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CANCER RESEARCH UK | CAMBRIDGE CENTRE

CRUK Cambridge Centre MRes rotation project

Rotation Project Title	Novel factors in the DNA damage response
Head of Laboratory (PI) Name	Svetlana Khoronenkova
Second supervisor if applicable	Luca Pellegrini
Programme	Cellular and Molecular Biology
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Laboratory Location	Department of Biochemistry

Project Outline	**Aims and objectives** Our laboratory is interested in identifying novel factors that are essential for the cell's ability to faithfully respond to DNA damage and facilitate its repair. This response to DNA damage lies at the heart of genome stability and provides the first line of cellular defence against cancer. We recently identified several novel factors of uncharacterized function that confer resistance to a variety of DNA damaging treatments. In this rotation project, we aim to unravel the cellular roles of TCF25, a predicted basic helix-loop-helix (bHLH) transcription factor, and its functions in the DNA damage response. Importantly, expression of *TCF25* is reduced in many cancers including ovary and prostate.
Experimental plan	The aim of this rotation project is to investigate the importance of TCF25 for cell survival following DNA damage. The applicant will establish *TCF25* knockout (ko) cell lines using CRISPR/Cas9 and complement the ko's with wild type or bHLH-domain deficient *TCF25*-encoding vectors. The sensitivities of these cell lines to genotoxic stress will be assessed using biochemical and cell biological approaches. Proteins interacting with mutant vs wild-type TCF25 will be identified using immunoprecipitation followed by mass spectrometry.
Main Techniques	Techniques to be utilised include: • mammalian tissue culture • CRISPR/Cas9 genome editing • cellular fractionations • clonogenic cell survival and single gel electrophoresis (Comet) assays • immunofluorescence, immunoprecipitation and mass spectrometry
Key References	**Khoronenkova S & Dianov G (2015)** *ATM prevents DSB formation by coordinating SSB repair and cell cycle progression. Proc Natl Acad Sci 112, 3997.* **Khoronenkova S *et al.* (2012)** *ATM-dependent down-regulation of USP7/HAUSP by PPM1G activates p53 response to DNA damage. Mol Cell 45, 801.*