Optimal Delivery of Therapeutic Genes to Pancreatic Islets

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Thesis Abstract

Islet transplantation is a promising therapeutic option for Type 1 Diabetic (T1D) patients, with the ability to improve glycometabolic control and in select cases achieve insulin independence. Intraportally transplanted islets must reside in the hostile environment of the liver, where they are exposed to the instant blood mediated inflammatory reaction (IBMIR), alloimmunity, recurrence of islet specific autoimmunity, a highly toxic pro-inflammatory cytokine storm (e.g. IL-1β, IFN-α, IFN-γ and TNF-α) and hypoxia due to inadequate revascularization post-transplantation. The early loss of functional islet mass (50-70%) due to apoptosis following clinical transplantation contributes to islet allograft failure. Strategies to prevent apoptosis are therefore highly desirable to enhance islet survival for transplantation.

In Chapter 3, the ability of Adenoviral (Ad) and Adeno-Associated Viral (AAV)-based vectors expressing a green fluorescent protein (GFP) reporter gene to transduce isolated human and rat pancreatic islets was investigated. Specific interest was placed on tyrosine mutant AAV-based vector types, which have not been previously explored in human and rodent pancreatic islets. Ad efficiently transduced isolated human and rat pancreatic islets while AAV failed to transduce human islets and showed a varied ability to transduce rat islets. The results in this chapter demonstrate that Ad vectors are more efficient at transducing isolated islets than AAV-based vector types.

Chapter 4 aimed to characterise an Ad-based vector encoding an anti-apoptotic molecule termed Insulin-like Growth Factor-II (Ad-IGF-II). Ad-IGF-II effectively transduced rat pancreatic islets without affecting islet viability or function and did not induce uncontrolled islet cell proliferation. The results in this chapter suggest that Ad-IGF-II is an effective and non-toxic vector type for use in an islet gene therapy setting.

In Chapter 5 and Chapter 6, the influence of local human IGF-II over expression on rat pancreatic islet cell survival in vitro and in vivo was examined, respectively. Over expression of IGF-II in islets resulted in enhanced islet survival in vitro and in an in vivo marginal mass islet transplant model. Transplantation of IGF-II over expressing islets under the kidney capsule of diabetic NOD-SCID mice restored euglycemia in 78% of recipients, compared to 46% and 18% of untransduced and Ad-GFP transduced control islet recipients, respectively.
In summary, this thesis demonstrated that compared to AAV, Ad is currently the optimal vector for use in an islet gene therapy setting. Moreover, over expression of IGF-II did not affect the viability or insulin secreting capacity of islets. Finally, the induced expression of anti-apoptotic IGF-II led to enhanced islet survival *in vitro* and improved transplant outcomes in an *in vivo* marginal mass islet transplant model, indicating that IGF-II gene transfer is a potentially powerful tool to improve islet survival post-transplantation.
**Thesis Declaration**

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Amy Hughes and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below*) resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.


Signed Amy Hughes
Publications, Presentations and Awards

Publications

Invited Reviews


Published Manuscripts (1) and Manuscripts in Preparation (2)


2. **Hughes, A**, Jessup CF, Drogemuller, CJ, and Coates PTH, Tyrosine mutations in AAV2 and AAV8 Capsids is Insufficient to Enhance Gene Delivery to Isolated Human Pancreatic Islets

Published Abstracts


Presentations


Awards

2012  Pfizer Young Investigator Award, Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra
2012  Medical Staff Society Research Prize, Medical Grand Round, Royal Adelaide Hospital
2012  Faculty of Health Sciences Postgraduate Travelling Fellowship, University of Adelaide
2010  International Travel Grant, The Transplantation Society of Australia and New Zealand
2009  Trevor Prescott Memorial Scholarship, The Freemasons Foundation, Adelaide
2009  Amgen Young Investigator Award, The Transplantation Society of Australia and New Zealand, Annual Scientific Meeting, Canberra
2009  Faculty of Health Sciences Postgraduate Divisional Scholarship, University of Adelaide
2008  The Queen Elizabeth Research Foundation Honours Scholarship, Adelaide
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**Abbreviations**

