Investigating the role of FAM109A/CCSER1 in cell division and oesophageal cancer

Lead Investigator: Pier Paolo D’Avino
Department of Pathology
http://www.path.cam.ac.uk/research/investigators/davino/

Co-Investigator: Rebecca Fitzgerald
MRC Cancer Unit, Hutchison MRC Research Centre
http://www.mrc-cu.cam.ac.uk/research/rebecca-fitgerald

This PhD project in the Cellular and Molecular Biology Programme offers a unique opportunity for a highly motivated student to carry out research in a multi-disciplinary group of clinicians and cell biologists and to learn a wide range of post-genomic, advanced cell imaging and genome editing techniques relevant to cancer research.

Project Description

Background and Aims

Oesophageal cancers are the 8th most common cancer worldwide and the 6th most common cause of cancer death with only 15% surviving 5 years and one of cancer highlighted by CR-UK with unmet needs. There are two main subtypes of oesophageal cancer: adenocarcinoma (OEAC) and squamous cell carcinoma (OSCC). Oesophageal cancers develop slowly from a metaplastic condition called Barrett’s oesophagus (BE). Recent genomic analyses of paired BE and OEAC samples have indicated that OEAC can develop from BE via two different mechanisms: the genome-doubled (tetraploid) pathway and the non-genome-doubled pathway (1, 2). The genome-doubled pathway involves the loss of p53 and subsequent tetraploidy, whereas the non-genome doubled pathway features the progressive loss of tumour suppressor genes, including initially CDK2NA and SMAD4, and then p53. Ultimately, both pathways result in the amplification of oncogenes, genomic instability and the formation of an invasive cancer.

The laboratory of the Co-Investigator has found that more than 60% of OEAC present deletions and rearrangements in the FAM109A (also known as CCSER1) gene and there is a highly significant correlation between the presence of FAM109 deletions and tetraploidy in these cancers. Furthermore, FAM109 deletions/rearrangements are also found specifically in highly advanced
dysplastic BE cases. A preliminary study indicated that RNAi depletion of FAM109A caused cytokinesis failure and multinucleation (3). Interestingly, we have found that OEAC cell lines carrying rearrangements in the FAM109A gene show significant levels of multinucleation. Together, these findings lead to the hypothesis that deletion of FAM109A after loss of p53 might be the event triggering polyploidy in BE cells, which could subsequently lead to OEAC via the genome-doubled pathway. To investigate this possibility, we propose to:

1. Analyse in detail the function(s) and regulation of FAM109A in cell division;
2. Characterise FAM109A alterations in OAEC and assess whether the introduction of similar mutations in BE cells can lead to tetraploidy, genomic instability, and carcinogenesis.

Objectives

1. Analysis of the function and regulation of FAM109A in cell division

Although a preliminary study indicated a requirement for FAM109 in cell division (3), its exact function(s) are still unknown. Thus, to fully understand the role(s) of FAM109A in cell division we will deplete this protein in immortalised (non-cancerous) RPE cells and analyse the entire process of mitosis by time-lapse and immunofluorescence microscopy. The laboratory of the Lead PI has RPE cell lines that express fluorescent markers that will allow detailed visualisation in living cells of components of the mitotic machinery (spindle, chromosomes, centrosomes, etc.). These time-lapse experiments will reveal in which mitotic events FAM109A is required and, based on these results, we will then investigate whether FAM109A depletion affects the localization of other mitotic proteins involved in the same processes by immunofluorescence. In parallel, we will tag FAM109A with the Green Fluorescent Protein (GFP) using CRISPR/Cas9 gene editing and use this cell line to analyse FAM109A::GFP dynamics in living cells by time-lapse microscopy. This FAM109A::GFP cell line will also be used to isolate FAM109A::GFP and its associated partners (i.e. the FAM109A interactome) at different stages of cell division by affinity purification using GFP nanobodies followed by protein identification by mass spectrometry (MS). MS analysis will also identify post-translational modifications (i.e. phosphorylation and ubiquitination) of FAM109A at different mitotic stages, thus providing important insights into the regulation of this protein during mitosis. The outcome of these experiments will reveal in detail the precise role of FAM109A in cell division, including its dynamics, regulation, and protein interaction network. The laboratory of the Lead Investigator is at the forefront of cell division research and has expertise in all the described techniques [see refs (4, 5)].

2. Characterisation of FAM109A alterations in OAEC and their potential role in the establishment of tetraploidy, genomic instability, and carcinogenesis

The FAM109 gene is predicted to have at least five different transcripts coding for five different proteins (Ensembl database). How the different deletions identified in OEAC samples affect the expression of different FAM109A isoforms and their role in the development of BE into OEAC are, however, unclear. To address these questions, we will first characterise the expression of FAM109A in a large collection of OEAC tissue samples (whole genome shotgun – WGS - with matched RNA sequences will be available for 500 cases as part of the International Cancer Genome Consortium, ICGC) and cell lines (collected and maintained by the laboratory of the Co-Investigator). The protein level will be confirmed for cases of interest by Western Blot in cell lines and immunohistochemistry in tissue sections. Several commercial FAM109A antibodies are available and we could also generate additional ones against specific FAM109A epitopes if these prove necessary based on the RNAseq data. To understand if these mutants have any effect on cell division, we will carry out a combination of FAM109A mutant over-expression and RNAi complementation analysis in non-cancerous RPE cells followed by analysis of cell division using the microscopy techniques described in objective 1. Finally, we will introduce these FAM109A mutations in BE cells already carrying mutations in the p53 gene.
via CRISPR/Cas9 gene editing in order to test if these mutants can promote carcinogenesis and genomic instability using functional assays to evaluate cell kinetics, invasion and motility already up and running in the laboratory of the Co-Investigator. Finally, generation of organoids from primary tissues with matched WGS data will be also available to confirm our studies in cultured cell lines. The laboratory of the Co-Investigator has vast experience in the identification and characterisation of mutations in OEAC through the ICGC project.

Concluding remarks

In conclusion, this project will provide fundamental insights into the role of FAM109A in cell division and will address whether mutations in this gene have any role in the development of BE into OEAC. As deletion and rearrangements of FAM109A have also been found in pancreatic and lung cancers, our findings will be relevant also for other cancer pathologies.

References


Applications

To apply for this studentship please see http://www.cambridgecancercentre.org.uk/studentships

For general enquiries please contact: Tina Thorn tina.thorn@cruk.cam.ac.uk

For further information or questions relating to this project please contact:

Pier Paolo D’Avino ppd21@cam.ac.uk