

Optimal Delivery of Therapeutic Genes to Pancreatic Islets

Amy Hughes

Thesis submitted in fulfilment for the degree of
Doctor of Philosophy

In

The Department of Medicine
Faculty of Health Sciences
The University of Adelaide

September 2012

Table of Contents

Thesis Abstract	viii
Thesis Declaration	x
Publications, Presentations and Awards	xi
Acknowledgements	xvii
Abbreviations	xix
CHAPTER 1: LITERATURE REVIEW	1
1.1 Introduction	1
1.2 The pancreas	1
1.3 Islet of Langerhans	2
1.3.1 The β -cell and glucose homeostasis	2
1.4 Diabetes Mellitus	2
1.4.1 Type 1 Diabetes	4
1.4.2 Immunology of Type 1 Diabetes	4
1.4.3 Type 2 Diabetes	5
1.4.4 Current treatments for Type 1 Diabetes	6
1.4.5 Islet Transplantation	7
1.5 Barriers to successful islet transplantation	9
1.6 Concepts and methods of gene therapy	11
1.6.1 Viral-mediated gene transfer to pancreatic islets	11
1.6.1.1 Adenoviral Vectors	14
1.6.1.2 Adeno-Associated Viral Vectors	15
1.6.1.3 Herpes Simplex Viral Vectors	16
1.6.1.4 Retroviral vectors	16
1.6.2 Non-viral mediated gene transfer to pancreatic islets	17
1.7 Alternative strategies towards islet survival	18
1.8 Gene therapy towards islet survival	19
1.9 Insulin-like growth factor-axis	24
1.9.1 Insulin-like Growth Factor-I	26
1.9.2 Insulin-like growth factor-II	26

1.9.3 Insulin-like growth factor-II expression	27
1.9.4 Insulin-like growth factor-II signalling	27
1.9.5 Insulin-like growth factor receptors	29
1.9.6 Insulin-like growth factor binding proteins	29
1.10 Apoptosis	30
1.10.1 Necrosis	30
1.10.2 Morphology of Apoptosis	31
1.10.3 Mechanisms of Apoptosis	31
1.10.3.1 Extrinsic (death receptor) pathway	34
1.10.3.2 Intrinsic (mitochondrial) pathway	34
1.10.3.3 Perforin/Granzyme Pathway	35
1.10.3.4 Execution Pathway	35
1.10.4 Apoptosis in Type 1 Diabetes	36
1.10.5 Apoptosis in islet transplantation	36
1.11 Thesis summary	37
1.12 Thesis aims and hypothesis	38
CHAPTER 2: MATERIALS AND METHODS	39
2.1 MATERIALS	39
2.1.1 Replication deficient Adenoviral-based vectors	39
2.1.2 Adeno-Associated Viral (AAV)-based vectors	39
2.1.3 Animals	40
2.1.4 Cytokines	40
2.1.5 Antibodies	40
2.1.5.1 Primary antibodies	40
2.1.5.2 Secondary antibodies	40
2.1.6 FACS reagents	41
2.1.7 Molecular biology reagents	41
2.1.8 Tissue culture reagents	42
2.1.9 Kits	42
2.1.10 Miscellaneous reagents	43
2.1.11 Equipment	45

2.2 CELLULAR TECHNIQUES	46
2.2.1 Maintenance of cell lines	46
2.2.2 Description of cell lines	46
2.2.3 Cell quantitation	46
2.2.4 Cryopreservation and storage of cell lines	48
2.2.5 Thawing frozen cell lines	48
2.2.6 Subculture of cell lines	49
2.2.7 Changing cell culture medium	49
2.3 MOLECULAR METHODS	49
2.3.1 RNA extraction	49
2.3.2 Reverse transcription using Oligo dT	50
2.3.3 Polymerase Chain Reaction	51
2.3.4 Agarose gel electrophoresis	53
2.3.5 Quantitative real-time PCR using TaqMan® primers	53
2.3.6 Viral DNA purification	54
2.4 ADENOVIRAL METHODS	55
2.4.1 Large-scale Adenoviral production	55
2.4.2 Virus purification	56
2.4.3 Adenoviral titre determination by flow cytometry	56
2.5 ISLET METHODS	57
2.5.1 Culture conditions	57
2.5.2 Islet dissociation	57
2.5.3 Islet quantification	57
2.5.4 Viral transduction	58
2.5.5 Cytokine treatment of islets	59
2.5.6 Glucose stimulated insulin release assay of islets	59
2.5.7 Insulin-like growth factor-1 receptor blocking	59
2.5.8 Western blotting analysis	59
2.6 FLOW CYTOMETRY	60
2.6.1 Annexin V/Propidium Iodide staining	60
2.6.2 GFP detection	60
2.6.3 7-AAD staining	60

