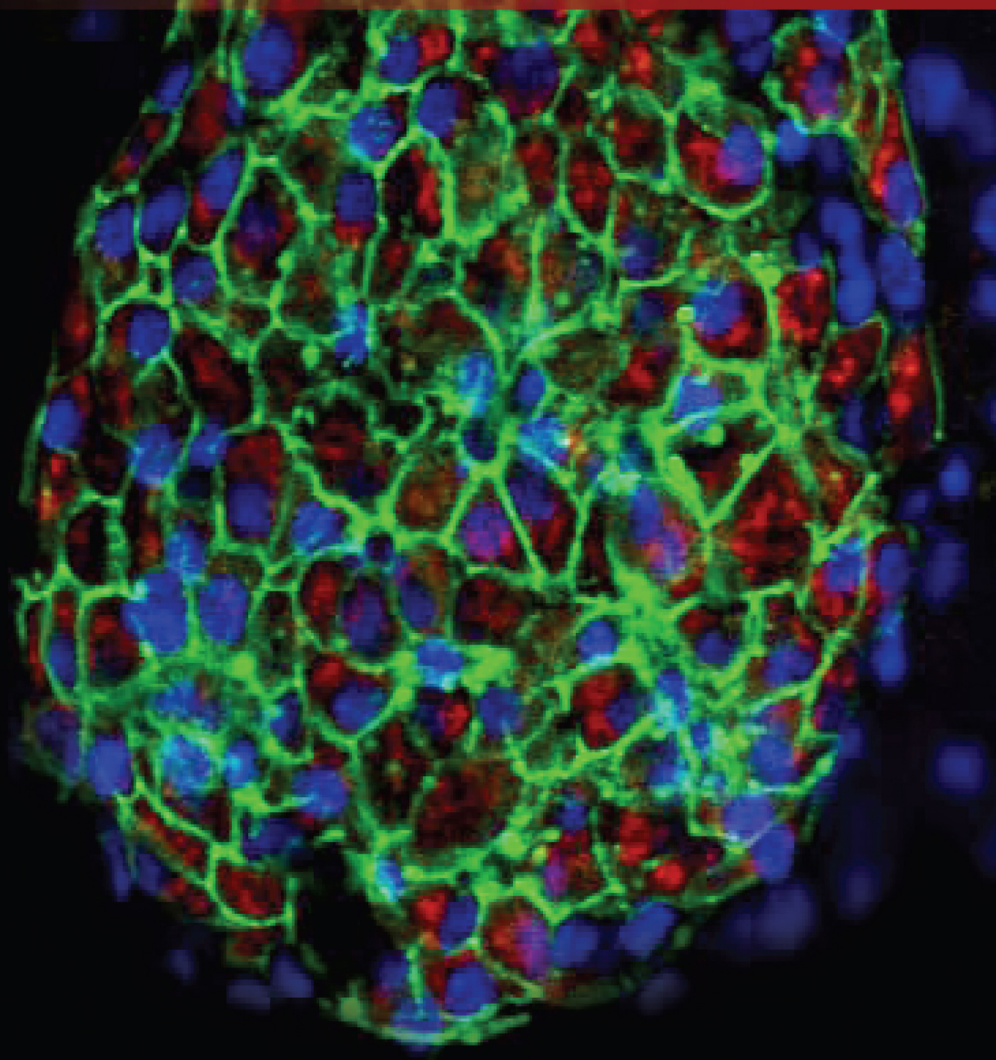
eISBN: 978-1-68108-365-0
ISBN: 978-1-68108-366-7

eISSN: 2468-7545
ISSN: 2468-7537

CURRENT AND FUTURE DEVELOPMENTS IN PHYSIOLOGY

VOLUME 1

CONTROL OF PANCREATIC BETA CELL FUNCTION AND PLASTICITY IN HEALTH AND DIABETES



Editor:
David J. Hill

Bentham Books

Current and Future Developments in Physiology

(Volume 1)

Control of Pancreatic Beta Cell Function and Plasticity in Health and Diabetes

Edited by

David J. Hill

Lawson Health Research Institute
St. Joseph's Health Care,
268 Grosvenor Street, London,
Ontario N6A 4V2,
Canada

Current and Future Developments in Physiology

Volume # 1

Control of Pancreatic Beta Cell Function and Plasticity in Health and Diabetes

Editor: David J. Hill

ISSN (print): 2468-7537

ISSN (online): 2468-7545

ISBN (online): 978-1-68108-365-0

ISBN (print): 978-1-68108-366-7

advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of the U.A.E. as applied in the Emirate of Dubai. Each party agrees that the courts of the Emirate of Dubai shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.
3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Ltd.
Executive Suite Y - 2
PO Box 7917, Saif Zone
Sharjah, U.A.E.
Email: subscriptions@benthamscience.org

CONTENTS

FOREWORD

The incidence of both type 1 (T1D) and type 2 diabetes (T2D) has increased over the past few decades and is frequently described as an epidemic. It is now recognized that loss of β-cell mass occurs in both forms of diabetes, through autoimmune destruction in the case of T1D and by exhaustion in the case of T2D. Despite current treatments, blood glucose levels are not restored to normal and this leads to serious injury in several organ systems. Ideally it should be possible to treat or reverse both forms of diabetes by re-establishing sufficient functioning β-cells to maintain normoglycemia. There is general agreement that the cells in the adult pancreas can regenerate, but the routes by which this occurs remain controversial. New β-cells are produced early in life by replication of existing cells, but it is less clear how β-cells could be produced naturally or induced in adults through post-injury ductal neogenesis, activation of resident progenitors/stem cells or transdifferentiation of other non-β-cells (*e.g.*, α-cells or acinar cells). A further complication is understanding to what extent these processes are influenced by environmental conditions.

It has recently been appreciated that terminally differentiated and stem/progenitor cells can differentiate into other cells in the pancreas, consistent with the idea of plasticity in cell fate that depends in part on modification of developmental programs by environmental factors [1]. The fate of cells depends on the type of stress encountered, for example pregnancy, metabolic demand due to hyperglycemia and insulin resistance (glucotoxicity), or ectopic expression or deletion of lineage specific factors.

The importance of restoring lost β-cell mass in T1D has been demonstrated by transplanting islets from cadaveric donors. Unfortunately, this solution is only temporary, requires the recipient to be on powerful immunosuppressive drugs and has side effects. Further constraints are the requirement for 2-3 pancreata/recipient and the limited number of pancreata available for transplantation. Hence, a strong research effort is underway to find other sources of β-cells. These include transplanting pig islets, promoting transdifferentiation of non-β-cells into functioning, insulin-producing β-like cells or enhancing proliferation, maintenance and function of remaining β-cells. Several attempts have been made to develop functioning β-cells from embryonic or adult pluripotent stem cells. However, most reports described the production of immature β-like cells from human pluripotent stem cells that did not respond normally to glucose. A recent report from the Melton group described the *in vitro* production from human embryonic stem cells of glucose-sensitive, insulin-producing β-cells that resemble mature β-cells [2]. It has been pointed out that the basis of this (and other) attempts to find new sources of β-cells comes from a vastly improved and expanded knowledge of pancreas developmental biology [3] and the conditions required to change cell fate, areas

which are addressed in this e-book. As with any discovery, there is good news and bad news. The use of embryonic stem cells still raises ethical concerns and there are technical issues to overcome before such a therapy can be considered for use in the clinic. Others must replicate these findings. It must also be remembered that these cells do not have the exact genetic profile and they are not identical copies of β-cells *in situ* [3]. It is unclear what will be the long term effects of transplanting such cells in patients. As with any "replacement" β-cells, they must be protected from the host's immune system. To do this, they will likely be placed in protective capsules that are themselves the target of a fibrotic process in the body that blocks the exchange of nutrients and insulin across the membrane. In short, it will be a while before these cells are ready for prime time.

Many questions remain unanswered regarding β-cell plasticity. Is it only a subset of cells of a certain lineage (*e.g.*, acinar) that respond to external stress and express transcription factors that promote self renewal or multi-potency directly or through de-differentiation? An ever present danger is the potential for these processes to go unchecked if fate constraints are lost, leading to cancer. It has been pointed out that it is difficult to evaluate transdifferentiation *per se* because the characteristics that reflect the extent of the new cell's maturity and stability of the phenotype are unclear [1]. Does the phenotype of these new cells differ from that of the cells that occur during development?

The editor, Dr. David Hill, has brought together experts in the field who provide extensive and sometimes provocative state-of-the-art discussions of various key aspects of β-cell function and plasticity in health and diabetes. The book is divided into three sections. In the first section entitled: "Origins and developmental biology of β-cells", Dr. Bertrand Duvillié and colleagues (Paris) review advances in understanding the hierarchy of transcription factors and discuss the influence of growth factors, partial oxygen pressure and nutrients in the intrauterine environment on β-cell maturation. This is followed by Aaron Cox's (Houston) discussion of the role of aging on β-cell development and how the decline in β-cell proliferation is affected by age-related impairment in signal transduction, altered cell cycle progression and epigenetic regulation of genes is likely to affect attempts to regenerate β-cells. Part 1 ends with a chapter on β-cell mass across the spectrum of aging, health and diabetes by Dr. Manami Hara and colleagues (Chicago). They discuss the heterogeneous distribution and function of β-cells among and within individuals and the need to identify markers of dysfunctional β-cells.

