Book of Abstracts

Organised by thematic area

133 abstracts · 6 thematic areas

Contents

METABOLISM IN ISLET CELLS 113

THEMATIC AREA 01

ENDOCRINE–EXOCRINE CROSSTALK 01

7 abstracts in this area

P.01 Loss of Cav1.2 disrupts intra-islet communication and glycemic regulation in Zebrafish

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POSTER Keywords: *Cav1.2, pancreas, intra-islet communication, zebrafish*

Pancreatic islet function relies on a highly coordinated interplay between β-, α-, and δ-cells to maintain glucose homeostasis and regulate insulin secretion. Increasing evidence suggests that communication between these endocrine cell types is essential for optimal hormone release and glycemic control. Calcium signaling, particularly via L-type voltage-gated Ca^{2+} channels (Cav), plays a critical role in stimulus–secretion coupling in the pancreas. Here, we investigated the role of δ- and α-cells in regulating β-cells using a zebrafish model lacking functional Cav1.2 (isl m458−/−). To address this, we combined genetic models with in vivo and ex vivo calcium imaging and glucose measurements. Previous work from our lab showed that islm458−/− mutants exhibit severe hyperglycemia and altered β-cell calcium dynamics, suggesting a cell-autonomous role of Cav1.2 in β-cell function. To further explore the regulation mediated by δ-cells, we performed in vivo calcium imaging. Under wild-type conditions, δ-cells display rhythmic Ca^{2+} oscillations. In contrast, these oscillations were significantly impaired in the mutants, suggesting disrupted δ-cell activity and altered paracrine regulation. In line with recent observations in mouse models, genetic ablation of δ-cells induced spontaneous ectopic Ca^{2+} activity in βcells and lowered their glucose activation threshold. Strikingly, δ-cell ablation also partially rescued the hyperglycaemic phenotype observed in these mutants, indicating that δ-cells play a critical role in regulating β-cell activity in a non-cellautonomous manner. Unlike β- and δ-cells, α-cells do not rely on L-type Ca^{2+} channels for their exocytotic machinery. Consistent with this, ex vivo calcium imaging revealed significantly increased Ca^{2+} spiking activity in α-cells in the mutants, even under hyperglycaemic conditions. Moreover, α-cell Ca^{2+} activity persisted following pharmacological inhibition of L-type channels with isradipine. Together, these findings suggest that loss of Cav1.2 disrupts intra-islet communication, leading to impaired glycemic control. Whether this regulation occurs through paracrine signalling or gap junction-mediated communication remains to be determined.

P.02 Activity of Pancreatic Cells in Health and Type 1 Diabetes

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ORAL COMMUNICATION Keywords: *T1D, pancreatic tissue slice, calcium imaging*

The pancreas is an essential organ responsible for metabolism and blood glucose homeostasis. A great deal of effort has been devoted to understanding the physiology of the pancreatic islets of Langerhans, particularly β cells. Pancreatic tissue slice preparation allows for the investigation of all cell types present within pancreatic tissue while preserving tissue architecture, including both intra- and intercellular interactions. The major advantage of this approach is that it enables the study of small and disrupted islets, which are commonly found in type 1 diabetes. Employing the pancreatic tissue slice method in combination with high-resolution Ca^{2+} imaging using laser-scanning microscopes allows for the simultaneous tracking of activity responses in multiple cell types at single-cell resolution. Human pancreatic tissue slices from healthy lean donors and type 1 diabetic donors were challenged with low glucose to reveal α cell activity, high glucose to activate β cells, and an amino acid cocktail mimicking post-meal plasma conditions to stimulate acinar cells and trigger a cascading response in ductal cells. To confirm the location of endocrine cells, I performed post-staining using antibodies against endocrine cell hormones. Through this study, I confirmed the lack of β-cell function in type 1 diabetic donors and successfully characterized multiple response phenotypes of pancreatic cells, including both endocrine and exocrine cells. The characterization of phenotypes in human pancreatic tissue slices at high spatial and temporal resolution has provided valuable insights. Single-cell resolution calcium activity imaging in pancreatic tissue has revealed distinct cellular phenotypes.

P.03 Pregnancy Drives a Regenerative Phenotypic Shift in Islet-Resident Macrophages in Mice

Willems Lien[1], *Petroons Bo*[1], *Bourgeois Stephanie*[1], *Van Mulders Annelore*[1], *Pierreux Julie*[1], *Coenen Sophie*[1], *Degroote Laure*[1], *Van Craenenbroeck Jolien*[2], *Bardet Pauline*[2], *Kancheva Daliya*[2], *Rolain Iris*[2], *Laoui Damya*[2], *Van Ginderachter Jo*[2], *Staels Willem*[1], *De Leu Nico*[1]

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POSTER Keywords: *Macrophages, pregnancy, beta-cell regeneration, CITE-Seq, Diabetes*

BACKGROUND & OBJECTIVES

Diabetes arises from dysfunction or loss of insulin-producing β cells. Current therapies improve glycemic control but do not restore endogenous β-cell mass, leaving patients vulnerable to long-term complications. Defining the molecular cues that enable adult β-cell regeneration is therefore essential. Islet-resident macrophages (mφ), historically viewed as drivers of β-cell dysfunction and death in diabetes, are increasingly recognized as heterogeneous populations with context-dependent roles in tissue homeostasis and repair. We investigated how pregnancy, a physiological model of robust β-cell proliferation, reshapes islet mφ composition and function.

