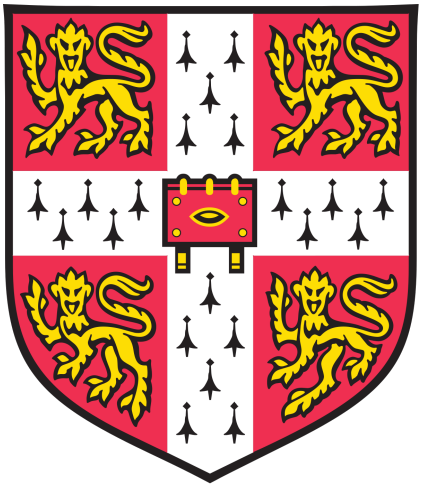
Genome-wide search for regulators of the first cell lineage decision in the mouse: a CRISPR/Cas9 endeavour



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DECLARATION

I hereby declare that the contents of this thesis are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other University. 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. This thesis does not exceed the 60.000 word limit set by the Faculty of Biology.

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ABSTRACT

In vitro cell lines derived from the early embryo retain the identity and lineage restrictions of their embryonic counterparts: embryonic stem cells (ESCs) are pluripotent and similarly to ICM contribute to all tissues in the embryo proper, being generally excluded from the extraembryonic layer. On the other hand, trophoblast stem cells (TSCs) resemble TE and are restricted to the extra-embryonic lineage. Despite the efforts of several groups, it is still not possible to completely convert ESCs to TSCs. The molecular players that underpin this restriction in ESCs remain largely unknown. I therefore proposed to identify suppressors of ESC differentiation to trophoblast in an unbiased approach, using CRISPR/Cas9 genome-wide loss of function screening.

I developed a reporter cell line that constitutively expressed *Cas9* from the *Rosa26* locus, and was engineered with *knock-in* of *T2A-Venus* into the *Elf5* locus (*Elf5::Venus* ESCs). *Elf5* is a stringent TSC marker that fails to be activated in most published methods for ESC conversion to TSC. Proof-of-concept experiments used *Oct4* knockout via CRISPR/Cas9 to validate this line for both efficient gene editing and faithful tracking of TSC differentiation. *Elf5::Venus* ESCs were then transduced with a genome-wide lentiviral gRNA library for CRISPR/Cas9 genetic screen. Mutant cells were differentiated in TSC media for fourteen days, at which point Venus positive and negative populations were sorted. gRNA representation was subsequently analysed by next generation sequencing. At a false discovery rate of 10%, I could identify 42 genes whose loss of function allowed ESC conversion to TSC. I could validate 22 genes with different phenotype strength, implicated in the maintenance of the first cell lineage decision. Importantly, the screen identified four components of the non-canonical Polycomb Repressive Complex 1.1 as strong lineage regulators. The role of two of these genes, *Bcor* and *Rnf2*, was further explored in ESCs for their differentiation profile as well as transcriptional changes that underline their mechanism. Preliminary data for *in vivo* chimera assays showed that derivatives of ESCs deficient for *Bcor* or *Rnf2* could be found within extraembryonic tissues in chimeric embryos. Further studies however will be necessary to assess both reproducibility and cell identity, and evaluate if this lineage restriction is indeed impaired *in vivo* in knockout ESCs.

Overall, my studies demonstrate the power of CRISPR/Cas9 screen, highlighting new layers of regulation for the first cell lineage decision and its maintenance in ESCs. I believe further characterisation of remaining screen hits will continue to elucidate the molecular mechanisms of TE and ICM segregation in early embryos.

TABLE OF CONTENTS

LIST OF FIGURES