The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Exploiting proteomic mass spectrometry analysis to understand the role of translation elongation factor eEF2 in oncogenesis through detailed characterisation of its function and regulation
Short Project Title:	Characterisation of function and regulation of translation elongation factor eEF2 and its role in oncogenesis

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Jyoti Choudhary
Other supervisory team members:	Dr Mercedes Pardo Professor Jon Pines

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Biology
Primary Team:	Functional proteomics

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Aberrant protein translation is a widespread characteristic of tumour cells and has been associated with many oncogenes and tumour suppressors (Roumeliotis et al., 2017). This convergence on the protein translation machinery is independent of the genetic make-up of the cancer. Therefore, therapeutic agents targeting the protein synthesis apparatus are potentially more robust to intra-tumoural heterogeneity and constitute a promising class of antitumor drugs.

Elongation factor-2 (eEF2) is a conserved protein essential for protein biosynthesis. A single histidine residue in eEF2 (His-715 and His-699 in humans and yeast, respectively) is modified to form diphthamide (Uthman et al., 2013), the target of ADP-ribosylation by several bacterial and viral toxins. The diphthamide modification is involved in translocation of the ribosome along the mRNA (Namy et al., 2006). Diphthamide modified eEF2 controls the selective translation of Internal Ribosome Entry Site-dependent protein targets and is crucial for survival under oxidative stress conditions (Arguelles et al., 2014).

Diphthamide is generated by seven DPH proteins; cells deficient in these enzymes lack diphthamide and are toxin-resistant. Mutation of diphthamide synthesis pathway has been associated with altered translational fidelity and leads to ribosomal -1 frameshifting in both yeast and mammals. Lack of diphthamide-eEF2 is associated with cancer and it also results in defects in mouse development (Schaffrath et al., 2014). Loss of diphthamide renders cells hypersensitive to TNF-mediated apoptosis, suggesting that diphthamide may have a role for in modulating NF-κB, death receptor, or apoptosis pathways. In yeast it leads to increased sensitivity to cytotoxic drugs. Collectively, these studies show the complexity of eEF2 regulation and role in diverse cellular processes.

Over expression of eEF2 and its phosphorylated form has been reported in various types of cancer. Notably, eEF2 and its kinase are both modulated and modulators of the cell cycle and overexpression of eEF2 has been shown to promote G2/M transition. Additionally, the eEF2 kinase which phosphorylates

and inactivates eEF2, has been suggested as a potential therapeutic target (Liu and Proud, 2016). As such, a better understanding of eEF2 function, regulation and activity could lead to the identification of novel cancer therapeutic avenues.

PROJECT AIMS

- Understand eEF2 regulation through identification of binding partners and characterisation of the spectrum of post translational modifications
- Understand translational programs regulated by eEF2 and the diphthamide modification by identifying target proteins
- Define translational aberrations caused by loss of eEF2 and diphthamide modification
- Examine how protein biosynthesis is altered by eEF2 deregulation in hepatocellular carcinoma cancer cells using protein correlation network analysis

RESEARCH PROPOSAL

1. The PhD candidate will perform protein interaction studies on eEF2 in cancer cell lines by mass spectrometry-based approaches. This will be achieved through two complementary strategies: affinity purification and APEX2 proximity labelling, followed by mass spectrometry analysis. These two approaches used in parallel are useful in capturing temporal and spatial aspects of the interaction network landscape (Hesketh et al., 2017). Proximity labelling is particularly suited for identifying weak or transient interactions, which modulatory enzymes are often involved in, that might not survive traditional co-purification approaches. The affinity purification is compatible with methods of chemical cross-linking. These experiments will yield structural and organisational information on eEF2 protein interactions. We anticipate that these experiments will identify regulators of eEF2 activity and other proteins involved in the regulation of translation.

2. We have previously described the use of size fractionation to resolve protein complexes (Pardo et al., 2017). Following affinity purification the complexes will be separated and analysed by mass spectrometry. In depth mass spectrometry data acquisition and analysis methods will be used to characterise the primary structure in detail. The cross-talk between post translational modifications will be explored.

3. Based on data from interaction studies, selected proteins will be validated biochemically by reverse immunoprecipitation and/or functionally via RNA interference. Confirmed candidates will be characterised functionally in detail using dedicated assays.

4. The candidate will characterise the consequences of loss of eEF2 on translational fidelity. We will use unnatural amino acid labelling with azidohomoalanine (AHA) that incorporates into newly synthesised proteins instead of methionine (McShane et al., 2016). AHA contains an azide group that enables capture of proteins via click chemistry, followed by mass spectrometry analysis. This analysis will enable the identification of proteins and/or pathways that might be modulated by eEF2 and DPH6. Furthermore, we will use a novel database search pipeline that has been developed recently by our group to describe the defects in translational fidelity caused by loss of eEF2 (Weisser and Choudhary, 2017). We will use protein correlation analysis to uncover functional relationships between cellular processes and/or protein complexes affected. We will also explore the existence of signals in the RNA sequence that might mediate the translational defects observed.

