WRISG 2021 ABSTRACT BOOKLET

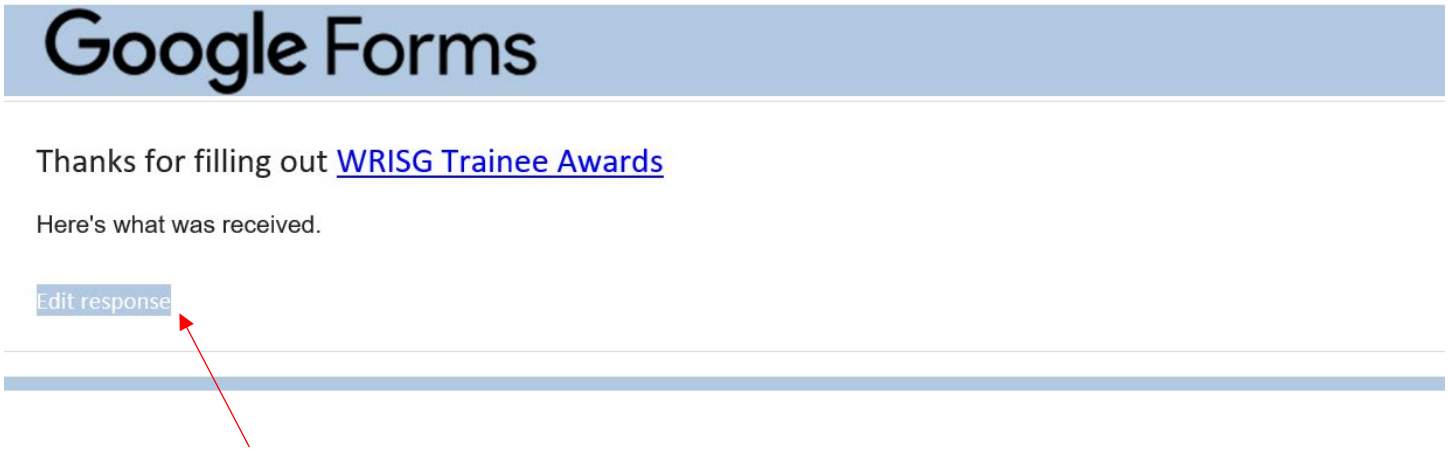
November 3-5, 2021

Skamania Lodge, Stevenson, WA

Voting Instructions for Trainee Awards

Please go to the following link to vote for the WRISG Trainee Awards, sponsored by the Diabetes Research Connection.

This form can be edited by utilizing the email link that will be sent to you once you submit the form the first time.



Click Here in Confirmation Email to Edit Response

Diabetes Research Connection

SESSION A: NOVEL MECHANISMS OF INSULIN SECRETION

Chairs: Sangeeta Dhawan and Patrick Fueger

DOC2b Enhances β-Cell Function via a Novel Tyrosine Phosphorylation-Dependent Mechanism

Diti Chatterjee Bhowmick[1], Arianne Aslamy[2], Supriyo Bhattacharya[3], Eunjin Oh[1], Miwon Ahn[1], and Debbie C. Thurmond[1]

1. *Department of Molecular and Cellular Endocrinology, Diabetes and Metabolic Research Institute, Beckman Research Institute of City of Hope, Duarte, CA*
2. *Department of Medicine, Cedars-Sinai Medical Center, West Hollywood, CA*
3. *Integrated Genomics Core, Center for Informatics, Department of Computational and Quantitative Medicine, City of Hope, Duarte, CA*

Double C2 Domain B (DOC2b) is required for SNARE assembly and glucose-stimulated insulin secretion (GSIS) in β-cells. However, the underlying mechanism of GSIS regulation by DOC2b remains unknown. Our biochemical analysis using clonal β-cell lines and primary human islets revealed that DOC2b is tyrosine phosphorylated within 2 minutes of glucose stimulation. We found that the Src family kinase (SFK) inhibitor SU6656 reduces tyrosine-phosphorylated DOC2b levels in DOC2b- enriched β-cells. Furthermore, our biochemical and RNAi analysis revealed that YES kinase is required for glucose-stimulated tyrosine phosphorylation of DOC2b in β-cells. Biochemical and functional analysis using $DOC2b^{Y301}$ mutants showed that Y301 phosphorylation is required for the glucose-stimulated interaction between DOC2b and YES and for DOC2b-mediated enhancement of GSIS in β-cells. Our co-immunoprecipitation studies established increasing association of the ERM family scaffolding proteins with DOC2b following glucose stimulation and pervanadate treatment in DOC2b- enriched β-cells, indicating that DOC2b regulates ERM-mediated mobilization of insulin secretory granules. Taken together, these results demonstrate the first glucose-induced post-translational modification of DOC2b in β-cells, pinpointing the kinase, site of action, and downstream signaling branch, and reveal the master regulatory role of SFK at various steps in GSIS. This work will enhance the development of novel therapeutic strategies to restore glucose homeostasis in diabetes.

Promoting Mitofusin 2 Activity Mediates Hyper Basal Secretion Induced Mitochondria Proton Leak

Jennifer Ngo[1, 2], Anthony Molina[3], Linsey Stiles[2], Nathaniel Miller[2], Fernanda Cerqueira[4], Siyouneh Bagadasharian[2], Brett Roach[2], Karel Erion[2], Sam B.Sereda[5], Jakob D. Wikstrom[6], Evan Taddeo[2], David Chan[7], Barbara E. Corkey[5], Marc Liesa[2, 8, 9], Orian S. Shirihai[2, 8, 9]

1. *Department of Chemistry & Biochemistry, UCLA, Los Angeles, CA*
2. *Division of Endocrinology, Diabetes and Hypertension, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA*
3. *Division of Geriatrics, Gerontology and Palliative Care, Department of Medicine, University of California, San Diego, San Diego, CA*
4. *University of the State of Rio de Janeiro, Laboratory of Physiology and Microcirculation (BIOVASC, Rio de Janeiro, Brazil*
5. *Obesity Research Center, Department of Medicine, Boston University School of Medicine, Boston, MA*
6. *Department of Dermato-Venereology, Karolinska University Hospital, Stockholm, Sweden*
7. *Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA*
8. *Department of Molecular & Medical Pharmacology, UCLA, Los Angeles, CA*
9. *Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA*

Pancreatic islets from type 2 diabetic obese subjects are characterized by decreased glucose stimulated insulin secretion (GSIS) and increased basal, or unstimulated, secretion. Increased basal secretion was proposed to be caused by increased sensitivity of islets to lipid-induced insulin secretion, facilitated by the high lipid environment in obesity. In this study, we identify the mitochondrial fusion protein Mfn2 determines hyperinsulinemia in pancreatic islets. We show that Mfn2 expression in islets is decreased in obesity, and beta-cell specific removal of Mfn2 recapitulates the features of obese prediabetic islets. Beta-cell specific removal of Mfn2 increases basal secretion by two-fold, while enhancing fatty acid oxidation and removing the oscillatory nature of GSIS. Additionally, Ca2+ uptake and capacity in Mfn2 impaired cells were strongly reduced. We hypothesize that altered Ca2+ homeostasis triggers the opening of the mitochondrial permeability transition pore (mPTP), leading to swelling and impaired uncoupled respiration. Conversely, promoting fusion activity pharmacologically and overexpression of Mfn2 lowered hyper basal secretion and reduced mitochondrial proton leak. Similarly, knockdown of UCH-L1 recapitulated features of beta cells deficient in Mfn2 but overexpression of UCH-L1 preserved Mfn2 content under high lipid conditions, suggesting Mfn2 expression is regulated by UCH-L1. Our findings thus identify Mfn2 bridges obesity and diabetes by inducing hyperinsulinemia in response to increased fatty acid metabolism in the obese state.

Changes in Peri-islet Extracellular Matrix Stiffness Regulate Insulin Secretion Via Mechanotransduction Signaling

Chelsea Garcia[1], Nikki Farnsworth[1], Keifer Holcomb[1], Meghana Murthy[1]

1. Chemical & Biological Engineering Department, Colorado School of Mines, Golden, CO

In the pancreas the islet is surrounded by extracellular matrix (ECM) that regulates cell survival and insulin secretion by providing mechanical and biochemical cues to the β-cells. During the onset of T1D, ECM molecules laminin and collagen are degraded leading to a decrease in matrix crosslinking and a decrease in peri-islet ECM stiffness. While changes to the peri-islet ECM have been well documented in T1D, the role of ECM changes to T1D pathogenesis are largely unknown. Changes to ECM stiffness have been correlated to changes in insulin secretion; however, the mechanisms of mechanotransduction regulating insulin secretion have not been studied in the islet. We hypothesized that increasing matrix stiffness from 0.1-10MPa would increase islet glucose sensitivity by increasing phosphofructokinase (PFK) activity. We determined the effect of changes in ECM stiffness on islet function and insulin secretion dynamics by using a novel reverse thermal gel (RTG) scaffold with encapsulated mouse islets. Rheological analysis was conducted on 5 and 10wt% RTGs and yielded increasing storage moduli at 40°C within the range of 0.0009-0.01455MPa. Glucose-stimulated insulin secretion (GSIS) was measured on isolated mouse islets encapsulated in 5wt% and 10wt% RTG and unencapsulated mouse islets for 24 hours. We found that insulin secretion increased as the matrix stiffness increased in both basal (2mM) and high glucose (20mM) conditions. Insulin secretion at 20mM glucose normalized to 2mM glucose, or the stimulation index (SI), decreases with matrix stiffness indicating dysfunction to insulin secretion. PFK activity analysis showed an increase in PFK activity in islets encapsulated in stiffer RTGs. The results from this work support a role for ECM mechanical properties in regulating islet function and define the relationship between mechanotransduction and metabolism in pancreatic islets. This work provides insight into how islet dysfunction is mediated by changes in ECM stiffness, as in pancreatitis and T2D.

What Role Do Highly Functional β-cell Subpopulations Play in Islets? A Theoretical Study

JaeAnn M. Dwulet[1], Jennifer K. Briggs[1], Richard K.P. Benninger[1]

1. Barbara Davis Center for Diabetes, Department of Bioengineering, University of Colorado Denver, Aurora, CO

The electrical activity of the pancreatic islet responds in a homogenous manner. At high glucose, Ca2+ activity of the islet is globally activated, whereas low glucose conditions cause global suppression of activity. This coordinated behavior arises from the highly-coupled state of the islet, despite heterogeneity of individual β-cells. Using multicellular computational models of mouse and human islet electrophysiology, we investigated how highly functional subpopulations of β-cells can influence global activity of islets. We mimicked experimental conditions of optogenetic silencing or laser ablation and investigated how these populations impacted the response of the islet. When a small population (10%) of highly metabolically active cells were silenced via hyperpolarization, large areas of the islet were silenced, similarly to experimental data. However, when these highly metabolic cells were absent from the simulation, the islet remained globally active and had near normal duty cycle. These results suggest a small highly metabolic subpopulation is not critical for maintaining islet dynamics. Next, we examined how β-cells with Ca2+ oscillations that precede the rest of the islet (early phase cells) influence Ca2+ dynamics. When these early phase cells were removed, there was little change in Ca2+ activity and Ca2+ oscillation frequency. In contrast, removing cells with delayed Ca2+ oscillations reduced the frequency of the islet, although only when a large number (>30%) of cells were removed. These results indicate that we can reproduce experimental measurement for how targeted hyperpolarization or ablation affects islet dynamics. Nevertheless, these highly functional subpopulations do not have significant impact on Ca2+ oscillations. NIH R01 DK106412 HIH R01 DK102950 NIH F31 DK126360

Effects of Naturally Occurring Genetic Variations in Incretin Receptors on Glucose Homeostasis

Anirudh Gaur[1], Elinor L. Lewis[1], Marcus Flisher[2], Ryan G. Hart[2], Mark O. Huising[2,3], Jennifer L. Whistler[1,3]

1. Center for Neuroscience, University of California-Davis, Davis, CA, USA.
2. Department of Neurobiology, Physiology and Behavior, College of Biological Sciences, University of California, Davis, Davis, CA.
3. Department of Physiology and Membrane Biology, School of Medicine, University of California Davis, Davis, CA.

Glucose homeostasis is critical for human health and its disruption is characteristic of type 2 diabetes. Insulin released by B-cells is the primary driver of glucose homeostasis. Glucose stimulated insulin secretion is augmented by the actions of incretin hormones including Glucagon-Like peptide 1 (GLP-1), which activates GLP-1 receptor (GLP-1R) in islets producing the "incretin effect". Two common genetic variations in GLP-1R, R131Q and G168S, are known to alter the incretin effect. Specifically, R131Q mutation increases while G168S mutation decreases the effect of GLP-1. We found that these genetic variations do not affect the affinity of GLP-1 nor its efficacy for G-protein signaling, presenting a "pharmacological mystery" as to how genetic variations at GLP-1R alter incretin function. In this study, we assessed whether genetic variation in GLP-1R affects receptor trafficking thereby producing altered incretin responses. HEK-293 cells stably expressing wild type (WT), R131Q or G168S GLP-1R variants were used to quantify receptor endocytosis and post- endocytic sorting to lysosomes. We found that WT GLP-1R was degraded after endocytosis through its interaction with GPCR-associated sorting protein-1 (GASP-1), a protein that is highly expressed in islets. Importantly, we found that R131Q variant recycled after endocytosis and G168S variant degraded more rapidly compared to WT GLP-1R. To examine the role of GASP-1 mediated GLP-1R degradation on incretin function, we used CRISPR to generate rat INS-1 cells with a disruption in GASP-1. We found that deletion of GASP-1 in cells had no effect on either acute GLP-1R signaling nor insulin secretion in response to incretin drug. However, prolonged incretin drug pretreatment produced a loss of incretin response in WT cells while GASP-1 KO cells showed preservation of the incretin effect. Taken together, these finding suggests that GASP-1 mediates post-endocytic degradation of GLP-1R in B-cells and that altered trafficking of the GLP-1R variants may underlie the differential incretin response in humans who carry these mutations.

