Genetic interrogation of histone lysine demethylation by UTX

Principal supervisor's name: Dr **Iva Tchasovnikarova**
Principal supervisor's email address: it257@cam.ac.uk

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

Department for student registration: Biochemistry
Department or institute where research will take place: The Gurdon Institute

Postgraduate scheme:

- **MRes + PhD (1 + 3-year non-clinical applicants)**
- **Part-time MRes + PhD (2 + 3-year clinical applicantsy)**

MRes project outline:

UTX is a histone lysine demethylase which removes the repressive histone modification H3K27me3. UTX is one of the most frequently mutated epigenetic factors in human cancers. Loss-of-function mutations in UTX are found in multiple cancer types; for example, inactivating mutations in UTX account for 29% of bladder carcinomas and loss of UTX induces spontaneous leukaemia in mice. Despite the importance of UTX as a tumour suppressor, many outstanding questions remain regarding its mechanism of action. For example, UTX lacks a DNA-binding domain, and so how it is recruited to its target loci remains unclear. Indeed, size-exclusion chromatography shows that only ~30% of UTX protein co-elutes with its known binding partners within the COMPASS complex, suggesting that UTX exerts its function through unidentified co-factors.

The rotation project will build on the results of a novel high-throughput, genome-wide assay – TRACE – in which genetically barcoded constructs integrated randomly across the genome report on the chromatin environment in which they are situated through GFP expression. By comparing GFP expression in the presence and absence of UTX, we have identified hundreds of genomic loci responsive to UTX function. The aim of the rotation project is to generate fluorescent reporters at the leading sites identified by TRACE, and exploit them to identify collaborative factors required for UTX-mediated gene regulation and to delineate the protein domains required for function.

MRes experimental plan:

1. Use CRISPR/Cas9-mediated knock-in to generate individual clones carrying GFP reporters at the leading sites identified by TRACE;
2. Use CRISPR/Cas9 to generate UTX knockout lines in the reporters;
3. Express mutant versions of the demethylase in UTX knockout lines to delineate the protein domains required for function;
4. Carry out a genome-wide, FACS-based CRISPR/Cas9 screen to identify novel factors required for UTX function.

Main Techniques

- Mammalian cell culture
- Lentivirus production
- Genetically-encoded fluorescent reporters
- CRISPR-mediated genome editing
- Flow cytometry / FACS
- Molecular cloning

PhD project outline:

UTX is one of the most frequently mutated epigenetic factors in human cancers. A key challenge is to understand the molecular basis of the phenotypes observed upon UTX loss-of-function mutations and their reliance on catalytic activity. Harnessing TRACE, you will generate cell-based reporters of UTX function, which you will exploit to understand its mechanism of action, the role that demethylation plays in gene regulation and the functional interplay between UTX and its catalytically-inactive paralogue UTY.

PhD experimental plan:

1) Interrogate TRACE-generated reporter lines to gain mechanistic insight into UTX function. Individual GFP reporter clones responsive to UTX depletion will be subjected to a battery of genetic assays to identify collaborative factors required for UTX-mediated gene regulation and to delineate the protein domains required for function. These will be complemented by co-immunoprecipitation experiments to define the physical interactions between these collaborative factors and UTX, the functional relevance of which will be studied using phenotypic, biochemical and genomics approaches.

2) Generate reporters responsive to the non-catalytic function of UTX. As the tumour suppressive functions of UTX do not require its catalytic activity, you will also apply TRACE to study the catalytic-independent functions of UTX and its catalytically-inactive paralogue UTY. You will overexpress UTY and wild-type and catalytically-inactive UTX mutants in the male monocytic cell line THP-1, which lacks functional UTX and UTY proteins, and perform TRACE in a comparative manner to identify reporters whose expression is specifically regulated independently of catalytic activity. Individual clones carrying GFP reporters identified by TRACE will be genetically interrogated to dissect the underlying mechanism of action.

3) Identify novel tumour suppressors acting alongside UTX. To examine the tumour-suppressive function of collaborative factors identified in your genetic screens, you will manipulate their abundance in UTX-mutant cancer cell lines and perform colony-forming assays. You will also perform colony-forming assays in CD34+ HSPCs depleted of collaborative factors.

Main techniques:

- Mammalian cell culture
- Lentivirus production
- Genetically-encoded fluorescent reporters
- CRISPR-mediated genome editing
- Flow cytometry / FACS
- Molecular cloning
- Colony-forming assays

Key references:

Tchasovnikarova IA, Marr SK, Damle M, Kingston RE (2022) TRACE generates fluorescent human reporter cell lines to characterize epigenetic pathways. Molecular Cell, 82 (2): 479-491.

Wang L and Shilatifard A (2019) UTX Mutations in Human Cancer. Cancer Cell, 35 (2): 168-176.