

PhD Project Proposal

Funder details

Studentship funded by: MRC DTP

Project details

Project title: Enabling novel E3 ligases for targeted protein degradation therapeutics.

Supervisory team

Primary Supervisor: Dr Agnieszka Konopacka

Associate Supervisor(s):

Secondary Supervisor: Prof. Swen Hoelder

Divisional affiliation

Primary Division: Cancer Therapeutics

Primary Team: Induced Proximity Therapeutics, Centre for Protein Degradation

Site: Sutton

Project background

Targeted protein degradation (TPD) has transformed cancer drug discovery by enabling removal of harmful proteins using small molecule degraders. PROTACs (Proteolysis Targeting Chimeras) and MGDs (Molecular Glue Degraders) induce formation of ternary complex between the drug, an E3 ligase and the target, followed by ubiquitination and degradation in proteasome (1,2). This catalytic process translates to lower drug doses and long-lasting therapeutic effect, better efficacy due to eliminating all protein functions, lower toxicity and resistance. It also allows to target previously undruggable proteins without enzymatic functions, like transcription factors.

Whereas there are over 600 E3 ligases in human cells, most degraders utilize just a few well characterized E3s, mostly VHL and CRBN. This poses significant chemical and biological limitations, including poor physicochemical properties or toxicity of the drugs, drug resistance and restricted pool of degradable target proteins (1). Therefore, exploring novel E3 ligases is necessary to overcome those issues and further expand the potential of TPD.

E3 ligases are heterogenous group of proteins with a complex and poorly understood biology and different tissue expression and activity. They are classified as HECT (homologous to E6-associated protein C-terminus), RING (Really Interesting New Gene) or U-box proteins. The three classes differ in mechanism of Ubiquitin transfer and composition of protein complexes (8). Recruitment of novel E3s for TPD has been also limited by poor availability of chemical matter. Nonetheless, recent studies have demonstrated activity of several novel E3s with PROTACs and MGDs, including DCAF15, DCAF16, KEAP1, RNF4, HERC4 (6,7), and the area is rapidly expanding. Aberrant expression and activity of E3 ligases is often a characteristic of cancer cells (3,5,4), which can be exploited therapeutically to generate cancer- or tissue-selective degraders.

Identifying novel E3 ligases will allow to expand degradable target range, improve target and tissue selectivity, identify drugs with better therapeutic properties and to overcome resistance. This project will use a combination of genetic manipulation, cell and chemical biology approaches to explore therapeutic potential novel E3 ligases for cancer applications and will contribute to discovery of novel drug modalities at the ICR.

Project aims

- Characterize therapeutic potential of 1 – 3 novel E3 ligases in AML, MM or solid cancer models using cell biology and genetic manipulation techniques
- Testing E3 ligase activity in cells using genetic degron tagging
- Identify endogenous substrates for a selected E3 ligase and investigate substrate recognition mechanism

Research proposal

Unparalleled opportunities presented by targeted protein degradation (TPD) resulted so far in successful degradation of nearly a hundred of different targets with PROTACs and molecular glue degraders (MGD) and a fast progress of many into the clinic. Most degraders however utilize just a fraction of E3 ligases expressed in human tissues, most commonly von Hippel-Lindau (VHL) and Cereblon (CRBN). Despite good activity of those ligases in most tissues, degradation of some targets still presents a challenge, especially if selectivity is important. Importantly, identification of novel chemical binders with better phys-chem properties, tolerance and less tumor resistance would be very valuable (). Thus, exploring additional E3 ligases for TPD applications might offer opportunity to increase the target scope, improve target and tissue selectivity, overcome resistance, and discover ligands with better physicochemical properties and therapeutic window. The aim of this project is to unlock this untapped potential by enabling novel E3 ligases for TPD applications through biological and mechanistic characterisation in relevant oncology models and to pave the way for discovery of novel PROTACs and MGDs.

Aim 1. Characterize therapeutic potential of 1 – 3 novel E3 ligases in cancer models using cell biology and genetic manipulation techniques

Multiple tumor cells have aberrantly high expression of certain E3 ligases. Recruiting those E3s by PROTACs and MGDs can be utilized to enhance therapeutic window and for tissue selective degradation. In the first year the project will focus on assessing therapeutic potential of 2 - 3 novel E3 ligases selected based on literature and available transcriptomics and proteomics data (DepMap, TNMplot), and drug tractability score (internal bioinformatics analysis). This will be achieved by verifying differential E3 ligase expression and activity in different cancer cell lines vs. healthy tissues using WB or fluorescent immunostaining and ubiquitination assays. In the next step we will select one E3 ligase for more detailed evaluation as a therapeutic target and as a potential effector for PROTACs and MGDs, looking the effects of E3 ligase KO on cancer cell viability and downstream signalling pathway.