Somatostatin Signaling Drives Filamentous-Actin Reorganization in Primary Mouse Beta Cells

Ryan Hart[1], Jessica Huang[1], Mark O. Huising[1,2]

1. *Department of Neurobiology, Physiology and Behavior, University of California Davis, Davis, California*
2. *Department of Physiology and Membrane Biology, University of California Davis School of Medicine, Davis, California*

Within the islet, Somatostatin (SST) secreted by the delta cells is a potent inhibitory hormone responsible for attenuating insulin secretion from the beta (β) cells of islet in a paracrine manner. This is accomplished through activation of the Gαi coupled Somatostatin Receptors (SSTRs) on β cells. Somatostatin mediated inhibition is characterized by decreased cyclic adenosine monophosphate (cAMP) and calcium (Ca2+) through Gαi mediated inhibition of adenylyl cyclase and activation of G protein coupled inward rectifying potassium channels respectively. However, these secondary messengers do not adequately explain the mechanistic underpinnings of SST signaling responsible for the attenuation of insulin exocytosis. To identify downstream effectors of SST in β cells, mouse islets were treated with 100 nM SST for 6 hours. These islets were subsequently dissociated, and their β cells subjected to bulk RNA-Sequencing and compared to non-treated control cells. Analysis of the Gene Ontology of upregulated genes revealed genes associated with "Actin Reorganization" as the most upregulated genes in response to SST treatment. Filamentous (F)-actin reorganization processes are controlled by the Rho Family of GTPases, including CDC42 and Rac1 which are necessary for insulin secretion in β cells. The upregulated genes were mostly associated with F-actin bundling and unidirectional polymerization. These processes are often mediated by RhoA, a GTPase that commonly antagonizes the activities of CDC42 and Rac1. To investigate this interaction, I conducted live cell imaging of SSTR-expressing HEK293T and INS1E cells in cells expressing FRET sensors reporting on either Rac1, CDC42 or RhoA GTPase activity. In both HEK293T cells and INS1E cells, treatment with SST activated RhoA. These results begin to reveal a model in β cells wherein SST inhibition of insulin secretion is accomplished not only by inhibiting both and Ca2+ and cAMP secondary messengers, but additionally through modulation of F-actin associated processes required for insulin exocytosis.

Spatial Organization of First Phase Calcium Response to Glucose in Mouse and Human Pancreatic Islets

Vira Kravets[1], Wolfgang Schleicher[1], Maura Sticco-Ivins[1], Richard Benninger[1]

1. University of Colorado Denver, Department of Bioengineering

Dynamics of glucose-stimulated insulin secretion is disrupted in diabetes. Insulin secretion is biphasic, and loss of the first phase is an early sign of type1, and a hallmark of type2 diabetes. First step to understanding mechanisms of this dysfunction is investigation of insulin secretion dynamics on a cellular and islet levels. Calcium (Ca2+) uptake by beta cells allows for visualization of the pattern of insulin secretion, since the two processes are coordinated. Beta cells are heterogeneous, and some cells respond sooner that others to glucose stimulation. We have shown that there exist a transient (~24h) beta cell state in which "first responders" cells act as triggers of the islet's response to glucose. Challenging an existing paradigm in the field, that first phase response to glucose in the islet is random and not spatially organized, we hypothesized that beta cells located closer to the first responder cells would have earlier response to glucose compared to more distant cells. We sought to compare such spatial organization in mouse vs human islets, and healthy vs diabetic cases. We measured Ca2+ dynamics in intact mouse islets using beta-cells specific expression of the Ca2+ sensor GCaMP6s. In human islet withing acute pancreatic slices we measured Ca2+ dynamics using calcium sensitive dye, Rhod2 AM. We then fixed islets and slices and performed immuno-staining for insulin, glucagon and somatostatin to identify different cell types. In 8/10 mouse islets we found linear dependence of the first-phase Ca2+ response to glucose on the distance from the first responder cells. Two islets had domains withing which there were local first responding cells, and withing which the response time vs distance to the first responder was also linear. In human islets there were more such domains. We also found correlation of the location of the first responder cells with proximity to the alpha cells. In diabetic human islets we saw loss of spatial organization of the Ca2+ dynamics.

Heterogeneity in Incretin Responses within Pancreatic Islets

Michelle Y. Chan[1], Marcus Flisher[1], Mark O. Huising[1]

1. Department of Neurobiology, Physiology, and Behavior, University of California Davis, Davis, CA

Incretins, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), potentiate insulin secretion stimulated from β cells by nutrients. Incretins mediate their insulinotropic effects by activating Gαs-coupled receptor signaling. cAMP kinetics depend on the balance between adenylyl cyclases (ACs) and phosphodiesterases activity. Incretin mimetics and dipeptidyl peptidase-4 inhibitors, which prevent the degradation of endogenous incretins, are prescribed to millions of Americans. However, while GLP-1 based drugs remain effective in improving insulin secretion in diabetes, the insulinotropic effects of GIP are lost in T2D patients3. This suggests that differences in the responses to GIP and GLP-1 exist and are accentuated in diabetes, despite the confocal microscopy and a genetically encoded cAMP FRET sensor, CAMPER. To investigate incretin-mediated cAMP signaling, intact islets were perfused with GIP and Exendin-4, an agonist of GLP-1R. GIP elicited a transient cAMP response in β cells while Exendin-4 alone elicited a sustained cAMP response that persists long after the peptide was washed out. This effect is not due to the order of stimulation. To investigate the intrinsic ability of β cells to generate cAMP, β cells from β-CAMPER mice were stimulated with 1uM of the direct AC activator forskolin. Perfusion with forskolin revealed considerable heterogeneity among the ability of individual β cells to generate cAMP, in which a subset of β cells take much longer to return to baseline cAMP activity. The observed heterogeneity was not explained by small variances in CAMPER levels. These findings demonstrate that β cells exhibit distinctly different cAMP responses to either incretin and display notable β cell-intrinsic heterogeneity in their capacity for cAMP generation.

SESSION B: NON-BETA CELLS IN HEALTH AND DISEASE

Chairs: Rocky Baker and Sakeneh Zraika

NKX2.2 Maintains α Cell Identity by Directly Regulating Cell Specific Gene Transcription

Elliott P. Brooks[1], Lori G. Sussel[1]

1. Barbara Davis Center for Diabetes, Department of Pediatrics, University of Colorado Denver,Aurora, CO

Although β cell loss is a major feature of diabetes, there is growing evidence that α cell dysfunction also occurs. However, less is known about how α cell function and identity is regulated. The transcription factor NKX2.2 was shown to maintain functional identity of β cells via direct transcriptional regulation. NKX2.2 is also expressed in adult α cells, but nothing is known about NKX2.2's role in the maintenance of α cell identity or how its function compares between α and β cells. To approach this in vivo, I generated α cell-specific NKX2.2 knockout mice. Adult mice lacking NKX2.2 specifically in α cells have decreased α cells, decreased glucagon expression and increased bihormonal cells, suggesting that NKX2.2 promotes α cell identity while repressing β cell identity. These results are opposite from NKX2.2's role in β cells, suggesting that NKX2.2 has cell-specific molecular activities. To explore NKX2.2's molecular mechanism in α cells, I performed NKX2.2 knockdown RNA-seq and NKX2.2 ChIP-seq in α cells. These experiments revealed that, in concordance with the NKX2.2 α cell KO phenotype, NKX2.2 directly activates many α genes and represses many β genes. To begin to understand NKX2.2's cell specific activities, I compared genomic locations of direct target NKX2.2 binding sites between α cells and β cells. I found that NKX2.2 predominantly binds to promoters in α cells, whereas it predominantly binds to introns and putative enhancers in β cells. Therefore, I hypothesize that cell-specific genome occupancy at regulated genes is a mechanism for how NKX2.2 has different and sometimes opposite regulatory functions in α and β cells. I'm currently exploring how NKX2.2 is directed to these unique sites in α and β cells. These results bring me towards uncovering mechanisms of α cell maintenance by NKX2.2 but also how NKX2.2 can have different islet cell specific functions.

Identifying the Role of RFX6 in Human Alpha Cell Function

Vy Nguyen[1], Krissie Tellez[1,2], Austin Bautista[3], Charles A. Chang[1], Mollie S.H. Friedlander[1], Robert Whitener[1], Yan Hang[1], Patrick E. MacDonald[3], Seung K. Kim[1,4]

1. *Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA*
2. *Division of Cardiology, Department of Medicine, University of California San Diego, La Jolla, CA; Division of Biological Sciences, Section of Molecular Biology, University of San Diego, La Jolla, CA*
3. *Department of Pharmacology and Alberta Diabetes Institute, University of Alberta, Edmonton, AB, Canada*
4. *Stanford Diabetes Research Center, Stanford University School of Medicine, Stanford, CA*

Regulatory factor X-box binding 6 (RFX6) is a highly conserved transcription factor that has been found to be associated with different forms of diabetes, including T1D, T2D, and Maturity Onset Diabetes of the Young (MODY). RFX6 has been characterized in human beta cells and shown to regulate expression of Ca2+ channel subunits. RFX6 mRNA levels in human alpha cells exceed those in beta cells and appear to be dynamically regulated in both T1D and T2D (Brissova 2018, Segerstople 2016). However, we have limited understanding of how this transcription factor regulates human alpha cell activity, reflecting general limitations of existing methods for targeted studies of human islet alpha cells. Using innovative tools to study glucagon secretion and alpha cell function developed by our lab and others, we have tested the hypothesis that RFX6 plays a critical role in regulating primary human alpha cell function. Our lab and others have successfully conducted genetic studies to elucidate mechanisms of endocrine function and regulation in human pseudoislets by the dispersion and reaggregation of manipulated islet cells. Here we report further refinement of pseudoislet genetics using magnetic bead-based separation ("Mag-Sep"). Isolation of CD26+ cells permit enriched genetic targeting of human alpha cells; isolated alpha cells can be efficiently infected with a lentivirus-based shRNA construct targeting RFX6, then reaggregated with the remainder of the islet cells after several days in culture. shRNA targeting of alpha cells in pseudoislets reduced RFX6 and decreased expression of putative targets. RFX6 knockdown Mag-Sep pseudoislets generated in this manner were also used for electrophysiological, secretion, and transplantation experiments. Preliminary data from these experiments indicate that RFX6 regulates glucagon secretion and motivate further interrogation of RFX6-dependent mechanisms of alpha-cell regulation and function.

Contribution of δ Cells to the Glucose Set Point

Jessica L. Huang[1], Sharon Lee[1], Mohammad Pourhosseinzadeh[1], Mark O. Huising[1,2]

1. *Department of Neurobiology, Physiology & Behavior, University of California Davis, Davis, CA*
2. *Department of Physiology and Membrane Biology, University of California Davis, Davis, CA*

Pancreatic islets are composed of several types of endocrine cells that coordinate to maintain blood glucose homeostasis. While β cells and α cells are thought to be the main drivers of glucose homeostasis in the islet through their secretion of insulin and glucagon respectively, the contribution of δ cells and SST secretion to establishing the glucose set point has not been studied. Our lab has demonstrated that β cells co-secrete the peptide hormone Urocortin 3 (Ucn3) with insulin, and that Ucn3 triggers somatostatin secretion from neighboring δ cells to provide feedback inhibition. We have further demonstrated that expressing Ucn3 in β cells directly causes an increase in basal glucose levels, likely by triggering somatostatin secretion from δ cells to create a negative feedback loop that attenuates insulin secretion. Therefore, I hypothesize that pancreatic δ cell secretion of somatostatin plays an important role in establishing the glycemic set point through control of β cell activity. To address this hypothesis, I specifically ablated somatostatin-expressing δ cells in mice using diphtheria toxin. δ cell ablation led to an immediate drop in basal glucose levels as well as increased glucose tolerance. The increase in glucose tolerance was accompanied by an increase in the fold change of insulin secreted after administration of glucose. Control experiments where α cells were ablated instead had no effect on the glucose setpoint. Simultaneous collection of calcium and secretion data demonstrated that in the absence of δ cells, β cells secrete more insulin and also have a low glucose threshold for insulin response compared to controls. These data establish a role of δ cells in determining the glucose set point through their interaction with β cells.