The second section addresses "Factors controlling β-cell mass and function". Beginning with Dr. David Hill's (London, ON) discussion of gestational programming of β-cell mass and function in which he emphasizes that the period of gestation affects cell fate and β-cell function in the offspring. Using fetal growth retardation as a model, he notes several systems are adversely affected leading to decreased β-cell mass, proliferation, increased apoptosis and

later impaired glucose regulation. The mechanisms are discussed and the point is made that giving micronutrient supplements to the dam and β-cell trophic peptide hormones to the neonate decreases disease risk. The second article by Drs. Mulchand S. Patel and Saleh Mahmood (Buffalo) addresses modification of β-cell function by diet in neonates. They suggest that the feeding of carbohydrate rich food adversely affects islet structure and β cell function in suckling neonates leading to hyperinsulinemia attributable to increased β cell plasticity. The changes noted in developmental gene expression and β-cell function lead to obesity in the adult which could promote development of T2D. The third chapter in this section is by Dr. Brian T. Layden and colleagues (Chicago) in which they provide a discussion of intra-islet control of β-cell function and mass. They highlight the role of under-appreciated autocrine and paracrine factors that contribute to β-cell mass and function. The last chapter in this section by Dr. Jens HøiriisNielson and colleagues (Copenhagen) deals with beta cell adaptability during pregnancy. Although it is well known that β-cell mass expands during pregnancy, they describe in detail the many growth factors, transcriptional modifications and changes in gene expression that have recently been characterized. The mechanism involved in β-cell mass expansion during pregnancy remains unclear, in particular the degree to which neogenesis is involved in rodents and humans.

The third and last part of the book deals with "Generation of β-cells and future applications". Chapter 8 by Dr. Christine A. Beamish (London, ON) addresses the production of β-cells from embryonic and adult stem cells and progenitors as a source of replacement β-cells in diabetes. The plasticity of cell fate in the endocrine pancreas is discussed. The last chapter is by Dr. Tyler T. Cooper and colleagues (London, ON) in which they describe the latest thinking on the role of bone marrow-derived stem cells for β-cell regeneration.

It is now clear that T1D is far more complex than previously appreciated [4]. In particular, the long history of mostly immune-based therapies has not resulted in new treatments or cures. Despite more than 20 clinical trials, most focused on immune suppression, there has been a remarkable lack of success with respect to prevention or reversal of T1D [5]. The picture continues to evolve with respect to T2D as well, as a role for adipose tissue inflammation is now being investigated. To address this level of complexity, both forms of diabetes must be thought of in terms of their integrative biology. That is, how do genetic predisposition, inappropriate immune reactivity, inappropriate β-cell mass/function/regenerative capacity and environmental exposures come together to result in diabetes? We are entering a new era of big data obtained through readily available high throughput analyses and higher computer power. This has raised the possibility of personalized or so-called "precision medicine" to address the heterogeneity in the human population and reveal the many pathways by which diabetes likely occurs. A further new approach involves the acknowledgement that there is unlikely to be a single infectious agent akin to *helicobacter pylori* and ulcers in the

environment that causes diabetes. To address the multiplicity of environmental factors that undoubtedly influence diabetes and other chronic diseases, a new "omics" designation was coined, termed the exposome [6]. And finally, it must be said that timing is indeed everything. Thus, the timing of these interactions and exposures is crucial in determining the success of the early developmental program for appropriate β-cell mass/function or its potential re-capitulation following injury, inflammation or disease. The articles in this e-book provide up-to-date summaries of key elements affecting natural and induced forms of β-cell plasticity and its potential as a therapy for diabetes. The reader will no doubt enjoy this timely collection.

Dr. Fraser W. Scott
The Ottawa Hospital Research Institute
Department of Medicine
Department of Biochemistry, Microbiology and Immunology
University of Ottawa
Ottawa, Ontario, Canada

REFERENCES

[1] Puri S, Folias AE, Hebrok M. Plasticity and dedifferentiation within the pancreas: development, homeostasis, and disease. Cell Stem Cell 2015; 16(1): 18-31.
[http://dx.doi.org/10.1016/j.stem.2014.11.001] [PMID: 25465113]

[2] Pagliuca FW, Millman JR, Gürtler M, *et al.* Generation of functional human pancreatic β cells *in vitro*. Cell 2014; 159(2): 428-39.
[http://dx.doi.org/10.1016/j.cell.2014.09.040] [PMID: 25303535]

[3] Mfopou JK, Bouwens L. Diabetes: β cells at last. Nat Rev Endocrinol 2015; 11(1): 5-6.
[http://dx.doi.org/10.1038/nrendo.2014.200] [PMID: 25385037]

[4] Atkinson MA, von Herrath M, Powers AC, Clare-Salzler M. Current concepts on the pathogenesis of type 1 diabetesconsiderations for attempts to prevent and reverse the disease. Diabetes Care 2015; 38(6): 979-88.
[http://dx.doi.org/10.2337/dc15-0144] [PMID: 25998290]

[5] Lord S, Greenbaum CJ. Disease modifying therapies in type 1 diabetes: Where have we been, and where are we going? Pharmacol Res 2015; 98: 3-8.
[http://dx.doi.org/10.1016/j.phrs.2015.02.002] [PMID: 25771310]

[6] Wild CP. Complementing the genome with an exposome: the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 2005; 14(8): 1847-50.
[http://dx.doi.org/10.1158/1055-9965.EPI-05-0456] [PMID: 16103423]

PREFACE

An adaptive metabolic axis has been a major evolutionary advantage in allowing humans to colonize every part of the globe from arid deserts to permanent ice fields. Prior to an effective food supply chain, metabolic plasticity evolved to deal with seasonal famines balanced by times of plenty, and a greater diversity of diets than perhaps any other mammalian species. In the developed world there are new challenges to metabolic plasticity including food over-abundance, unbalanced diets, child and adult obesity, and an increasing rate of type 1 and 2 diabetes. A plasticity of pancreatic β-cell mass and function are key to metabolic adaption. The β-cell mass normally increases proportionally to fetal and child growth, in response to the added metabolic stress of pregnancy, and in response to the nutritional stress of an obesogenic diet. Yet, in the face of the autoimmune challenge of type 1 diabetes or the glucotoxicity of type 2 diabetes there is a net loss of β-cells with limited potential for endogenous regeneration. Thus lies the paradox. How can a highly physiologically-adaptive β-cell mass prove so difficult to manipulate following the pathological loss that accompanies diabetes?

Key to creating and testing strategies for the therapeutic manipulation of β-cell number is to know their developmental origins and normal ontogeny. The first section of this volume addresses current knowledge around the developmental origins of pancreatic β-cells, and how β-cell mass and proliferation change throughout the human lifespan. The second section explores the mechanisms responsible for β-cell plasticity, drawing from animal models and clinical studies revealing environmental, epigenetic, endocrine and paracrine regulators that contribute to the normal homeostatic processes, and the delicate balance of proliferation *vs*. apoptotic loss that optimizes β-cell mass during normal metabolic homeostasis. The final section examines the presence and potential of resident stem cells within the pancreas or bone marrow, β-cell progenitors, and the potential for pancreatic endocrine cell differentiation or trans-differentiation.

Underlying each of these chapters is the assumption that β-cells can potentially be replaced endogenously, but only through a thorough understanding of normal development and the exploitation of existing, but perhaps sub-optimal adaptive physiology. There is great reason for confidence. In humans there is reproducible histological evidence of β-cell turnover involving mitogenesis and apoptosis throughout life, including both children and adults with type 1 or type 2 diabetes [1 - 5]. The regenerative potential of human β-cells may normally be age-limited, since new cells were not generated in the short-term in patients aged over 50 following surgical reduction of pancreatic mass [6]. However, insulin release had improved between 2-4 years post-surgery, suggesting that even in older individuals a slower adaptive replacement of β-cells can occur [7]. Overcoming such physiological limitations to optimize

β-cell mass and function to match metabolic demand will likely be a major focus for diabetes research in the coming decade.

Dr. David J. Hill
Lawson Health Research Institute
St. Joseph's Health Care, 268 Grosvenor Street
London, Ontario N6A 4V2
Canada
Tel: 519 6466100 Ext. 64716
E-mail: david.hill@lhrionhealth.ca

REFERENCES

[1] Gepts W, De Mey J. Islet cell survival determined by morphology. An immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus. Diabetes 1978; 27 (Suppl. 1): 251-61. [http://dx.doi.org/10.2337/diab.27.1.S251] [PMID: 75815]

[2] Yoneda S, Uno S, Iwahashi H, *et al.* Predominance of β-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. J Clin Endocrinol Metab 2013; 98(5): 2053-61. [http://dx.doi.org/10.1210/jc.2012-3832] [PMID: 23539729]

[3] Keenan HA, Sun JK, Levine J, *et al.* Residual insulin production and pancreatic ß-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes 2010; 59(11): 2846-53. [http://dx.doi.org/10.2337/db10-0676] [PMID: 20699420]

[4] Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? Diabetologia 2005; 48(11): 2221-8. [http://dx.doi.org/10.1007/s00125-005-1949-2] [PMID: 16205882]

[5] Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Evidence of increased islet cell proliferation in patients with recent-onset type 1 diabetes. Diabetologia 2010; 53(9): 2020-8. [http://dx.doi.org/10.1007/s00125-010-1817-6] [PMID: 20532863]

[6] Menge BA, Tannapfel A, Belyaev O, *et al.* Partial pancreatectomy in adult humans does not provoke β-cell regeneration. Diabetes 2008; 57(1): 142-9. [http://dx.doi.org/10.2337/db07-1294] [PMID: 17959931]

[7] Menge BA, Breuer TG, Ritter PR, Uhl W, Schmidt WE, Meier JJ. Long-term recovery of β-cell function after partial pancreatectomy in humans. Metabolism 2012; 61(5): 620-4. [http://dx.doi.org/10.1016/j.metabol.2011.09.019] [PMID: 22079939]

