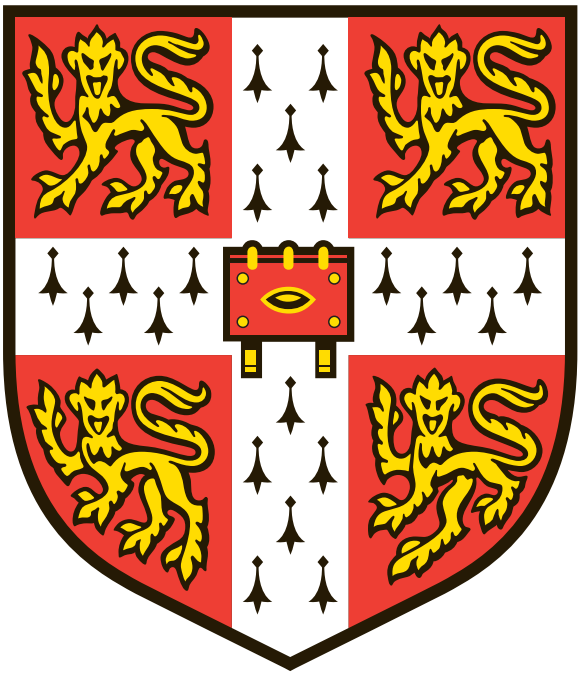
Application of CRISPR/Cas9 screening to study cancer drivers and to identify novel cancer vulnerabilities



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I would like to dedicate this thesis to maw, paw and Danielle;
I hope I can make you all as proud of me as I am of you.

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 Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

This dissertation does not exceed 60,000 words in length, exclusive of tables, footnotes, bibliography, and appendices.

Gemma Turner

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"Whit's fur ye'll no go by ye."

Abstract

The development of targeted therapies has had a significant impact on cancer survival rates. However, targeting cancers that are driven by loss of tumour suppressor genes remains a major challenge. One promising approach to treat these cancers is the exploitation of synthetic lethal interactions. Synthetic lethality describes an interaction between two genes, where loss of one gene alone does not affect viability but loss of both genes induces cell death. Inhibiting the synthetic lethal partner of a tumour suppressor gene should specifically kill tumour cells, and so these represent potential therapeutic targets. However, very few synthetic lethal interactions have been well-established.

The aim of this project was to systematically screen for synthetic lethal partners of known tumour suppressor genes. To do so, isogenic human induced pluripotent stem cell lines were generated, each carrying a loss-of-function mutation in a single tumour suppressor gene. These cells have a normal genetic background, thus making it simpler to accurately identify interactions. CRISPR/Cas9 technology was applied as it allows for large-scale, unbiased screening of genetic interactions. A genome-wide guide RNA library was prepared and implemented for knockout screening in the isogenic cell line panel. Analysis was performed to identify genes that were specifically essential for cell fitness/survival in the mutant lines. Particular focus was placed on four tumour suppressor genes that encode subunits of the PBAF/BAF complexes. Approximately 20% of human cancers harbour mutations in subunits of these complexes, so identifying dependencies associated with these could have broad therapeutic potential. Candidate synthetic lethal interactions with these genes were investigated using low-throughput assays in the stem cells and in a cancer cell line. The data obtained suggests that screening in stem cells produces highly variable results. Although potential vulnerabilities associated with all of the tumour suppressor genes were identified, further work is required to validate these and to assess the quality of the results.

In addition to genome editing, CRISPR/Cas9 has been adapted as a tool for controlling gene regulation. In collaboration with Dr Louise van der Weyden, I applied this technology to address another challenging area of cancer biology. Metastasis is the main cause of cancer mortality, yet we still have a poor understanding of the genes that control this process. Considering this, an *in vivo* CRISPR activation screen was performed to identify novel drivers of metastatic colonisation. A mouse melanoma cell line was transduced *in vitro* with a library designed to up-regulate expression of membrane proteins, which represent ideal drug targets.

These cells were then used in an *in vivo* experimental metastasis assay. Enrichment of guide RNAs in the lungs was assessed to identify genes that increased pulmonary metastatic colonisation when activated. Candidate genes were selected using three analysis strategies, and hits from each were tested. Several genes were successfully validated using the experimental metastasis assay. The most robust hit was studied further to explore its potential as a therapeutic target.

Collectively, the work described in this thesis demonstrates how CRISPR/Cas9 screening can be applied in different model systems to study genes that drive cancer and to explore novel therapeutic strategies.

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Abbreviations

AUC	area under the curve
BAF	BRG-/BRM-associated factor
BAGEL	Bayesian Analysis of Gene EssentiaLity
BF	Bayes factor
BFP	blue fluorescent protein
bp	basepair
Cas	CRISPR associated protein
CGaP	Cellular Genotyping and Phenotyping facility
CRISPR	Clustered Regularly Interspaced Palindromic Repeats
CRISPRa	CRISPR activation
CRISPRi	CRISPR interference
crRNA	CRISPR RNA
dCas9	deactivated/dead Cas9
DSB	double-strand break
FACS	fluorescence-activated cell sorting
FBS	fetal bovine serum
FDR	false discovery rate
gDNA	genomic DNA
GFP	green fluorescent protein
gRNA	guide RNA
GSH	glutathione
HDR	homology-directed repair
hESCs	human embryonic stem cells
indel	insertion/deletion
iPSCs/iPS cells	induced pluripotent stem cells
IRES	internal ribosomal entry site
KO	knockout
LB	Luria broth
LC-MS	liquid chromatography mass spectrometry
LOF	loss-of-function
M-FISH	multiplex fluorescence *in situ* hybridisation

MAGeCK	Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout
MEFs	mouse embryonic fibroblasts
MOI	multiplicity of infection
MRT	malignant rhabdoid tumour
MSI	microsatellite instability
MUT	mutant
ncBAF	non-canonical BAF
NHEJ	non-homologous end joining
NTC	non-targeting control
OCCC	ovarian clear cell carcinoma
PAM	protospacer-adjacent motif
PBAF	polybromo-associated BAF
PBS	phosphate-buffered Saline
PFA	paraformaldehyde
PrRc	precision recall
RNAi	RNA interference
ROC	receiver operator curve
RRA	robust rank aggregation
shRNA	short hairpin RNA
siRNA	short interfering RNA
SLI	synthetic lethal interaction
TBS-T	Tris-Buffered Saline supplemented with 0.1% Tween-20
TCGA	The Cancer Genome Atlas
tracrRNA	trans-activating crRNA
TSG	tumour suppressor gene
WSI	Wellcome Sanger Institute
WT	wildtype