A CHROMOSOME-SPECIFIC RECESSIVE GENETIC SCREEN FOR GENES INVOLVED IN *IN VITRO* DIFFERENTIATION IN MOUSE EMBRYONIC STEM CELLS

A dissertation submitted in fulfilment of the requirements 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 specially indicated in the text. None of the material presented herein has been submitted previously for the purpose of obtaining another degree.

Wei Wang

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ABSTRACT

Mouse embryonic stem (ES) cells are pluripotent cells that retain unlimited self-renewal potential. ES cells can also differentiate into all the three germ layers and many specified cell types *in vitro*. The *in vitro* differentiation of ES cells recapitulates early embryogenesis, thus serves as a valuable *in vitro* model for developmental studies. Little is known about the genes which are important in the ES cell differentiation process, partly due to the difficulty of generating homozygous mutations in ES cells. In this study, we have developed a method, which explored induced mitotic recombination and regional trapping mutagenesis method to accumulate large numbers of homozygous gene-trap mutations in a genomic region of interest in mouse embryonic stem cells. A cell line was engineered to undergo mitotic recombination and to capture the subset of gene-trap mutations that were generated on chromosome 11. A large panel of genome-wide gene-traps were generated in this cell line and those that are located on chromosome 11 were specifically selected via Cre/*loxP* mediated inversions. The inversions were then made homozygous by induced mitotic recombination. Using this system, 66 independent homozygous gene-traps on chromosome 11 have been isolated from a library of about 10,000 gene-trap clones. These homozygous clones have been assessed for their developmental potential by an *in vitro* differentiation assay. The differentiation of each of these lines has been assessed by RT-PCR using a panel of markers that are characteristic for the three germ layers and various differentiated cell types. Clones that show abnormal expression of one or more markers are verified using microarrays, western blotting and *in situ* hybridization. A homozygous mutation of ATP-citrate lyase (*Acly*) gene was found to block the differentiation of ES cells *in vitro*. Under retinoic acid induction, the embryoid bodies derived from the mutated cell line were still mostly composed of undifferentiated embryonic stem cells. This was confirmed by the high expression of the epiblast makers, such as *Oct3/4*, *Nodal* and *Nanog*. BAC rescue experiment has been carried out to reverse the phenotype of this cell line and make a causal link between the expression level of *Acly* and the

phenotype. Therefore, we have shown that we can create random homozygous gene-trap mutagenesis in a candidate region of the genome and use these clones for functional genomics study.

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