List of Contributors

Aaron R. Cox	McNair Medical Institute, Baylor College of Medicine, Houston, Texas
Amarnadh Nalla	Department of Biomedical Sciences, University of Copenhagen, Denmark Centre for Fetal Programming, Copenhagen, Denmark
Ananta Poudel	Department of Medicine, The University of Chicago, Chicago, USA
Angela H. Darmon	INSERM, U1016, Institut Cochin, Paris, France Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
Benjamin Broche	INSERM, U1016, Institut Cochin, Paris, France Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
Bertrand Duvillié	INSERM, U1016, Institut Cochin, Paris, France Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
Birgitte Søstrup	Department of Biomedical Sciences, University of Copenhagen, Denmark Centre for Fetal Programming, Copenhagen, Denmark
Brian T. Layden	Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois, USA
Christine A. Beamish	Department of Surgery, Islet Transplantation Laboratory, The Methodist Hospital Research Institute, Houston Texas, USA
David A. Hess	Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada
David J. Hill	Lawson Health Research Institute, St. Joseph's Health Care, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada
Jeanette Kirkegaard	Department of Biomedical Sciences, University of Copenhagen, Denmark Novo Nordisk A/S, Måløv, Denmark
Jens H. Nielsen	Department of Biomedical Sciences, University of Copenhagen, Denmark Centre for Fetal Programming, Copenhagen, Denmark
Jonas Fowler	Department of Medicine, The University of Chicago, Chicago, USA
Manami Hara	Department of Medicine, The University of Chicago, Chicago, USA
Mulchand S. Patel	Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA

Paul Richards	INSERM, U1016, Institut Cochin, Paris, France Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France
Ruth M. Elgamal	Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada
Saleh Mahmood	Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA
Signe Horn	Department of Biomedical Sciences, University of Copenhagen, Denmark Novo Nordisk A/S, Måløv, Denmark
Stephanie Villa	Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
Tyler T. Cooper	Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada
William L. Lowe	Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

Section 1: Origins and Developmental Biology of β-Cells

CHAPTER 1

Understanding the Developmental Biology of β-Cells as a Strategy for Diabetes Reversal

Bertrand Duvillié[1,2,*], Benjamin Broche[1,2], Angela Herengt Darmon[1,2] and **Paul Richards[1,2]**

[1] *INSERM, U1016, Institut Cochin, Paris, France*

[2] *Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France*

Abstract: In recent decades, intense efforts have been made to understand the cellular and molecular mechanisms controlling β-cell development. This process is well coordinated and consists of multiple steps. Many studies have tried to identify (i) molecular signals governing the proliferation of progenitors and (ii) their differentiation into mature pancreatic β-cells. A number of laboratories have focused on the role of transcription factors, and well constructed experiments have contributed to defining a hierarchy, highlighting the importance of each transcription factor in the interconnected network. Moreover, studies over the last 10 years have shown that the pancreatic mesenchymal cells, which are in contact with progenitors, influence pancreas organogenesis. Recent work has also indicated that the intra-uterine milieu influences gene expression and endocrine development. Indeed, nutrients, locally expressed growth factors and even the partial pressure of oxygen also control pancreas development. In a more applied setting, these understandings may improve our knowledge on the different forms of diabetes and, importantly, allow us to mimic a similar developmental process *in vitro*. This is because the precise understanding of each step *in vivo* seems to be necessary for designing protocols to generate β-cells from embryonic stem (ES) cells or induced pluripotent stem cells (iPS). These stem cell-derived β-cells should, in theory, provide new sources of insulin-secreting cells for transplantation into diabetic patients. A description of the recent advances in the field will be presented and illustrated in this chapter.

[*] **Corresponding author Bertrand Duvillié:** Institut Curie, Centre de Recherche, Laboratoire Eychene, INSERM U1021, CNRS UMR3347, Bât. 110, Centre Universitaire, 91405 ORSAY Cedex, France; Tel: (33) 1 69 86 30 74, Fax: (33) 1 69 86 30 51; E-mail: bertrand.duvillie@curie.fr

David J. Hill (Ed.)

Keywords: Beta cell, Development, Differentiation, FGF, HIF, Insulin, Oxygen, Pancreas, Proliferation, Reactive oxygen species.

GENETIC NETWORK CONTROLLING PANCREATIC DEVELOPMENT

The pancreas originates from the dorsal and ventral regions of the foregut endoderm, located directly behind the stomach. Signals from adjacent mesodermal structures, notochord, dorsal aorta and cardiac mesoderm are important for the emergence and early development of the pancreas [1 - 5]. During these early stages, *i.e.* E11.5 in the mouse, the pancreas is composed of an undifferentiated epithelium surrounded by mesenchyme. The epithelial fraction contains all the precursor cells that develop into the exocrine and endocrine compartments. A sequential expression of transcription factors is required to determine the commitment of these early progenitors to the different endocrine and exocrine cell types.

The Sequential Implication of Transcription Factors

These precursor cells express the gene *Pancreatic and Duodenal Factor 1* (*Pdx1*). This is a master gene as its deletion leads to the complete agenesis of the pancreas in the mouse. In humans, the paralogue of Pdx1 is called insulin promoting factor 1 (IPF1). Interestingly, a pancreatic agenesis was also described in a patient with a homozygous single nucleotide deletion in the codon 63 of IPF1. The role of Pdx1 is thus conserved between rodents and humans. Following Pdx1 expression, the transcription factor Neurogenin 3 (NGN3) is transiently expressed in endocrine progenitors. The disruption of *Ngn3* results in the absence of α−, β−, δ− and PP-cells [6]. Other transcription factors are involved more specifically in exocrine cell development, for example MIST1 [7]. The genetic network controlling pancreas development is represented in Fig. (**1**).

In addition to these complex genetic mechanisms, it has been shown that the local environment releases signals that determine not only proliferation of the precursor cells but also their differentiation. A detailed review of these signals will be presented in the next section.

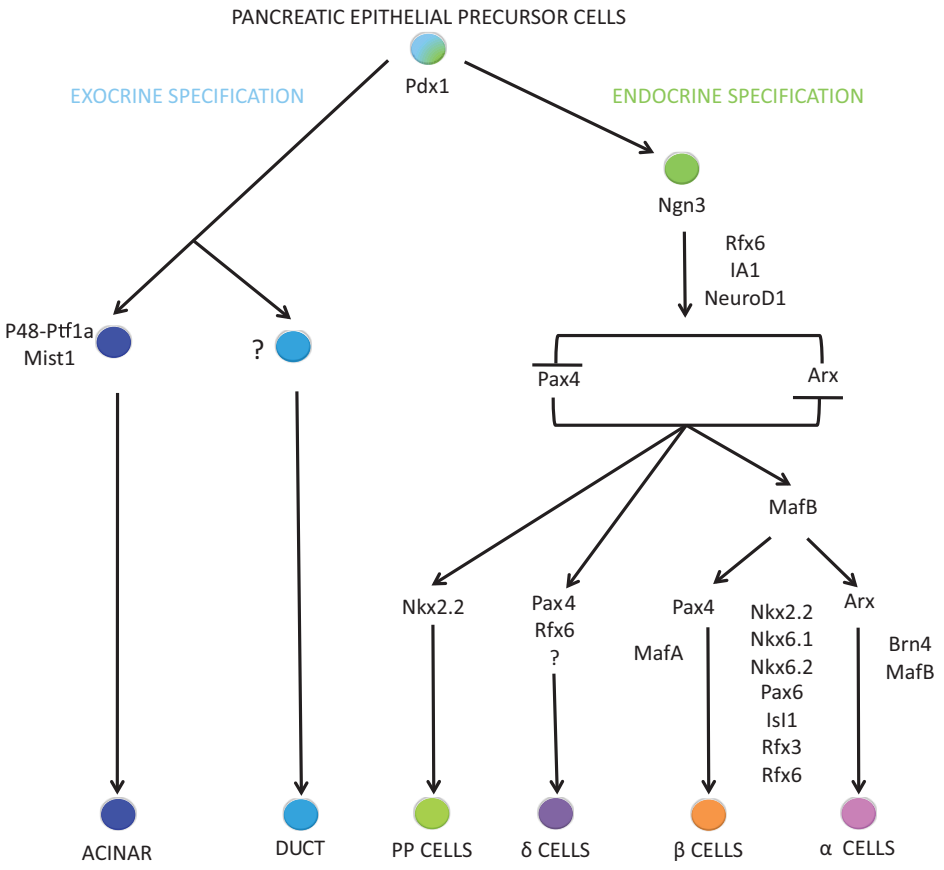


Fig. (1). The genetics of pancreas development [8].

THE PANCREATIC MESENCHYME AND GROWTH FACTORS

For many years the exact role of the mesenchymal cells that surround the epithelial precursors remained elusive and was the subject of much debate. In the laboratory of R Scharfmann, an *in vitro* model allowing the development of the embryonic pancreas in the presence or absence of the mesenchyme was designed [9]. In these conditions, more acinar cells developed in the presence rather than in the absence of the mesenchyme. To investigate the mechanism by which the mesenchyme exerts its effects, the expression of tyrosine kinase receptors was researched. A special interest was paid to the Fibroblast Growth Factors family (FGFs). There are four FGF receptors, called FGF1 to FGF4, with several isoforms. Of importance, the FGFR2IIIb isoform expression is specific to the

CHAPTER 2

Aging and β-Cell Proliferation, Molecular and Signaling Changes and What This Means for Targeted Regeneration

Aaron R. Cox*

McNair Medical Institute, Baylor College of Medicine, Houston, Texas, U.S.A

Abstract: Increased age confers a greater risk for the development of type 2 diabetes (T2D), and also has significant consequences for β-cell growth and regeneration. Pancreatic insulin-producing β-cells are long-lived, and exhibit very little turnover in adult life. The severe decline in β-cell proliferation contributes to a decreased capacity for β-cell regeneration with age. β-cell regeneration is dependent on mitogenic signals, receptor and downstream signal transduction, cell cycle progression, and epigenetic regulation of gene expression, all of which are significantly affected by increasing age. Studies suggest that circulating growth factors and their receptors are decreased with age, along with important intracellular signaling molecules, such as Pdx-1 and FoxM1. Cell cycle progression is inhibited by an increased expression of cell cycle inhibitors and a reduction in cell cycle kinase complexes (Cyclin/Cdks). Moreover, decreased expression of epigenetic silencers, such as polycomb group proteins, results in de-repression of the cell cycle inhibitor p16, and a significant reduction in β-cell proliferation. Collectively, these age-induced changes present obstacles for the design of β-cell regenerative therapies for diabetes; however, some reports suggest that even very old β-cells can re-enter cell cycle. Future studies will further define the effects of aging on β-cell proliferation and elucidate new drug targets for diabetes therapy.