METHODS

Pancreata from C57BL/6J mice (male, female, and pregnant gestational day (G)10.5, G14.5, G15.5, and G18.5) were harvested for immunoflorescence-based quantification of β-cell proliferation and mφ abundance. For phenotypic and molecular characterization, islets from male, female, and pregnant (G14.5) C57BL/6J mice were isolated (collagenase digestion and manual hand-picking) and analyzed via multicolor flow cytometry and CITE-Seq (combined single-cell transcriptomics and surface proteomics). Functional necessity is being assessed using an Fcgr1-Cre;R26-DTR mφ depletion model, with Diphtheria Toxin (DT) administered and pancreata harvested at G14.5.

RESULTS

β-cell proliferation peaked at gestational day (G)14.5. Contrary to earlier reports, total islet mφ numbers remained stable throughout pregnancy. Multicolor flow cytometry revealed a pregnancy-associated phenotypic shift characterized by reduced CD11c expression. High-resolution CITE-Seq profiling identified five intra-islet mφ subsets, including two trophic, potentially pro-regenerative populations (“Mac prolif” and Folr2+ mφ). Both subsets proportionally expanded at G14.5, indicating selective remodeling of the islet immune niche toward a regenerative state. Ongoing conditional mφ depletion at G14.5 is assessing whether these subsets are required for peak β-cell proliferation. Conclusion & Implications: Pregnancy drives a qualitative reorganization of the islet mφ compartment, favoring trophic, regenerative mφ subsets rather than an overall increase in mφ abundance. These findings highlight specific mφ populations as candidate regulators of β-cell expansion and promising targets for future regenerative therapies in diabetes.

P.04 Profibrotic and fatty acid signalling gene expression profiles underlying progressive pathology within the human CF pancreas

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ORAL COMMUNICATION Keywords: *Cystic fibrosis related diabetes, NanoString, gene expression, exocrine pancreas, fibrosis*

BACKGROUND & OBJECTIVES

Cystic fibrosis (CF) is associated with progressive pancreatic pathology impacting exocrine and endocrine function. 85% of children develop pancreatic exocrine insufficiency and at least 50% of adults develop CF-related diabetes. We recently published a morphological analysis in a post-mortem cohort of CF cases describing a timeline of disease progression from an initial fibrosis alone pattern (CFP1); through a mixed fibrosis / liposis pattern (CFP2); to near-total liposis (CFP3) [1]. We hypothesise profibrotic signalling as a potential key driver of exocrine and endocrine dysfunction in CF and aimed to elucidate changes in fibrosis-associated gene expression along this timeline.

METHODS

Bulk RNA was extracted from FFPE pancreatic tissue blocks obtained from 3 CFP1, 3 CFP2 and 3 CFP3 donors in addition to 3 control donors of comparable age without pancreatic pathology. Gene expression was quantified using NanoString 'Human Fibrosis V2' 770 gene panel with RStudio downstream analysis.

RESULTS

Comparison of all CF (n=9; age range premature–27 years' old) vs Control (n=3; age range 6-27 years) donors revealed 31 significantly upregulated and 58 downregulated genes ($p<0.05$). Upregulated genes included collagens, ACTA2 (encoding αSMA) and TGF-β in keeping with pancreatic stellate cell activation and FABP4 expressed in adipocytes and macrophages regulating fatty acid trafficking and metabolism. Upregulated KEGG pathways included inflammation and immune signalling pathways as well as cytoskeleton/ECM pathways. Aldolase-B and SEC61 Translocon Subunit Beta were highest-fold downregulated genes with pathways included ER processing and insulin signalling. Comparison of differential gene expression between each CF pattern and control donors revealed trajectories over the natural history of disease progression (Fig 1). COL1A1 and ACTA2 expression were highest in CFP1 but remained higher than controls in end-stage CF. Expression of fatty acid-related genes FABP4 and Fatty Acid Synthase (FASN) was highest in CFP2 and CFP3. Conclusions & Implications NanoString bulk RNA expression profiling of pancreata representative of the natural history of CF progression enabled elucidation of underlying signalling pathways and how these evolve over time. This will inform design of accurate in vitro model systems to determine mechanisms through which exocrine signalling impacts endocrine function and test novel therapeutic interventions.

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P.05 Pancreatic Gαs ablation reveals cell-type-specific GPCR networks and exocrine YAP activation with attempted β-cell regeneration

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ORAL COMMUNICATION Keywords: *Gαs signaling, GPCR, exocrine-endocrine crosstalk, YAP, β-cell regeneration*

BACKGROUND & OBJECTIVES

The G protein α-subunit Gαs integrates signals from numerous GPCRs, including GLP-1 and adrenergic receptors, across pancreatic cell types. While β-cell-specific Gαs deletion impairs insulin secretion, the organ-wide consequences remain poorly understood (1, 2). Here, we investigated the role of Gαs in coordinating endocrine and exocrine homeostasis using a conditional knockout model with pancreas-wide deletion from specification (PGsKO).

METHODS

PGsKO mice were generated by crossing Gαs-floxed mice with Pdx1-Cre. Metabolic phenotyping, histology, immunofluorescence, and morphometric analyses were performed at 4 and 10 weeks. GPCR expression was profiled by reanalyzing integrated public scRNA-seq datasets, cross-referenced with GPCRdb annotations.