- Measurement of E3 ligase protein levels by WB in selected AML, MM and solid cancer cell lines and healthy cells (fibroblasts, PBMCs)
- E3 ligase activity will be tested using *in vitro* ELISA or TR-FRET autoubiquitination assay.
- E3 ligase KO using CRISPR-Cas9 and degradation with dTAG in selected AML, MM and solid cancer cell line. The results will be confirmed by WB and HiBit (dTAG).
- Determination of the effects of E3 ligase KO/ degradation on cell viability will be performed using CTG assay.

Aim 2. Testing E3 ligase degradation activity

The main objective of the study is to assess potential of a novel E3 ligase for TPD application. Therefore, in the next stage the project will focus on assessing if the selected E3 ligase is able to degrade different targets following recruitment with a PROTAC.

- The experiments will require generating cell line overexpressing BromoTag-GFP protein in combination with inducible dTAG-E3 ligase.

- E3 ligase degradation activity will be assessed by measuring degradation potency of dTAG-BromoTag PROTAC in GFP fluorescence assay. Alternatively, a BRD4 – dTAG- or promiscuous kinase-dTAG PROTAC may be used to test degradation of BRD4 or selected endogenous kinases (BRD4-Hibit assay or WB).

Aim 3. Identify endogenous substrates for a selected E3 ligase and investigate substrate recognition mechanism

Majority of intracellular proteins are degraded *via* Ub-proteasome pathway. However, information about endogenous E3 ligase substrates is scarce and it is often context dependent. Identifying endogenous substrates for the selected E3 ligase will be an important starting point for designing E3 ligand discovery strategy. Characterising E3 ligase complex is critical for mechanistic understanding of E3 ligase function, design of screening assays and degraders.

Identifying therapeutically relevant endogenous substrates will be a cue for development of MGDs based on molecules enhancing the natural interaction. Endogenous substrates recruit E3 ligases *via* short peptide motifs called degrons, which are often shared by multiple proteins. For example, glycine loop recognized by CRBN ligase is shared by multiple Zinc Finger proteins. Hence identifying a degron recognized by a given E3 ligase is important for finding potential MGD targets. Importantly, mimicking the degron structure can also be used as a strategy for discovery chemical E3 ligase binders. For example, VHL binder was designed by mimicking hydroxyproline in HIF1 alpha degron.

Therefore, the next part of the project will focus on characterizing the interactome and endogenous substrates of the selected E3 ligase, and substrate recognition mechanism. The experiments will include:

- Proteomics profiling following E3 ligase degradation (E3 ligase-dTAG cells) to identify stabilised proteins. Rescue experiment with WB readout will include E3 ligase overexpression construct and/or inactive dTAG PROTAC.
- Identification of E3 ligase interactome and ubiquitinome (potential substrates) by proximity biotin labelling (TurboID) followed by mass spectrometry
- Substrate recognition mechanism studies will include expression of truncated E3 ligase target mutants with Hibit tag in cancer cells overexpressing E3 ligase, and degradation Hibit assay. If required, degron – E3 ligase binding will be confirmed by IP pull-down/WB.

Literature references

- [1] Yoon H et al (2024). Induced protein degradation for therapeutics: past, present, and future. *J Clin Invest.* 134(1):e175265
- [2] Garber K (2024). The glue degraders. *Nat Biotechnol* 42, 546–550
- [3] Tsherniak A et al (2017). Defining a Cancer Dependency Map. *Cell.* 170(3):564-576.
- [4] Bartha A et al (2021). TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. *Int. J. Mol. Sci.* 22(5), 2622.
- [5] Duan and Pagano (2021) Ubiquitin ligases in cancer: Functions and clinical potentials. *Cell Chem Bio* 28(7):918-933.
- [6] Kramer L and Zhang X (2022). Expanding the landscape of E3 ligases for targeted protein degradation. *Curr Res Chem Bio* 2, 100020.
- [7] Mutlu M et al (2024). Small molecule induced STING degradation facilitated by the HECT ligase HERC4. *Nat Commun* 15, 4584
- [8] Yang Q et al (2021). E3 ubiquitin ligases: styles, structures and functions. *Mol Biomed* 2(1):23.

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:

BSc or MSc in Cell Biology, Molecular Biology, Biochemistry

Intended learning outcomes:

- Critical thinking
- Literature and data analysis and interpretation
- Experiment design
- Range of biochemical, cell biology and genetic engineering techniques
- Knowledge of drug discovery, novel therapeutic modalities, cancer biology
- Data presentation
- Scientific writing

Advertising details

Project suitable for a student with a background in:

- Biological Sciences
- Physics or Engineering
- Chemistry
- Maths, Statistics or Epidemiology
- Computer Science