Keywords: Aging, β-cell regeneration, Cell cycle, Diabetes, Epigenetics, Molecular signals, Proliferation.

* **Corresponding author Aaron R. Cox:** McNair Medical Institute, Baylor College of Medicine, Houston, Texas, U.S.A; Tel/Fax: 832-824-0711; E-mail: racox@bcm.edu

David J. Hill (Ed.)

AGING AND DIABETES

Aging is associated with an increased risk for several diseases including metabolic syndrome and type 2 diabetes (T2D). The incidence of T2D increases with age from ~8% in middle age (40-59 years) to ~33% in older adults (>60 years) [1]. These rates increase substantially when accounting for individuals with prediabetes, defined as impaired fasting glucose or glucose tolerance. There are many factors which may contribute to the age associated increased risk of diabetes. Peripheral insulin sensitivity declines with age, and can be attributed to increased obesity, reduced physical activity, and decreased lean muscle mass [2 - 4]. These changes are moderately compensated by increased β-cell function and hyperinsulinemia [5]. However, aging is also associated with a progressive decrease in β-cell function [6 - 8], which may be impacted by decreased incretin levels [9, 10], Sirt1-mediated glucose stimulate insulin release [11], mitochondrial function and ATP content [12, 13], as well as increased oxidative stress [12] and glucolipotoxicity [14]. Moreover, isolated islets demonstrate greater sensitivity to glucose-induced β-cell apoptosis with increasing age [8]. Thus, chronic hyperglycemia and insulin resistance in the setting of aging and declining β-cell function collectively contribute to drive β-cell loss. Human post-mortem samples demonstrate increased rates of apoptosis and reduced β-cell mass in patients with T2D [15]. The inability of β-cells to adequately compensate through increased β-cell mass in the presence of insulin resistance leads to β-cell apoptosis and T2D. Elucidation of the mechanisms of aging that contribute to failed β-cell compensation will be critical for diabetes therapies. Aging is also an important factor in type 1 diabetes (T1D) given that over 50% of T1D cases occur in adults [16], and moreover, with insulin therapy and quality health care, the life expectancy of juveniles with T1D extends well into adulthood [17]. Considering the large adult population with T1D, age associated changes in β-cell function and mass may influenced the design of successful diabetes therapies.

β-CELL PROLIFERATION AND AGING

Pancreatic β-cell mass rapidly increases in young rodents and slowly expands with advancing age [18, 19]. Maintenance and expansion of β-cell mass is dependent on a fine balance between cell birth (self-replication and neogenesis)

and cell death (apoptosis). Postnatal β-cell neogenesis is extremely controversial, and while many suggest that β-cells arise from exocrine duct cell differentiation [20 - 22], there is strong evidence to suggest that postnatal β-cell growth exclusively occurs by self-duplication [23 - 27]. Thus, it is critically important to understand the underlying biology governing β-cell proliferation for developing diabetes therapies.

β-cell proliferation in the early postnatal period is ~20% in rodents [18, 28], declining dramatically in adolescence and into young adulthood [18, 19, 28, 29]. This decline stabilizes within the first 100 days of life to ~1-4% per day [18]. Based on this data, it was estimated that the lifespan of a β-cell was between 1 and 3 months [18]. Limited data at older ages prevented conclusive determination of the β-cell replication rate in older rats. Subsequently, Montanya *et al.* [19] measured intra-islet proliferation in rats up to 20 months of age. The authors determined that intra-islet cell proliferation stabilized at ~0.1% using a 6-h BrdU pulse (~0.4% per day) at 7 months of age. Mouse β-cell proliferation was extremely low at 1 year of age, ~0.04% per day, and remained constant at 19-months [28, 30]. Similarly, β-cell apoptosis in metabolically normal rodents is rare and decreases with age [28, 31, 32]. β-cell turnover is governed by the replication refractory period, which prevents cell cycle entry immediately following cell division, thus limiting the frequency a β-cell can divide [24]. This replication refractory period of β-cell turnover is lengthened with age [24, 33]. Collectively, these studies suggest that β-cells are largely post-mitotic and long-lived, contributing to a very slowly expanding adult tissue through self-renewal. Therefore, rodent β-cell turnover is minimal with increasing age.

Human β-cell proliferation also decreases with age [15, 34 - 37]. Meier *et al.* [34] measured β-cell proliferation by Ki67 in 46 tissue samples from children and young adults from 2 weeks to 21 years. In some infants, β-cell proliferation was quite high (~2.5%), and quickly declined with age. Surprisingly, pancreata from several individuals indicated no β-cell proliferation even at an early age (<10 years). Low replication rates were also observed in middle age [35]. By elderly age (~78 years), data from 17 non-diabetic individuals indicated that β-cell proliferation rates had fallen below 0.1% $Ki67^+$ β-cells [15]. While $Ki67^+$ expression is a static measurement at the time of pancreas collection, thymidine

CHAPTER 3

Human β-Cell Mass and Distribution in Health, Aging and Diabetes

Jonas L. Fowler, Ananta Poudel and **Manami Hara***

Department of Medicine, The University of Chicago, Chicago, USA

Abstract: Regulation of pancreatic β-cell mass is an essential matter to understand pathophysiology of diabetes. Physiological and pathological changes of β-cell mass associated with aging, obesity and diabetes have been reported for over a century. However, the degree of compensation or alteration significantly varies among literature. The difficulty in studying the human pancreas is its large size and uneven distribution of β-cells/islets. Whole pancreas analysis has revealed intra-individual (regional) and inter-individual heterogeneity in β-cell mass, which hampers accurate quantification. Furthermore, physical β-cell loss is not the only contributing factor, but "dysfunctional" β-cells may be involved in insulin deficiency as well. Development of a practical stereological approach to quantify β-cell mass to overcome intra-individual and inter-individual heterogeneity would provide a standardized methodology in the field. Identification of marker(s) for quantifying dysfunctional β-cells that synthesize insulin but are deficient in insulin secretion should lead to a better understanding of β-cell pathophysiology.

Keywords: Aging, β-cell mass, Diabetes, Islets.

INTRODUCTION

Accurate quantification of β-cell mass in the human pancreas is challenging due to its large size. Furthermore, there is marked variability in the β-cell/islet distribution in the different regions of the pancreas. The head region is anatomically and developmentally distinct, and it exclusively contains a pancreatic polypeptide

* **Corresponding author Manami Hara:** Department of Medicine, The University of Chicago, Chicago, USA; Tel/Fax: 773-702-3727/773-834-0486; E-mail: mhara@uchicago.edu

David J. Hill (Ed.)

(PP) cell rich area, which can confound analysis of β-cell mass if not analyzed separately. Besides these regional differences, there is also marked variability among individuals that show no direct association with sex, age, BMI or type 2 diabetes (T2D). Starting with the basics of the human pancreas from its anatomy to intrinsic heterogeneity in β-cell mass, this chapter will review recent studies on β-cell mass and distribution in health, aging and diabetes and current difficulties of accurately assessing β-cell mass will be discussed.

HUMAN PANCREAS

Anatomy

The human pancreas is commonly divided into three main regions based on the anatomy, which are referred to the head, body, and tail. The head is localized closely to the duodenum on the right side of the abdomen. The superior mesenteric vessels beneath the neck are inferior and to the left of the head of the pancreas, while the uncinate process lies within the head posterior and to the right of the superior mesenteric vessels. The body region extends laterally to the left of the head, lying along the floor of the lesser sac, which is covered by peritoneum derived from the superior leaf of the transverse mesocolon. The tail region extends from the body laterally into the left side of the abdomen towards the spleen, where there is no coverage from transverse mesocolon derived peritoneum.

The head region is distinct from the rest of the pancreas both developmentally and anatomically [1] (Fig. **1**). The pancreas has two main sources of blood supply. The superior pancreaticoduodenal artery supplies the head region, while the splenic artery supplies both the body and tail. Hepatic ducts from the liver and cystic duct from the gall bladder merge into the common bile duct, which flows down to the head region of the pancreas and at the ampulla of Vater connects with the pancreatic duct. The ductal network is controlled by innervations from the celiac plexus and vagus. The sphincter of Oddi, a circular muscle band, functions as a valve to regulate the flow of pancreatic enzymes and bile into the duodenum, separating the pancreas from the intestinal environment.