Coordination Between Beta and Delta Cells Is Not Mediated by Gap Junctions

Mohammad S. Pourhosseinzadeh[1], Jessica L. Huang[1], Jaresley V. Guillen[1,2], and Mark O. Huising[1,2]

1. *Department of Neurobiology, Physiology & Behavior, University of California Davis, Davis, CA*
2. *Department of Physiology and Membrane Biology, University of California Davis, Davis, CA*

Beta and delta cell hormone secretion is tightly coupled, with somatostatin trailing insulin secretion by approximately 30 seconds in mice. However, the mechanism of this coordination is still largely debated. Our lab has demonstrated that beta cells release the peptide hormone Ucn3 to stimulate delta cells at high glucose, establishing a negative feedback loop whereby somatostatin secretion inhibits nearby beta cells. This is an example of bidirectional paracrine crosstalk between beta and delta cells. The alternative explanation suggests direct gap junction-mediated coordination, analogous to Connexin36 (Cx36)-mediated gap junctions that synchronize beta cells in the same islet. To resolve the mechanism(s) of crosstalk between beta and delta cells, I generated mice expressing the fluorescent calcium reporter GCaMP6 in both cell types to record their activities simultaneously within the same islet in real time. Under low glucose, delta cells exhibit rapid calcium spikes while beta cells are silent. Under high glucose, delta cells maintain these rapid oscillations, which are in many delta cells complemented by slow calcium oscillations coordinated with beta cells. These slow oscillations consistently precede the calcium responses in adjacent beta cells by an average of 20 seconds. Importantly, the uncoordinated rapid oscillations in delta cells occurred during nadirs in the beta cell calcium oscillations. This demonstrates a lack of inhibition from neighboring beta cells and is inconsistent with a model for gap junction coupling between beta and delta cells because gap junctions increase the barrier of activation for any individual cell within a network. This is supported by dispersed FACS-purified beta cell experiments demonstrating a lower threshold to depolarization. Additionally, Cx36 staining in the mouse islet demonstrates its absence on the delta cell plasma membrane, but an abundance on the beta cell plasma membrane. Altogether, my data provides strong support for a model of beta/delta coupling via paracrine signaling.

Delta Cell Mediated Inhibitory FFAR4 Signaling Potentiates Insulin and Glucagon Secretion

Marcus R. Flisher[1], Marine L. Croze[3], Arthur Guillaume[3], Caroline Tremblay[3], Glyn M. Noguchi[1], Sabrina Granziera[3], Kevin Vivot[3], Vincent C. Castillo[1], Scott A. Campbell[3], Julien Ghislain[3], Vincent Poitout[3,4], Mark O. Huising[1,2]

1. *Department of Neurobiology, Physiology, and Behavior, College of Biological Sciences, University of California Davis, Davis, CA, USA*
2. *Department of Physiology and Membrane Biology, School of Medicine, University of California Davis, Davis, CA, USA*
3. *Montreal Diabetes Research Center, CRCHUM, Montréal, QC, Canada*
4. *Department of Medicine, Université de Montréal, Montréal, QC, Canada*

The pancreatic islet is responsible for regulating blood glucose, primarily through beta cells, which respond to the local extracellular glucose concentration and release insulin into the blood stream to facilitate glucose uptake in peripheral tissues. Although glucose is the principal driver of insulin secretion, other locally released and circulating factors modulate beta cell activity to further tune the level of insulin secretion. Of these factors, free fatty acids (FAs) modulate islet cell exocytosis by directly binding to G-protein coupled receptors (GPCR) and altering secretion. Free fatty acid receptor 4 (FFAR4) is a GPCR whose activation potentiates both beta cell insulin secretion and alpha cell glucagon secretion and inhibits delta cell somatostatin secretion. Here, we find that inhibitory FFAR4 signaling acts directly on delta cells, thereby reducing somatostatin mediated negative feedback on beta and alpha cell activity and indirectly increasing insulin and glucagon secretion.

Presence of Alternatively Spliced Insulin Gene Product in Human Pancreatic Delta Cells

Rene van Tienhoven[1*], Maria J.L. Kracht[2*], Arno R. Van der Slik[3], Anouk H.G. Wolters[4], Ben N.G. Giepmans[4], Juan Pablo Romero Riojas[5], Michael S. Nelson[6], Francoise Carlotti[5], Eelco J.P. de Koning[5], Rob C. Hoeben[2], Arnaud Zaldumbide[2,8], and Bart O. Roep[1,5,8]

1. *Department of Diabetes Immunology, Arthur Riggs Diabetes & Metabolism Research Institute at the Beckman Diabetes Research Institute, City of Hope National Medical Center, Duarte, California, USA.*
2. *Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands.*
3. *Department of Immunology, Leiden University Medical Center, Leiden, Netherlands.*
4. *Department of Biomedical Sciences of Cells and Systems, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.*
5. *Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands.*
6. *Light Microscopy core, City of Hope, Duarte, California, USA*
7. ** Joined first author*
8. *† These authors contributed equally to this work*

Transcriptome analyses revealed insulin gene (INS) derived transcripts in non-beta endocrine islet cells. We studied alternative splicing of human INS mRNA in pancreatic islets and identified an alternatively spliced INS product. This variant encodes the complete insulin signal peptide and B-chain and an alternative C-terminus that is largely overlapping with a previously identified defective ribosomal product of INS. Immunohistochemical analysis revealed that the translation product of this INS-derived splice transcript was detectable in somatostatin-producing delta cells but not in beta cells, which was confirmed by light and electron microscopy. The exclusive presence of this alternatively spliced INS product in delta cells may be explained by its clearance from beta cells by insulin degrading enzyme (IDE) capturing its insulin B-chain fragment and a lack of IDE expression in delta cells. Our data demonstrate that delta cells can express an INS product derived from alternative splicing containing both the diabetogenic insulin signal peptide and B-chain in their secretory granules. We propose that this alternative INS product may play a role in islet autoimmunity and pathology, as well as endocrine or paracrine function or islet development and endocrine destiny, and trans-differentiation between endocrine cells. INS promoter activity is not confined to beta cells and should be used with care when assigning beta cell identity and selectivity.

Determination of Hybrid Insulin Peptide Mechanism of Formation in Type 1 Diabetes

Samantha Crawford[1], T. Aaron Wiles[1], Roger Powell[1], My Linh Dang[1], Jason Groegler[1], Anita Hohenstein[1,2], Janet Wenzlau[2], Kathryn Haskins[2], Thomas Delong[1]

1. *Skaggs School of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutical Sciences,University of Colorado Denver, Aurora, CO*
2. *Barbara Davis Center for Diabetes, Department of Immunology and Microbiology, University ofColorado Denver, Aurora, CO*

In Type 1 Diabetes (T1D) autoreactive T cells mediate the destruction of insulin producing beta cells. Recent findings from our lab points to hybrid insulin peptides (HIPs) as targets for autoreactive T cells in T1D. A HIP is the product of a transpeptidation reaction in which a new peptide bond is formed between a proinsulin fragment and another beta cell peptide. As a result, HIPs contain non- genomically encoded amino acid sequences, making them plausible targets for autoreactive T cells in T1D. Various diabetes-triggering CD4 T cells target HIPs in non-obese diabetic (NOD) mice. In human disease, HIP reactive T cells were identified not only in the residual pancreatic islets of T1D organ donors, but also in the peripheral blood of recent onset T1D patients. How HIPs form is currently poorly understood, however it is well established that proteases can drive the formation of new peptide bonds in a side reaction during peptide bond hydrolysis. We hypothesized that a protease in beta cells is responsible for the formation of various disease-relevant HIPs. Our goal was to determine what specific protease may be responsible for the formation of a disease-relevant HIP that is targeted by the diabetes-triggering CD4 T cell clone BDC-2.5 in NOD mice. To isolate such a protease, we fractionated HIP-containing beta cell granule lysates by size exclusion chromatography. We then incubated fractions with precursor peptides that are required for the generation of the HIP recognized by BDC-2.5. Formation of new 2.5HIP in the fractions was monitored through T cell ELISA assays. Experiments were done in the presence and absence of various protease inhibitors, to block HIP formation. This allowed us to identify a protease family that is responsible for the formation of the 2.5HIP. Subsequent mass spectrometric analyses on the HIP-forming fractions led to the identification of a protease responsible for the formation of the 2.5HIP in beta cells.

NOD Mouse CD4 T Cells Recognize a Novel Insulin B-chain Hybrid Peptide

James E DiLisio[1], Janet Wenzlau[1], Rocky L Baker[1], and Kathryn Haskins[1]

1. Department of Immunology and Microbiology, University of Colorado School of Medicine, Aurora, CO

Autoimmune type 1 diabetes (T1D) in humans and the NOD mouse is thought to be driven by T cells that recognize peptides originating from pancreatic islet β-cells. Insulin is considered a key antigenic target of autoreactive T cells and much work has focused on the insulin B-chain sequence, B:9-23. Our lab previously identified disease-relevant T cells in the NOD mouse and humans that respond to hybrid insulin peptides (HIPs), representing a unique type of post-translational modification formed by a fusion of insulin C-peptide fragments to peptides from various β -cell granule proteins such as chromogranin A. Our question in this study was whether ligands of T cell clones responsive to whole insulin or to insulin B:9-23 could include HIPs consisting of sequences from insulin B:9-23 bound to other granule protein sequences. Here we show that a subset of insulin-reactive T cells derived from the NOD mouse can respond to a novel B-chain hybrid peptide. MHC class II tetramers were designed with the new B-chain HIP and used to identify HIP-specific T cells in the islets of NOD mice. Peptide-elicited βNFI ELISpot assays enumerated T cell responses to the B-chain HIP in diabetic splenocytes. Lastly, injection of the B-chain HIP into 6-10 week-old NOD mice led to an expansion of tetramer-positive cells. This work highlights a novel subset of T cells in autoimmune diabetes that respond to insulin B-chain hybrid peptides and which might be targeted in antigen-specific immunotherapy.

SESSION C: BETA-CELL DYSFUNCTION AND TYPE 2 DIABETES

Chairs: Andrew Templin and Luke Wander

Unlocking the Therapeutic Potential of GPR92 to Combat Diabetes

Dayoung Oh[1], Camila de Souza Oleivera[1], Lorena Arango[1]

1. Touchstone Diabetes Center, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

We have deorphanized GPR92 as a receptor for farnesyl pyrophosphate (FPP) which is an intermediate product of cholesterol pathway. GPR92 is highly expressed in the islet macrophages, but not in the endocrine beta and alpha cells in the pancreatic islets. GPR92 expression level in islet macrophages is upregulated in the high fat diet-induced obese mice. GPR92 knockout (KO) mice exhibit glucose intolerance, reduced insulin and glucagon levels, and increased islet inflammation compared to WT mice, despite of paradoxical enlarged pancreatic islets. These results indicate that lack of GPR92 in islet can cause severe islet dysfunction led to disrupted glucose homeostasis. Out study suggests that GPR92 can be a potential target to improve islets dysfunctions related to diabetes progression.

Sex Specific Effects of Nr4a1 in the Beta Cell

Jacob A. Herring[1, 2], Kyle B. Kener[1, 3], Adam G. Wynn[1, 4], Matt C. Austin[1, 5], Courtney J. Smith[1], Jeffery S. Tessem[1]

1. *Department of Nutrition, Brigham Young University, Provo, Utah 84602, USA*
2. *Department of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah 84602,USA*
3. *Medical Education Program, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, Texas 79905, USA*
4. *Medical Education Program, Texas Tech School of Medicine, Texas Tech University Health Sciences Center, Lubbock, Texas 79430, USA*
5. *Medical Scientist Training Program, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA*

A central aspect of type 2 diabetes disease progression is impaired functional β-cell mass. The hyperglycemic and hyperlipidemic environment present in type 2 diabetes corresponds with impaired beta cell function. The orphan nuclear receptor Nr4a1 is critical for fuel utilization in various tissues, however little is known regarding its function in the β-cell. Nr4a1 expression is decreased in the β-cell of rodent models of type 2 diabetes, as well as in primary human islets from type 2 diabetics. Here we demonstrate sex specific effects of β-cell specific Nr4a1 deletion under high fat chow feeding. While female and male βNr4a1-/- mice were no different than wild type controls when fed a standard chow diet, a clear sex dependent difference is observed when fed a high fat diet. High fat fed male βNr4a1-/- have improved fasting and non-fasting blood glucose levels, high fat fed female βNr4a1-/- mice have impaired glucose tolerance as early as two months after beginning high fat feeding. Given the sex specific differences in glucose tolerance, we demonstrated that treatment of INS-1 832/13 cells and mouse islets with estradiol resulted in increased expression of Nr4a1 and not the other two Nr4a family members. Our data suggest that Nr4a1 is critical for estrogen mediated maintenance of beta cell function, and that loss of Nr4a1 under conditions of obesity and overnutrition in females results in impaired beta cell compensation and function. Funding for this project was provided by the NIDDK Grant R15DK124835-01A1

Cholesterol Accumulation in Islets Upregulates Expression of the Mitochondrial Cholesterol Transporter Steroidogenic Acute Regulatory (StAR) Protein and Reduces Islet Viability

Rehana Akter[1], Meghan F. Hogan[1], Nathalie Esser[1], Joseph J. Castillo[1], Rebecca L. Hull[1], Sakeneh Zraika[1], Steven E. Kahn[1]

1. Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, VA Puget Sound Health Care System and University of Washington, Seattle, WA

Diabetes is associated with elevated levels of circulating cholesterol, which can exert deleterious effects on β-cell function/viability via its intracellular accumulation. In steroidogenic tissues, the mitochondrial cholesterol transporter StAR moves cholesterol from the outer to inner mitochondrial membrane for subsequent metabolism. We have demonstrated that StAR is expressed in β-cells and is upregulated under conditions of islet amyloid formation, resulting in increased mitochondrial cholesterol content and decreased mitochondrial function. To determine whether excess cholesterol per se can increase StAR expression and reduce islet viability, we cultured mouse islets for 24 hours in 11.1 mM glucose media with increasing concentrations of cholesterol (0-0.5 mM complexed with cyclodextrin). We then quantified expression of Star and other genes involved in cholesterol synthesis (Hmgcr; HMG-CoA reductase), uptake (Ldlr; LDL receptor) and efflux (Abca; ATP binding cassette subfamily A member 1), as well as islet viability. Islet cholesterol content increased with increasing cholesterol concentrations (27.1±1.73, 55.3±0.47 and 72.7±5.18 µg/mg protein for 0, 0.25 and 0.5 mM respectively; n=3, $p<0.001$ by ANOVA). This was associated with increased Star mRNA expression (1.00±0.06, 3.03±0.88 and 7.02±1.48 for 0, 0.25 and 0.5 mM, respectively; n=3, p=0.014). Further, mRNA expression of Hmgcr tended to decrease (Hmgcr: 1.04±0.22, 0.75±0.26 and 0.28±0.04 for 0, 0.25 and 0.5 mM respectively; n=3, p=0.088), whereas expression of Ldlr and Abca1 did not change (Ldlr: 1.67±0.84, 1.05±0.68 and 0.54±0.26; n=3, p=0.508; Abca1: 1.10±0.3, 1.88±0.72 and 1.07±0.10; n=3, p=0.413). These changes were associated with a dose-dependent decrease in cell viability (100±15.7%, 68.0±12.6% and 57.3±9.6% for 0, 0.25 and 0.5 mM respectively; n=2, p=0.025). In summary, islet cholesterol accumulation is associated with increased StAR expression and decreased cell viability. Collectively, these data suggest that elevated cholesterol levels in diabetes may contribute to β-cell dysfunction by increasing StAR expression and mitochondrial cholesterol accumulation.

