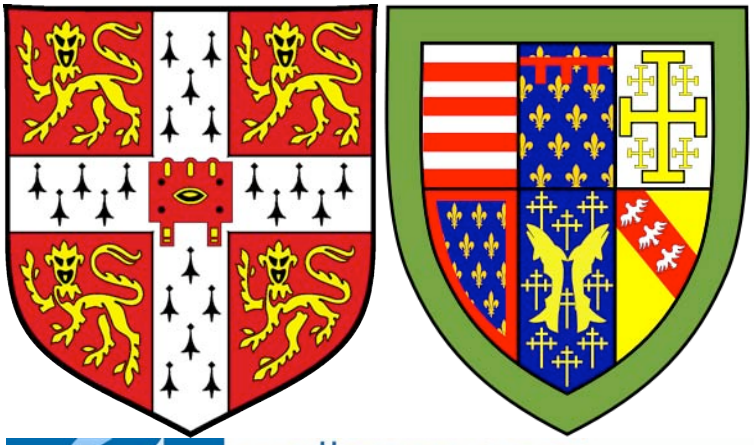
REPROGRAMMING OF T CELLS TO NATURAL KILLER-LIKE CELLS UPON BCL11B DELETION

A Dissertation submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

By Peng Li



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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. I confirm that this thesis does not exceed 300 single sided pages of double spaced text, or 60,000 words.

Peng Li

For always being there, Dad,

Mum, and Wife

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T cells develop in the thymus and play critical roles in immunity. In mice, the transcription factor Bcl11b is required for fetal thymocyte development and for double-positive thymocyte selection. Using a *Bcl11b-tdTomato* knock-in reporter mouse, I found that *Bcl11b* was T-cell-restricted, and was expressed from very early thymocytes to all mature T cells.

To study the functions of Bcl11b in adult T cells, I used a *Bcl11b* conditional knockout mouse strain and demonstrated that Bcl11b was indispensable for early T cell development and for the maintenance of T cell identity. Deletion of Bcl11b caused early T cells to lose their T cell potential and differentiate to natural killer (NK)-like cells in T cell cultures. Similarly, acute loss of Bcl11b in committed and mature T cells resulted in the reprogramming from T cells to induced-T-to-natural killer (ITNK) cells in a cell-autonomous manner. ITNKs derived in vitro and in vivo exhibited many NK cell features, such as expression of NK cell surface markers and lysis of NK tumor targets. In addition, ITNKs derived in vivo were able to prevent the outgrowth of tumour cells in a mouse model.

The gene expression profiles of ITNKs were also similar to that of regular NK cells, and not their parental T cells. ITNKs upregulated NK cell-associated genes while downregulated T cell genes, suggesting that Bcl11b might regulate the T-versus-NK cell fate choice. Furthermore, results from chromatin immunoprecipitation assays confirmed that the canonical Notch signaling directly regulated Bcl11b transcription level.

In summary, I showed that Bcl11b is essential for T cell development and is currently the only known transcription factor critical for the maintenance of T cell identity. Finally, it is believed that human ITNKs may potentially be exploited for therapeutic use in cancer treatments.

LIST OF ABBREVIATIONS

β-gal	β-galactosidease
ACT	Adoptive cell transfer
AGM	Aorta-gonand-mesonephros
AIDS	Acquired immune deficiency syndrome
BAC	Bacteria artificial chromosome
BCA	BioCinchominic Acid
Bcl	B-cell lymphoma/leukaemia
BCR	B cell receptor
BM	Bone marrow
BSA	Bovine serum albumin
ChIP	Chromatin immunoprecipitation
CKO	Conditional knockout
CLP	Common lymphoid progenitors
CMP	Common myeloid progenitors
COUP-TF	Chicken ovalbumin upstream promoter transcription factor
CRAC	Calcium release-activated $Ca2^{+}$
CTIP	COUP-TF interacting protein
CTLs	Cytotoxic T lymphocytes
DAG	Diacylglycerol
DMSO	Dimethyl sulfoxide
DN	Double negative ($CD4^{-}CD8^{-}$)
Dox	Doxycyclin
DP	Double positive ($CD4^{+}CD8^{+}$)
dpc	Days post-coitum
eGFP	Enhanced green fluorescent protein
ENU	N-ethyl-N-nitrosourea
ER	Estrogen receptor
ES	Embryonic stem
ETP	Early T cell precursors
FACS	Fluorescent-activated cell sorting
FCS	Fetal calf serum
FDG	Fluorescein di-β-D-galactopyranoside
Flt	Fms-like tyrosine kinase receptor
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte/macrophage colony-stimulating factor
GMP	Granulocyte macrophage progenitors
HD	Huntington's disease
HDAC	Histone deacetylase
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigens
HRP	Horseradish peroxidase
HSC	Hematopoietic stem cell
IFN	Interferon
IHC	Immunohistochemistry
IL	Interleukin
IRES	Internal ribosome entry site
iPS	Induced pluripotent stem
IP3	Inositol trisphosphate

ISP	Immature single-positive
ITAMs	Immunoreceptor tyrosine-based activation motifs
ITK	Inducible T cell kinase
ITNK	Induced T-to-natural-killer
LAK	Lymphokine-activated killer
LCK	Lymphocyte protein-tyrosine kinase
Lin	Lineage
M-CSF	Macrophage colony-stimulating factor
MEP	Megakaryocyte erythroid progenitors
MHC	Major histocompatibility complex
MTA	Metastasis-associated proteins
NCoR	Nuclear receptor co-repressor
NICD	Notch intracellular domain
NK cells	Natural killer cells
NKP	Natural killer cell precursor
NKT cells	Natural killer T cells
NSC	Neural stem cells
NuRD	Nucleosome remodeling and histone deacetylase
NZB	New Zealand black
OHT	4-hydroxytamoxifen
PAGE	Polyacrylamide gel electrophoresis
PB	PiggyBac
PI	Phosphatidylinositol
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLCγ1	Phosphorylation of phospholipase C γ 1
polyA	Polyadenylation signal
pre-BCR	pre-B cell receptors
Pre-TCR	Pre-T cell receptors
qRT-PCR	Quantitative real-time reverse transcription PCR
RNAi	RNA interference
RT	Room temperature
SCF	Stem cell factor
SCID	Severe combined immune deficiency
SIRT1	Sirtuin 1
SMRT	Silencing mediator for retinoid and thyroid hormone receptor
SP	Single positive
T-ALL	T-cell adult leukemia/lymphoma
TCR	T cell receptor
tdTomato	Tandem dimmer Tomato
Tet	Tetracycline
TetR	Tet repressor protein
TGF	Transforming growth factor
Th2	T-helper-2
TIL	Tumour-infiltrating lymphocyte
TNF	Tumour necrosis factor
TNKP	T/NK progenitor
TRE	Tetracycline response element
tTA	Tetracycline-controlled transactivator
UTR	Untranslated region
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

Zap-70	Z-chain associated protein kinase
ZFN	Zinc finger nuclease

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