RESULTS

PGsKO mice developed severe diabetes from 4 weeks with reduced β-cell fraction (75% reduction) and increased α-cell numbers per islet (33% increase). Islets exhibited disorganized architecture and increased E-cadherin, suggesting compensatory adhesion. Beyond the endocrine compartment, we uncovered profound exocrine defects: acinar cells were hypertrophic, displayed loss of apical-basal polarity (mislocalized zymogen granules and amylase), and showed robust nuclear YAP reactivation—a conserved mechanism previously linked to Gαs loss in skin (3). Acinar hypertrophy was most pronounced near islets, implicating disrupted islet–acinar crosstalk. To map potential signaling pathways, we generated a comprehensive atlas of Gαs-coupled GPCRs across pancreatic cell types. This revealed cell-type-specific networks, including opposing adrenergic (Gαi in β-cells, Gαs in α-cells) and parallel adenosine receptor (Gαs) architectures, positioning Gαs as a central hub integrating paracrine and hormonal inputs. Remarkably, despite severe dysfunction, PGsKO pancreata showed signs of attempted β-cell regeneration, including extra-islet insulin-positive clusters and Pdx1 re-expression in ducts. Conclusions & Implications: Pancreatic Gαs is a non-redundant hub essential for both endocrine and exocrine integrity. Its deletion causes multicompartment failure and reveals latent regenerative attempts, albeit insufficient to reverse diabetes. Our GPCR atlas identifies candidate pathways—such as adrenergic and adenosine signaling—that may bias cell fate decisions. Targeting these networks could unlock regenerative potential from non-β-cell sources, offering new strategies for diabetes therapy.

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P.06 FLRT3 identifies a ductal-derived pancreatic population with high endocrine differentiation potential

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IDIBELL - UB

POSTER Keywords: *Ductal cells, Pancreas development, Endocrine differentiation*

Single-cell transcriptomic analysis of the murine pancreatic ductal tree led to the identification of a previously uncharacterized cell population expressing FLRT3, predominantly derived from medium and large ducts. Notably, organoids derived from FLRT3^{+} cells display the highest efficiency of differentiation into hormone-expressing cells (including insulin, glucagon and

somatostatin) following our differentiation protocol that mimics embryonic signaling cues. FLRT3 (Fibronectin Leucine-Rich Transmembrane Protein 3) is a transmembrane protein mainly studied in the context of neurodevelopment, where it regulates processes such as repulsive and adhesive axonal guidance and participates in fibroblast growth factor–related signaling during embryogenesis and neuronal growth. However, the role of FLRT3 in the pancreas remains largely unexplored. To investigate its function in vivo, we are using a FLRT3CreERT2;R26-tdTomato transgenic mouse line and have optimized the tamoxifen-induction protocol to achieve efficient recombination of FLRT3$^+$ cells in the pancreatic ducts. During embryonic pancreas development, we detected FLRT3 expression between E11 and E15, predominantly within the tip domain. Ongoing studies aim to extend this characterization to later developmental stages to understand the transition of FLRT3 expression from the embryonic tip domain to the ductal compartment in the adult pancreas. In addition, we are analyzing FLRT3 expression in a streptozotocin-induced diabetes model to determine how FLRT3$^+$ cells respond to β-cell loss and pancreatic injury. Together, this work aims to provide new insights into the role of FLRT3 in pancreatic biology and to explore its potential contribution to the endocrine compartment.

P.135 Acute pancreatitis disrupts glucose-dependent calcium dynamics in pancreatic islets

Polona Kovacic, Jasmina Kerčmar, Lidija Križančić Bombek, Eva Paradiž Leitgeb, Jan Kopecky, Viljem Pohorec, Maruša Plesnik Rošer, Maša Skelin Klemen, Andraž Stožer, Jurij Dolenšek
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POSTER Keywords: *Acute pancreatitis; Tissue damag; Calcium dynamics; Glucose stimulation; Beta-cell function*

Acute pancreatitis (AP) is classically viewed as an exocrine pancreatic disease, yet its impact on endocrine cell function during the early phase of injury remains insufficiently defined. Here, we investigated whether cerulein-induced AP alters glucose homeostasis and pancreatic islet calcium signaling in mice. AP was induced by repeated cerulein injections, while control animals received physiological solution. The following day, intraperitoneal glucose tolerance testing was performed and pancreatic tissue was collected for structural and functional analyses. Successful induction of pancreatic injury was confirmed by a marked increase in amylase activity in the AP group compared with controls, together with differences in relative body weight. Pancreatic tissue was further characterized by Live/Dead assay. Systemic metabolic status was assessed by intraperitoneal glucose tolerance testing, total glucose area under the curve, fasting glucose, fasting insulin, insulin-to-glucose ratio, insulin sensitivity index, beta cell function, and disposition index. These analyses indicated that AP was accompanied by changes in selected metabolic readouts. To assess the effects of AP on endocrine cell function at the tissue level, we analyzed intracellular calcium dynamics in pancreatic islet cells during glucose stimulation. AP significantly altered both activation and deactivation kinetics in a glucose-dependent manner. Activation delay differed at 7, 8, 10, and 16 mM glucose, with no difference at 12 mM, whereas deactivation delay was altered at 7, 8, and 10 mM glucose. Moreover, AP increased active time across all glucose concentrations, likely driven by the higher oscillation frequency observed in AP islets, while

oscillation duration was generally shorter. Coactivity, however, remained unchanged between groups. Together, these findings show that AP is associated not only with exocrine injury, but also with pronounced alterations in islet calcium dynamics and intercellular functional organization. Our results identify pancreatic islet activity as a sensitive functional readout of pancreas-wide disease and support the concept that endocrine dysfunction emerges early during acute pancreatitis.