Unlocking β-cell replication through the manipulation of αE-catenin function.

Mark Andrade[1], Patricia Kensel-Hammes[1], Ava M. Hudson[1], Sina Gharib[1], Laura Crisa[1,2], Vincenzo Cirulli[1,2]

1. Department of Medicine, University of Washington, Seattle, WA, USA
2. Department of Pharmacology, University of Washington, Seattle, WA, USA
3. Institute of Stem Cells & Regenerative Medicine, University of Washington, Seattle, WA, USA

During pancreas development, $Pdx1^{+}$ multipotent progenitors engage Wnt and SHH pathways in both early and late stages of differentiation to give rise to exocrine and endocrine cell lineages. We previously reported that de-repression of SHH signaling in multipotent $Pdx1^{+}$ progenitors, achieved by deleting αE-catenin (a known repressor of SHH), results in the accumulation of bipotent $Sox9^{+}$ progenitors that are unable to enter an endocrine differentiation pathway and exhibit high rates of proliferation. To investigate whether downregulation of αE-catenin at later stages of pancreatic development could impact the ability of β-cells to replicate through the de-repression of SHH and/or Wnt signaling, we generated $Ins1^{CRE}$/αE-$catenin^{-/-}$ mice. Morphometric analysis of the pancreas of these mutant mice revealed a significant increase of the β-cell mass, an increased frequency of proliferating β-cells, with islet clusters appearing less compact, suggesting a reduced stability of αE-catenin/E-cadherin junctions. Furthermore, a metabolic analysis revealed that $Ins1^{CRE}$/αE-$catenin^{-/-}$ mice are glucose intolerant. Quantitative expression profiling of genes previously implicated in the regulation of β-cell development revealed that pancreatic islets from $Ins1^{CRE}$/αE-$catenin^{-/-}$ mutant mice exhibit a significant upregulation of Wnt effectors Frizzled and Wnt5a, genes of the Calcineurin/NFATC1 and cMyc pathways, as well as regulators of SHH signaling such as Patched-1, Gli1 and Gli3. Conversely, DYRK1B, a potent inhibitor of the SHH signaling, is downregulated. In separate experiments, we also assessed the competency of β-cells from $Ins1^{CRE}$/αE-$catenin^{-/-}$ mutant mice to respond to physiologic growth stimuli during pregnancy. These studies revealed that pregnant $Ins1^{CRE}$/αE-$catenin^{-/-}$ mutant mice presented more than double (i.e., 2.4 folds) the number of proliferating β-cells compared to control pregnant mice. These results indicate that targeting the function of αE-catenin in β-cells may provide a powerful mean for simultaneously increasing intracellular pools of SHH and Wnt effectors, while suppressing counter-regulatory inhibitors of SHH to support the activation of pro-regenerative programs.

Islet Amyloid Formation is Associated with Increased Islet Capillary Diameter and Increased Pericyte Density in a Transgenic Mouse Model

Joseph J. Castillo[1], Daryl J. Hackney[1], Rehana Akter[1], Alfred C. Aplin[1], and Rebecca L. Hull[1]

1. University of Washington and VA Puget Sound Health Care System, Seattle, WA

The islet vasculature, composed of endothelial cells and pericytes, is an important regulator of beta cell function and survival. We have shown that islet capillaries from human type 2 diabetes (T2D) donors are thickened and fragmented around areas of amyloid deposition (which accumulates between beta cells and islet capillaries). In the present study, we utilized a human islet amyloid polypeptide (hIAPP) transgenic (TG) mouse model that develops islet amyloid deposits to investigate hIAPP aggregation's vascular effects *per se*. One year old high- fat fed hIAPP TG mice were injected with lectin-Dylight 647 to label endothelial cells. Pancreata were harvested and intravitally stained with thioflavin S to visualize hIAPP aggregates. Analyzing islet capillary structure morphometry using confocal microscopy revealed capillaries adjacent to amyloid deposits had a significantly larger mean diameter than those that were not (7.3±0.28 µm vs. 6.3±0.19 µm [mean±SE]; n=8 mice, 5 islets/mouse, ~200 capillary structures/islet; p=0.01), consistent with our T2D findings. Since pericytes control capillary constriction, we suspected pericyte dysfunction, which has been associated with T2D. Therefore, we next examined islet pericyte coverage using NG2 immunostaining in the same TG mice. We found that the number of pericytes per capillary was significantly increased in islet capillaries adjacent to amyloid (3.2±0.24 vs. 1.7±0.10; n=6 mice/group, 5 islets/mouse, ~160 capillary structures/islet; p=0.0001). Therefore, increased capillary diameter occurring adjacent to amyloid deposition may be due to dysfunctional islet pericytes, since they are known to proliferate and differentiate into myofibroblasts (a sign of dysfunction associated with impaired constriction and dilation in a fibrosis model). We take these data together to interpret that amyloid formation has detectable detrimental impacts on islet vasculature on a microenvironmental scale, which results in a dysmorphic phenotype that likely diminishes islet function.

Transplantation of Stem Cell Derived Beta-like Cells (sBCs) Triggers a Senescence Associated Secretory Phenotype (SASP) Marked by CD9

Ali H. Shilleh[1], Prabha Yadav[1], **Holger A. Russ[1]**

1. Barbara Davis Center for Diabetes, Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO

Cadaveric islet transplantation has been suggested as a cure for type 1 diabetes, but inadequate donor supply is a key challenge. We and others have recently developed a step wise differentiation protocols for large-scale production of stem cell derived insulin producing beta-like cells (sBCs). sBCs can correct diabetes in preclinical animal models, demonstrating the promise of this stem cell-based approach. Here investigated sBC heterogeneity and identify a small but distinct CD9 subpopulation in vitro. CD9, in conjunction with ST8SIA1, has been shown to mark functionally distinct beta cell subpopulations (β1 to 4) in human cadaveric islets and subpopulation distribution is increased towards CD9+ in diabetic patients. We further identify a SASP like phenotype in CD9+ sBC as revealed by flow cytometric (FCM) and immune fluorescence analysis (IF) for markers, including p21 and IL-6. Additionally, senescence was confirmed by β-gal staining. Single cell RNA sequencing analysis of sBCs further corroborated these findings. Similarly, CD9+ primary human beta cells exhibit markers of SASP. Engraftment of sBCs in immune deficient mice results in a marked ~35% increase in CD9+ SASP sBCs detected by FCM and IF analysis. SASP beta cells have recently been found to be enriched in mouse models of both type 1 and 2 diabetes. Ablation of SASP beta cells using senolytics results in disease prevention or reversal, respectively in these models. Our findings provide important insights for current cell therapy efforts, showing the emergence of sBCs with a SASP phenotype *in vivo*, a beta cell phenotype previously implicated in T1D diabetes onset and progression.

Correlation Between Perturbed β-cell Intracellular Calcium Dynamics and Maturity Marker Expression After Prolonged Exposure to Hyperglycemia

Donghan Shin[1], Marcus Flisher[1], Sharon Lee[1], Mark O. Huising[1]

1. *Department of Neurobiology, Physiology, and Behavior, University of California Davis, Davis, CA*

Numerous studies have demonstrated that β-cells undergo dedifferentiation during the progression of type 2 diabetes (T2D) due to the metabolic stresses that hyperglycemia imposes on β-cells. Dedifferentiated β-cells downregulate the expression of key transcription factors and other maturity markers, while also upregulating genes that are not normally expressed by mature β-cells. Since these cells generally display diminished functional capacity, dedifferentiation is widely regarded as a contributing mechanism of β-cell failure in T2D. Typically, β-cell states are assessed by surveying a panel of presumptive maturity markers. However, there is considerable heterogeneity among β-cells under diabetic conditions with regards to the markers they express. Furthermore, the correlation between different maturity markers and how they are associated with β-cell function is not always clear. Therefore, we quantified β-cell function in real-time within intact and dispersed islets of transgenic mice expressing the genetically encoded calcium sensor GCaMP6. Prolonged exposures to hyperglycemic conditions either by in-vivo treatment with the insulin receptor antagonist S961, or ex-vivo islet culture in high glucose media (≥ 16.8mM) rapidly changed the intracellular calcium dynamics of β-cells under glucose stimulation. We correlated these changes to the reduced expression of Ucn3 and increased expression of Aldh1a3 in a subset of mouse β-cells. To connect these in vitro findings with the human condition, we quantified the lack of co-expression of Ucn3 and Aldh1a3 by immunofluorescence in human islets using pancreatic tissue sections from both non-diabetic and diabetic donors. This confirmed previous reports of reduced Ucn3 and increased Aldh1a3 in keeping with their status as established markers of β-cell maturity and dedifferentiation respectively. Building on these observations, we tested the hypothesis that Aldh1a3 is more likely to be upregulated in the Ucn3 negative subset of β-cells found in the islets of diabetic donors.

SESSION D: BETA-CELL DEATH AND HNF1A-MODY

Chairs: Thomas Delong and Christine Doucette

Membrane-bound LGR4 Its Soluble Form (LGR4-ECD) As Novel Regulators ß- cell Survival Proliferation

Joanna Filipowska[1], Nagesha G. Kondegowda[1], Nancy L. Rivera[1], Rupangi C. Vasavada[1]

1. Arthur Riggs Diabetes and Metabolism Research Institute, Department of Translational Research and Cellular Therapeutics. City of Hope, Duarte, CA

We have shown that RANK (Receptor activator of NF-κB)/RANK ligand (RANKL) interaction inhibits β- cell proliferation and survival, which is reversed by Osteoprotegerin. Recently, the G protein-coupled receptor LGR4 (leucine-rich repeat-containing G-protein-coupled receptor 4), which classically binds R-spondin, was identified as a novel receptor for RANKL in osteoclast precursors. LGR4 is expressed in rodent and human β-cells, but its role in β-cells remains unknown. We postulated that LGR4, through its stoichiometry with RANKL and RANK, is involved in regulating β-cell survival and proliferation. Knockdown of *Lgr4 in vitro*, in INS1 cells or rodent islets, is detrimental for β-cell proliferation and survival in basal and cytokine-stimulated conditions. In contrast, overexpression of *Lgr4* in INS1 cells protects them against cytokine-induced cell death. β-cell-specific *Lgr4* knockout (cko) mice, generated to assess its role *in vivo*, exhibit normal blood glucose homeostasis but have significantly reduced β-cell proliferation and survival compared to wild-type (WT) controls. When exposed to multiple low dose streptozotocin treatment or high fat diet, *Lgr4*-cko mice exhibit increased β-cell death and reduced β-cell proliferation, respectively, compared to WT-control mice. We hypothesized the LGR4-extracellular domain (LGR4-ECD), which is the soluble form of LGR4 that inhibits RANKL/RANK interaction in osteoclasts, will benefit β-cells. Indeed, LGR4-ECD significantly enhances β-cell proliferation in young (8-12-week) and aged (1-year) rodent islets, as well as in human islets from subjects without (46±16 years), and with Type 2 diabetes (51±8 years). Furthermore, LGR4-ECD significantly promotes mouse and human β-cell survival against cytokine-induced cell death. Thus, LGR4 and LGR4-ECD are positive regulators of β-cell proliferation and survival. Future studies will determine the mechanisms by which this pathway modulates β-cell homeostasis and its therapeutic potential in the setting of diabetes. Acknowledgements: Funding: JDRF postdoctoral fellowship #3- PDF-2020-936-A-N to JF; NIH/R01DK102893, COH Wanek Family Project to Cure T1D to RCV. Human Islets: IIDP

Laminin Interactions with the Islet Protect Against Cytokine-mediated β-cell Death via Protein Kinase C δ Downregulation

Meghana Shivananda Murthy[1,2], Nikki L. Farnsworth[1,2], Keifer Holcomb[1], Drake Hampton[1], and Richard K. P. Benninger[2,3]

1. *Department of Chemical and Biological Engineering, Colorado School of Mines, Golden, CO*
2. *Barbara Davis Center for Childhood Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO*
3. *Department of Bioengineering, University of Colorado Anschutz Medical Campus, Aurora, CO*

Type 1 diabetes (T1D) is an autoimmune disease characterized by the loss of insulin producing pancreatic islet β-cells. Islets are surrounded by a specialized extra cellular matrix (ECM) that interacts with islet via integrins to support islet function and viability. ECM components, such as laminin, have previously been shown to promote islet survival Integrin activity has been linked to changes in PKCδ activity. We hypothesize that loss of ECM interactions with islet mediates β-cell death via upregulation of PKCδ. Isolated mouse islets from WT mice and mice with a β-cell specific KO of PKCδ (PKCδ-βKO) were encapsulated in a reverse thermal gel (RTG) functionalized with laminin-10 (RTG-lam), lysine (RTG-lys) or RTG and were treated with TNF-α 10ng/ml, IL-1β 5ng/ml, IFN-γ 100ng/ml for 24 hours. MIN6 cells were cultured on ECM with or without laminin and treated with cytokines. Cytokine-mediated β-cell death was reduced in islets encapsulated in RTG-lam ($p=0.027$), compared to RTG only and free cultured islets. PKCδ translocation and activity was increased at the cell membrane in cytokine-treated MIN6 cells culturedwith laminin ($p=0.039$) compared to those without laminin as measured by westernblot and FRET sensor. Mouse PKCδ-βKO islets were significantly ($p=0.02$) protected against cytokine-induced death compared to WT controls. Our results implicate a role for ECM-islet interactions protecting against cytokine-induced islet death via downregulation of PKCδ at the cell membrane. This study presents a novel mechanism of β-cell death and will guide the development of therapies to protect against the onset of T1D.

