early translation events.

To investigate this further, dTAG-degradation models for NCBP1/2 that are reported to have a role in the pioneer round of mRNA translation, or eIF4G2 or eIF3D that have been implicated in eIF4E-independent translation[9] will be constructed. The effects of depleting these proteins will be evaluated using outputs outlined in **AIM 2** and also using direct transfection of synthetic reporter mRNAs. **OUTPUT:** Data generated will contribute to assessing the mechanisms by which tumour cells might rely on the pioneer round of protein synthesis versus maintenance of established oncogenic protein synthesis, and how these functions may impact on eIF4E-targeted degradation strategies and potential as future therapeutic targets.

Available Resources
The candidate will join a team with extensive expertise in dTAG-models, rescue genetics and protein synthesis[3,7]. A robust pipeline for developing dTAG-degradation and genetic rescue models is in place. The candidate will have the opportunity to work with state-of-the-art tools, including several automated systems. The team maintains a productive collaboration with the ICR Centre for Protein Degradation.

Risks and Contingencies
Most models and assays are established coupled with existing validated protocols and pipelines to ensure continuity for generating additional models. The mutant library rescue approach has the highest risk as it is new to our laboratory. Should challenges arise, the candidate will switch to structure-guided individual mutations.

Overall outcomes and impact
This project will build on our understanding cancer cell dependencies on the interplay between eIF4E-mediated mRNA nuclear export and eIF4-dependent protein synthesis, and the role of eIF4E in the pioneer round of translation which has not been previously described. These advances will either validate existing approaches or uncover new therapeutic strategies.

The functional relevance of ligand-binding pockets identified by fragment-based screening or DNA-encoded libraries of hard-to-drug targets often remains unclear. From experience, using specific mutants can have unpredictable outcomes, however, our mutant library strategy offers a more scalable and precise paradigm for validating the functional importance of novel ligand-binding sites on proteins of interest, either across the entire protein or focused on regions surrounding candidate pockets.

Literature references

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2. Kovalski, J. R., Kuzuoglu-Ozturk, D. & Ruggero, D. Protein synthesis control in cancer: selectivity and therapeutic targeting. *The EMBO Journal* **41**, e109823 (2022).
3. D'Agostino, S., Davies, C., Powers, M. V. & Clarke, P. A. Advances in understanding and targeting eIF4E activity. *Biochemical Society Transactions* (2025) doi:10.1042/bst20241045.
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5. Wu, S. & Wagner, G. Deep computational analysis details dysregulation of eukaryotic translation initiation complex eIF4F in human cancers. *Cell systems* **12**, 907-923.e6 (2021).
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7. Sharp, S. Y. *et al.* Integrating fragment-based screening with targeted protein degradation and genetic rescue to explore eIF4E function. *Nat Commun* **15**, 10037 (2024).
8. Mars, J.-C., Culjkovic-Kraljacic, B. & Borden, K. L. B. eIF4E orchestrates mRNA processing, RNA export and translation to modify specific protein production. *Nucleus* **15**, 2360196 (2024).
9. Mahé, M., Rios-Fuller, T., Katsara, O. & Schneider, R. J. Non-canonical mRNA translation initiation in cell stress and cancer. *NAR Cancer* **6**, zcae026 (2024).

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: ICR's standard minimum entry requirement of an undergraduate Honours degree (First or 2:1)

Intended learning outcomes:

- Knowledge and skills required to construct and use dTAG-degradation models
- Skills associated with running genetic rescue experiments and reporter assays
- Knowledge of global gene expression analysis e.g. RNAseq and proteomics
- Experience working with small molecule tool compounds and compound libraries
- Experience and skills associated with drug target validation
- Exposure to state-of-the-art drug discovery

Advertising details

Project suitable for a student with a background in:

X Biological Sciences

☐ Physics or Engineering

☐ Chemistry

☐ Maths, Statistics or Epidemiology

☐ Computer Science