2.6.4 Ki67 staining	60
2.7 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)	61
2.7.1 Rat insulin ELISA	61
2.7.2 Human insulin ELISA	61
2.7.3 Human IGF-II ELISA	61
2.8 GRIESS REACTION FOR NITRIC OXIDE DETERMINATION	62
2.9 ANIMAL METHODS	62
2.9.1 Albino wistar rat islet isolation	62
2.9.2 Streptozotocin diabetes induction	64
2.9.3 NOD-SCID kidney capsule islet transplant	65
2.10 IMMUNOHISTOCHEMISTRY	65
2.10.1 Islet cytopins	65
2.10.2 Paraffin embedding of human islet cell suspensions	66
2.10.3 Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)	66
2.10.4 Antigen retrieval	66
2.10.5 Insulin staining of islets	66
2.10.6 Fluorescent confocal microscopy of transduced islets	67
2.11 STATISTICAL ANALYSIS	67
2.12 SOLUTIONS AND BUFFERS	68
CHAPTER 3: COMPARISON OF ADENOVIRAL AND ADENO-ASSOCIATED VIRAL TRANSDUCTION OF HUMAN AND RODENT PANCREATIC ISLETS	75
3.1 Introduction	75
3.2 Results	79
3.2.1 Ad-GFP transduction induces GFP expression in rat islets	79
3.2.2 Ad-GFP transduction does not affect rat islet viability or function	79
3.2.3 GFP expression is localized to the perimeter in Ad-GFP transduced islets	82
3.2.4 Ad-GFP transduction induces GFP expression in human islets	82
3.2.5 Ad-GFP transduction does not affect human islet viability	82
3.2.6 GFP expression profile of AAV-GFP transduced rat pancreatic islets	86
3.2.7 GFP expression profile of AAV-GFP transduced rat islets with vector dose 6.25x10 ⁸ , 1.25x10 ⁹ , 2.5x10 ⁹ and 5x10 ⁹ vg	89

3.2.8 AAV-GFP based vectors transduce rat islets with various levels of efficiency	91
3.2.9 GFP expression is localized to the islet perimeter in AAV2/1 transduced rat islets	91
3.2.10 AAV-GFP transduction does not affect viability or glucose stimulated insulin secretion of rat islets	91
3.2.11 AAV-GFP based vectors failed to transduce isolated human islets	95
3.2.12 GFP expression profile of AAV-GFP transduced human islets	95
3.2.13 GFP expression profile of AAV-GFP transduced HEK 293 cells	95
3.2.14 Immunohistochemical staining for heparan sulphate proteoglycan and integrin $\alpha\beta 5$ in human pancreatic islets	99
3.3 Discussion	102
CHAPTER 4: CHARACTERISATION OF AN ADENOVIRAL-BASED VECTOR ENCODING HUMAN INSULIN-LIKE GROWTH FACTOR-II	107
4.1 Introduction	107
4.2 Results	110
4.2.1 Sequencing of human IGF-II from Ad based vector (Ad-IGF-II)	110
4.2.2 Microscopic evaluation of Ad-GFP and Ad-IGF-II transduced HEK 293 cells	110
4.2.3 Human IGF-II transgene expression in Ad-IGF-II transduced HEK 293 cells	113
4.2.4 Secretion of human IGF-II by Ad-IGF-II transduced HEK 293 cells to examine secretion of folded protein	113
4.2.5 Transduction of isolated rat islets with Ad-GFP	113
4.2.6 Rat islet viability following Ad-IGF-II transduction	117
4.2.7 Characterisation of rat islet function following Ad-IGF-II transduction	117
4.2.8 Evaluation of Ad-GFP β -cell transduction in isolated rat islets	117
4.2.9 Determination of human IGF-II secretion in Ad-IGF-II transduced rat islets	121
4.2.10 Determination of islet proliferation in Ad-IGF-II transduced rat islet	121
4.3 Discussion	124

