Post-transcriptional regulation of Aurora kinase in the cell cycle and cancer

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Principal supervisor's CRUK CC theme: Cell and Molecular Biology Programme

Department for student registration: Pharmacology
Department or institute where research will take place: Pharmacology.

Postgraduate scheme: **MRes + PhD (1 + 3-year non-clinical applicants only)**

MRes project outline:

We have recently published a study of translational regulation of the oncogenic AURKA kinase, showing that an alternative polyadenylation event (APA) resulting in a shortened 3'UTR leads to more efficient translation and higher expression of the protein (Cacioppo et al., 2023). Our results suggest the hypothesis that a shift in APA determines cell cycle regulation of AURKA levels via one or more miRNAs (let-7a) acting on the 3'UTR. They also provide a molecular rationale for AURKA overexpression upon 3'UTR shortening, as observed in Triple Negative Breast Cancer (TNBC). However, our study was carried out in a cell line highly amenable to cell cycle studies (U2OS) but has not been confirmed in breast tissue cells, nor in any disease model.
With this MRes project we aim to test key elements of the AURKA APA hypothesis in the non-tumorigenic human breast epithelial cell line, MCF10A. We will use our previously developed biosensors for AURKA translation, and quantitative PCR methods to measure mRNA isoform usage, to profile cell cycle regulation of AURKA APA and expression in MCF10A cells. We will compare our findings in MCF10A cells with breast cancer cell lines (such as MDA-MB-231, MCF7).
Our specific objectives will be to answer the following questions:
• Do the long and short 3'UTRs show differential expression in the cell cycle in MCF10A?
• Do they mediate different rates of AURKA translation?
• Are they differentially sensitive to miRNA (let-7a) overexpression/knockdown?
• How do 3'UTR usage, miRNA sensitivity and AURKA expression compare in cancer cells?

MRes experimental plan:

Weeks 1-3
Optimising cell culture conditions for MCF10A and breast cancer cell lines
Expression of cell cycle sensor in single cell microscopy assays
Weeks 3-6
Cell cycle synchronisation
mRNA expression analysis (RT-qPCR)
Protein expression (quantitative immunoblot)
Weeks 5-8
Translation efficiency assay (comparing cell cycle phases)
Weeks 9-12
Translation efficiency assay (miRNA overexpression and suppression)

Main techniques:

Human cell culture (non-transformed and transformed)
Plasmids/RNA transfection
Widefield fluorescence microscopy using biosensors
Timelapse imaging
Quantitative image analysis
RNA sample preparation and analysis RT-qPCR
Immunoblotting

PhD project outline:

AURKA overexpression contributes to cancer progression, but despite development of highly effective kinase inhibitors, none has made it to the clinic. One reason may be AURKA's kinase-independent roles (such as N-MYC stabilization in neuroblastoma), pointing to a need for new cancer-relevant functional assays. We are working with collaborators to develop alternative therapeutic tools targeting AURKA, including protein-protein interaction (PPI) inhibitors of N-MYC-AURKA (Boi et al., 2021) and Targeted Protein Degradation (TPD) tools such as PROTACs (Wang et al., 2021).

We propose a PhD project to generate organoid models of clinically-relevant AURKA overexpression for improved testing of new pharmacological tools.
Our bioinformatic investigations show a strong link between APA and AURKA overexpression in breast cancers (Cacioppo et al., 2023 & in preparation). The project will therefore build on the characterisation of AURKA APA in breast epithelial MCF10A cells undertaken during the MRes: We will force expression of the short 3'UTR form of AURKA in MCF10A through CRISPR/Cas9 editing to mutate the distal polyadenylation site (dPAS). Since MCF10A cells are amenable to 3D-culture, we will grow spheroids and assess the consequences of AURKA APA for 3D-architecture, cell physiology and behaviour. We will also transform MCF10A parental and dPAS-deleted cells to compare the contribution of AURKA APA at different steps in cancer progression. We will then use the MCF10A-dPAS model for testing new therapeutic tools targeting AURKA. The efficacy of such strategies in reversing phenotypic effects of AURKA overexpression will be assessed. In addition to PPI inhibitors and TPD tools we aim to assess use of potential therapeutic miRNA tools for modulation of AURKA levels.

