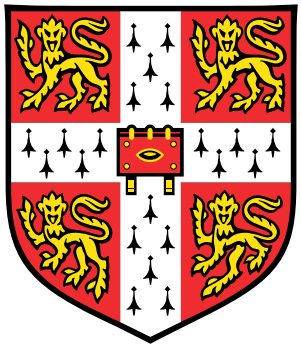
Identifying mediators of malignant transformation in human cancer using genome-wide forward genetic screening approaches



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I would like to dedicate this thesis to my parents, grandparents and my brother Jack for their ongoing love and support, and to Ben, Lily and Martha for their reassurance and encouragement during the writing process.

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Declaration and specified in the text. Additionally, this thesis does not exceed the prescribed word limit for the relevant Degree Committee.

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Abstract

Malignant transformation is the transition of a cell from a normal state of proliferative homeostasis to a state of abnormal over-proliferation, acting as the initial step of tumourigenesis. This process is governed by the mutation of genes controlling cellular processes such as cell division, DNA replication and growth signaling. In this project, genome-wide forward genetic screening approaches were used to identify novel candidate genes involved in transformation. The model system used was the transformation-sensitive murine cell line NIH3T3, in which genes were assessed for their ability to initiate the formation of transformed foci of proliferation when mutated *in vitro*.

Firstly, the NIH3T3 genome was sequenced to characterise its genetic background and identify possible reasons for its transformation-sensitive phenotype. This was accompanied by Multiplex - Fluorescence *In Situ* Hybridisation to investigate large-scale genomic alterations. These approaches identified specific indels and single nucleotide variants in known cancer-associated genes, and large-scale genomic alterations, both of which may contribute to the transformation sensitivity of the cell line. The karyotype was discovered to be highly abnormal and heterogeneous, suggesting high chromosomal instability and continuing evolution of the line. This work has provided valuable insight into the limitations of this model and has implications for its use in this project and beyond.

The first genetic screening approach used was a pooled CRISPR-Cas9 genome-wide knockout screen, identifying candidate tumour suppressor genes by generating loss-of-function mutations. Genes causing an increase in proliferative focus formation when knocked out were identified by sequencing the guide RNA population and identifying those that were overrepresented in the cultured cells using the algorithm Model-based Analysis of Genome-wide CRISPR-Cas9 Knockout. This identified putative genes associated with transformation, which were then compared with existing mutation data from human cancer sequencing efforts. This screen successfully identified some known cancer-associated tumour suppressor genes, along with potential novel candidates. As a complementary approach, a genome-wide transposon-based screen was also conducted, activating genes by inserting the CMV promoter at random throughout the genome using a *PiggyBac*-based transposon. Recovery of the insertion sites in the final cell population to locate sites that are overrepresented is currently in progress, aiming

to identify putative oncogenes.

While further work to validate the candidates identified is needed, this work has made some progress towards identifying novel transformation-associated genes. If these genes can be validated, they may provide useful insights into the biology of early tumourigenesis, informing further research and possible therapeutic targets.

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