Characterization of primate-specific systems regulating aspects of the DNA damage response

Principal supervisor's name: Dr **Michael Imbeault**
Principal supervisor's email address: mi339@cam.ac.uk

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

Department for student registration: Genetics
Department or institute where research will take place: Genetics.

Postgraduate scheme: **MRes + PhD (1 + 3-year non-clinical applicants only)**

MRes project outline:

The Imbeault lab is focused on the study of KRAB zinc finger proteins (KZFPs), the largest family of transcription factors in the human genome with 350 protein-coding members. These epigenetic silencers target transposable elements and restrict their replication potential. We have discovered that this process also plays a role in the long term domestication of transposable elements and the evolution of gene regulatory networks. While little is known about the function of most evolutionary conserved KZFPs, the few examples we have showcase they can have surprisingly important functions in regulating core biological processes such as genomic imprinting, pancreas development and inflammation. The proposed MRes project aims to investigate KZFPs we have identified as playing a role in regulating the DNA damage response in primates.

For example, we have recently identified a primate-specific KZFP at the root of a p53-responsive dynamic system. It is itself upregulated early during the DNA damage response and it is repressing transposable element targets containing p53 binding sites. Initial studies in MCF7 breast cancer cells reveal that this system is controlling the expression of an important component of the ubiquitin-proteasome pathway related to regulation of death-associated kinases. The MRes project aims to further characterize the dynamics of this system and probe the function of its targets in relationship with time after DNA damage events. Its main objective is to quantify the dynamics at the RNA and protein level of all components of the system and phenotypically characterize the effects of its dysregulation. This is the first step to characterize the functional effects of deletion of this KZFP in cancer, notably in Uterine corpus endometrial carcinoma (UCEC) where it is often lost.

MRes experimental plan:

The main approach will aim to transcriptionally modulate KZFPs and their targets. You will use CRISPRi (a fusion of Cas9 and the KRAB domain to epigenetically silence a genomic locus) using designed guide RNAs to silence either the KZFP itself or its transposable element targets. You will then assay gene expression, first using qRT-PCR and in later stages RNA sequencing. This will be performed in different cell lines with various basal expression levels of the KZFP. The inducibility of the system will be tested at early and late time points following DNA damage

insults such as gamma-ray irradiation or wusing double-strand breaks inducing chemical agents. Proteomic analysis of KZFPs and target genes will be performed to gain insight in dynamics - this will be potentially followed by microscopy-based observation of specific components and markers.

PhD project outline:

Related to the MRes plan described above, the best p53-inducible KZFP we have currently identified is part of a larger, yet to be functionally characterized system. The genomic locus where it is found contains both a second protein-coding KZFP paralog and a locus coding for their antisense at the RNA level. This 3-component system has recently been picked up in a codeletion CRISPR screen looking for pairs of genes controlling cell proliferation (see reference below) - the KZFP pair is the second hit in terms of magnitude, with an effect size comparable to the gold standard CDKN2A/CDKN2B. Both protein-coding KZFP in the locus have affinity for transposable element targets that harbor p53 binding sites, yet they target different genomic loci. Furthermore, we find transposable elements of the same family in close proximity of the promoter of both KZFPs, hinting at a regulatory feedback loop. Importantly, the pair has been found to be deleted in 25% of Uterine corpus endometrial carcinoma (UCEC).

The PhD project aims to perform an in-depth characterization of the full system to improve our understanding of its implication in biology and cancer. The major objective is to perform a functional analysis of all components of the system (protein-coding KZFPs, antisense non-coding RNA, transposable element targets), either in isolation or in combination. This project is an exciting opportunity to understand how a primate-specific system can partially rewire an evolutionary conserved process with important implications for human health.

PhD experimental plan:

As a continuation of the MRes, many aspects of the workflow will be similar but expanded in scale and complexity. CRISPR based approaches will be used to build experimental systems that will be assayed with genome-wide transcriptomic and epigenetic techniques. To supplement results obtained with CRISPRi, full knockouts of components of the system studied will also be performed. The deletion of specific transposable elements within the genome that have been identified as important is planned. On top of RNA-seq transcriptomic analysis and genome-wide quantification of relevant epigenetic marks, 3D conformation capture assays will be performed to understand causal links between both.

Phenotypic characterization will be performed to assess the impact of dysregulation on cell proliferation. Initially cancer cell lines will be used with a planned move to primary samples for validation. For example, epigenetic characterization of cancer samples obtained through collaboration with the clinic is an axis that is envisioned, as is the validation by qRT-PCR in primary cells of dysregulated genes found in model cell lines. Collaboration within and outside our group to datamine large cancer genomic datasets to correlate patterns observed experimentally is an important component of the project.

Main techniques:

CRISPR/Cas9, CRISPRi, ChIP-seq, qRT-PCR, RNA-seq, Tagmentation, Bioinformatics, microscopy, Western blot

Key references:

Parrish PCR, Thomas JD, Gabel AM, Kamlapurkar S, Bradley RK, Berger AH. Discovery of synthetic lethal and tumor suppressor paralog pairs in the human genome. Cell Rep. 2021 Aug 31;36(9):109597. doi: 10.1016/j.celrep.2021.109597. PMID: 34469736; PMCID: PMC8534300.

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