PhD experimental plan:

Years 1-2 We will engineer mutation of the distal polyadenylation site of endogenous AURKA (AURKA-dPAS) in MCF10A cells by CRISPR/CAS9 editing, to create MCF10A-dPAS cells able to express only the short 3'UTR isoform of AURKA mRNA (Cacioppo et al., 2023). We will characterise proliferation and growth properties of the cells to assess the contribution of APA-dependent AURKA overexpression (AURKAoe). We will switch to 3D-culture conditions to create a novel organoid model for breast tissue with clinically relevant levels of AURKAoe.
Years 2-3 We will transform MCF10A cells through expression of SV40 largeT/telomerase/oncogenic Ras or by induction of extra centrosomes with Plk4 overexpression (Godinho et al., 2014). We will grow spheroids from wild-type MCF10A, MCF10A-dPAS and transformed MCF10A-dPAS cells and assess 3D-architecture, metabolic activity and stress responses, to better understand the oncogenic potential of APA-dependent AURKAoe.
Years 2-3 The MCF10A-dPAS AURKAoe model will then be used to test new therapeutic tools in modulating AURKA expression/function and in reversing phenotypic effects of AURKAoe in cell physiology and behaviour. We will test
• alisertib-based PROTACs effective in reducing cellular AURKA levels that we recently

described (Wang et al., 2021)
• novel protein-protein interaction (PPI) inhibitors of the N-MYC-AURKA interaction
• PROTACs based on PPI inhibitors, available to us through our collaborative network
• miRNA tools predicted to manipulate AURKA levels through 3'UTR binding

Main techniques:

3D (organoid) mammalian cell culture
Widefield fluorescence microscopy of living cells (and organoids)
Use of biosensors, quantitative image analysis
CRISPR/Cas9 cloning, cell sorting, immunoblot, RNA quantification
Degradation and ubiquitination assays (using HiBIT luminescence, plate-reader)
Proliferation assays & metabolic assays (mitochondrial function, metabolomics)

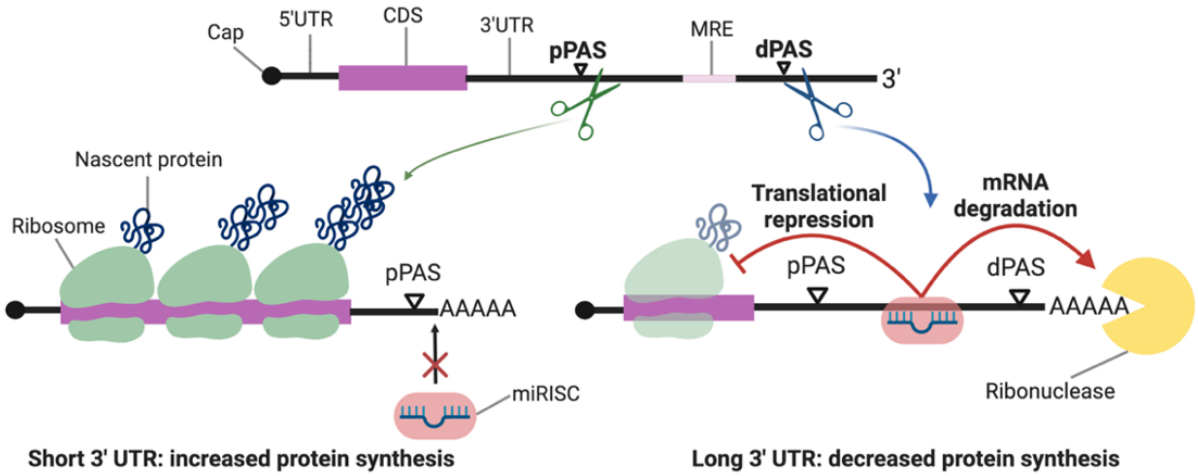
Key references:

Boi D, Souvalidou F, Capelli D, Polverino F, Marini G, Montanari R, Pochetti G, Tramonti A, Contestabile R, Trisciuoglio D, Carpinelli P, Ascanelli C, Lindon C, De Leo A, Saviano M, Di Santo C, Costi R, Guarguaglini G, Paiardini A (2021). PHA-680626 Is an Effective Inhibitor of the Interaction between Aurora-A and N-Myc. Int. J. Mol. Sci. 22 13122

Cacioppo R, Akman H, Tuncer T, Erson-Bensan A, Lindon C (2023). Differential translation of mRNA isoforms underlies oncogenic activation of cell cycle kinase Aurora A. Elife 12 RP87253

Godinho S, Picone R, Burute M, Dagher R, Su Y, Leung CT, Polyak K, Brugge JS, Théry M, Pellman D (2014). Oncogene-like induction of cellular invasion from centrosome amplification. Nature 510, 167

Wang R, Ascanelli C, Abdelbaki A, Fung A, Rasmusson T, Michaelides I, Roberts K, Lindon C (2021). Selective targeting of non-centrosomal AURKA functions through use of a targeted protein degradation tool. Commun. Biol. 4 640



Coupling of APA and miRNA-mediated mRNA regulation.