Examination of Pancreatic Transdifferentiation in the Livers of Animals

by Que Tran La

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of:
Professor Ann M. Simpson and
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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Que Tran La declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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List of Publications

1. La, Q.T., Ren, B., Logan, G.J., Cunningham, S.C., Khandekar, N., Nassif, N.T., O'Brien, B.A., Alexander, I.E., and Simpson, A.M., *Use of a Hybrid Adeno-Associated Viral Vector Transposon System to Deliver the Insulin Gene to Diabetic NOD Mice.* Cells, 2020. **9**(10): p. 2227.

2. Ren, B., La, Q.T., O'Brien, B.A., Nassif, N.T., Tan, Y., Gerace, D., Martiniello-Wilks, R., Torpy, F., Dane, A.P., Alexander, I.E., and Simpson, A.M., *Partial pancreatic transdifferentiation of primary human hepatocytes in the livers of a humanised mouse model.* The Journal of Gene Medicine, 2018. **20**(5): p. e3017.

List of Presentations

1. La, Q.T., Ren, B., Logan, G.J., Cunningham, S.C., Khandekar, N., Nassif, N.T., O'Brien, B.A., Alexander, I.E., and Simpson, A.M., D The reversal of diabetes in non-obese-diabetes (NOD) mice using adeno associated viral vector (AAV) and furin-cleavable human insulin (*INSFUR*) gene . In: Australasian Diabetes Congress, Sydney, 2019.

2. La, Q.T., Ren, B., Logan, G.J., Cunningham, S.C., Khandekar, N., Nassif, N.T., O'Brien, B.A., Alexander, I.E., and Simpson, A.M., Delivery of The Insulin gene Using an Integrating Adeno-Associated Viral Vector (AAV) to Diabetic NOD mice. In: The American Diabetes Association Scientific 79th Scientific Session, San Francisco, USA, 2019.

3. La, Q.T., Ren, B., O'Brien, B.A., Nassif, N.T., Alexander, I.E, and Simpson, A.M., Reversal of diabetes in a humanised mouse model. In: Australasian Cell and Gene Therapy Society Biannual Meeting, Sydney, Australia, 2017.

4. La, Q.T., Ren, B., O'Brien, B.A., Nassif, N.T., Alexander, I.E, and Simpson, A.M., Pancreatic transdifferentiation in the livers of a humanised mouse model engineered to express insulin. In: New Horizons Conference, Sydney, Australia, 2015.

Format of the Thesis

This is a thesis by compilation. The first chapter is the introduction to the research study and the review of the literature. Chapter 2 describes the experiments, general materials and methods, followed by chapter 3, 4 and 5 showing detailed methods, results and the discussion of each subset of experiments. Chapter 4 is a published peer reviewed journal article. The last chapter of this thesis is the general discussion and the recommendation for future studies.

Publication Included in the Thesis

Title: Use of a Hybrid Adeno-Associated Viral Vector Transposon System to Deliver the Insulin Gene to Diabetic NOD Mice

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Author Contributions

Ann M. Simpson[1], Ian E. Alexander [3,4], and Bronwyn A. O'Brien [1,2] initiated the concept of this study and secured funding.

Ann M. Simpson[1] and Que T. La [1,2] wrote the draft article and all authors contributed to review of the final article and editing.

Binhai Ren [1,2] produced the lentiviral vector and with Que T. La [1,2] , contributed to experimental design, performed the animal experiments, immunohistochemistry, RT-PCR analysis and data analysis.

Grant Logan [3] designed the AAV vectors, contributed to experimental design and performed data analysis.

Sharon C. Cunningham [3] designed the AAV/*piggyBac* vectors and contributed to experimental design and data analysis.

Neeta, Khandekar [3] produced the AAV vectors and performed VCN and data analysis.

Najah T. Nassif [1,2] contributed to the experimental design and performed primer design and data analysis.

Bronwyn A. O'Brien [1,2] contributed to experimental design and data analysis.

All authors contributed to the interpretation of the data.

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Table of Contents

List of Figures and Tables

List of Abbreviations

9330175E14Rik	RIKEN cDNA 9330175E14 gene
AAV	Recombinant adeno-associated virus
Ad	Adenovirus
Adar	adenosine deaminase, RNA-specific
Adar1	Double-stranded RNA-specific adenosine deaminase 1
AIHW	Australian Institute of Health and Welfare
APC	Antigen presenting cells
Apol9a	apolipoprotein L 9a
Apol9b	apolipoprotein L 9b
Arx	Aristaless related homeobox
ATP	Adenosine triphosphate
BGL	Blood glucose levels
bHLH	basic helix-loop-helix
B-H-p-value	Benjamini-Hochberg-P-value
Ca^{2+}	Calcium ion
Ccl5	Chemokine (C-C motif) ligand 5
CD28	Cluster of Differentiation 28
CD40	Cluster of Differentiation 40
Cdc42	Cell division cycle 42
cDNA	Complementary deoxyribonucleic acid
CMRI	Westmead Children Medical Research Institute
CSII	Continuous subcutaneous insulin infusion
Cxcl10	chemokine (C-X-C motif) ligand 10
Cxcl11	chemokine (C-X-C motif) ligand 11
Ddx58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
Ddx60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60
Dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58
DNA	Deoxyribonucleic acid
eGFP	Enhanced green fluorescent protein