5. Building upon work from the previous point, the candidate will perform AHA labelling in combination with chemical tagging-based protein quantification to characterise changes in translation fidelity that occur in response to diverse stimuli or different conditions, namely oxidative stress, treatment with cytotoxic drugs, malignant transformation, with a focus on programmed ribosomal frameshifting. We will use this data to

annotate the genome with examples of this phenomenon important for the regulation of cellular expression and hence regulation of cellular processes.

This PhD will offer a broad range of training in state of the art proteomics mass spectrometry, molecular biology, cell biology, tissue culture, as well as relevant assays. Bioinformatics skills will also be gained as there is an important component of large scale data analysis. There will also be exposure to the protein structure field through interactions with Protein Structure groups. This will ensure that the PhD provides a highly multidisciplinary training.

LITERATURE REFERENCES

ARGUELLES, S., CAMANDOLA, S., CUTLER, R. G., AYALA, A. & MATTSON, M. P. 2014. Elongation factor 2 diphthamide is critical for translation of two IRES-dependent protein targets, XIAP and FGF2, under oxidative stress conditions. *Free Radic Biol Med,* 67**,** 131-8.

HESKETH, G. G., YOUN, J. Y., SAMAVARCHI-TEHRANI, P., RAUGHT, B. & GINGRAS, A. C. 2017. Parallel Exploration of Interaction Space by BioID and Affinity Purification Coupled to Mass Spectrometry. *Methods Mol Biol,* 1550**,** 115-136.

LIU, R. & PROUD, C. G. 2016. Eukaryotic elongation factor 2 kinase as a drug target in cancer, and in cardiovascular and neurodegenerative diseases. *Acta Pharmacol Sin,* 37**,** 285-94.

MCSHANE, E., SIN, C., ZAUBER, H., WELLS, J. N., DONNELLY, N., WANG, X., HOU, J., CHEN, W., STORCHOVA, Z., MARSH, J. A., VALLERIANI, A. & SELBACH, M. 2016. Kinetic Analysis of Protein Stability Reveals Age-Dependent Degradation. *Cell,* 167**,** 803-815 e21.

NAMY, O., MORAN, S. J., STUART, D. I., GILBERT, R. J. & BRIERLEY, I. 2006. A mechanical explanation of RNA pseudoknot function in programmed ribosomal frameshifting. *Nature,* 441**,** 244-7.

PARDO, M., BODE, D., YU, L. & CHOUDHARY, J. S. 2017. Resolving Affinity Purified Protein Complexes by Blue Native PAGE and Protein Correlation Profiling. *J Vis Exp*.

ROUMELIOTIS, T. I., WILLIAMS, S. P., GONCALVES, E., ALSINET, C., DEL CASTILLO VELASCO-HERRERA, M., ABEN, N., GHAVIDEL, F. Z., MICHAUT, M., SCHUBERT, M., PRICE, S., WRIGHT, J. C., YU, L., YANG, M., DIENSTMANN, R., GUINNEY, J., BELTRAO, P., BRAZMA, A., PARDO, M., STEGLE, O., ADAMS, D. J., WESSELS, L., SAEZ-RODRIGUEZ, J., MCDERMOTT, U. & CHOUDHARY, J. S. 2017. Genomic Determinants of Protein Abundance Variation in Colorectal Cancer Cells. *Cell Rep,* 20**,** 2201-2214.

SCHAFFRATH, R., ABDEL-FATTAH, W., KLASSEN, R. & STARK, M. J. 2014. The diphthamide modification pathway from Saccharomyces cerevisiae--revisited. *Mol Microbiol,* 94**,** 1213-26.

UTHMAN, S., BAR, C., SCHEIDT, V., LIU, S., TEN HAVE, S., GIORGINI, F., STARK, M. J. & SCHAFFRATH, R. 2013. The amidation step of diphthamide biosynthesis in yeast requires DPH6, a gene identified through mining the DPH1-DPH5 interaction network. *PLoS Genet,* 9**,** e1003334.

WEISSER, H. & CHOUDHARY, J. S. 2017. Targeted Feature Detection for Data-Dependent Shotgun Proteomics. *J Proteome Res,* 16**,** 2964-2974.

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: e.g. BSc or equivalent in specific subject area(s)	Biology, Biochemistry or Chemistry
Intended learning outcomes:	Mass spectrometry Chemical Biology Molecular Cell Biology Cancer

	Genomics Proteomics Data analysis