CHAPTER 5: THE ANTI-APOPTOTIC ACTIVITY OF INSULIN-LIKE GROWTH FACTOR-II IN AN <i>IN VITRO</i> MODEL OF CYTOKINE INDUCED APOPTOSIS	128
5.1 Introduction	128
5.2 Results	131
5.2.1 Pro-inflammatory cytokines Interleukin-1 β and Interferon- γ induce cell death in human and rat pancreatic islets <i>in vitro</i>	131
5.2.2 Assessment of human and rat islet morphology following IL-1 β and IFN- γ pro-inflammatory cytokine exposure	131
5.2.3 Pro-inflammatory cytokines IL-1 β and IFN- γ induce DNA damage in isolated rat islets	134
5.2.4 Pro-inflammatory cytokines IL-1 β and IFN- γ impair the glucose stimulated insulin secretory ability of rat islets	134
5.2.5 Nitric oxide expression in rat islets following pro-inflammatory cytokine exposure	134
5.2.6 Ad-IGF-II transduction of rat islets protects against pro-inflammatory cytokine induced cell death <i>in vitro</i>	138
5.2.7 Ad-IGF-II transduction of human islets does not protect against IL-1 β and IFN- γ pro-inflammatory cytokine induced cell death <i>in vitro</i>	138
5.2.8 Ad-IGF-II transduced rat islets display a decreased number of TUNEL positive apoptotic cells following pro-inflammatory cytokine exposure <i>in vitro</i>	138
5.2.9 Characterisation of Ad-IGF-II transduced rat islet function following IL-1 β and IFN- γ pro-inflammatory cytokine exposure	143
5.2.10 The anti-apoptotic effect of IGF-II is neutralized by blocking the IGF-1R	143
5.2.11 IGF-II activates the PI3K/Akt pathway to inhibit islet apoptosis	143
5.3 Discussion	147
 CHAPTER 6: A MARGINAL MASS ISLET TRANSPLANT MODEL TO STUDY THE ABILITY OF AD-IGF-II TRANSDUCED RAT ISLETS TO IMPROVE ISLET SURVIVAL IN DIABETIC NOD-SCID MICE	 152
6.1 Introduction	152

6.2 Results	156
6.2.1 Optimization of diabetes induction in NOD-SCID mice	156
6.2.2 Gender differences confer susceptibility to STZ-induced diabetes weight loss in NOD-SCID mice	156
6.2.3 Generation of an <i>in vivo</i> marginal mass islet transplant model	156
6.2.4 NOD-SCID islet transplant procedure	161
6.2.5 Effect of Ad-IGF-II transduced rodent islets in a marginal mass islet transplant model	161
6.2.6 Confirmation of diabetes in NOD-SCID mice following transplantation	161
6.3 Discussion	168
CHAPTER 7: CONCLUDING REMARKS AND FUTURE DIRECTIONS	172
References	179
Appendix	222

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. Intra-portal 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-1 β , 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.

***Hughes A**, Jessup C, Drogemuller C, Mohanasundaram D, Milner C, Rojas D, Russ GR, Coates PT. Gene therapy to improve pancreatic islet transplantation for Type 1 diabetes mellitus. *Curr Diabetes Rev.* 2010 Sep;6(5):274-84.

Signed Amy Hughes

Publications, Presentations and Awards

Publications

Invited Reviews

1. **Hughes, A**, Jessup, C, Drogemuller, C, Mohanasundaram, D, Milner, C, Rojas, D, Russ, G.R and Coates, P.T, Gene Therapy to Improve Pancreatic Islet Transplantation for Type 1 Diabetes Mellitus, 2010 Curr Diabetes Rev, 6, 274-84

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

1. **Hughes A**, Mohanasundaram D, Kireta S, Jessup C, Drogemuller C, Coates PTH. Insulin-like Growth Factor-II Prevents Proinflammatory Cytokine-Induced Apoptosis and Significantly Improves Islet Survival After Transplantation. *Transplantation*. 2013;95: 00-00.
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