THEMATIC AREA 02

GENOMICS & EPIGENOMICS OF ISLET CELLS

33 abstracts in this area

P.07 Compensatory de-novo β-cell differentiation in the Neurod1-deficient pancreas

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ORAL COMMUNICATION Keywords: *pancreatic islet, mouse model, single-cell RNA-sequence, beta cells, diabetes*

The transcription factor NEUROD1 is one of the earliest markers of endocrine progenitors and a key determinant of β-cell identity. NEUROD1 is necessary for the maintenance of functional β cells. Mutations in the NEUROD1 gene in humans are linked to maturity-onset diabetes of the young (MODY 6) and increased susceptibility to the acute-onset of type I diabetes mellitus. In mouse models, Neurod1 deletion during pancreatic development causes severe neonatal diabetes and postnatal Using a Neurod1 conditional deletion model (Neurod1CKO), we previously demonstrated that Neurod1 deficiency disrupts H3K27me3 histone modification patterns, compromising endocrine cell potential, differentiation, and functional properties of endocrine cells1. Surprisingly, approximately 40% of Neurod1CKO mice survive to adulthood, although they have impaired glucose tolerance. In our research, we aimed to identify the cellular origin and molecular programs responsible for the denovo generation of functional β-cells. Single-cell RNA sequencing of pancreatic cells at postnatal day 9 (P9) and adulthood was combined with immunohistochemical analyses to investigate cellular composition and molecular changes in the Neurod1CKO pancreas. Single-cell transcriptomic analysis revealed reprogramming within both α- and β-cell populations to compensate for Neurod1 loss. Comparative analysis of endocrine cells from P9 and adult pancreas identified a distinct β-cell subpopulation in Neurod1CKO mice that arises postnatally. Initially limited to a few cells at P9, this population expands substantially, forming a significant component of the adult endocrine pancreas and supporting the survival of Neurod1CKO mice. Molecular characterization showed that these de-novo differentiated β cells share key molecular features with control β cells but display significantly increased expression of insulin and genes involved in insulin biosynthesis and secretion. In addition, these cells showed increased expression of markers associated with cellular stress responses, anti-apoptotic pathways, together with markers linked to β-cell dysfunction. Our findings suggest that the pancreas retains the capacity for postnatal endocrine plasticity, enabling compensatory denovo β-cell differentiation that partially restores insulin-producing cell mass. This adaptive mechanism highlights regenerative potential within the endocrine pancreas and may provide insights into cellular reprogramming strategies for diabetes therapy. This research was supported by the Czech Science Foundation GA 25-15876S, and the institutional support RVO: 86652036.

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P.08 Cell-specific DNA methylation in human α- and β-cells regulates gene expression and impacts type 2 diabetes

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ORAL COMMUNICATION Keywords: *α-cells, β-cells, DNA methylation, epigenetic editing , T2D*

BACKGROUND

α- and β-cell dysfunction contributes to type 2 diabetes (T2D). We aimed to characterize the global DNA methylome and transcriptome of α- and β-cells from donors with or without T2D, and by using epigenetic editing, investigate the causative impact of methylation on transcription of α- and β-cell specific genes. We also investigated the impact of pre-T2D/T2D on the methylomes and transcriptomes, followed by functional validation of top candidate genes.

METHODS

Human islets from 24 donors with or without T2D were sorted into α- and β-cell fractions, followed by whole-genome bisulfite sequencings to study methylation genome-wide, and RNA-sequencing. Epigenetic editing in EndoC-βH1 β-cells was achieved by CRISPR-dCas9-DNMT3A or CRISPR-dCas9-TET1 together with guide-RNAs targeting α- versus β-cell differentially methylated regions (DMRs). Functional validation was performed by manipulation in human islets, EndoC-βH1 β-cells and INS-1 β-cells.

RESULTS

We discovered 22,544 DMRs annotated to 7,975 genes in α- versus β-cells, like INS, GCG,PDX1, and PCSK1, with ~50% showing differential expression. CRISPR-dCas9-DNMT3A based epigenetic editing increased INS and TH DNA methylation, while CRISPR-dCas9-TET1-based editing decreased GCG methylation, each altering INS, TH or GCG expression and content in β-cells. Pre-T2D/T2D-associated DMRs in α- and β-cells overlap 12-18% of T2D-associated GWAS candidates. By integrating β-cell DNA methylomes and transcriptomes from donors with or without T2D, with comprehensive functional analyses in human islets and β-cells, we discovered that ONECUT2 has a key role in β-cell function and T2D pathogenesis. Specifically, ONECUT2 is epigenetically upregulated in β-cells from donors with pre-T2D/T2D and elevated in GK-rat islets. ONECUT2 overexpression in human islets and β-cells down-

regulated gene-sets impacting insulin secretion and glucose homeostasis, and reduced mitochondrial activity, ATP/ADP-ratio, and insulin secretion. Silencing ONECUT2 in β-cells rescued the perturbed insulin secretion. Lastly, we provide a web-tool allowing exploration of epigenetic dysregulations contributing to impaired insulin secretion.