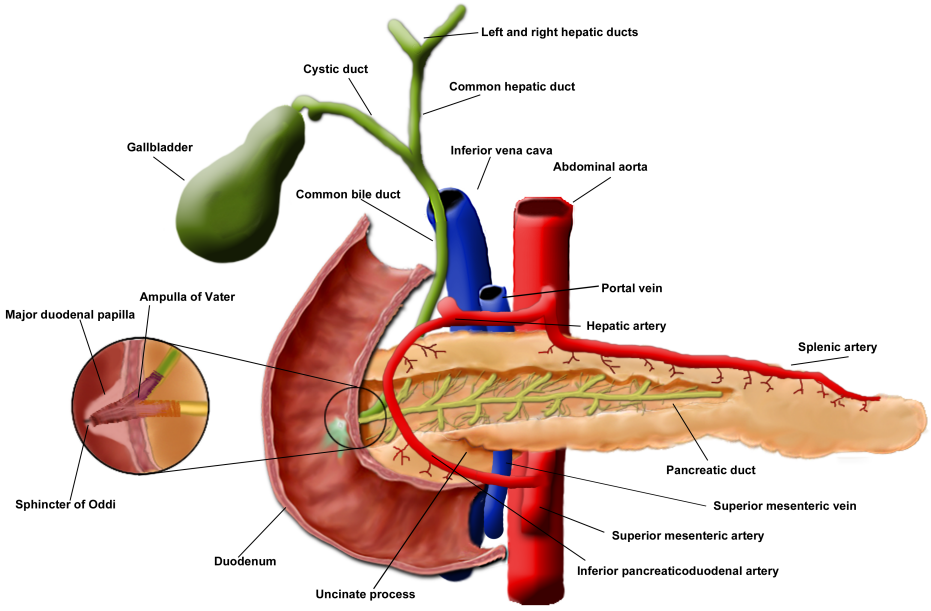


Fig. (1). Head region of the human pancreas (Reproduced from [1]).

Regional Differences in β-Cell/Islet Mass

The pancreatic islet is a highly vascularized micro-organ that is composed of multiple cell types including relatively large populations of β-, α- and δ-cells and small populations of PP- and ε-cells. There is an intrinsic regional difference in β-cell mass and correspondingly islet mass [2, 3]. While it is comparable between the head and body region, there is a gradual increase in β-cell and islet mass toward the tail region resulting in a ~2-fold difference. This regional difference in β-cell/islet density has important implications for the assessment of β-cell mass. Random inter-specimen comparisons between different regions may confound study accuracy. For example, a study of the tail region alone where the measured values are normalized using the pancreas volume or weight could result in overestimation of β-cell/islet mass, whereas using only the head or body regions could result in underestimation.

Section 2: Factors Controlling β-Cell Mass and Function

CHAPTER 4

Gestational Programming of β-Cell Mass and Pancreatic Function in the Next Generation

David J. Hill*

Lawson Health Research Institute, St. Joseph's Health Care, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada

Abstract: The gestational environment can have profound effects on the future health of the offspring, including a greater risk of type 2 diabetes and of cardiovascular diseases. Whilst the function of numerous tissues that can impact on future metabolism are altered by an adverse fetal environment, including the hypothalamic control of appetite and the release of glucocorticoids, hepatic function, and the insulin sensitive tissues such as skeletal muscle and adipose, some of the most definitive data concerns changes in the phenotype and function of the pancreatic β-cells. A number of animal models of intrauterine growth restriction (IUGR) have been utilized to study the long-term effects on the offspring, such as a reduced maternal calorie intake, a reduced protein content of the diet, uterine vessel occlusion, and nicotine administration. Changes to the pancreatic β-cells are remarkably similar and include a reduced tissue mass, lower rate of proliferation, increased developmental apoptosis, less plasticity following damage postnatally, higher sensitivity to cytotoxic cytokines, and reduced glucose-stimulated insulin release. These changes persist into adulthood and result in impaired glucose tolerance, Similar changes are also seen in offspring from pregnancies complicated by maternal diabetes. The mechanisms responsible for the altered β-cells function include changes to the mTOR signaling pathway, epigenetic changes altering the expression of key genes involved with β-cell growth and insulin synthesis, and changes in the rate of telomere shortening resulting in premature cellular aging. These pathways may also be influenced by environmental toxins during pregnancy. Nutritional intervention by micronutrient supplementation of the mother, or treatment of the newborn with peptide hormones trophic for the β-cells can reverse the pancreatic phenotype and reduce the risk of adult metabolic disease.

* **Corresponding author David J. Hill:** Lawson Health Research Institute, St. Joseph's Health Care, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada; Tel/Fax: 519 6466100 Ext. 64716; E-mail: david.hill@lhrionhealth.ca

David J. Hill (Ed.)

Keywords: β-cell mass, Epigenetics, Fetal programming, Gestational diabetes, Intra-uterine growth restriction, MTOR, Pregnancy, Type 2 diabetes.

INTRODUCTION

The pre-conceptual and gestational environment can have profound effects of the future health of the offspring, including a greater risk for type 2 diabetes, cardiovascular diseases and other chronic diseases [1]. This was convincingly shown in cohort studies of individuals exposed to the Dutch hunger winter of 1944/45 where severe maternal calorie restriction during first trimester resulted in a reduced birth weight and a higher rate of offspring obesity by 19 years of age, associated with diabetes and vascular disorders in later life [2]. Surprisingly, similar disease risks are observed in the offspring of women who were obese or had diabetes prior to conception, or who developed hyperglycemia, with or without gestational diabetes [3 - 6]. The impact of relative under- or over-nutrition of the fetus on future health has been shown to involve programmed changes to a number of key tissues involved with energy homeostasis, including adipose, liver, and muscle [7], as well as changes to the neuronal architecture of the hypothalamus resulting in altered production of peptides that determine appetite, such as NPY [8], responsiveness to leptin [9] and the basal levels of cortisol and tissue glucocorticoid receptors [10]. Fetal programming of future metabolic diseases is therefore likely to represent an accumulated effect of developmental changes across multiple tissues. However, some of the most profound and well-studied determinants involve changes to the phenotype and function of the pancreatic β-cells. This chapter will review some of the animal models used and the mechanisms proposed for environmental programming of the developing endocrine pancreas prior to birth, and some of the corrective strategies so far investigated.

ANIMAL MODELS OF FETAL PROGRAMMING OF THE ENDOCRINE PANCREAS THROUGH NUTRITIONAL DEFICIT

A number of animal models that disrupt maternal nutritional availability or uteroplacental function have been used to represent the human small-fo--gestational age term infant, as reviewed previously [11]. These include reduced

calorie availability to the pregnant rat or mouse [12, 13], uterine vessel occlusion to reduce utero-placental blood flow [14] and nicotine administration to the mother [15]. In each case the resulting changes to endocrine pancreas morphology and function are remarkably similar. We and others have utilized a maternal low protein diet (LP) made isocalorific by the addition of carbohydrate from gestation to either parturition, or continued until weaning. If LP diet was given to rats or mice throughout pregnancy it resulted in a lower β-cell mass and mean islet size in the offspring at birth, and this was further exacerbated at weaning [16 - 19]. This was due to less β-cell proliferation together with an increased rate of developmental apoptosis. Further analysis showed that the cell cycle kinetics of β-cell replication had been altered with an extended G1 phase [16]. Whilst some recovery of β-cell mass was possible if the LP dietary insult was removed at parturition, severe deficits remained throughout life if the insult was extended to weaning [17]. Glucose-stimulated insulin release was reduced and β-cells were more susceptible to cytokine-induced cell death *in vitro* [16, 20]. These deficits were transmitted to the F2 generation through females, even when the F1 offspring received a normal diet through gestation. Once adult at 130 days of age the offspring of the LP-fed rats were glucose intolerant with peripheral glucose resistance [19]. A direct human correlate of the maternal LP diet model in rodents is the reduced birth weight associated with restricted maternal protein intake experienced by vegetarian women in rural India, as characterized by the Pune Maternal Nutrition Study [21, 22].

Exposure to LP diet during gestation also had phenotypic effects on the anatomy of the microvasculature of the pancreas. This is important as paracrine signaling occurs across the basement membrane juxtaposing the β-cell and the capillary endothelium, mediated by integrins and by various peptide growth factors, including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) and [23 - 25]. The presence of VEGF-A is necessary to maintain a fenestrated islet endothelium that allows for rapid glucose sensing from the circulation and the export of secreted insulin [24]. We reported that intra-islet vascular volume and the abundance of the VEGF receptor were both lower in the offspring of LP-fed animals at birth [26]. Islet microvacular density remained compromised in the pancreas of the offspring until adulthood. Similarly, the

CHAPTER 5

Malprogramming of β-Cell Function by a Dietary Modification in the Immediate Postnatal Period

Mulchand S. Patel* and **Saleh Mahmood**

Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York, Buffalo, New York 14214, USA

Abstract: The development of the structure and function of the endocrine pancreas is known to be influenced by altered nutritional experience during the fetal period. Nutritional modifications in the suckling period are also recognized as contributing factors to developmental programming of the endocrine pancreas. In this chapter we describe the malprogramming of rat pancreatic islet structure and β cell functions in response to an increased intake of carbohydrate-derived calories in a milk formula (HC) during the suckling period. Alterations in β cell function of HC rat pups result in the development of hyperinsulinemia due to β cell plasticity in the immediate postnatal period. These modifications include: altered islet architecture and increased insulin-producing mass, increased insulin secretion capacity with a leftward shift in glucose-stimulated insulin secretion, insulin secretion in the absence of glucose and/or Ca^{2+}, increased gene transcription of several genes crucial for β cell development and function, and increased parasympathetic input, as well as malprogramming of orexigenic circuitry in the hypothalamus. Interestingly, these alterations in β cell function are maintained even after weaning of HC rats on a standard rodent chow, resulting in adult-onset obesity due to development of hyperphagia. It is possible that early introduction of carbohydrate-rich infant supplemental foods could contribute to modified β cell functions in infants which could, in turn, over a longer period predispose to the development of childhood obesity and/or adult-onset obesity and its associated metabolic complications including type 2 diabetes.

* **Corresponding author Mulchand S. Patel:** Department of Biochemistry, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214, USA; Tel: 716-829-3074, Fax: 716-829-2725; E-mail: mspatel@buffalo.edu

David J. Hill (Ed.)

Keywords: Artificial rearing of rat pups, β cell neogenesis, β cell proliferation, Hyperinsulinemia, Hypothalamic programming, Insulin secretion, Metabolic programming, Nutritional modification, Obesity, *Pdx-1* gene expression.