1. **Hughes, A**, Mohanasundaram, D, Drogemuller, CJ, Jessup, CF, Coates PTH, Anti-Apoptotic Insulin-like Growth Factor-II Gene Therapy Protects Islets from Cytokine Induced Cell Death, American Journal of Transplantation, Volume 12, Issue Supplement s3, pages 27 – 542, May 2012
2. **Hughes, A**, Jessup, CF, Drogemuller, Mohanasundaram, D, Milner, CR, Rojas, D, Russ, GR, Coates, PTH, Transduction of Rat Pancreatic Islets with Wildtype Adeno-Associated Virus (AAV) Serotype 2, Pseudotype AAV2/8. AAV2/1 and Surface-Exposed Tyrosine Mutant AAV Vectors – A comparative study, American Journal of Transplantation, Volume 90-Supplement, pp:1-1078, July 2010
3. **Hughes, A**, Jessup, CF, Drogemuller, Mohanasundaram, D, Milner, CR, Rojas, D, Russ, GR, Coates, PTH, Adenovirus-Mediated Transduction of Isolated Pancreatic Islets Using Insulin-Like Growth Factor-II to Promote Islet Survival Post-Transplantation, American Journal of Transplantation, Volume 90-Supplement, pp:1-1078, July 2010

4. **Hughes, A**, Jessup, CF, Drogemuller, Mohanasundaram, D, Milner, CR, Rojas, D, Russ, GR, Coates, PTH, Transgenic Expression of Insulin-like Growth Factor-II (IGF-II) in Pancreatic Islets to Prevent Apoptosis, Xenotransplantation, Vol. 16, Issue 6, page 553 December 2009

Presentations

A. Hughes, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Adenovirus-Mediated Transduction of Pancreatic Islets using Insulin-like Growth Factor-II (IGF-II) to Prevent Apoptosis. Oral presentation. Australian Society for Medical Research, Annual Scientific Conference, 2009

A. Hughes, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Adenoviral-Mediated Transduction of Pancreatic Islets using Insulin-like Growth Factor-II (IGF-II) to Prevent Apoptosis. Oral presentation. Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Canberra, 2009

A. Hughes, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Transgenic expression of Insulin-like Growth Factor-II in pancreatic islets to prevent apoptosis. Oral Presentation. Australian Diabetes Society (ADS) and the Australian Diabetes Educators Association (ADEA) Annual Scientific Meeting, Adelaide, 2009

A. Hughes, A.J. Kupke, C.J. Drogemuller, D.M. Mohanasundaram, C. Mee, C.R. Milner, C.F. Jessup, P.T.H. Coates. Investigation of cytokine-induced early apoptosis in isolated islets of langerhans. Oral Presentation. Annual Immunology Retreat, Australasian Society for Immunology, Adelaide, 2009

A. Hughes, C.J. Drogemuller, C.J. Jessup, P.T.H. Coates. Optimal Delivery of Therapeutic Genes to Pancreatic Islets. Invited Oral Presentation, T cell Laboratory, Weatherall Institute of Molecular Medicine, Oxford, UK, 2009

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Transgenic expression of insulin-like growth factor-II (IGF-II) in pancreatic islets to prevent apoptosis. Poster Presentation. Joint International Pancreas and Islet Transplantation Association (IPITA) and International Xenotransplantation Association (IXA) Meeting, Venice, Italy, 2009

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Investigation of cytokine-induced early apoptosis in isolated islets of langerhans. Oral Presentation. The Queen Elizabeth Hospital Annual Research Day, Adelaide, 2009

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Comparison of wildtype adeno-associated virus (AAV) serotype 2 vectors to pseudotyped AAV vectors for the transduction of rat pancreatic islets. Oral Presentation. Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Canberra, 2010

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Transduction of Rat Pancreatic Islets with Wildtype Adeno-Associated Virus (AAV) Serotype 2, Pseudotype AAV2/8, AAV2/1 and Surface-Exposed Tyrosine Mutant AAV Vectors – A Comparative Study. Poster Presentation. XXIII International Congress of the Transplantation Society (TTS), Vancouver, Canada, 2010