CONCLUSION

We identified cell-specific epigenetic patterns in α- and β-cells controlling gene expression, evidenced by epigenetic editing. Our data support ONECUT2 as a key transcription factor in β-cells and T2D, regulating genes involved in mitochondrial function, glucose homeostasis, and insulin secretion.

P.09 Epigenetic adaptation of beta cells across lifespan and disease: age-related demethylation is advanced in type 2 diabetes

Elisabetta Manduchi[1], *Hélène Descamps*[1], *Jinping Liu*[1], *Jonathan Schug*[1], *Tong Da*[1], *Deeksha Lahori*[1], *Hilana El-Mekkoussi*[1], *Michelle Lee*[2], *Eseye Feleke*[3], *Diana Bernstein*[1], *Chengyang Liu*[4], *Ali Naji*[4], *Benjamin Glaser*[3], *Klaus H. Kaestner*[1], *Dana Avrahami*[5]

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ORAL COMMUNICATION Keywords: *DNA-methylation aging T2D beta-cells*

Although the prevalence of type 2 diabetes (T2D) increases with age, most adults maintain normoglycemia despite rising insulin resistance, largely due to the adaptive capacity of pancreatic beta cells to meet increased metabolic demand. However, persistent insulin resistance can lead to beta cell dysfunction and T2D onset. Here, leveraging cell-type-specific methylome data from the Human Pancreas Analysis Program (HPAP), we investigate the epigenomic basis of beta cell adaptation by mapping genome-wide DNA methylation (DNAm) patterns across the human lifespan. In healthy donors, we identify progressive age-related demethylation enriched in cis-regulatory elements at beta cell identity and function genes, suggesting that epigenetic remodeling supports functional adaptation to metabolic demand over time. In contrast, alpha cells show the opposite trajectory, with subtle, age-related hypermethylation1. In T2D beta but not alpha cells we observed further demethylation compared to healthy controls, underscoring a unique capacity of beta cells to respond to changes in metabolic demand1. Together, our findings suggest that DNAm remodeling in healthy beta cells reflects a long-term adaptation to metabolic demand, which in T2D is accelerated as part of a compensatory response that ultimately fails under sustained insulin resistance.

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P.10 Location matters, small endocrine object beta cells show transcriptomic and functional difference from beta cells within larger islets

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POSTER Keywords: *Pancreas, endocrine objects, beta cells, insulin, spatial transcriptomics*

BACKGROUND

The function of insulin-producing beta cells within the islets of Langerhans has been well studied in both healthy and diseased states. More recently, our group and others have shown that numerous beta cells also exist outside the islets as single cells or small clusters (extra-islet). Notably, these single beta cells or small clusters are almost completely absent in individuals with T1D, and because of technical limitations, they have remained largely unexamined. This study set out to address a major knowledge gap by investigating these single beta cells and small clusters in both health and disease.

METHODS

Spatial transcriptomic data was generated using the 10X Genomics Visium HD pipeline. Publicly available snRNA-seq datasets were analysed using Seurat in R. Immunohistochemistry was used to validate transcriptomic data analysis. Beta cells in cultured human pancreatic slices were labelled using the calcium indicator GCaMP6s under the control of an insulin promoter. Following exposure to different metabolic stimuli, calcium signalling responses were captured in situ by confocal time-lapse imaging for functional analysis.

RESULTS

Spatial transcriptomic and snRNA-seq data analysis identified cell clusters corresponding to single beta cells/small clusters. Differentially expressed genes were identified when comparing extra-islet and islet beta cells. Single beta cells/small clusters exhibited reduced responsiveness to metabolic stimuli compared to beta cells within islets.

CONCLUSION

Our preliminary findings indicate that single beta cells and small clusters in the human pancreas exhibit distinct transcriptional and functional profiles compared with beta cells located in larger islets. Ongoing studies are focused on determining the underlying mechanisms driving their loss in T1D.

P.11 Human pancreatic islet co-expression networks reveal a role for ZKSCAN1 in beta cell identity and function

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ORAL COMMUNICATION Keywords: *Co-expression networks, SC-islet differentiation, Transcription factors*

BACKGROUND & OBJECTIVES

Pancreatic beta cells are the sole source of insulin and are essential for maintaining blood glucose levels. While key pancreatic lineage transcription factors are well characterized, the contribution of broadly expressed transcription factors to beta cell identity remains poorly understood. Here, we aimed to investigate how tissue-specific and ubiquitously expressed transcription factors jointly regulate beta cell identity.

METHODS

We constructed a gene regulatory network using >200 human donor-derived islet bulk RNA-sequencing datasets to identify gene co-expression modules responsible for beta cell specific functions (WCGNA). We further defined islet specificity for each transcription factor in our network and shortlisted candidate transcription factors that were part of modules responsible for beta cell specific functions. Selected transcription factors were subjected to a loss-of-function screen, followed by transcriptomic differential gene expression analysis. We identified ZKSCAN1 as a ubiquitously expressed transcription factor belonging to gene regulatory modules specific to the beta cells. We used an in vitro stem cell to beta cell differentiation model to study the function of ZKSCAN1 using transcriptomic, genomic and functional assays.