Cleavage of Protein Kinase C δ by Caspase-3 Mediates Cytokine-Induced β-Cell Death

Jillian Collins[1], Richard Benninger[2,3], Nikki L. Farnsworth[1,2]

1. *Department of Chemical and Biological Engineering, Colorado School of Mines, Golden, Colorado, USA*
2. *Barbara Davis Center for Childhood Diabetes, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA*
3. *Department of Biological Engineering, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA*

In type 1 diabetes (T1D), autoreactive immune cells secrete cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ) that can initiate β-cell apoptosis in pancreatic islets. Protein kinase C delta (PKCδ) plays a role in mediating cytokine-induced β-cell apoptosis; however, the exact mechanisms of PKCδ regulation in β-cell death and role in T1D are unknown. In other cell types, PKCδ mediates apoptosis by translocating to the nucleus and being cleaved by caspase-3. We hypothesize PKCδ mediates cytokine-induced β-cell apoptosis by translocating to the nucleus and cleaved by caspase-3. Islets from WT mice or mice with a β-cell specific knockout of PKCδ were cultured for 24hr with cytokines (10ng/ml TNFα, 5ng/ml IL-1β, 100ng/ml IFN-γ), a caspase-3 inhibitor (Z-DEVD-FMK, 33μg/ml), or with an adenovirus to produce GFP- tagged PKCδ, GFP only, GFP with a constitutively active nuclear localization sequence, and a PKCδ mutant that cannot be cleaved by caspase-3 (CM-GFP-PKCδ). Human islets were treated with 10μM PKCδ inhibitor (δV1-1) for 24hr with or without cytokines. Translocation was determined by GFP colocalization with a nuclear stain. Apoptosis was determined by fluorescent live/dead staining. PKCδ activity was determined with a FRET-based sensor in MIN6 cells. Decreasing PKCδ activity protected β-cells in mouse ($p=0.003$) and human ($p=0.020$) islets. FRET showed PKCδ activity increased in nuclei of cytokine treated islets ($p=0.039$) and a higher nuclear/cytosolic ratio of GFP-tagged PKCδ was measured at 3hr ($p=0.052$) and 24hr ($p<0.001$). Islets treated with caspase inhibitor and CM- GFP-PKCδ islets were protected from cytokine-induced death. Inhibiting PKCδ protected islets from cytokine-induced β-cell death. Pro-inflammatory cytokines induce nuclear translocation and activity, but nuclear localization alone is insufficient for apoptosis. Caspase-3 cleavage is required for cytokine-induced apoptosis. Our data suggests targeted inhibition of PKCδ has the potential toprevent or delay β-cell death in T1D onset.

TNFα-Induces RIPK3 Mediated β-Cell Necroptosis When Caspases Are Inhibited

Christopher J. Contreras[1], Noyonika Mukherjee[1,2], Li Lin[1], Meghan F. Hogan[3], Andrew A. Oberst[4], Steven E. Kahn[3], **Andrew T. Templin[1,2,5,6]**

1. *Lilly Diabetes Center of Excellence, Indiana Biosciences Research Institute, Indianapolis, IN*
2. *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN*
3. *Division of Metabolism, Endocrinology and Nutrition, Department of Medicine, VA Puget Sound Health Care System and University of Washington, Seattle, WA*
4. *Department of Immunology, University of Washington, Seattle, WA*
5. *Center for Diabetes and Metabolic Diseases, Indiana University School of Medicine, Indianapolis, IN*
6. *Department of Medicine, Roudebush VA Medical Center and Indiana University School of Medicine, Indianapolis, IN*

Necroptosis is a lytic and immunogenic form of cell death that occurs downstream of TNFα signaling. Receptor interacting protein kinase 3 (RIPK3) mediates TNFα-induced necroptosis when caspase activity is inhibited, but this pathway has not been examined in β-cells. Since TNFα signaling is associated with β-cell loss in diabetes and we found RIPK3 is expressed in INS-1 and mouse islet cells, we hypothesized β-cells are susceptible to necroptosis. To test this hypothesis, INS-1 and mouse islet cells were treated with TNFα (40 ng/ml) in the absence or presence of the pan-caspase inhibitor zVAD for 24 hours. In INS-1 cells, TNFα increased cell death and caspase activity, consistent with apoptosis. When caspases were inhibited with zVAD, TNFα no longer increased caspase activity, but induced a similar degree of cell death, consistent with necroptosis. When TNFα-induced cell death was amplified with a SMAC mimetic, we again observed similar levels of cell death with and without caspase inhibition. Overexpression of RIPK3 increased INS-1 cell death under TNFα and zVAD treatment. Using co-immunoprecipitation, we found RIPK3 interacts with the necroptosis effector protein MLKL. In mouse islet cells, TNFα alone did not significantly increase either cell death or caspase activity after 24 hours. However, when caspases were inhibited, TNFα increased islet cell death to a similar degree as with combination TNFα, IL1β (10 ng/ml) and IFNγ (100 ng/ml) treatment. To further investigate the mechanism of β-cell death under caspase inhibition, we applied the RIPK3 inhibitor GSK'872. Addition of GSK'872 did not alter TNFα-induced cell death in INS-1 or mouse islet cells. However, GSK'872 reduced TNFα-induced cell death when caspases were inhibited with zVAD. These data indicate that TNFα induces β-cell death via RIPK3-mediated necroptosis when caspases are inhibited. Additional studies are required to understand the factors that may promote β-cell necroptosis in the setting of human diabetes.

Inflammatory Stress-Induced Endogenous-dsRNAs Drive β-cell to IFN-I State

Kevin K. Chi[1], Amy Leung[2], Parijat Senapati[2], Dustin E. Schones[2]

1. *Department of Diabetes Complications and Metabolism, Beckman Research Institute, City of Hope, Duarte, CA USA; Irell & Manella Graduate School of Biological Sciences, City of Hope, Duarte, CA USA.*
2. *Department of Diabetes Complications and Metabolism, Beckman Research Institute, City of Hope, Duarte, CA USA.*

Proinflammatory cytokine exposure, which precedes the onset of type 1 diabetes (T1D), rapidly alters transcriptional outputs of cells. Recent work has shown that proinflammatory signaling networks activate transcription of retrotransposons, including endogenous retroviruses and Alus. These elements can, in turn, generate double-stranded RNAs (dsRNAs) that are recognized by sensors of type-I-interferon signaling, potentially triggering apoptosis. We are examining how proinflammatory cytokine exposure, through expression of dsRNAs from retrotransposons, contributes to β-cell death. Chromatin accessibility profiling using ATAC-sequencing and transcriptome profiling with RNA-seq revealed that proinflammatory cytokine treatment with TNF and IFN-I increased chromatin accessibility and transcription of retrotransposons. Antiviral dsRNA-sensors such as MDA5, RIG-I, and TLR3 were furthermore upregulated in proinflammatory cytokine treated cells. These data suggest that proinflammatory cytokines can promote a heightened anti-viral state, which in conjunction with cytokine-induced elevated expression of dsRNAs may drive β-cell death and/or dysfunction. Finally, transcriptomes of T1D islets, compared to those from non-diabetic islets, show increased expression of dsRNA-forming Alu elements, which are dramatically unedited in T1D islets compared to islets from normal individuals. Altogether, our results demonstrate that proinflammatory stress in beta cells both activates the expression of retrotransposons and upregulates antiviral sensors. These results provide a novel mechanism through which β-cells in T1D islets are triggered for destruction.

Genetic Engineering of Stem Cell Derived Pancreatic Beta-like Cells Confers Protection from Autoimmune Diabetes

Roberto Castro Gutierrez[1], Jorge Santini-Gonzalez[2], Aimon Alkanani[1], Kristen McDaniel[1], Edward A. Phelps[2], Aaron Michels[1], Holger A. Russ[1]

1. *Barbara-Davis Center for Diabetes, Aurora, CO*
2. *University of Florida, Gainesville, FL*

Type 1 diabetes (T1D) results from an autoimmune attack and loss of pancreatic beta cells mediated by diabetogenic $CD8^+$ T cells. We and others have described the generation of an abundant source of functional stem cell derived beta-like cells (sBCs) that can revert diabetes after transplantation into preclinical animal models. However, the possibility of re-occurrent autoimmunity is a remaining hurdle to overcome. Our goal is to protect sBCs from autoimmune diabetes to provide cell therapy alternatives for people suffering from T1D. We have generated an *in vitro* assay that allows for the analysis of sBCs – T cell interactions in an isolated system. Our data shows that upon pro- inflammatory cytokine exposure, sBCs upregulate Human Leukocyte Antigen (HLA) class I receptors which allow for their recognition by diabetogenic CD8+T cells. To avoid this, we employed genetic engineering to delete HLA and overexpress PD-L1 receptors in sBCs. These modifications resulted in complete protection from diabetogenic CD8+T cell activation *in vitro*. To confirm this protection *in vivo,* we transplanted genetically engineered sBCs into a human HLA-matched diabetic mouse model (NOD.b2m.HHD). Preliminary results show that grafts overexpressing PD-L1 exhibit greater survival compared to controls. Here, we present *in vitro* and *in vivo* platforms to study immune-beta cell interactions in an autoimmune human context by (i) co-culture of sBCs with human diabetogenic CD8+T cells and (ii) a humanized HLA-matched diabetic mice. We specifically present evidence that manipulation of HLA and PD-L1 receptors provide sBCs protection from autoimmune diabetes. Taken together, we postulate potential cell therapy modalities for patients suffering from T1D.

The HNF-1aG319S Variant Shifts Beta-Cell Metabolism Towards Fat Oxidation in MIN6 Beta-Cells and Mouse Islets

Taylor S. Morriseau[1, 2], Kristin L. Hunt[1, 3], Cuilan Nian4, Vernon W. Dolinsky[1, 2], Francis Lynn[4], Christine A. Doucette[1, 2, 3]

1. *Diabetes Research Envisioned and Accomplished in Manitoba (DREAM) Theme of the Children'sHospital Research Institute of Manitoba, Winnipeg, MB*
2. *Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, MB*
3. *Department of Physiology and Pathophysiology, University of Manitoba, Winnipeg, MB*
4. *BC Children's Hospital Research Institute, Vancouver, BC*

Background: 40% of Indigenous youth with type 2 diabetes (T2D) in Manitoba harbour a variant in the HNF-1alpha gene. The G319S variant is thought to drive pancreatic beta-cell dysfunction; however, youth-onset T2D is a relatively recent phenomenon. We hypothesize the G319S variant impairs beta- cell insulin secretion when exposed to modern dietary carbohydrate stress but is protective in the context of traditional foods rich in fat and protein. Methods: CRISPR/Cas9 was used to knock-in the G>A.955 substitution into MIN6 beta-cells (G319S-MIN6) and C57/BL6 mice. Mice were weaned onto (1) standard chow, (2) a "traditional" high-fat, low-carbohydrate (HFLC) diet, or (3) a "modern" high- fat, high-carbohydrate (HFHC) diet for 12- or 24-weeks. Beta-cell function was assessed by glucose- stimulated insulin secretion (GSIS) and oxygen consumption rates for glucose or palmitate oxidation. Results: In the absence of dietary manipulation, a consistent reduction (>2.8-fold) in basal insulin secretion was observed in MIN6 cells and islets expressing the G319S-variant. The suppression of basal insulin may be driven by increased fatty-acid beta-oxidation (1.5-fold), which also protects G319S-MIN6 from palmitate-induced impairments in GSIS. Given this propensity for fatty-acid metabolism, G319S-expressing mice retained both glucose tolerance and GSIS when fed a HFLC diet that otherwise impaired wild-type mice at 12-weeks-of-age. Conversely, a HFHC diet elevated weight gain and impaired GSIS in G319S-expressing mice at 24 weeks-of-age. Conclusion: The G319S variant appears to shift beta-cell metabolism towards fat oxidation which may suppress fasting insulin and reduce systemic glucose utilization. To align nutritional intake with this metabolic shift, the consumption of a HFLC diet appears to normalize insulin secretion and glucose tolerance in G319S carriers, although studies to assess long-term effects are underway. Conversely, a HFHC diet worsens metabolic outcomes across all genotypes. These studies may inform nutritional interventions for children with T2D while ultimately supporting community efforts to access traditional foods.

