

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

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

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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

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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.

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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 (<i>E. coli</i>)
Mlh3	mutL homolog 3 (<i>E. coli</i>)
MMS	Methyl Methane Sulfonate
MMuLV	Moloney Murine Leukemia Virus
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
Msh2	mutS homolog 2 (<i>E. coli</i>)
Msh3	mutS homolog 3 (<i>E. coli</i>)
Msh4	mutS homolog 4 (<i>E. coli</i>)
Msh5	mutS homolog 5 (<i>E. coli</i>)
Msh6	mutS homolog 6 (<i>E. coli</i>)
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	phosphoglycerate kinase
Pms1	postmeiotic segregation increased 1 (<i>S. cerevisiae</i>)
Pms2	postmeiotic segregation increased 2 (<i>S. cerevisiae</i>)
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