RESULTS

Our results indicate that the islet-specific regulatory network modules are regulated by both ubiquitous and cell-type specific transcription factors. We identify ZKSCAN1 as a ubiquitously expressed transcription factor tightly co-expressed with PDX1 and MAFA. Loss of ZKSCAN1 did not impair pancreatic lineage specification but led to aberrant activation of a neuronal transcriptional program in mature beta-like cells as well as downregulation of genes belonging to pathways associated with transmembrane transport. Functionally, ZKSCAN1-deficient cells exhibited reduced glucose stimulated insulin secretion and other functional defects. Conclusions & Implications Our findings identified ZKSCAN1 as a ubiquitous transcription factor that suppresses alternative lineage programme and maintains β-cell identity and function. Our study highlights that ubiquitously expressed transcription factors can acquire context-dependent regulatory roles in specialized cell types, providing new insights into beta-cell biology and potential mechanisms underlying beta-cell dysfunction.

P.12 Trans-Ancestral Genetic Architecture of Type 1 Diabetes: Non-HLA Loci Link Insulin Secretion and Resistance to Disease Risk and Clinical Progression

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ORAL COMMUNICATION Keywords: *type 1 diabetes, GWAS, insulin secretion, insulin resistance, polygenic risk scores*

BACKGROUND & OBJECTIVES

Type 1 diabetes (T1D) has a strong genetic predisposition, yet trans-ancestral non-HLA genetic links to insulin secretion/resistance, and their integrative effect on T1D risk and related clinical features remain incompletely understood, especially in non-Europeans.

METHODS

We conducted a trans-ancestral GWAS meta-analysis of 26,198 T1D cases and 36,733 controls across diverse populations (including European, Finnish, African, African American, and East Asian individuals) to identify non-HLA risk loci. We assessed their associations with clinical features, integrated epigenetic profiles, mapped implicated genes to identify tissue and cell-type specificity, conducted pathway enrichment analyses, and built polygenic risk scores (PRS) to evaluate their utility in Chinese cohorts.

RESULTS

We identified 69 trans-ancestral non-HLA risk regions, including three novel signals: rs2179781 in AHI1, rs10117059 near TRAF1, and rs7184802 near ADCY7. Approximately one-third of variants influenced insulin secretion/resistance related indices. Through Bayesian fine mapping integrated with bioinformatics annotation strategies, we prioritized 136 candidate functional variants. Based on integrative analysis using Roadmap Epigenomics data, these variants exhibited enhanced regulatory potential, as evidenced by DNase I hypersensitivity, characteristic H3 histone modifications, and active chromatin states in key metabolic tissues (pancreatic islets, liver, skeletal muscle, and adipose tissue). These variants were mapped to 447 candidate genes, which display tissue-specific expression and are significantly enriched in pathways related to insulin secretion and resistance—a pattern not observed for HLA risk genes. Using a PRS derived from trans-ancestral non-HLA risk variants, we achieve an area under the curve (AUC) of 0.729 (0.708-0.749), which improved to 0.878 (0.864-0.892) when combined with the HLA risk regions in a Chinese cohort. Furthermore, the trans-ancestral non-HLA PRS was an independent risk factor, high PRS was associated with lower glucose-responsive C-peptide levels in newly diagnosed (most significant for 180min time point; Ptrend=9.52E-05) but not long-standing T1D individuals. Conclusions & Implications: Our findings expand the trans-ancestral genomic landscape of T1D, reveal a shared non-HLA genetic architecture linking T1D susceptibility to insulin secretion defects and resistance, and support the independent utility of trans-ancestral non-HLA PRS in predicting T1D onset and progression in non-European populations.

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P.13 Dorsal or Ventral Islets: What Sets Endocrine Fate?

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ORAL COMMUNICATION Keywords: *Transcription factors, gene regulatory networks, endocrine pancreas specification*

Pancreas development begins with two spatially and molecularly distinct progenitor niches: a dorsal and a ventral bud that later fuse to form the mature organ. Endocrine differentiation is initiated by activation of NEUROG3, the master regulator of endocrine fate, giving rise to five principal endocrine cell types: β, α, δ, PP (γ), and ε (ghrelin) cells. Within mature islets of Langerhans, β-cells predominate, followed by α-cells, with δ-, PP-, and ε-cells present at lower proportions. A limited set of transcription factor axes orchestrates endocrine cell fate allocation and ultimately shapes islet cell ratios. For example, the PAX4–ARX regulatory axis, downstream of NEUROG3, is a central determinant of α- versus β-cell identity. In addition, it is well established that islets derived from dorsal and ventral regions of the pancreas differ in cellular composition and function: dorsal islets are enriched in β-cells with robust glucose responsiveness, whereas ventral islets exhibit increased PP-cell representation and β-cells with comparatively blunted glucose-stimulated insulin secretion. Here, we identify Single-minded homolog 1 (SIM1) as a previously unrecognised regulator of dorsal endocrine pancreas identity. SIM1 is expressed prior to NEUROG3 activation in both stem cell–derived islet-cell (SC-islet) differentiation systems and human donor-derived pancreatic progenitors, as revealed by scRNA-seq analysis. Surprisingly, loss of SIM1 results in mis-specification of endocrine cell ratios, yielding a ventral-like islet composition accompanied by impaired glucosestimulated insulin secretion and blunted calcium influx in the β-cells. In SC-islet models, SIM1 deficiency does not significantly alter α-cell numbers but leads to reduced expression of GCG mRNA, indicating transcriptional alterations despite preserved lineage allocation. These findings point to a broader impact on endocrine identity and function in SIM1-deficient SC-islets. Collectively, our data uncover a novel role for SIM1 in establishing dorsal endocrine pancreas identity and highlight the existence of additional, previously underappreciated transcriptional regulators that fine-tune pancreatic endocrine cell fate and β-cell functional competence.