A Human Pluripotent Stem Cell Model of HNF4α/MODY1 Provides Mechanistic Insights into Disease Phenotypes

Kim-Vy Nguyen-Ngoc[1], Medhavi Mallick[1], Vivian Lin[1], Winnie Gong[1], Han Zhu[1], Maike Sander[1]

1. *Departments of Pediatrics and Cellular & Molecular Medicine, Pediatric Diabetes Research Center, University of California, San Diego, La Jolla, CA*

Heterozygous mutations in the transcription factor HNF4α cause maturity onset diabetes of the young (MODY). Clinically, HNF4α/MODY1 mutations are associated with postnatal hyperinsulinemic hypoglycemia, evolving into diabetes later in life. To gain insight into disease mechanisms of HNF4α/MODY1, we introduced the heterozygous *HNF4α*R141X point mutation into human pluripotent stem cells (hPSC) and differentiated *HNF4α*$^{R141X/+}$ and control cells into pancreatic islet cells (SC-islet). We observed no impact of the *HNF4α*R141X mutation on islet cell differentiation. However, *HNF4α*$^{R141X/+}$ SC-islets exhibited insulin hypersecretion in basal and high glucose, and in KCl-depolarized conditions, which was reversed by diazoxide treatment. These findings are consistent with the HI phenotype and treatment in the MODY1 patients. To understand *HNF4α*-dependent gene regulatory programs in beta cells, we compared transcriptomes and chromatin accessibility in *HNF4α*$^{R141X/+}$ and control SC-islets at single cell level. Beta cell-specific analyses revealed reduced expression of *NEUROD1* and ion channels, consistent with reported insulin hypersecretion in loss-of-function models for these genes. In addition, *HNF4α*$^{R141X/+}$ SC-beta cells exhibited reduced expression of pro-survival genes (*BCL2L1*, *SERPINA1*, *HSPA5*, *ANKS4B*), which are known to compensate for ER stress. Through gene regulatory network analyses, we identified *BCL2L1* and *SERPINA1* as direct target genes of HNF4α in beta cells. In agreement with these molecular findings, *HNF4α*$^{R141X/+}$ SC-beta cells were more prone to undergo apoptosis in response to thapsigargin-induced ER stress, a phenotype that was rescued by supplementing SERPINA1 protein. These results suggest that beta cells in HNF4α/MODY1 patients are more susceptible to stress-induced cell death, providing a possible mechanism for progression to diabetes. Overall, our hPSC-based HNF4α/MODY1 model recapitulates key aspects of the human phenotype. Furthermore, by providing mechanistic insight into *HNF4α*-regulated cellular processes in beta cells, our work identifies opportunities for therapeutic intervention.

A Primary Human Model of HNF1α Deficiency Recapitulates HNF1A-MODY Phenotypes

Mollie S.H. Friedlander[1], Romina J. Bevacqua[1], Charles A. Chang[1], Vy M. Nguyen[1], and Seung K. Kim[1,2,3,4,5]

1. *Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA*
2. *Department of Medicine, Stanford University School of Medicine, Stanford, CA*
3. *Department of Pediatrics, Stanford University School of Medicine, Stanford, CA*
4. *Stanford Diabetes Research Center, Stanford University School of Medicine, Stanford, CA*
5. *JDRF Center of Excellence, Stanford University School of Medicine, Stanford, CA*

Haploinsufficiency of HNF1A results in one of the most common forms of Maturity Onset Diabetes of the Young (HNF1A-MODY), yet there are still large gaps in our understanding of how the transcription factor HNF1α regulates pancreatic islet cell function. Hnf1a mutant rodents do not mimic human phenotypes, demonstrating a need for alternative HNF1A-MODY models to facilitate understanding and targeting of the pathways responsible for human disease. Here, we aimed to develop a primary human islet cell model to elucidate HNF1α-dependent mechanisms of pancreatic islet regulation. Specifically, we (1) created a platform to suppress expression of HNF1A in human pancreatic islet cells with shRNAs, (2) assessed the consequences of HNF1A suppression (HNF1AKD) on islet gene expression and function in vitro, and (3) characterized the long-term consequences of HNF1α deficiency in vivo through transplantation of HNF1AKD pancreatic islets to murine hosts. We efficiently and reproducibly reduced HNF1A expression in primary human pancreatic islet cells via lentiviral delivery of shRNA and pseudoislet formation. In vitro, HNF1AKD correlated with dysregulation of genes critical for β- and α- cell function. Additionally, HNF1AKD pseudoislets transplanted to murine kidney capsules secreted significantly less insulin than controls after 4 weeks in vivo. Blunted glucose-stimulated insulin secretion in transplanted HNF1AKD pseudoislets was rescued by sulfonylurea treatment, mirroring HNF1A-MODY patients' sensitivity to this class of antidiabetic medication. In summary, we have developed a primary human pancreatic model of HNF1α deficiency that recapitulates HNF1A-MODY phenotypes. These studies could accelerate understanding of HNF1α functions in islet β- and α- cells, and could advance development of new therapeutic approaches for diabetes.

SESSION E: STRATEGIES FOR BETA-CELL REPLACEMENT

Chairs: Senta Georgia and Zong Wei

Muiltiomic Single Cell Analysis Identifies Mechanisms of Human Pancreatic Endocrine Cell Specification and Beta Cell Maturation

Han Zhu[1], Gaowei Wang[1], Kim Vy Nguyen-Ngoc[1], Dongsu Kim[1], Jenna Kovsky[1], Michael Miller[2], Sebastian Preissl[2], Kyle J. Gaulton[1,4], Maike Sander[1,3,4]

1. *Department of Pediatrics, Pediatric Diabetes Research Center, University of California, San Diego, USA*
2. *Center for Epigenomics, University of California, San Diego, USA*
3. *Department of Cellular & Molecular Medicine, University of California, San Diego, USA*
4. *Institute for Genomic Medicine, University of California, San Diego, USA*

Human pluripotent stem cell (hPSC)-derived islet cells (SC-islets) hold great promise for diabetes therapy, both as a transplantable cell source and as a model for identifying disease mechanisms. SC-islets consist of endocrine cells that resemble islet endocrine cell types, including alpha-, beta-, and delta-like cells. In addition, endocrine cells with intestinal enterochromaffin features (SC-ECs) are produced, which have been suggested to be islet-aberrant. The gene regulatory mechanisms that specify endocrine cell populations during hPSC differentiation have remained largely unknown. Moreover, it is unclear how closely hPSC-derived endocrine cells resemble their primary counterparts. We hypothesized that such knowledge will help manipulate cell fates and cell states during *in vitro* differentiation. Here, we combined single-cell genomics and gene regulatory network (GRN) analysis to gain insight into the gene regulatory programs governing SC-islet differentiation. Our analysis identified developmental trajectories and predicted transcription factors that define each cell type. The known intestinal transcription factor CDX2 was predicted to regulate SC-EC-specific genes, in particular genes of the serotonin synthesis pathway. In agreement with these findings, CRISPR/Cas9- mediated deletion of *CDX2* in hPSCs reduced the number of endocrine cells expressing serotonin synthesis genes, showing that predictions from single-cell genomics can inform directed cell fate manipulations. Furthermore, through integration of single-cell data from SC-islets with similar data from primary juvenile and adult human islets, we identified maturation-related transcriptional programs which are less active in SC-beta cells than in primary beta cells. These programs include mitochondrial biogenesis genes downstream of the transcription factor estrogen-related receptor gamma (ERRγ) and cell proliferation genes downstream of the androgen receptor (AR). Validating these predictions, activation of ERRγ and the AR in SC-islets improved mitochondrial activity and increased SC-beta cell numbers, respectively. Together, our findings demonstrate the power of single- cell genomics for informing beta cell differentiation strategies from hPSCs.

Groucho Co-repressor Proteins Regulate β Cell Development and Proliferation by Repressing *Foxa1* in the Developing Pancreas

Alexandra Theis[1], Ruth A. Singer[2], Diana Garofalo[2], Lori Sussel[1,2]

1. *Barbara Davis Center, Graduate Program in Cell Biology, Stem Cells, and Development, University of Colorado Anschutz Medical Campus, Aurora, CO*
2. *Naomi Berrie Diabetes Center, Cell and Molecular Biomedical Science Graduate Program, Columbia University Medical School, New York City, NY*

Pancreas development is controlled by a complex network of transcription factors (TFs) that promote spatiotemporal expression of genes during organogenesis. In addition, genetic regulation by cell- specific TFs often depends on their interactions with co-activators/-repressors, such as the Groucho- related gene (GRG) co-repressor family. GRGs function with TFs to recruit epigenetic modifiers, such as HDACs, and repress target genes by creating closed chromatin that is inaccessible to transcriptional machinery. *Grgs* are expressed early in pancreas development and are maintained throughout adulthood. Studies in other contexts demonstrate GRGs interact with essential endocrine cell TFs, including NKX2.2, PAX6, and FOXA2, yet the molecular mechanisms behind GRG function in pancreas development had not been fully characterized. Here, we used complex mouse genetics and transcriptomic analyses to determine that GRG3 is essential for pancreatic β cell development, and that in the absence of *Grg3*, there is compensatory upregulation of *Grg4*. *Grg3/4* double mutant mice are extremely hyperglycemic at birth with early lethality occurring in the majority of pups. Transcriptome analysis on e18.5 pancreata revealed dysregulation of genes involved in pancreas development and β cell function. Surprisingly, canonical liver genes are ectopically expressed in mutant pancreata, including *Foxa1*, a master regulator of the liver program. Additionally, *Neurod1*, an essential β cell transcription factor and predicted target of *Foxa1*, is downregulated in *Grg3/4* mutants. Loss of *Neurod1* in β cells inhibits the proliferative expansion of the β cell population, which is phenocopied in *Grg3/4* mutant mice displaying a significant decrease in the percentage of proliferating β cells perinatally. These results implicate GRGs as critical cofactors in the transcriptional network controlling pancreas gene expression during β cell development. Funded by NIH F31 DK122634-01 (AT) and NIH R01 DK082590 (LS).

Integrin-Linked Kinase (ILK) Regulates β-cell Mass Development and Function

David Vaisar[1], Patricia Kensel-Hammes[1], Sina Gharib[3], Laura Crisa[1,2], Vincenzo Cirulli[1,2]

1. *Department of Medicine**
2. *Department of Pharmacology**
3. *Computational Medicine Core, Center for Lung Biology**

**Institute of Stem Cells and Regenerative Medicine, University of Washington, Seattle, WA, USA*.

Our laboratory has previously uncovered unique functions of extracellular matrix receptors of the Integrin family in the development and function of islet β-cells. We have demonstrated that β1 integrins are required for the development of a normal β-cell mass, owing to their signaling properties in regulating cell replication. More recently, building on these discoveries, we designed new experiments to identify downstream effectors of β1 integrin signaling regulating β-cell development, proliferation, and endurance to metabolic stressors. Among molecules that are recruited by β1 integrins upon binding to their cognate ECM ligands, ILK (integrin-linked kinase) is of significant interest, due to its involvement in multiple developmental processes that include cell growth, differentiation, survival and function. Hence, we generated $Ins1^{Cre}ILK^{-/-}$ mutant mice that lack ILK in β-cells, and conducted a full morphometric assessment of their β-cell mass and insulin secretory function, under normal and a high fat diet (HFD). The results of these studies revealed that $Ins1^{Cre}ILK^{-/-}$ mutant mice exhibit a reduced β-cell mass, and significant glucose intolerance in spite of an increased insulin content. This phenotype was significantly more evident in males when compared to female mutants, and was exacerbated by HFD. We further observed that the islet area populated by ILK^{null} β-cells, but not that occupied by α-cells, is less vascularized when compared to control mice. In an attempt to identify mechanisms underlying the observed phenotypes, we conducted experiments of scRNA-seq on islets from $Ins1^{Cre}ILK^{-/-}$ and WT mice. Preliminary analysis of these data reveled that ILK^{null} β-cells exhibit numerous alterations in the expression of genes that control the trafficking of secretory vesicles and the production of pro-angiogenic factors. Collectively, these results point to an important function of ILK in β-cell development and functional homeostasis through effects on hormone exocytosis and, indirectly, on vascular development and/or remodeling.

Control of Pancreatic Islet Cell Identity and Function by Tissue Macrophages

Samanda Valente[1], Jessica O'Sell[1], Stephanie Pardike[1], Sina Gharib[3], Vincenzo Cirulli[1,2], Laura Crisa[1,2]

1. *Department of Medicine, University of Washington, Seattle, WA, USA*
2. *Department of Pharmacology, University of Washington, Seattle, WA, USA*
3. *Computational Medicine Core, Center for Lung Biology, University of Washington, Seattle, WA, USA*
4. *Institute of Stem Cells & Regenerative Medicine, University of Washington, Seattle, WA, USA*

The perinatal stage of pancreas development marks a time characterized by maximal proliferation of pancreatic islet cells, establishing the endocrine mass required for the maintenance of glucose homeostasis throughout life. We have reported on distinct myeloid cell populations in the murine pancreas, and uncovered a temporally restricted requirement for CCR2+ myeloid cells in the perinatal proliferation of the endocrine pancreatic epithelium. Hence, using CCR2-specific depletion models, we have shown that loss of this myeloid population leads to a striking reduction in β-cell proliferation, dysfunctional islet phenotypes and glucose intolerance in newborns. In follow-up metabolic assessments, CCR2-depleted newborns were weaned to either standard chow or high-fat diets. We now find that loss of CCR2+ myeloid cells in perinatal life, although transient, significantly worsens tolerance to glucose loads in adult female mice fed with standard chow. Further functional analysis of pancreatic islets isolated from these mice reveals reduced insulin content and secretion in response to glucose. To investigate cellular and molecular mechanisms underlying these defects, we performed scRNAseq experiments using islets from females isolated at 4 weeks and 6 months after CCR2+ myeloid cell depletion. Islets from age- and gender-matched wild type mice served as controls. These experiments revealed remarkable changes in islet cell populations associated with perinatal loss of CCR2+ myeloid cells which included increased expression of inflammasome and ER stress cell markers in beta cells, an abnormally high representation of de-differentiated beta-like populations, and pro-proliferative/bi-hormonal phenotypes of alpha cells. Some of these phenotypes resembled those found in aging 6 months old islets of either control or depleted mice. These findings disclose long-lasting effects of early pancreatic myeloid cell niches on the islets' ability to endure metabolic stress later in life, and highlight the importance of CCR2+ myeloid cells in the development and/or maintenance of mature endocrine islet populations.

