Investigating growth factor-specific PI3K signalling activation in a PIK3CA-H1047R HeLa cell disease model

Student: Oliwia Mruk; School of Life Sciences, University of Dundee
Supervisor: Dr Ralitsa Madsen; MRC-PPU, School of Life Sciences, University of Dundee

Introduction

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway is crucial for many cellular processes. Although the hyperactivation of this pathway is the cause of many cancers and developmental disorders, known as PIK3CA-related overgrowth spectrum (PROS), the underlying mechanisms are not well understood. Development of a more comprehensive PI3K signalling map is key to addressing these gaps and, in the future, allow for the development of less toxic drug alternatives, improving patient outcomes.

This project investigated the pathway dynamics of the PIK3CA-H1047R mutation. HeLa cells containing varying doses of the mutation (WT (wild-type), 1x H1047R, 2x H1047R and inactive (introduced frameshift)) expressing the FOXO-based KTR reporter (biosensor of AKT activity downstream of PI3K) were engineered by Dr Madsen for the purposes of investigating the dose-dependent effects of the mutation and growth factor stimulation.

Aims of the project

The project's aim was to explore the PI3K signalling pathway activation through investigating:

1. if/how the identity of the signalling pathway in response to GFs (growth factors) changes in disease-causing mutations; as compared through WT (wild-type) versus CRISPR-engineered PIK3CA-H1047R HeLa cells,
2. what the differences between the growth factor (IGF-1 and insulin) responses involved in the pathway are.

Additionally, this project provided an opportunity to examine a new experimental setup and, thus, verify the reproducibility of the live-cell imaging workflows previously performed.

Methods used

The methods used during this project relied heavily on tissue culture of CRISPR-engineered HeLa cell line clones, stably expressing a FOXO-based KTR reporter. All cell lines were cultured in complete medium (DMEM, with the addition of 10% Fetal Bovine Serum and L-glutamine) at 37°C and 5% CO_2. The expression of the reporter was verified using a fluorescence microscope. A genotyping protocol for phenotype verification was performed as well. Preparation for imaging experiments included:

Day 1: Seeding of clones (WT, 1x H1047R, 2x H1047R, inactive) 24h beforehand at 30,000 or 40,000 cells/ml in a 35mm µ-Dish (Ibidi), complete medium,

Day 2: Starvation of cells 2h before experiment commencement using FluoroBrite™ DMEM (with the addition of L-glutamine and Penicillin-Streptomycin).

The computational methods relied on the use of Fiji, Matlab, Python and R; pipelines previously developed by Dr Madsen and collaborator Dr Alix Le Marois (Francis Crick Institute).

Results and outcomes of studentship

By the end of the studentship, 3 datasets, comparing IGF-1 and insulin, using widefield fluorescence microscopy were generated. The results were quantified using the aforementioned analysis pipeline and the generated results revealed:

1. differences in signalling responses to IGF-1 and insulin,
2. observed blurring of response in H1047R clones,
3. the experimental design is reproducible across different microscopy setups.

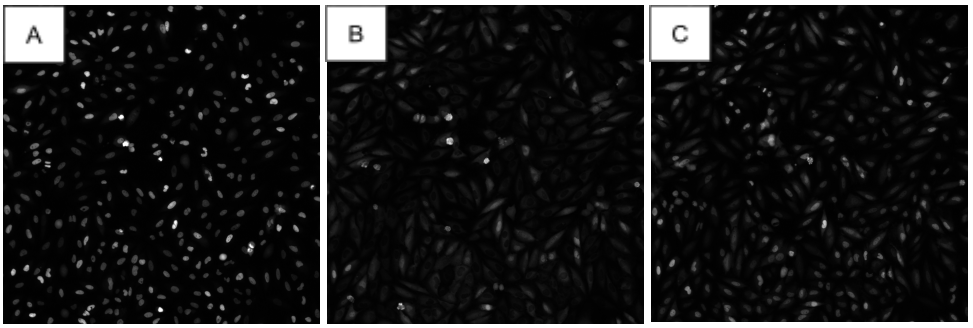

Figure 1. Images of WT HeLa cell responses to IGF-1 at A. time point 1 (baseline), B. time point 16 (60 minutes after the addition of IGF-1), C. time point 36 (60 minutes after the addition of alpelisib, alpha-specific PI3K inhibitor), experimental run two

Departures from original plan

While the original plan was the measurement of PIP3/PI(3,4)P2 upon growth factor stimulation using total internal reflection fluorescence (TIRF) microscopy, we experienced some technical issues with the setup. Although some images were produced, they were not sufficient for analysis. All subsequent experiments were shifted to single-cell measurements of AKT activity through the use of a KTR reporter, mentioned in the Methods section. The comparison of growth factors, due to time limitations, included IGF-1 and insulin, instead of the previously proposed EGF versus epigen.

Future directions in which the project could be taken

My project focused on comparisons of signalling dynamics downstream of the receptor tyrosine kinases' (RTKs) ligands, IGF-1 and insulin. The next, crucial step in understanding the complex spatiotemporal dynamics of PI3K signalling is the investigation of PI3K activation upon concentration changes of these ligands. In order to construct a comprehensive PI3K signalling map, the engineering and testing of further disease models is necessary; an example being the engineering of the E545K mutation in HeLa cells. Further cell models better mimicking the conditions present *in vivo* can also be considered for future work.

Value of studentship

Working under the supervision of Dr Madsen has been an unforgettable educational experience. I got to practice my existing skills in the lab and learn entirely new procedures, including tissue culture with HeLa cells and iPSCs (induced pluripotent stem cells), genotyping and running microscopy experiments, following best practice. The studentship was also a unique opportunity for me to improve computational biology skills alongside my work in the lab, allowing me to better prepare for my final year Honours project and further opportunities. Additionally, Dr Madsen's hands-on approach gave me an insight into the work of a researcher; this included management of the lab, meetings with collaborators, research paper preparations and PROS community engagement. The studentship proved very beneficial to the Madsen Lab as well; the acquired data will ultimately contribute to the generation of PI3K dynamic signalling maps required for the development of predictive models of the pathway input-output relationships. My PI3K/AKT signalling diagram will also be featured on the lab's interactive website.

Figure 2. The Madsen Lab members; Grey Yin (helping visiting student), Dr Ralitsa Madsen (supervisor) and Oliwia Mruk. From left to right

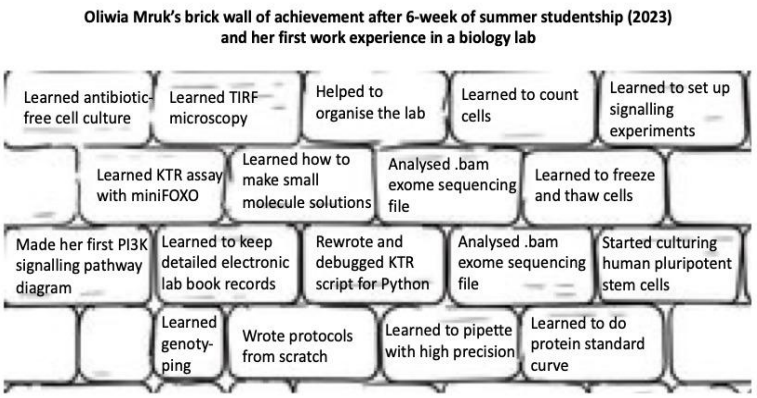

Figure 3. Oliwia's achievements after the 6-week Biochemical Society research studentship, generated by Dr Madsen