P.14 Targeting miR-125b-5p in β-cells: Novel approaches to modulate lysosomal function and insulin secretion

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POSTER Keywords: *microRNAs, miRNA inhibitors, lysosomal function, autophagic flux, insulin secretion*

BACKGROUND & OBJECTIVES

MicroRNAs are small RNAs that repress gene expression post-transcriptionally, essential for βcell function. We previously showed that glucose-regulated miR-125b-5p (miR-125b) inhibits glucose-stimulated insulin secretion (GSIS) and identified the lysosomal hydrolases transporter M6PR (mannose-6-phosphate receptor) as a target [1]. β-cell lines and mice overexpressing miR-125b in β-cells (miR-125bTg) display enlarged lysosomes with reduced Cathepsin D (CatD) content. We hypothesise that miR-125b disrupts lysosomal function via M6PR and that its inhibition could restore βcell function and glycaemic control. Here, we examine the role of miR-125b in β-cell lysosomal function via M6PR and explore novel strategies to counteract its effects.

METHODS

Lysosomal CatD content and morphology were analysed by immunofluorescence and TEM in EndoCβH3 and MIN6 cells with siRNA-mediated M6PR knockdown. M6PR expression and autophagy were assessed by Western blotting (WB) and imaging of an RFP-EGFP-LC3 reporter in miR-125bTg islets. GSIS was quantified by HTRF. Islet miR-125b levels (RT-qPCR) and glucose tolerance were assessed following subcutaneous eGLP1-conjugated miR-125b inhibitor injection [2].

RESULTS

In β-cell lines, M6PR knockdown reduced lysosomal CatD content, caused lysosomal enlargement, and impaired GSIS, mirroring the effects of miR-125b overexpression. Short-term β-cell-specific miR-125b overexpression did not alter autophagy despite reduced M6PR. In contrast, lifelong overexpression increased p62 and LC3-II and reduced autophagolysosomes, suggesting impaired autophagic flux. Responses to chloroquine and rapamycin were preserved, indicating lysosomal dysfunction rather than defective autophagy initiation. Transfection of commercial miR-125b inhibitors, miR-125b–M6PR target site blockers (TSBs) and CRISPR/Cas13 targeting miR-125b-M6PR, and gymnotic delivery of an eGLP1-miR-125b inhibitor restored M6PR levels upon miR-125b overexpression in MIN6 cells. In vivo, eGLP1-miR-125b inhibitors reached β-cells and improved glucose tolerance in miR-125bTg mice. Conclusions & Implications: Our data points to M6PR as a downstream effector of miR-125b in lysosomal regulation and insulin secretion, which we aim to demonstrate through rescue experiments using miR-125b–M6PR TSBs and CRISPR/Cas13. eGLP1-miRNA inhibitors effectively reach β-cells, with TSBs and CRISPR/Cas13 arising as promising tools to increase target and tissue specificity.

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P.15 GPCR and purinergic signaling shape pancreatic lineage specification through transcriptional and translational integration

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POSTER

The G-protein coupled receptor (GPCR) family comprises a large number of proteins regulating several cellular processes during development and in the adult organism. Several GPCRs and their downstream effectors are expressed during pancreas development. We have previously shown that GPCR sphingosine-1-phosphate receptor 2 (S1pr2) signaling is necessary for the specification of the acinar and endocrine lineages in the mouse. Endocrine specification, in particular, completely depends upon the function of the Gai subunit. However, the role of GPCRs and the related purinergic receptors in pancreas progenitor differentiation and lineage allocation remains largely unexplored. We leveraged air-to-liquid interface (ALI) organotypic cultures of 14.5 dpc mouse embryonic pancreata and used selective inhibitors or activators, to systematically perturbed different nodes of GPCR and purinergic signalling to assess their role in pancreas specification. We then analyzed the survival, proliferation and differentiation patterns of the pancreatic progenitors at selective appropriate time points using immunofluorescence and quantitative image analysis. Additionally, RNA-Seq and Ribo-Seq analyses were performed to determine the response of the cells at the transcriptional and translational levels. Differential RNA abundance and translational efficiency were quantified and integrated to estimate changes in translational output. In all cases, this integration markedly reshaped the apparent transcriptional regulatory landscape. Compared to RNA-level analysis, the number of differentially regulated genes was substantially reduced. This reflected the loss of many RNA-level changes but also the emergence of regulated genes that did not appear as such at the transcriptional level. In some cases, there was even a reversion of the apparent direction of regulation. The same pattern appeared after system level genes set enrichment analyses were performed. In summary we found that distinct nodes of GPCR and purinergic receptor signalling differentially promote or inhibit specific lineages through the integration of transcriptional and translational regulation.