INTRODUCTION

Metabolic Programming After Birth

Numerous lines of evidence from animal models, epidemiological studies and clinical investigations indicate that altered nutritional exposures during critical periods of early development (fetal and immediate postnatal periods) can have permanent effects at the cellular, molecular, and biochemical levels in several tissues [1 - 7]. During this rapid growth period, the endocrine pancreas in rodents undergoes both normal structural and biochemical maturation of the endocrine functions [8, 9]. Altered nutrition during these early phases of life can induce permanent adaptations in these processes which continue to be expressed in adulthood [6, 10 - 14]. This phenomenon was initially proposed by Barker [15] as 'Fetal Origins of Adult Disease' followed by other similar terms and more recently referred to as 'Developmental Origins of Health and Disease'. Altered nutritional experiences during the fetal period can be exerted due to maternal malnutrition (such as caloric restriction, protein deficiency, calorie plus protein deficiency, vitamin deficiency), maternal obesity and maternal diabetes.

Accumulated evidence indicates that nutritional alterations (undernutrition, overnutrition and altered milk composition) in the immediate postnatal period (referred to as the suckling period) alone can malprogram metabolic capacities of tissues (*e.g.* pancreatic β cells and hypothalamus) with increased risk for the development of metabolic disorders in adulthood. Human milk is the natural choice for infants because it not only provides nutrients for optimal growth but is also the source of bioactive compounds and immunoglobulins for immunity development during the immediate postnatal period [16]. Commercially available infant formulas are devoid of the latter components. The health benefits of breast-feeding over infant formula feeding are widely recognized, including optimal growth during childhood and longer term benefits such as lower rates of cardiovascular risk and obesity in adulthood [16, 17]. During the suckling period,

over-nourishment may be caused by infant milk-formula feeding (bottle feeding with unrestricted supply of milk formula) with or without early introduction of carbohydrate-enriched infant foods. Although exclusive breast-feeding of babies for the first six months is recommended by the American Dietetic Association [18], formula feeding (aka bottle feeding) and early introduction of complementary infant foods are widely practiced in Westernized societies. It is quite possible that feeding formula alone may result in over-feeding causing increased weight gain and possible metabolic programming of infants. Rodent models of overnutrition by intake of excess maternal milk during the suckling period (*e.g.* by reducing the litter size) are well characterized for metabolic programming of the hypothalamus and pancreatic β cells, resulting in excess weight gain during the immediate postnatal period and predisposition for the development of adult on-set obesity [19 - 22].

Unlike human milk and infant milk formula with a higher level of calories derived from fat, supplementary infant foods (fruits, juices, cereals) are highly enriched with carbohydrate-derived calories but have few calories from fats. Hence, early introduction of infant foods prior to 6 months of age can increase carbohydrate-derived calorie ingestion. The focus of our research has been on metabolic programming effects in β cells due to increased intake of carbohydrate *via* a milk-substitute formula by rat pups during the suckling period [23 - 25]. To investigate this phenomenon we have employed a rat model (referred to as Pup-in-a-Cup model) in which rat pups are artificially reared by intra-gastric feeding of a milk formula high in carbohydrate-derived calories during the suckling period. Based on the observations on the HC rat model, as presented in this chapter, it is possible that early introduction of sugar-dense supplementary foods for human infants could be a contributing factor to metabolic programming of β cells and hence in the etiology of obesity in childhood and adulthood.

EXPERIMENTAL APPROACHES: 'PUP-IN-A-CUP' RAT MODEL

To feed a modified rat milk-substitute formula enriched in carbohydrate-derived calories to neonates during the suckling period, we took advantage of an artificial rearing technique employing intragastric feeding of rats [26 - 28]. On postnatal day 4, pups born to normal dams consuming standard laboratory chow *ad libitum*

CHAPTER 6

New Concepts in the Intra-Islet Control of β-Cell Function and Mass

Brian T. Layden[1,2,*], Stephanie Villa[1] and **William L. Lowe[1]**

[1] *Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA*

[2] *Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois, USA*

Abstract: The regulation of pancreatic β-cell function and mass is critical to the maintenance of euglycemia. β-cells integrate numerous signals from the host to secrete appropriate amounts of insulin and maintain tight control of blood glucose levels. Together with glucose; nutrients, amino acids, hormones, and metabolic by-products contribute to this physiologic response. Within the islet microenvironment, where β-cells reside, there exists a network of interacting pathways that contribute to insulin secretion and regulation of β-cell mass. While factors within these pathways are often sourced from digestive processes and peripheral tissues, intra-islet-derived factors are also important components in the ability of -βcells to accurately integrate metabolic demands with β-cell function. In recent years, many biologic factors have been found to have previously unappreciated autocrine and paracrine roles within the islet. Moreover, differences have been described between signaling within rodent and human islets that are important for informing our understanding of autocrine/paracrine signaling between species. In this review, we highlight these new findings and future directions for this field of study.

Keywords: Autocrine, β-cells, β-cell function, β-cell mass, Diabetes, Glucose, Insulin, Islets, Nutrient-sensing, Paracrine.

[*] **Corresponding author Brian T. Layden:** Division of Endocrinology, Metabolism, and Molecular Medicine, Department of Medicine, Northwestern University, Chicago, IL, USA; Tel/Fax: 312-503-0006/312-908-9032; E-mail: b-layden@northwestern.edu

David J. Hill (Ed.)

INTRODUCTION

Defining the Importance of β-Cell Function and Mass

The Islets of Langerhans are composed of multiple cell types. β-cells, the most abundant cell type within islets, produce and secrete insulin in response to glucose. The metabolism of glucose initiates biochemical signaling cascades that ultimately result in β-cell depolarization, insulin granule fusion, and insulin secretion. The secretion of insulin, which is synthesized primarily in pancreatic islets, is largely dependent on extracellular glucose levels and tightly regulated to maintain euglycemia. While glucose is the primary mediator of insulin secretion, other factors (such as nutrients, cytokines, and hormones) fine-tune insulin secretion based on the physiologic needs of the organism. Deficient insulin secretion is central to the pathogenesis of diabetes mellitus (DM) and results in diminished uptake of glucose by peripheral tissues, which leads to hyperglycemia. Understanding the additional pathways involved in the regulation of insulin secretion and maintenance of glucose homeostasis has provided novel mechanistic insight into DM and pathways to target for its treatment.

While blood glucose levels are the primary driver of insulin secretion, changes in insulin sensitivity that accompany physiologic conditions such as pregnancy [1] and obesity [2] require the pancreas to increase its capacity for insulin secretion. To support this demand, β-cells have the capacity to expand their overall mass through the regulation of β-cell proliferation and apoptosis, β-cell hypertrophy, and/or β-cell neogenesis [3]. However, the pathways and mechanisms (proliferation/apoptosis, hypertrophy or neogenesis) that predominate are still debated. Regardless, the end result of β-cell mass expansion is to provide increased insulin secretory capacity under conditions of increased insulin demand. Because of this, identification of endogenous and exogenous factors that promote β-cell mass expansion provides insight into mechanisms used to compensate for deficient insulin secretion and its alteration in states such as type 2 diabetes (T2D) and gestational diabetes mellitus (GDM).

Known Factors Affecting β-Cell Function and Mass and Their Mode of Action

As noted above, glucose is the primary insulin secretagogue, and elevated glucose has been shown to increase β-cell mass by inducing β-cell proliferation in both mouse and human islets [4, 5]. Along with glucose, other nutrients (fatty acids and amino acids) and hormones such as glucagon-like peptide-1 (GLP-1) have a documented role in insulin secretion and β-cell mass regulation (Table 1). In general, these nutrients and hormones are derived either from dietary sources or by secretion from a peripheral tissue and delivered to islets through the systemic circulation. Thus, these factors act as endocrine hormones. However, hormones can also be secreted and signal locally through a paracrine (action on neighboring cells) or autocrine (action on cells the factor was secreted by) effect.

Table 1. Partial listing of extracellular signaling factors affecting insulin secretion and β-cell proliferation.

	Signal type	Mode of action at β-cell	β-cell effect
Insulin	Autocrine	Receptor tyrosine kinase	Potentiate GSIS?
GLP-1 [6]	Endocrine/Paracrine	GPCR	Potentiate GSIS Stimulate β-cell proliferation Protect against β-cell death
Glucose-dependent insulinotropic polypeptide (GIP) [6]	Endocrine	GPCR	Potentiate GSIS Stimulate β-cell proliferation Protect against β-cell death
Somatostatin [7]	Paracrine	GPCR	Inhibit GSIS
Amino acids [8]	Endocrine	GPCR/ Intracellular metabolism	Inhibit or potentiate GSIS (depending on amino acid type)
Ghrelin [7]	Endocrine/Paracrine/Autocrine	GPCR	Inhibit GSIS
Vasoactive intestinal polypeptide (VIP) [7]	Neuropeptide	GPCR	Potentiate GSIS

CHAPTER 7

β-Cell Adaptability During Pregnancy

Jens Høiriis Nielsen[1,2,*], **Signe Horn**[1,3], **Jeannette Kirkegaard**[1,3], **Amarnadh Nalla**[1,2] and **Birgitte Søstrup**[1,2]