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Adenovirus-Mediated Transduction of Isolated Pancreatic Islets Using Insulin-Like Growth Factor-II to Promote Islet Survival Post-Transplantation. Poster Presentation. XXIII International Congress of the Transplantation Society (TTS), Vancouver, Canada, 2010

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Introduction to the Renal and Transplantation Immunobiology Laboratory. Invited Oral Presentation. Diabetes Research Institute (DRI), University of Miami, Florida, 2010

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Evaluation of wildtype adeno-associated virus (AAV) serotype 2, pseudotype AAV2/8, AAV2/1 and surface-exposed tyrosine mutant AAV vectors and their ability to transduce isolated rat pancreatic islets. Oral Presentation. The Queen Elizabeth Hospital Annual Research Day, Adelaide, 2010

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Adenovirus-Mediated Transduction of Pancreatic Islets using Insulin-like Growth Factor-II (IGF-II) to Prevent Apoptosis. Oral presentation. Australian Society for Medical Research, Annual Scientific Conference, 2011

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, C.R. Milner, D. Rojas, G.R. Russ, P.T.H. Coates. Optimal Delivery of Insulin-Like Growth Factor-II to Rat Pancreatic Islets using a Replication Deficient Adenoviral Construct. Poster Presentation. Faculty of Health Sciences, Postgraduate Research Conference, Adelaide, 2011

A. Hughes, C.F. Jessup, C.J. Drogemuller, D. Mohanasundaram, P.T.H. Coates. Adenoviral Overexpression of Insulin-like growth factor II Protects Pancreatic Islets from Pro-Inflammatory Cytokine Induced Apoptosis and Necrosis. Poster Presentation. Australasian Society of Immunology, Adelaide, 2011

A. Hughes, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Anti Apoptotic Insulin-Like Growth Factor-II Gene Therapy Protects Islets from Cytokine Induced Cell Death. Poster Presentation. American Transplant Congress 2012, Boston, USA, 2012

A. Hughes, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Transgenic Expression of Insulin-Like Growth Factor-II (IGF-II) in Pancreatic Islets Offers a Novel Therapeutic Strategy to Improve Islet Cell Survival Post-Transplantation. Oral Presentation. Australian Society for Medical Research, Annual Scientific Conference, 2012

A. Hughes, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Anti-Apoptotic Insulin-Like Growth Factor-II (IGF-II) Gene Transfer Offers a Novel Therapeutic Strategy to Improve Islet Cell Survival Post-Transplantation. Oral Presentation. Transplantation Society of Australia and New Zealand Annual Scientific Meeting, Canberra, 2012

A. Hughes, D. Mohanasundaram, C. J. Drogemuller, C. F. Jessup P.T.H. Coates. Insulin-Like Growth Factor-II Decreases Islet Apoptosis *In Vitro* and Improves Islet Transplant Function in a Minimal Mass Model. Poster Presentation. 24th International Congress of The Transplantation Society, Berlin, Germany, 2012

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

Acknowledgements

Firstly, I would like to thank my supervisors Associate Professor Toby Coates, Dr. Claire Jessup and Chris Drogemuller for their time, guidance and support. Thank you for reviewing all my abstracts, all my thesis drafts and all my manuscript drafts. In particular I would like to give my sincere gratitude to Dr. Coates. Toby I thank you for giving me the opportunity to complete my PhD in the laboratory. Three years ago my life became richer and more fulfilling, and then it became “amazing” simply because I was given the opportunity to work day in and day out in the field of islet transplantation, working with a remarkable endocrine cell that so elegantly exists within the pancreas to release different hormones in response to blood nutrient levels. Toby I also cannot forget Brunello di Montalcino as a wine first experienced in Venice, but to the end of time, it will remain in my heart as a reminder of your generosity.

