CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Investigating the role of FAM190A in cell division and oesophageal cancer**
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Second supervisor if applicable	Rebecca Fitzgerald (MRC Cancer Unit)
Programme	Cellular and Molecular Biology
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Laboratory Location	Department of Pathology

Project Outline	**Aims and objectives** 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 the cancers highlighted by CR-UK with unmet needs. There are two main subtypes of oesophageal cancer: adenocarcinoma (OAC) 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 OAC samples have indicated that OAC 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 oncogene amplification, loss of tumour suppressors, genomic instability and the formation of an invasive cancer. The laboratory of the Co-PI (Rebecca Fitzgerald; MRC Cancer Unit) has found that more than 50% of OAC 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. Characterise *FAM109A* alterations and expression in OAC cancer cell lines and primary cancer samples. 2. Analyse in detail the function(s) and regulation of FAM109A in cell division.
Experimental plan	**1. Characterisation of *FAM190A* alterations and expression in OAC cancer cell lines and primary cancer samples.** The *FAM109* gene is predicted to have at least five different transcripts coding for five different proteins (Ensembl database). How the different rearrangements identified in OAC samples affect the expression of different FAM190A isoforms and their role in the development of BE into OEAC are, however, unclear. To start addressing these questions, the student will characterise the molecular re-arrangements in the *FAM190A* gene in a large collection of OAC tissue samples and

	cell lines collected and maintained by the laboratory of the Co-PI. In parallel, the students will analyse *FAM190A* expression at both mRNA and protein level in the same samples through the analysis of RNA sequencing (RNAseq) data and by Western Blot. **2. <u>Analysis of the function and regulation of FAM109A in cell division</u>** Although a preliminary study indicated a requirement for FAM109 in cell division (3), its exact function(s) are still unknown. To fully understand the role(s) of FAM190A in cell division we will deplete this protein by RNAi 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. In parallel, we will tag FAM190A with the Green Fluorescent Protein (GFP) using CRISPR/Cas9 gene editing and use this cell line to analyse FAM190A::GFP dynamics in living cells by time-lapse microscopy.
Main Techniques	The student will employ a wide range of techniques including: • bioinformatics analysis of Whole Genome Sequence (WGS) and RNAseq data • cell culture • molecular biology • RNA interference, microscopy and gene editing
Key References	**Ross-Innes CS, Becq J, Warren A, Cheetham RK, Northen H, O'Donovan M, et al.** *Whole-genome sequencing provides new insights into the clonal architecture of Barrett's esophagus and esophageal adenocarcinoma. Nature genetics. 2015;47(9):1038-46.* **Stachler MD, Taylor-Weiner A, Peng S, McKenna A, Agoston AT, Odze RD, et al.** *Paired exome analysis of Barrett's esophagus and adenocarcinoma. Nature genetics. 2015;47(9):1047-55.* **Patel K, Scrimieri F, Ghosh S, Zhong J, Kim MS, Ren YR, et al.** *FAM190A deficiency creates a cell division defect. Am J Pathol. 2013;183(1):296-303.*