[1] *Department of Biomedical Sciences, University of Copenhagen, Denmark*

[2] *Centre for Fetal Programming, Copenhagen, Denmark*

[3] *Novo Nordisk A/S, Måløv, Denmark*

Abstract: Pregnancy is a physiological condition associated with β-cell mass expansion occurring in response to increased insulin demand. If the insulin resistance is not compensated by proper augmented insulin production gestational diabetes will occur. As reviewed herein, pregnancy induced hormonal changes have occupied scientists since the beginning of the last century where important discoveries of the hormonal regulation of metabolism during pregnancy have been accomplished. Of the multiple hormonal and metabolic changes the somatolactogenic hormones, placental lactogens (PL) and placental growth hormone (GH-V) are the most described and are found to have dual roles by induction of insulin resistance and promotion of β-cell function and expansion. More recently, the direct effects on isolated pancreatic islets and the influence of signaling pathways involved in the adaptation of β-cell growth and function during pregnancy have been elucidated. This has identified contributions of a number of known peptide hormones and growth factors (EGF, NGF, HGF, IGFs, GLP-1) and steroid hormones (progesterone, estrogens, glucocorticoids). In addition, glucokinase has been found to be essential for the both proliferation and glucose stimulated insulin secretion during pregnancy. Some transcriptional activators and repressors (FoxM1, HNF4α, Myc, Bcl6, Men1) have been implicated in β-cell growth and survival, but also systemic factors like betatrophin, serotonin and osteoprotegerin have been reported to stimulate β-cell proliferation during pregnancy. Gene expression studies and proteomics of islets from pregnant rodent have furthermore revealed upregulation of a number of genes (*e.g.* cyclophilin B, stathmins, dlk-1, trefoil factor-3 and several others) that may influence β-cell growth and function during pregnancy although the mechanisms driving these changes are not yet known. Similarly,

* **Corresponding author Jens Høiriis Nielsen:** Department of Biomedical Sciences, University of Copenhagen, Denmark; Tel: +45 28757721; E-mail: jenshn@sund.ku.dk

David J. Hill (Ed.)

circulating factors in serum from pregnant women have been identified. Among the stimulating factors are peptide fragments of alpha-1 antitrypsin, kininogen-1, apolipoprotein-1, fibrinogen alpha chain and angiotensinogen. An intriguing question remains about the origin of the increased β-cell mass in pregnancy. In humans, studies have primarily reported an increase in the number of small islets, suggesting that neogenesis as the primary driver of β-cell mass expansion in human. In rodents, however, β-cell replication is believed to be the primary mechanism, although increased expression the neogenesis marker, neurogenin-3, has also been reported in pancreas of pregnant rodents. Interestingly, recent studies have suggested that the apparent loss of β-cells occurring during development of diabetes may be due to de-differentiation rather than cell death, suggesting contributions from mechanisms going beyond neogenesis and replication. In summary, gestational diabetes (GDM) is associated with lack of appropriate adaptation of the β-cells that may be due to a reduced pre-pregnancy β-cell mass, lack of stimulating hormones and growth factors or appearance of β cytotoxic metabolites or factors. This chapter reviews the existing knowledge of multiple factors and put forward new mechanisms of pregnancy induced β-cell mass expansion, which are not yet completely understood.

Keywords: Diabetes, Growth hormone, Insulin, Pancreatic β-cells, Pregnancy, Prolactin.

INTRODUCTION

Pregnancy is a unique physiological condition associated with expansion of the β-cell mass in response to an increased insulin demand. As type 2 diabetes (T2D) is characterized by insulin insufficiency due to a lack of compensatory β-cell mass expansion, a better understanding of the mechanisms involved in β-cell adaptation during pregnancy, may help to elucidate the pathogenesis and lead to interventions in the treatment of T2D. Pregnancy is characterized by increased food intake, weight gain, changes in metabolism towards "facilitated anabolism" after food intake and "accelerated starvation" during fasting, in order to maintain optimal supply of nutrients to the fetus [1]. In normal pregnancy, these metabolic changes lead to an increase in the postprandial plasma glucose level which elicits an exaggerated insulin secretion in particular in late pregnancy. The physiological link between pregnancy and the mechanisms failing during development of T2D are further underscored by an increased risk of developing gestational diabetes (GDM). Interestingly, certain genetic forms of diabetes are often diagnosed during pregnancy [2]. As will be described in this chapter the adaptation of the β-cells to

pregnancy is a complex process triggered by a series of known, and yet unknown, systemic stimuli. β-cell adaptation is accomplished by increased β-cell function such as enhanced glucose stimulated insulin secretion (GSIS), as well as, by the formation of new β-cells most likely by a combination of replication and neogenesis. The changes in β-cell mass during normal and diabetic conditions are illustrated in Fig. (**1**) [3].

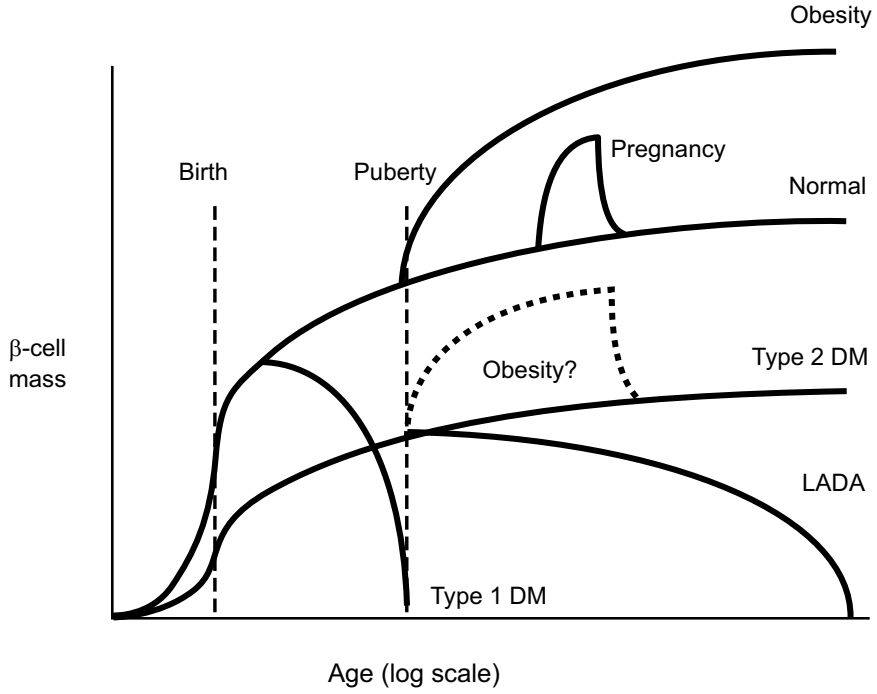


Fig. (1). Schematic illustration of changes in β-cell mass in response to increased insulin demand *i.e.* perinatal period, intrauterine growth retardation, obesity, pregnancy and type 1 diabetes (DM), type 2 diabetes (DM) and Latent Autoimmune Diabetes in Adults (LADA or type 1½ diabetes) [3].

Morphological Changes

Changes in the morphology of the endocrine pancreas during pregnancy in both humans and animals were described already in the early 20th century [4]. In 1930, Akehi [5] found hypertrophy, proliferation and newly formed islets during pregnancy in rabbits, which was followed by rapid atrophy and reduction of β-cells after parturition. In the same year, Macleod [6] described a diabetic woman that had a marked reduction in her insulin requirement in the last trimester,

Section 3: Generation of β-Cells and Future Applications

CHAPTER 8

β-Cells from Embryonic and Adult Stem Cells and Progenitors

Christine A. Beamish*

Department of Surgery, Islet Transplantation Laboratory, The Methodist Hospital Research Institute, Houston Texas, USA

Abstract: Diabetes is a chronic autoimmune disease, causing the destruction of the insulin-producing β-cells of the pancreatic islet and leading to glycemic dysregulation. Exogenous insulin administration and glucose testing moderately rectifies hyperglycemia, but does not provide adequate fine tuning necessary for complete prevention of hypoglycemia acutely, nor micro- and macro-vascular complications in the long-term. Islet transplants have shown great promise for this dynamic glucose regulation, but a shortage of cadaveric-sourced cells, and lifelong immune suppression requirements vastly restrict this technique from being widely available to patients with the disease. Therefore alternative sources of insulin-producing cells are needed. In this chapter, the role of stem cell biology in the current context of diabetes therapy is discussed, including an assessment of human embryonic and human induced pluripotent stem cells for the restoration of β-cell mass. Additionally, the existence of putative resident stem cells, and possible fluidity in lineage fate determination within endocrine pancreas- related cell types is examined.

Keywords: β-cell, Diabetes, Pancreas, Plasticity, Progenitor cell, Regeneration, Stem cell.

INTRODUCTION

Type 1 diabetes mellitus is an autoimmune disease of the endocrine pancreas, involving an interplay of immune, genetic, and environmentally-mediated factors

* **Corresponding author Christine A. Beamish:** Department of Surgery, Islet Transplantation Laboratory, The Methodist Hospital Research Institute, Houston Texas, USA; Tel: 713-363-9193; Fax: 713-441-3240; E-mail: cbeamish@houstonmethodist.org

David J. Hill (Ed.)

[1 - 3]. While the overarching goal of diabetes therapy would ostensibly restrict the autoimmune destruction of insulin-producing β-cells and retain existing β-cell mass in susceptible individuals, the primary impediment to this approach is that the disease only presents with overt symptoms of hyperglycemia after β-cell mass is lost below a clinical threshold (~10% remaining), and long after disease initiation [4]. Furthermore, while research advances actively target a variety of parameters including auto-antibody presence, diabetes-correlative genetics such as the presence of the HLA alleles DR3-DQ2/DR4-DQ8 [3], and viral exposure [2], <20% of patients have known risk factors prior to diagnosis (such as first-degree relatives with diabetes) [5], thus limiting the ability to predict diabetes incidence in all but few patients [6, 7]. Therefore, resolving the immune destruction is only part of the solution, and β-cell replacement strategies are imperative.