Early De Novo DNA Methylation Patterning Regulates Beta Cell Function and Survival Programs

Nazia Parveen[1], Jean K. Wang[1], Sangeeta Dhawan[1]

1. Arthur Riggs Diabetes and Metabolism Research Institute, City of Hope, Duarte, CA

DNA methylation is an important epigenetic module that controls the cellular transcriptional programs to direct their phenotype. Dynamic, stage-specific changes in DNA methylation patterning coordinate the gene-expression patterns associated with cell differentiation during development. Our prior work has shown that the establishment of new DNA methylation patterns is essential for functional maturation of beta cells in postnatal life, while maintenance of existing methylation patterns restricts beta cell identity. While maintenance of DNA methylation in pancreatic progenitors is essential for their survival during differentiation, the requirement for new DNA methylation patterns in pancreatic progenitors has not been investigated. To address this, we generated pancreatic progenitor specific ablation of the de novo DNA methyltransferase, Dnmt3a, an enzyme that establishes new DNA methylation patterns, using Pdx1-Cre (Dnmt3aPKO). RNA-sequencing of the Dnmt3aPKO islets reveals that early loss of Dnmt3a alters the beta cell identity, function, and survival programs. In addition, we observe the upregulation of genes associated with dopamine signaling and disruption of imprinted gene expression in the KO islets. Several of these gene-expression changes are distinct from those observed in beta cell specific KO of Dnmt3a, suggesting the unique contribution of early patterning in adult beta cell phenotype. Genome-wide profiling of DNA methylation patterns using reduced representation bisulfite sequencing (RRBS) in the Dnmt3aPKO islets points to disruption of similar programs associated with cell identity and function. These data also reveal interesting differences in the DNA methylation patterns in male and female Dnmt3aPKO mice. In conclusion, our data suggest that stage specific DNA methylation patterning during beta cell differentiation controls specificaspects of the mature beta cell phenotype.

The Roles NKX2.2 and NKX6.1 in Human Islet Cell Fate Determination

Christopher Schaaf[1], Roberto Castro-Gutiérrez[1], Patrick Sean McGrath[1, 2], Holger A. Russ[1, 2], Lori Sussel[1]

1. *Barbara Davis Center for Diabetes, University of Colorado Anschutz Medical Campus, Aurora, CO 80045*
2. *Disease Modeling Core, University of Colorado Anschutz Medical Campus, Aurora, CO 80045*

Extensive rodent studies have paved the way to the establishment of step-wise protocols that guide the differentiation of human pluripotent stem cell populations to the islet cell lineages. NKX2.2 and NKX6.1 are two transcription factors that are essential for islet cell differentiation and function in mice. NKX2.2 is expressed throughout the pancreas and ultimately becomes restricted to a subset of endocrine lineages. Null mutations in Nkx2.2 result in a complete absence of beta-cells and severely reduced numbers of alpha-cells. NKX6.1 is also expressed early during pancreas development and gradually becomes restricted to the beta-cell lineage; beta-cell numbers are severely reduced in mice carrying null mutations of Nkx6.1. Both NKX2.2 and NKX6.1 proteins are conserved in humans and are expressed in the developing pancreas, albeit temporally different when compared to mice. To identify their respective roles in human islet cell specification, we have generated Nkx2.2 and Nkx6.1 knockout hESC lines and will characterize the ability of these lines to differentiate into the different islet lineages. We hypothesize that deletion of Nkx2.2 will disrupt differentiation of alpha- and beta- cell program, and Nkx6.1 deletion will disrupt beta-cell formation and skew differentiation either towards duct and/or alpha-cell fates. These studies will not only inform the molecular mechanisms regulating these important islet cell fate decisions, but also have the potential to improve thedirected differentiation of human islet cells.

Type 2 Diabetes-Associated Gene *PAX4* Is Required for Human Endocrine Cell Development

Nicole A.J. Krentz[1,2], Marta Pérez Alcántara[2], Fernando Abaitua[2], Jila Ajeian[3], Varsha Rajesh[1], Alokkumar Jha[1], Hanice Sun[1]m Benoite Campon[3], Claire E. Duff[3], Daniela Moralli[2] Benjamin Davies[2], Anna L. Gloyn[1,2,3,4]

1. *Department of Pediatrics, Stanford School of Medicine, Stanford University, CA, USA*
2. *Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK*
3. *Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), University of Oxford, Oxford,UK*
4. *Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford, UK*

Protein coding variants that causally influence type 2 diabetes (T2D)-risk are a powerful tool to identify disease mechanisms. Genome-wide association studies have identified a population specific (Southeast Asian) coding allele in the transcription factor *PAX4* (p.R192H) that alters T2D-risk. In mice, loss of *Pax4* results in diabetes due to impaired beta-cell formation but well documented differences between mouse and humans means that uncertainty remains over the role of *PAX4* in humans. To determine the impact of *PAX4* loss on human endocrine cell development, we coupled CRISPR-Cas9 genome editing in human induced pluripotent stem cells (hiPSC) with *in vitro* differentiation along the endocrine lineage. We generated isogenic homozygous knockout (*PAX4*$^{-/-}$; n=3) and unedited wildtype (*PAX4*$^{+/+}$; n=3) hiPSC clones, differentiated them in triplicate, and performed bulk RNA-seq at three key stages of endocrine development. *PAX4* loss significantly reduced expression of the pan-endocrine gene *CHGA* (log_2FC = -2.9, p_{adj}= 5.8E-8) and endocrine progenitor marker *FEV* (log_2FC = -4.6, p_{adj} = 1.5E-5), suggesting a defect in endocrine cell formation. Conversely, there was an upregulation of alpha-cell genes *ARX* (log_2FC = 2.3, p_{adj} = 2.0E-6) and *GCG* (log_2FC = 1.1, p_{adj} = 1.5E-05), supporting a role for human *PAX4* in alpha- versus beta-cell lineage determination. To understand if PAX4 is required for mature human beta-cell function, transient transfection of siRNAs was used to reduce expression of *PAX4* to 51.2% of control EndoC-BH1 cells and no differences in glucose-stimulated insulin secretion were observed. Our data support a critical role for PAX4 in endocrine cell specification in humans. *PAX4* knockout cells have a gene regulatory signature consistent with an increased formation of alpha-cells at the expense of the beta-cell lineage. Together, our data suggests that T2D-risk alleles at the *PAX4* locus are likely to work through defects in endocrine cell development.

Identifying the NKX6.1 Interactome in Beta Cells

Connor C. Littlefield[1], Nathanael C. Jensen[1], Jonathon T. Hill[2], John C. Price[3], Jeffery S. Tessem[1]

1. *Department of Nutrition, Dietetics and Food Science, Brigham Young University, Provo, Utah 84602, USA*
2. *Department of Physiology and Developmental Biology, Brigham Young University, Provo, Utah 84602, USA*
3. *Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602, USA*

The beta cell transcription factor NKX6.1 plays a critical role in pancreatic progenitor cell differentiation, mature beta cell proliferation and insulin secretion. The pathways regulated by NKX6.1 that results in these phenotypes are still being defined. Given that transcription factors generally function in complexes, we sought to determine proteins that interact with NKX6.1 and may be harnessed to expand functional beta cell mass. INS-1 832/13 beta cells were transduced with AdCMV-NKX6.1-BioID, AdCMV-GFP-BioID or left untreated. Biotinylated proteins were isolated and the NKX6.1 interactome was defined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We defined 176 biotinylated proteins in cells transduced with AdCMV-NKX6.1-BioID that were not observed with AdCMV-GFP-BioID or naturally biotinylated proteins from untreated lysates ($P \leq .05$, n=9). Categorizing the 176 proteins by function using the DAVID Bioinformatics Database revealed 35 functional annotation clusters under medium stringency. The greatest enriched clusters included the terms mitochondrion (22 proteins, P=1.9E-7), ribonucleoprotein (17 proteins, P=9.1E-4), metal-binding (38 proteins, P=1.4E-5), nucleotide-binding (37 proteins, P=3.3E-10), snoRNA binding (5 proteins, 3.8E-5), transcription (24 proteins P=1.4E-6), DNA damage (8 proteins, P=3.5E-4), and response to glucose (6 proteins P=3.2E-3). Among the enriched transcription factors, we validated that PDX1, MEF2D, NR2C2, CPT2, and SIRT7 were biotinylated in response to transduction with AdCMV-NKX6.1 using biotin-targeted immunoprecipitation and western blot. To examine if these transcription factors are likely to interact with NKX6.1 on the genome, we analyzed the frequency of proximal binding sites for NKX6.1 and our candidate genes. NR2C2 and PDX1 binding sites in close proximity to NKX6.1 binding sites were statistically overrepresented in the genome ($P \leq .01$). These data define potential NKX6.1 binding partners in INS-1 832/13 cells, as wells as suggest a non-canonical role for NKX6.1 outside of the nucleus. Funding for this project was provided by the Beatson Foundation

SESSION F: NEW TECHNOLOGIES AND DISEASE MODELS

Chairs: Holger Russ and Hung Ping Shih

ECM Signaling and Cell-Cell Adhesion Coordinate the Development of Islet Architecture and Functional Maturation

Wilma Tixi[1], Maricela Maldonado[1], Ya-Ting Chang[1], Ryan G. Hart[2], Amy Chiu[1], Jeffrey D. Serrill[1], Michael Nelson[3], Janel L. Kopp[4], Mark Huising[2], Sangeeta Dhawan[1], and Hung-Ping Shih[1]

1. *Department of Translational Research and Cellular Therapeutics, Arthur Riggs Diabetes and Metabolic Research Institute, Beckman Research Institute, City of Hope, Duarte, CA, USA*
2. *Department of Physiology and Membrane Biology, School of Medicine, University of California Davis, Davis, CA, USA*
3. *Light Microscopy Core, Beckman Research Institute, City of Hope, Duarte, CA, USA*
4. *Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, British Columbia, Canada*

Pancreatic islets are critical for blood glucose homeostasis. Their destruction or dysfunction causes the loss of glucose regulation that characterizes diabetes. Islets form 3-dimensional (3D) aggregates consisting of unique cellular composition, cell polarity, cell-to-cell contacts, and interactions with blood vessels. In adult islets, insulin-secreting β-cells are organized in rosette-like clusters around blood vessels with three distinct morphological and functional domains: apical, lateral and basal. The islet aggregation and rosette-like structures are thought to play a central role for endocrine cell functions. However, it remains unclear how islets establish and maintain the aggregation and rosette- like clusters during development. To investigate how islets cluster and develop their distinct morphological structures, we utilized 3D imaging techniques, including whole mount lightsheet, airyscan, and live-imaging to study how the endocrine cells are clustered in developmental stages. We found that the formation of islet clusters is a developmental process occurring from late embryonic to early postnatal stages. During this process, blood vessels serve as the organizing center and presumably provide extracellular matrix (ECM) to establish the basal polarity of β-cell rosettes. Islet-specific inactivation of ECM signaling promoted the formation of larger islets, while ablation of cell-cell adhesion disrupted islet clustering and weakened cellular junctions in β-cell rosettes. Mice deficient in ECM signaling and cell-cell adhesion are glucose intolerant and have impaired islet hormone secretion. Gene expression profiling and histological analysis revealed mis-regulation of the endocrine cell maturation process in these mutant mice, suggesting that the establishment of islet clusters and β-cells rosettes is tightly linked with the functional maturation process. In vitro targeting of these adhesion molecules might provide an avenue to improve stem cell differentiation protocols for β-cell replacement and regeneration therapies for diabetes.