ER	Endoplasmic reticulum
FAH	Fumarylacetoacetate hydroxylase
FDR	False detection rate
FFO	Full flow occlusion
Foxa2	Forkhead box A2
G6pc	Glucose-6-phosphatase catalytic
Gbp3	Guanylate binding protein 3
Gbp6	Guanylate binding protein 6
GDM	Gestational diabetes mellitus
GK	Glucokinase
GLUT2 (Slc2a2)	Glucose transporter 2
GLUT4 (Slc2a4)	Glucose transporter 4
Gm4951(Isg15)	predicted gene 4951
GPCRs	G protein coupled receptors
Gyg	Glucogenin
Gys2	Glycogen synthase 2
h	Hour
H4IIE/ND	Rat liver cell line (H4IIE) transfected with NeuroD1 gene
H4IIEins/ND	Rat liver cell line (H4IIE) transfected with INSFUR and NeuroD1 genes
HDAd	Helper-dependent adenoviral vector
HK3	Hexokinase3
HIV	Human immunodeficiency virus
HLAs	High-risk human leukocyte antigens
HMD/INSFUR	Lentiviral vector carrying INSFUR gene
HMGA2	AT-hook2 gene
HNF1	Hepatocyte Nuclear Factor 1
HNF6	Hepatocyte Nuclear Factor 6
HSV	Herpes simplex virus
IDF	International Diabetes Federation
Ifi44	Interferon-induced protein 44
Ifit1	Interferon-induced protein with tetratricopeptide repeats 1

Ifit3	Interferon-induced protein with tetratricopeptide repeats 3
IFN-γ	Interferon γ
Ikbke	Inhibitor of nuclear factor kappa B kinase subunit epsilon
Ikbkg	Inhibitor of nuclear factor kappa B kinase regulatory subunit gamma
IL-1β	Interleukin 1β
INS-FUR	*Furin-cleavable insulin*
i.p	intraperitoneal
IPA	Ingenuity Pathway Analysis
IPCs	Insulin-producing-cells
IRF	Interferon regulator factor
Irf7	Interferon regulatory factor 7
Isg15	ISG15 ubiquitin-like modifier; Predicted gene 9706
ITRs	Inverted terminal repeats
kb	Kilobase
kg	Kilogram
Keap1-Nrf2	Kelch-like ECH-associated protein 1-nuclear factor (erythroid-derived 2)-like 2
LSP	Apolipoprotein E (ApoE) enhancer and the human α1-antitripsin (hAAT) promoter
LTRs	Long terminal repeats
Ly6a	Lymphocyte antigen 6 complex, locus A
Ly6c1	lymphocyte antigen 6 complex, locus C1; lymphocyte antigen 6 complex, locus C2
MafA	*V-maf musculoaponeurotic fibrosarcoma oncogene homolog A*
MafB	*V-maf musculoaponeurotic fibrosarcoma oncogene homolog B*
Map3k7	Mitogen-activated protein kinase 7
min	Minute
mRNA	Messenger ribonucleic acid
mmol	Millimole
MSCV	Mouse stem cell virus
Mx1	MX dynamin-like GTPase 1

Mx2	MX dynamin-like GTPase 2
NeuroD1	*Neuronal differentiation 1*
ng	nanogram
Ngn3	*Neurogenin3*
Nkx2.2	*Nierenberg and Kim 2 homeobox 2*
Nkx6.1	*Nierenberg and Kim 6 homeobox 1*
Nlrc5	NLR family, CARD domain containing 5
NOD	Non-obese diabetic
NOD/scid	Non-obese-diabetic/ severe combined immunodeficiency
NTBC	2-(2-nitro-4-trifluoro-methylbenzoyl) 1,3-cyclohexedione
Oas1a	2'-5' oligoadenylate synthetase 1A
Oasl1	2'-5' oligoadenylate synthetase-like 1
Oasl2	2'-5' oligoadenylate synthetase-like 2
Pax4	*Paired box 4*
Pax6	*Paired box 6*
PC1	*Processing endopeptidase 1*
PC2	*Processing endopeptidase 2*
PCA	Principal component analysis
Pck1	Phosphoenolpyruvate carboxykinase 1, cytosolic gene
PCR	Polymerase chain reaction
Pdx1	*Pancreatic duodenal homeobox1*
PKR	Protein Kinase R
Pklr	Pyruvate kinase liver and red blood cell gene
Ptf1	Pancreatic specific transcription factor 1
Ptf1a (or P48)	Pancreatic specific transcription factor 1a
Rsad2	Radical S-adenosyl methionine domain containing 2
Rtp4	Receptor transporter protein 4
RT-PCR	Reverse transcriptase polymerase chain reaction
RT-qPCR	Quantitative reverse transcriptase polymerase chain reaction
SAP	Sensor augmented pump
s.c	Subcutaneous
SCID	Severe combined immunodeficiency