°C - Degrees Celsius

1x PBS - 1 x Phosphate Buffered Saline

1xHBSS - 1x Hanks Buffered Salt Solution

4E-BP1 - Eukaryotic initiation factor binding protein

4E-eIF4E - Eukaryotic initiation factor

AAV - Adeno-associated viruses

Ad - Adenovirus

Ad-GFP - Adenoviral-Green Fluorescent Protein

Ad-IGF-II - Adenoviral-Insulin like Growth Factor-II

ALS - Acid-labile subunit

Apaf-1 - Apoptosis-protease activating factor-1

APC - Antigen presenting cell

BAD - Bcl-associated death promoter

Bcl-2 - B-cell lymphoma 2

BGL - Blood glucose levels

BLAST - Basic local alignment search tool

bp - Base pairs

CAR - Coxsackie Adenovirus Receptor

CITR - Collaborative Islet Transplant Registry

cm - Centimeter
CPE - Cytopathic effects

DAPI - 4',6-diamidino-2-phenylindole

DISC - death-inducing signaling complex

ELISA - Enzyme linked immunosorbent assay

Expect-value - E-value

FADD - Fas-associated death domain

FasL - Fas-Fas ligand

FCS - Foetal calf serum

FOXO - Forkhead transcription factor

GAD65 - Glutamic acid decarboxylase

GFP - Green fluorescent protein

GLUT2 - Glucose transporter 2

GSIS - Glucose stimulated insulin secretion

GSK-3β - Glycogen synthase kinase 3β

HEK - Human Embryonic Kidney

hIL-1Ra - Human Interleukin-1 Receptor Antagonist

h - Hour

HPRT-1 - Hypoxanthinephosphoribosyltransferase 1

HSPG - Heparan sulphate proteoglycan

HSV - Herpes Simplex Virus

i.p - Intra peritoneal
IBMIR - Instant blood mediated inflammatory reaction
IEQ - Islet equivalents
IFN-γ - Interferon-gamma
IGF - Insulin-like Growth Factor
IGF-1R - Insulin-like Growth Factor-I receptor
IGF-1R/IR - Insulin-like Growth Factor-I receptor/Insulin receptor
IGFBP - Insulin-like Growth Factor binding protein
IGF-I - Insulin-like Growth Factor-I
IGF-II - Insulin-like Growth Factor-II
IGF-IIIR - Insulin-like Growth Factor-II receptor
IL-10 - Interleukin-10
IL-1β - Interleukin-1β
IL-4 - Interleukin-4
iNOS - Inducible nitric oxide synthase
IR - Insulin receptor
IRS-2 - insulin receptor substrate 2
kbp - Kilo base pairs
kDa - kilo dalton
lamR - Laminin receptor
M - Molar
MAPK - Mitogen activated kinase
PS - Phosphatidyl serine
PVDF - Polyvinyl difluoride
rIGF-II - Recombinant IGF-II
RIP - Receptor-interacting protein
RT - Room temperature
RT-PCR - Real-time PCR
SCID - Severe combined immune deficiency
SDS-PAGE - Sulfate polyacrylamide gel electrophoresis
SEM - Standard error of the mean
SFA - Sulphation factor activity
SI - Stimulation index
STAT-1 - Signal transducer and activator of transcription-1
STZ - Streptozotocin
SW - Starting weight
T1D - Type 1 Diabetes
T2D - Type 2 diabetes
TNF - Tumour necrosis factor
TRADD - TNF receptor-associated death domain
TRAF2 - TNF-R-associated factor 2
TSC - Tuberous sclerosis gene product
TUNEL - Terminal deoxynucleotidyl transferase dUTP nick end labelling
VEGF - Vascular endothelial growth factor

vg - Vector genome

μg - Microgram

μl - Microliters