Since its advent in 1989 by Lacy, Ricordi, Scharp and associates [8, 9], islet transplants have improved drastically as a treatment for specific T1D patients. Much of the improvement came in the year 2000 with the Edmonton Protocol pioneered by Shapiro and colleagues [10], allowing exogenous insulin independence effected by a higher islet mass from two donors, and the use of steroid-free immunosuppression *via* sirolimus, tacrolimus, and daclizumab, an IL2-receptor antagonist antibody [11]. Grafts now last up to 5 years [12, 13]. In addition to freedom from injections, benefits from islet transplantation include significant decreases in micro- and macro-vascular complication rates, decreases in severe hypoglycemic episodes, increased circulating C-peptide titers, and improvement in HbA_{1c} [13, 14]. Importantly, these benefits are noted even if the graft fails. The obstacle to this therapy being offered more widely is primarily due to lack of islets, as well as risks associated with life-long immunosuppression for allogeneic grafts [13]. This shortage of islets is multi-factorial: many available pancreata do not meet minimum criteria for transplant, including donor age and metabolic profile; islets may be damaged irreparably during isolation; and tragically, the majority of islets die in the first days after transplant [15], primarily resulting from an instant blood mediated immune reaction (IBMIR) [16]. Some research has focused on mitigating the factors responsible for this process, such as modulating platelet-monocyte interactions [17] or by blocking complement activation [18]. The remaining islet death results from the loss of blood supply,

causing hypoxia, ischemia-reperfusion injury [19], and amyloid deposition as time progresses. Islet vascularization is a critical determinant of cell survival in the long-term [20]. Cost of the procedure is a final regional consideration, resulting from islet transplants being designated as "experimental therapy" in the United States, and hence only (and rarely) covered by private insurance or research funding, although this is not the case in other countries, such as Canada [21].

Alternate sources of insulin-producing β-cells would alleviate the need for cadaveric-sourced human tissue with their associated sequelae, and significantly alter morbidity and mortality outcomes in the future. The potential role of stem cell biology will be examined to this end, including assessments of both exogenous and endogenous stem and progenitor cell populations and their applications to human medicine.

GENERATION OF β-CELLS FROM STEM CELL SOURCE

The *de novo* generation of insulin-producing β-cells has long been a goal for diabetes therapy. Given the expansive data generated from lineage tracing experiments, it is now possible to ascertain the sequence of steps necessary in the developmental biology of the β-cell, from primitive gut endoderm through to functional, glucose-responsive, β-cell [22]. The generation of β-cells from stem cell source has generally co-opted these developmental procedures.

A stem cell may be defined by two functional properties, namely an unlimited ability for self-renewal, and the capacity to generate multiple cell types (*e.g.* multi-potentiality). This may be, but is not limited to, a response to injury or other stimulus. A progenitor cell, alternatively, demonstrates some restriction in self-renewal capability and which is usually uni-potent [23, 24]. The distinction between these two cell states is often fluid and vaguely indeterminate in the literature, but which may yield important and definitive outcomes for medicine. Similarly, subdivisions in definition exist for the somatic, or tissue-specific, stem cells *vs.* embryonic stem cells, namely that the latter demonstrates the capacity to generate progeny across all primary germ layers. However, precedents exist to suggest that this is not always rigidly obeyed, as has been demonstrated by hematopoietic and neural stem cells forming tissues from germ layers other than

CHAPTER 9

Induction of β-Cell Regeneration by Human Postnatal Stem Cells

Tyler T. Cooper, Ruth M. Elgamal and **David A. Hess***

Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute; Department of Physiology & Pharmacology, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada

Abstract: The International Diabetes Federation estimates 382 million people are currently living with diabetes mellitus worldwide; and with increasing rates of obesity in an aging population this number is predicted to increase to 592 million by 2035. The inability to ameliorate the causes of diabetes has motivated researchers to develop novel approaches aimed at providing curative therapies to replace current symptomatic management using exogenous insulin. Accordingly, postnatal or adult stem cell transplantation has recently emerged as a promising therapeutic strategy following reports detailing the stimulation of islet regeneration in preclinical and early clinical studies. Postnatal bone marrow (BM) and umbilical cord blood (UCB) sources contain progenitor cells of hematopoietic, endothelial, and mesenchymal lineages; and each have demonstrated islet regenerative functions in animal models of diabetes. In the context of this chapter, we summarize accumulating evidence from preclinical and clinical studies describing transplantation of these specific postnatal lineages to stimulate the regeneration of endogenous insulin secreting β-cells, and how these stem cells may be used to provide paracrine support alongside the transplantation of allogeneic islets.

Keywords: Allogeneic transplantation, Autologous transplantation, β-cells, Bone marrow, Diabetes mellitus, Endothelial progenitor cells, Hematopoietic progenitor cells, Hypoxia, Insulin, Islet angiogenesis, Islet neogenesis,

* **Corresponding author David A. Hess:** Molecular Medicine Research Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute, Department of Physiology & Pharmacology, Western University, 1151 Richmond St, London, Ontario, Canada, N6A 5B7; Tel/Fax: 519.931.5777x.24118; E-mail: dhess@robarts.ca

David J. Hill (Ed.)

Islets of langerhans, Islet regeneration, Multipotent stromal cells, Pancreas, Paracrine signals, Progenitor cells, Stem cells, Transplantation, Umbilical cord blood.

INTRODUCTION

Diabetes mellitus is generally characterized by the body's inability to maintain controlled glycemic levels due to pancreatic β-cell death or dysfunction [1]. Type 1 diabetes (T1D), also referred to as "juvenile onset diabetes" or "insulin-dependent diabetes", arises as a result of the autoimmune destruction of insulin-secreting β-cells in the islets of Langerhans. T1D is mediated by an inflammatory infiltrate composed of $CD4^+$ and $CD8^+$ T-lymphocytes, B-lymphocytes, macrophages, and NK-cells [2, 3]. β-cell-specific autoimmune depletion leaves T1D patients with an inadequate supply of endogenous insulin, requiring exogenous insulin to control hyperglycemia. In contrast, T2D, often referred to as "late-onset diabetes" or "non-insulin-dependent diabetes", occurs as a result of insulin insensitivity or resistance in peripheral tissues (skeletal muscle, liver, and adipose), and is associated with a combination of risk factors including obesity, sedentary lifestyle, environmental stimuli, and/or genetics [4]. In the early stages of T2D, prolonged hyperglycemia stimulates β-cells to over secrete insulin and results in β-cell exhaustion and apoptosis [5], culminating in reduced β-cell mass as T2D develops [6, 7]. Regardless of these pathological differences, T1D and T2D, ultimately result in the loss of β-cell mass over time; leading to inadequate insulin secretion within islets and the requirement for exogenous insulin therapy. Due to the insulin deficiency created by diabetes, restoration of physiological insulin secretion is essential to treating both T1D and T2D and its subsequent complications.

Dealing with the Complications and Consequences of Diabetes Mellitus: An Emerging Global Crisis?

In 2013, the International Diabetes Federation (IDF) estimated 382 million people are currently living with diabetes mellitus worldwide; and this number is predicted to increase to 592 million by 2035 [4, 8]. Specifically, >90% of diagnosed individuals have T2D, while <10% have T1D. Due to increasing rates of obesity and aging population demographics worldwide, diabetes mellitus has

reached pandemic proportions. Since the discovery of insulin by Banting and Best in the early 1920s [9], and despite the recent development of improved insulin administration (automated insulin pumps) [10], both T1D and T2D patients ultimately develop serious comorbidities and secondary complications associated with inadequate control of glycemia. The most severe complications include increased rates of cardiovascular diseases (heart attack, coronary and peripheral artery disease, stroke), vision loss/blindness, kidney failure, nerve damage, problems with pregnancy, and depression. According to the Public Health Agency of Canada, individuals with diabetes are 3 times more likely to develop cardiovascular disease, 12 times more likely to be hospitalized with end-stage renal failure, and 20 times more likely to require non-traumatic lower limb amputation [11]. Although it is difficult to assess the economic burden of diabetes worldwide, the American Diabetes Association estimated that the total cost of diabetes in 2012 exceeded $245 billion in the USA ($176 billion in direct medical costs and $69 billion in reduced productivity) [12]. These staggering numbers have prompted researchers to aggressively investigate improved and potentially curative therapies to combat both T1D and T2D.

The Advent of Cellular Therapies for Diabetes: The Edmonton Protocol

Recently, cell-based therapies have emerged as a frontrunner to provide curative therapies for diabetes using islet replacement to restore functional β-cell mass [13]. In 2000, the pioneering efforts of the Edmonton protocol provided 'proof-o--concept' that portal vein transplantation of cadaveric human islets, combined with modern immunosuppressive therapy, could lead to insulin independence in patients with severe T1D [13, 14]. Although results at 1 year were initially promising with 7 of 7 patients achieving insulin-independence after the transplantation of islets from at least 2 donor pancreata, islet rejection and continued autoimmune assault resulted in the return to insulin therapy in the majority of patients [13]. In addition, islet survival and function was compromised by isolation procedures and engraftment in the hepatic site. Widespread application of this approach remains limited due to an extreme shortage of donor pancreas tissue available for transplantation. Finally, due to the requirement for life-long immunosuppression and increased risk of infections, islet transplantation is only indicated for brittle T1D patients [15].

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V

David J. Hill

David Hill (D.Phil., FCAHS) is Scientific Director of the Lawson Health Research Institute and Integrated Vice President, Research London Health Sciences Centre and St. Joseph's Health Care London, Ontario, Canada. He holds the Lawson Professorship in Diabetes Research, and is a Professor in the Departments of Medicine, Physiology and Pharamcology, and Paediatrics at Western University, Canada. Educated at the Universities of Nottingham and Oxford, Professor Hill is a past Chair of the National Board of the Canadian Diabetes Association, and is presently a Board Member for Research Canada and of Health Care Canada. He is a recipient of the Canadian Diabetes Association Frederick G. Banting Award, the Medal of the Society for Endocrinology from the UK, and is a Fellow of the Canadian Academy of Health Sciences. Dr. Hill has published some 250 research papers, and has most recently focused on the targeted generation of new insulin producing ß-cells in the pancreas, the fetal programming of type 2 diabetes, and strategies to prevent gestational diabetes and early-onset type 2 diabetes in obese youth.