sec	Second
Sp100	Nuclear antigen Sp100
SPINK 2	Serine protease inhibitor Kazal-type 2
STZ	Streptozotocin
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TAC	Transcriptome Analysis Console
Tgtp1	T cell specific GTPase 1
Tgtp2	T cell specific GTPase 2
Th1	T helper1
Th2	T helper 2
Tlr3	Toll-like receptor 3
Tu	Transduction unit
Usp18	Ubiquitin specific peptidase 18
VCN	Vector copy number
vg	Viral genome
VSV-G	Vesicular stomatitis virus envelope glycoprotein G
Wnt	Wingless-related integration site
Xaf1	XIAP associated factor 1
Zbp1	Z-DNA binding protein 1
α-cell	Alpha cell
β-cell	Beta-cell
δ-cells	Delta-cell

Abstract

Type I diabetes mellitus (T1D) is a chronic metabolic disorder resulting from the autoimmune attack and destruction of the pancreatic β-cells, leading to insulin deficiency and hyperglycaemia. The condition is currently managed by insulin therapy, which delays but does not fully prevent the long-term complications of the disease. The only cure for T1D is pancreas or islet transplantation; however, due to the lack of available organs and the complications from immunosuppression, transplantation is not widely applied. Gene therapy is one treatment being considered to treat and/ or cure T1D.

In earlier studies, our group delivered a lentiviral vector (HMD) carrying the furin-cleavable human insulin gene (*INS-FUR*) to streptozotocin (STZ)-induced diabetic rats, non-obese-diabetic mice, pancreatectomised diabetic pigs and humanized FRG mice using intervallic infusion of the vectors in full flow occlusion (FFO), a surgical technique that isolated the liver from the circulation. Reversal of diabetes with normal glucose tolerance and the expression of key β-cell transcription factors in the liver tissue of the transduced animals were achieved.

The main aim of the current study was to explore the possibility of reproducing the results of the HMD/*INS-FUR* transduction using less invasive transduction techniques. Traditional AAV8 vectors that have a higher tropism toward the hepatocytes were used to deliver the transgene *INS-FUR,* the β-cell transcription factors *Pdx1* or *NeuroD1*. The liver specific promoter (LSP) was also incorporated into the vector system to limit the expression of the transgene(s) to the livers and allow the vectors to be delivered by a simple intraperitoneal injection.

The expression of *Pdx1* or *NeuroD1* in the livers of the STZ-induced diabetic non-obese-diabetic and severe immune incompetent (NOD/scid) mice by the AAV8-LSP system did not cure diabetes. The delivery of the *INS-FUR* gene by AAV8-LSP led to hypoglycaemia in both STZ-induced diabetic NOD/scid mice and autoimmune non-obese-diabetic (NOD) mice. The transcriptome analysis of the livers of the NOD mice transduced by

AAV8-*INS-FUR* alone showed that severe hypoglycaemia that was observed in the animals may have been caused by an activation of the glycolysis pathway and an inhibition of the gluconeogenesis pathway. Using the same AAV8-LSP vector system to deliver *INS-FUR* with β-cell transcription factors (*Pdx1* and/ or *NeuroD1*) caused hypoglycaemia in diabetic NOD/scid mice, while the combination of *INS-FUR* and *Pdx1* was ineffective in treating diabetes in NOD mice. The combination of the AAV8-*INS-FUR* and FFO surgery or the empty HMD vector could not reverse diabetes in NOD mice either. In addition, the transcriptome analysis showed that the AAV8 transduction could also generate an antiviral immune reaction, but the AAV8 transduction with or without the FFO surgery may not cause any liver disease.

In this project the hybrid AAV8/*piggyBac* that could facilitate the integration of the transgene into the host genome was also used to deliver the *INS-FUR* gene. The hybrid system had both the transposon and *transposase* constructs of the *piggyBac* system incorporated into the AAV8-LSP vectors (AAV8/*piggyBac-INS-FUR)*. The delivery of the *INS-FUR* gene by the AAV8/*piggyBac* could not normalise the random blood glucose levels in the NOD mice, but normal glucose tolerance was achieved. By comparison NOD mice that received FFO surgery after the AAV8/*piggyBac-INS-FUR* transduction had normal blood glucose levels, normal glucose tolerance and possibly glucose-responsive insulin secretion without the expression of any endogenous β-cell transcription factors. It was thought that the stable blood glucose levels were achieved because the FFO surgery may have helped to reduce the number of *transposases* and allowed a more stable integration of the *INS-FUR* gene to the host genome. In addition, the results suggested that the clinical desirable outcomes (normal blood glucose levels and glucose tolerance) could be achieved without the expression of β-cell transcription factors. This is the first *in vivo* study using the hybrid AAV8/*piggyBac* system to treat T1D. The study suggested that AAV8/*piggyBac* system may be further developed to become an alternative therapy for the disease.