Thank you to all the past and present members of the lab. Tim Searcy (LF), thank you for every minute I was blessed to be in your presence. Our friendship has been witness to laughter, happiness, adventures, tears and frustrations and for this I will be eternally grateful. Chris Hope, my dear friendly friend, thank you for being by my side as we hold on tight and ride the PhD roller coaster together. You are the epitome of what a good scientist represents and have never been anything but a truly exceptional friend to me. The beautiful Jodie Nitschke, from the moment I met you I have been mesmerized by your soul which at every given moment radiates nothing but pure “amazing-ness”. Thank you for all your help in the lab, thanks for - saturday morning ‘agarose gel master class’, for your guidance with all things sequencing and PCR, for your help with preparing buffers and reagents (the list could go on and on). Julie Johnston (Jules), there has never been a question that you cannot answer. Your scientific knowledge and understanding truly rivals that of a senior post-doc. I have never been anything but in awe of you, and thankful for our friendship. Dr. Darling Rojas-Canales, thank you for your friendship, for your mentorship, patience and advice. I hold close to my heart over four years of “TIL/ITF/RTIL/TQEH/RAH” memories. Darling, I am so excited to watch your post-doctoral career because you are destined for great things. Plinio Hurtado (Plin), your love of life is contagious and I thank you for allowing me to share that passion with you. Ernesto Hurtado (E), I have enjoyed our conversations (mainly about desert and wine bars). The field of science gained an exceptional advocate when you entered the realm of medical research and I wish you the best of luck in all your future endeavors.

I thank Dr. Daisy Mohanasundaram for her patience in teaching me how to perform rat islet isolations. Daisy, I thank you for your help with the islet transplants performed in this thesis, of which Chapter 6 would not have been possible without your help. Finally, I would like to give my special thanks to Svjetlana Kireta, Clyde Milner, Dr. Michael Collins, Matthew Stephenson, Dr. Natasha Rogers, Daniella Penko and Kisha Sivanathan for their friendship and help in the laboratory.

Thank you to the donors and donor families, whom without their generosity, this research would not have been possible. I am also greatly appreciative of the support given by The Australian Islet Consortium, The University of Adelaide, CNARTS and The Transplantation Society of Australia and New Zealand. I would like to extend my sincere gratitude to the Trevor Prescott/Freemasons foundation for their commitment to health, their community involvement and awarding me the prestigious Trevor Prescott Memorial Scholarship for my PhD studies in 2009.

I want to thank my parents, Lynn and Greg Hughes from the bottom of my heart for their endless love and support throughout my academic education. You will be in my heart forever and always. Thank you to my siblings Sarah, Michelle, Matt, Joe, Hannah and Finlay. There is no other love like the love I feel for all of you. Special thanks to my niece Isabella, you are still my angel sent from god to brighten all our lives, and my nephew Callum, you might not even realize it yet, but every day you remind me that life is “amazing”.

Lauren and Erin, I have had the honor of knowing you since our very first day in undergraduate biotechnology. Thank you for the unrelenting support, laughter and fun times. You have both witnessed my countdown, for three years now. Finally, the countdown is over. Dr. Helena Ward, thank you for your support, friendship and advice. I am looking forward to our purple-themed dinner to celebrate ‘the PhD’.

To my PhD, you have been my friend, my every day, my early mornings, and my weekend accomplice (eating far too much take away laksa from Rundle Street) while running ELISAs late into the evening. For the last three years you have been the first thing on my mind in the morning and the last thing on my mind in the evening. I will miss you, but without you I would not know the true meaning of dedication, the meaning of determination and the meaning of strength.

Abbreviations

°C - Degrees celcius

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-3 β - Glycogen synthase kinase 3 β

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-IIR - Insulin-like Growth Factor-II receptor

IL-10 - Interleukin-10

IL-1 β - Interleukin-1 β

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

ml - Millilitre

mm - Millimeter

mM - Millimolar

MOI - Multiplicity of infection

mRNA - messenger RNA

mTOR - Mammalian target of rapamycin

NF- κ B - nuclear factor kappa B

nm - Nanometer

NO - Nitric oxide

NOD - Non-Obese Diabetic

NOD-SCID - Non-Obese Diabetic Severe combined immune deficiency

p70S6K - ribosomal protein S6 kinase

pAkt - Phospho-Akt

PCR - Polymerase Chain Reaction

PDK - phosphoinositol dependent kinase-1

Pfu - Plaque forming units

PI - Propidium Iodide

PI3K - Phosphoinositide-3-kinase

PKB - Protein kinase B

Post-tx - Post-transplant

Pre-tx - Pre-transplant

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