Elucidating the Role of PTPN2 in Human Pancreatic Beta Cells

J. Quinn Matuschek[1], Ali H. Shilleh[1], Roberto Castro-Gutierrez[1], Shane Williams[1], Maria Hansen[1], Holger A. Russ[1] and Taylor M. Triolo[1]

1. Barbara Davis Center for Diabetes, Department of Pediatrics, University of Colorado Anschutz, Aurora, CO

Type 1 diabetes (T1D) is an autoimmune disease marked by permanent loss of insulin-producing pancreatic beta cells. Patients are unable to maintain glucose homeostasis and present with clinically symptomatic hyperglycemia. Accumulating evidence suggests that pancreatic beta cells may contribute to their own eradication, a novel hypothesis that has yet to be thoroughly investigated. To overcome challenges in studying primary human beta cells, we and others have demonstrated generations of functional, glucose responsive human stem cell derived beta cells (sBCs) by direct differentiation from human pluripotent stem cells (hPSCs). While genetic risk for T1D is typically associated with high-risk HLA genotypes, genome wide association studies have identified additional immune associated risk genes. Protein tyrosine phosphatase non-receptor 2 (PTPN2) is expressed in T cells, B cells, and in pancreatic beta cells and has been implicated in the early onset of T1D. Mice with a beta cell specific KO of PTPN2 exhibit impaired glucose stimulated insulin secretion (GSIS) and increased susceptibility to stress induced beta cell death. Little is known about the function of PTPN2 in the human pancreatic beta cell. To study PTPN2 in the human context, we generated CRISPR/Cas9 mediated clonal PTPN2 KO hPSC lines with a homozygous deletion in exon 5, abolishing the functional domain of PTPN2. We produced PTPN2 KO sBCs via direct differentiation and are characterizing differences between sBCs from KO and WT cell lines. We are in the process of characterizing metabolomic, proteomic and hormone content analyses between the two cell lines. We have exposed these cell lines to disease associated stress conditions to model T1D in vitro. By combining state of the art stem cell and genome editing technologies, we anticipate elucidating the role of the T1D susceptibility gene, PTPN2, on human pancreatic beta cell function.

Defining Genetic Effects on Cell Type-Specific Cis-Regulatory Programs and Function in Pancreatic Islets using Single Cell Multimodal Profiling

Hannah M. Mummey[1], Katha Korgaonkar[2], Jackie Newsome[1], Paola Benaglio[2], Michael Miller[3], Jocelyn Fox[4], Mark McCarthy[5], Bing Ren[3,6], Anna Gloyn[7], Patrick MacDonald[4], Sebastian Priessl[3], Kyle J. Gaulton[2]

1. *Bioinformatics and Systems Biology Program, University of California San Diego, La Jolla, CA*
2. *Department of Pediatrics, University of California San Diego, La Jolla, CA*
3. *Center for Epigenomics, Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA*
4. *Department of Pharmacology, University of Alberta, Edmonton, AB*
5. *Department of Human Genetics, Genentech Inc., South San Francisco, CA*
6. *Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA*
7. *Department of Pediatrics, Stanford University, Stanford, CA*

Genetic variants influencing diabetes risk identified by genome-wide association studies (GWAS) are primarily non-coding and affect cis-regulatory elements (cREs) in pancreatic islets. A detailed understanding of cis-regulatory programs in pancreatic islet cells, including heterogeneity in the regulatory programs of each cell type, is therefore crucial for deciphering the functional impact of diabetes risk variants. Recently developed assays that generate multimodal single nucleus RNA-seq (snRNA-seq) and Assay for Transposase-Accessible Chromatin using sequencing (snATAC-seq) profiles from the same cells enable high-resolution mapping of islet cis-regulatory programs in individual cell types. Our group has generated deeply-sequenced multimodal data in 30 non-diabetic pancreatic islet samples from the Alberta Diabetes Institute using 10x multiome assays. We will present a high- resolution map of islet cell type and sub-type regulation created from these multimodal data. In a preliminary analysis, we are clustering single cell profiles from all samples together to define islet cell types and sub-types. For sub-types within each islet cell type, we are further characterizing their epigenomic and transcriptional properties. Finally, we are defining islet cell type and subtype cis- regulatory programs including cREs, transcriptional regulators, target genes of cRE activity, and gene expression levels, and annotating diabetes risk variants with these regulatory programs. In on-going work, we plan to identify diabetes risk variants that affect islet cell type cis-regulatory programs directly using quantitative trait locus (QTL) mapping, as well as link islet cell type and sub-type cis- regulatory programs to function by integrating Patch-seq data into our multimodal map.

Vitamin D-Dependent Chromatin Accesibility Dynamics in Pancreatic Islet Dysfunction

Liu Wang[1], SM Bukola Obayomi[1], and **Zong Wei[1]**

1. Department of Physiology and Biomedical Engineering, Mayo Clinic Arizona, Scottsdale, AZ

In type 2 diabetes (T2D), inflammation induces massive changes in beta cell transcriptome and epigenome, resulting in eventual dysfunction and death of beta cells. Accordingly, strategies designed to protect beta cells from inflammatory stress at transcriptional and epigenetic level could be attractive for combating diabetes. Through genome-wide CRISPR screening in human stem cell differentiated islet like organoids, we identified a novel mechanism connecting signal- dependent transcription by vitamin D receptor (VDR) and fine-tuning of chromatin accessibility by a balance between two SWI/SNF complexes, BAF and PBAF. Our studies revealed that VDR recruits BAF and PBAF complexes through two bromodomain proteins, BRD9 and BRD7, respectively. The balance between BAF-BRD9 and PBAF-BRD7 determines the VDR-driven anti-inflammatory and pro- survival response in pancreatic beta cells. In a dual regulatory mechanism, inhibition of the VDR-BRD9 interaction in combination with ligand activation of VDR cooperate to dismiss the BAF-BRD9 complex and shift the balance to the activating PBAF-BRD7 complex to induce a coordinated transcriptional response. Notably, pharmacologically potentiated VDR signaling by a synthetic ligand in combination with a BRD9 inhibitor can partially restore beta cell function and glucose homeostasis in various T2D mouse models. Tissue specific genetic models further demonstrated the functional role of VDR and BRD9 in beta cell stress response in vivo. Together, our results revealed that an unexpected epigenetic balance between the bromodomain readers has a major impact on beta cell survival and glucose homeostasis, and demonstrated the therapeutic potential of targeting inflammatory/metabolic stress through synergistic modulation of hormone receptors and chromatin accessibility.

The Role of UCP2 in the Maturation of Stem Cell-Derived Beta-like Cells

Maria S. Hansen[1], Fiona Docherty[1], Prabha Yadav[1], Ali Shilleh[1], Roberto Castro-Gutierrez[1], Balachandar Nedumaran[1], Holger A. Russ[1]

1. *Barbara Davis Center for Diabetes, Department of Pediatrics, University of Colorado Denver,Aurora, CO*

The use of human pluripotent stem cells (hPSCs) for the generation of b-like cells (sBCs) holds great promise for transplantation therapies for type 1 diabetes. We and other laboratories have established suspension culture-based protocols for *in vitro* differentiation of hPSCs into glucose responsive sBCs. Although the resulting sBCs all express insulin, our single cell RNAseq data suggests there are distinct sBC populations that separate mature sBCs from immature sBCs. Fluorescence-activated cell sorting using a surface marker associated with maturation, followed by detailed analysis comparing these two populations, validated the increased maturation and functionality in the mature sBC population. Interestingly, multiple data sets show that Uncoupling Protein 2 (UCP2) expression is highly increased in immature sBC cell populations at the transcript level. Immunofluorescence analysis confirms the expression of UCP2 protein only in immature, but not in mature sBCs nor cadaveric human b-cells. Previously, UCP2 has been described as a gatekeeper of the metabolic switch from glycolysis to oxidative phosphorylation (OXPHOS), which is also a key event in the functional maturation of b-cells. In order to directly test the role of UCP2 in human b-cell development and function, we have generated homo- and heterozygous UCP2 knockout hPSC lines using CRISPR/Cas-mediated gene editing techniques. Currently we are evaluating the effects of UCP2 deletion on sBCs using complementary quantitative assays. In addition, we are using Genipin, a small molecule inhibitor of UCP2, to facilitate a dose-dependent temporal downregulation of UCP2 in established sBCs. We anticipate that downregulating UCP2 will facilitate a shift towards an OXPHOS-dependent metabolism and thereby allow for an improved maturation of sBCs.

Utilizing Multimodal Imaging Techniques to Investigate Beta Cell Structure and Function During the Pathogenesis of COVID-19

Janielle Cuala[1], Katelyn Millette[2], Carolyn Marks[3], Jason Junge[4], Peiyu Wang[5], Veronica Woo[1], Sean Kim[6], Daniel Liu[7], Scott Fraser[1, 2, 4, 5, 8], and Senta Georgia[1, 2, 9]

1. Keck School of Medicine, Medical Biophysics Department, University of Southern California, Los Angeles, CA
2. Keck School of Medicine, Development, Stem Cell, and Regenerative Medicine Department, University of Southern California, Los Angeles, CA
3. Core Center of Excellence in NanoImaging, University of Southern California, Los Angeles, CA
4. Translational Imaging Core, Universityof Southern California, Los Angeles, CA
5. Viterbi School of Engineering, Biomedical Engineering Department, University of Southern California, Los Angeles, CA
6. Dana and David Dornsife College of Letters, Arts, and Sciences, University of Southern California, Los Angeles, CA
7. School of Pharmacy, University of Southern California, Los Angeles, CA
8. Translational Biomedical Imaging Laboratory, Saban Research Institute, Children's Hospital Los Angeles, Los Angeles, CA
9. Department of Endocrinology, Children's Hospital Los Angeles, Los Angeles, CA

Understanding the complex nature of cellular function is considered a "grand challenge" in biology. Decades of research have provided exquisite details on the cellular components such as ions, small molecules, nucleic acids, proteins, complexes, organelles, and subcellular neighborhoods. Yet, we do not fully understand how these components work together to mediate cellular function. To solve this challenge, we are taking a unique and creative approach to creating a multimodal microscopy pipeline to investigate the relationship between beta cell ultrastructure and metabolism in the context of SARS-CoV2 (SCV2) infection. We hypothesize that SCV2 infection acutely compromises beta-cell function, replication, and survival by reprogramming cellular metabolism, thus leaving hosts susceptible to beta cell dysfunction and hyperglycemia acutely and/or post-resolution of infection. We used multiple imaging modalities to determine changes in cellularity, metabolism, and subcellular ultrastructure of the pancreatic islets throughout the progression of COVID19. First, with super- resolution microscopy, we quantified insulin degranulation within infected samples. Second, using electron microscopy, we detected organelle ultrastructural changes such as endoplasmic reticulum and mitochondrial swelling. Finally, with Fluorescence Lifetime Imaging Microscopy, we observe a shift towards more of a glycolytic profile, indicative of beta cell stress. Preliminarily, we conclude that the SCV2 infected beta cells present with hallmarks of beta cell stress such as insulin degranulation, organelle swelling, and impaired metabolic profile shifting towards glycolysis. The results may also provide more information on cellular pathways for developing interventions to help preserve the beta- cell mass and functions during and after SCV2 infection. Ultimately, these innovative methods can help understand how disease dictates both structure and function. In the future, we hope to correlate clinical measurements to molecular phenotype as a means of providing insight into how to improve long-term outcomes for patients with COVID19.

Alleviating SARS-CoV2 Infection in Beta Cells with ER Stress Pathway Antagonists

Youngjin Kim[1,2], **Sudipta Ashe**[1,2], Irene Chen[3,4], Albert Vallejo-Gracia[3,4], Michael Downey[1,2], Vincent C. Auyeung[1,2], Feroz R. Papa[1,2], Melanie Ott[3,4], Matthias Hebrok[1,2]

1. Department of Medicine, University of California, San Francisco, San Francisco, CA, USA
2. Diabetes Center, University of California, San Francisco, San Francisco, CA, USA
3. Gladstone Institute of Virology, Gladstone Institutes, San Francisco, CA, USA
4. Department of Medicine, University of California, San Francisco, CA, USA

Recent studies have highlighted the potential of SARS-CoV2 to preferentially target and infect insulin producing beta cells thus impairing their function and ultimately developing diabetes post an acute COVID-19 infection. Though the percentage of beta cells getting infected with SARS-CoV2 is low, it doesn't rule out the possibility of such cells serving as reservoirs for the virus. Further, the mechanism by which SARA-CoV2 causes loss of beta cell potential to release insulin and develop diabetes is still unknown. To investigate the potential mechanisms by which the virus targets and replicates in beta cells, causes beta cell impairment as well as testing therapeutic intervention targets, it is empirical to establish a coherent infection model and critically establish beta cell parameters upon infection. In this direction, we tested the potential of live SARS-CoV2 to infect both human islets as well as stem cell derived beta cells. In this study, we have established multiple parameters to conclusively establish SARS-CoV2 infection in beta cells. Our findings also suggest that ACE2 along with other cognate receptor-coreceptor are expressed in beta cells. Further, we have established that SARS-CoV2 infection causes loss of beta cell markers C-peptide, NKX6.1 and PDX1 which can lead to loss of beta cell function. RNAseq and GSEA on beta cells post SARS-CoV2 infection resulted in upregulation of interferon signaling pathways whereas the beta cell identity markers were downregulated. Viral infections typically lead to an increase in protein synthesis capacity of the cells that can overwhelm the ER folding capacity, resulting in unfolded protein accumulation and ER stress. This leads to activation of signaling pathways known as unfolded protein response (UPR), which homeostatically matches ER protein folding capacity to cellular secretory needs and functions to reduce the overall protein load by increasing ER's folding capacity and targets misfolded proteins to degradation. UPR consists of three arms activated by the transmembrane protein sensors IRE1, PERK and ATF6. We used kinase inhibitors, partial antagonists of IRE1α RNase (PAIRs), that partially antagonize the IRE1α RNase at full occupancy to reduce the ER stress mediated by SARS-CoV2 infection. Our data suggests that the viral load is reduced upon using combination of different ER stress inhibitors that could lead to development of future therapy against infection. Recognizing the biomolecular signatures that facilitate SARS-CoV2 infection and mechanisms the pathogen highjacks to favor its replication and transmission are prerequisites in understanding disease prevalence and achieve disease control. Towards this direction, our work focuses on generating a stable model for SARS-CoV2 infection in beta cells, identifying mechanisms by which the virus causes ER stress and thus establish a connection between COVID-19 and diabetes.