Genome-wide recessive screens for DNA mismatch repair genes in mouse ES cells

This dissertation is submitted for the degree of Doctor of Philosophy.

By Zikai Xiong

The Wellcome Trust Sanger Institute
Hughes Hall, University of Cambridge

DECLARATION

I hereby declare that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text.

None of the material presented herein has been submitted previously for the purpose of obtaining another degree.

Zikai Xiong

ACKNOWLEDGEMENT

I would like to take this opportunity to formally thank all the people who supported me during the course of my PhD project.

My deepest thank is to my PhD supervisor Dr Allan Bradley, for his enormous support and excellent advice through the whole project. It was great pleasure to work with Allan and achieve challenging tasks in his laboratory. I thank his patience as well as encouragement during the hard time of this project.

I have special thanks to all Bradley laboratory members for their communication and kind help. I am particularly grateful to my wife Dr Wei Wang for her huge support in both my life and career. We have had numerous scientific discussions and collaborations during these experiments. I learned recombinant retroviral techniques from her. Many thanks are also due to Oliver Dovey for his detailed guidance and contribution to array CGH experiments. I also want to thank Peter Ellis for his help on expression arrays and I am grateful to Gregory Lefebvre, Keith James and Robert Andrews for their help in bioinformatics analysis. I thank Dr Ge Guo and Dr Wei Wang (Male) for their instructions in ES cells' culture and molecular manipulations and valuable discussions. I also thank Dr Yue Huang for discussions at the beginning of the project. I thank Haydn Prosser for providing vectors and advice, Qi Liang for comfirming the recombination medicated cassette exchange ability of my cell line in her experiments. I am grateful to Frances Law and Alastair Beasley for their help in cells' culture and routine laboratory issues. I thank Shaun Cowley, Juan Cadinanos, Roland Rad, George Vassiliou, Antony Rodriguez, Nathalie Conte for valuable scientific discussions.

The advice and feedback from my PhD committee were precious. The members were Pentao Liu, Jessica Downs, Andrew Fraser, Derek Stemple and Allan Bradley, who shared their critical thinking and provided excellent advice for my project.

I must say "Thank you!" to my parents Yan Xiong and Huiqin Zhu. Their endless support never leaves me.

My 4-year full studentship is sponsored by the Wellcome Trust.
Registered charity number 210183

ABSTRACT

Genome-wide genetic screens on libraries containing homozygous mutant mouse embryonic stem (ES) cells make it feasible to examine all mouse genes expressed in ES cells for their role in any biological process which is active in ES cells. Bloom syndrome protein (Blm) deficient ES cells have high mitotic recombination and loss of heterozygosity rates, which allow cells with homozygous mutations to be generated in populations of mutated *Blm*-deficient ES cells. A genome-wide mutation library was generated in this genetic background using gamma radiation. Analysis of isolated clones from this library by high-resolution Comparative Genomic Hybridization (CGH) arrays revealed that each carried several duplications or deletions ranging in size from 0.1 to 50Mb. This mutation library provided good coverage of the mouse genome and it is a new genetic resource for conducting loss-of-function genetic screens in mammalian cells.

In humans, mutations in components of the DNA mismatch repair (MMR) system cause hereditary non-polyposis colorectal carcinoma (HNPCC, Lynch syndrome). Mutations in the *MSH2* and *MLH1* genes account for the majority of the cases. To extend our understanding of the MMR system a recessive screen was implemented in the mutation library to identify new components by selection in 6-thioguanine (6TG). This nucleotide analogue is incorporated into DNA and is recognized by the DNA MMR complex, leading to cell death in wild type cells while MMR-deficient cells are viable. In this screen, several independent mutants with homozygous deletions covering the MMR genes *Msh2* and *Msh6*, and one mutant with a heterozygous deletion at the *Dnmt1* gene were isolated. Mutants were also isolated in which a number of unknown genes were deleted. Joint analysis using genomic and transcriptional arrays discovered that a set of genes, including *Msh2* and *Msh6*, were deleted and silenced in the $6TG^R$ mutants. The most frequently silenced genes are highly likely to be involved in the MMR pathway.

This screening system confirmed that irradiation is efficient in generating genome-wide loss-of-function mutation libraries in the *Blm*-deficient cells, and combined with DNA- and RNA-based array analysis platforms, provides a new approach for phenotype-driven recessive genetic screens in mouse ES cells.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

6TG	6-Thioguanine
BAC	Bacterial Artificial Chromosome
Blm	Bloom syndrome homolog (human) gene
BME	β-Mercaptoethanol
bp	base pairs
Bsd	Blasticidin resistance gene
Bcyd	Blasticidin S HCl
CGH	Comparative Genomic Hybridization
DMSO	Dimethyl Sulfoxide
Dnmt1	DNA methyltransferase (cytosine-5) 1 gene
DSB	Double-Strand Break
ENU	N-ethyl-N-nitrosourea
FBS	Foetal Bovine Serum
FIAU	1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodouracil
GPS	Glutamine-Penicillin-Streptomycin
Hprt	Hypoxanthine guanine phosphoribosyl transferase 1
HSVtk	Herpes Simplex Virus type 1 thymidine kinase
IR	Ionizing Radiation
LET	Linear-Energy-Transfer
LOH	Loss of Heterozygosity
Mlh1	mutL homolog 1 (*E. coli*)
Mlh3	mutL homolog 3 (*E coli*)
MMS	Methyl Methane Sulfonate
MMuLV	Moloney Murine Leukemia Virus
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
Msh2	mutS homolog 2 (*E. coli*)
Msh3	mutS homolog 3 (*E. coli*)
Msh4	mutS homolog 4 (*E. coli*)
Msh5	mutS homolog 5 (*E. coli*)
Msh6	mutS homolog 6 (*E. coli*)
NCBI	National Center for Biotechnology Information
NMD	nonsense-mediated decay
OMIM	Online Mendelian Inheritance in Man
PBS	Phosphate Buffered Saline
PCNA	Proliferating Cell Nuclear Antigen
PCR	Polymerase Chain Reaction
PGK	phophoglycerate kinase
Pms1	postmeiotic segregation increased 1 (*S. cerevisiae*)
Pms2	postmeiotic segregation increased 2 (*S. cerevisiae*)
PTC	premature termination codon
Puro	puromycin N-acetyltransferase gene
RFLP	Restriction Fragment Length Polymorphism
RMCE	Recombination Mediated Cassette Exchange
SDS	Sodium dodecyl sulphate
SIGTR	Sanger Institute Gene Trap Resource
SSB	Single-Strand Break
SSLP	Simple Sequence Length Polymorphism