P.16 Insulin Deficiency Drives Epigenetic Reprogramming in Pancreatic α-Cells

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ORAL COMMUNICATION

Keywords: *α-cell plasticity, Insulin signaling, Epigenetic reprogramming, Chromatin remodeling, Cell identity*

Background/Objectives: Spontaneous cell reprogramming involves coordinated changes in chromatin accessibility, gene expression, and protein production. In diabetes, impaired insulin signaling triggers conversion of a small fraction of adult αcells (~2%) into insulin producers1-3. This limited conversion rate has led to the view that most α-cells are reluctant to reprogramming. Here, we investigated whether the broader α-cell population—despite not initiating insulin production—acquire epigenetic signatures indicative of cell fate conversion under insulin-deficient conditions, or instead are resistant to spontaneous reprogramming.

METHODS

α- and β-cells from Glucagon-Venus;Insulin-mCherry reporter mice treated with an insulin receptor antagonist were isolated by FACS and profiled by RNA-seq and ATAC-seq. α-cells from insulin-deprived or control mice were then cultured in vitro, with or without forced Pdx1 expression, to assess β-cell gene activation and insulin production.

RESULTS

Insulin signaling blockade induced widespread, reversible chromatin remodeling in α-cells, characterized by reduced accessibility at α-cell identity loci and increased accessibility at β-cell regulatory regions. These more accessible loci were enriched for β-cell transcription factor motifs—including Pdx1—consistent with a permissive chromatin landscape primed for β-cell gene activation. Interestingly, α-cells from insulin-deprived mice spontaneously upregulated β-cell transcription factors (Pdx1, Nkx6-1, MafA) and produced insulin when cultured in vitro. By contrast, α-cells from mice with intact insulin signaling required ectopic Pdx1 expression to achieve comparable β-cell gene activation (Fig. 1). Together, these data support a model in which loss of insulin signaling drives epigenetic reprogramming that broadly primes α-cells for insulin production. Conclusions/Implications: Insulin signaling acts as a gatekeeper of α-cell identity by maintaining chromatin states that restrict β-cell gene activation. Its loss remodels the epigenetic landscape, creating a permissive state for β-cell transcription factor induction and subsequent insulin production. Our observations highlight loss of insulin signaling as a driver of α-cell plasticity and suggest that bihormonal cells in human type 2 diabetes arise from insulin resistance–induced conversion, with epigenetic reprogramming potentially occurring across α-cells. These findings advance our mechanistic understanding of cell fate conversion and highlight the translational potential of exploiting α-cell epigenetic reprogramming to enhance insulin production and improve β-cell replacement strategies.

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P.17 Virus-Induced Noncanonical Translation of Non-Coding RNA: A Potential Source of Neoantigens in Type 1 Diabetes

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ORAL COMMUNICATION Keywords: *Type 1 Diabetes, Coxsackievirus B1, non-canonical translation, Ribo-seq, micropeptides*

BACKGROUND & OBJECTIVES

Type 1 Diabetes (T1D) is a chronic autoimmune disease that develops in genetically predisposed individuals following exposure to environmental triggers. The timing and mechanisms by which such environmental exposures contribute to disease onset remain uncertain. Among the proposed environmental triggers of T1D, viruses have emerged as relevant candidates, particularly Coxsackievirus B1 (CVB1)[1]. In parallel, most genetic variants associated with T1D localize in noncoding regions of the genome, including loci encoding long non-coding RNAs (lncRNAs). By acting on these non-coding regions, viral infections may induce the noncanonical translation of micropeptides, some of which could function as neoantigens that escape immune tolerance in T1D[2]. The aim of this study is to investigate how CVB1 infection alters the RNA translation landscape in pancreatic beta cells and its potential role in neoantigen generation.

METHODS

A pancreatic beta cell model (EndoC-βH1) infected with CVB1 was analyzed in biological triplicates against controls. To characterize virus-induced changes in RNA translation, we performed ribosome profiling (Ribo-seq), enabling a global analysis of actively translated RNAs. Using multiple bioinformatic approaches, we focused on detecting open reading frames (ORFs) with potential translation derived from noncoding regions and compared them between conditions.

RESULTS

Ribo-seq analysis reveals that CVB1 infection induces increased translation of genes involved in the splicing machinery, chromatin remodeling, and activation of TP53-associated pathways. Moreover, we detect active translation across a wide variety of ORFs annotated as non-coding, including lncRNAs. Among the most strongly differentially translated lncRNAs, relevant candidates are identified, including POLG-DT (Figure 1), previously identified in beta cells as a potential coding lncRNA after exposure to a synthetic analog of viral dsRNA[3]. Our results highlight the impact of CVB1 infection on the translatome of beta cells. Conclusions & Implications Taken together, these preliminary findings remark the importance of investigating the non-coding genome in the context of Coxsackievirus infection. Our study proposes a novel mechanism by which viral infections may generate neoantigens derived from noncoding regions, providing new insights into the initiation of autoimmune diseases and the role of viral infections in conditions such as T1D.

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P.18 Charting endocrine progenitors across species and organs

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ORAL COMMUNICATION Keywords: *Endocrinogenesis, Multiomics, Endocrine progenitors, Cross-species, Cross-organ*

Hormone-producing cells in the pancreas and intestine regulate whole-body metabolism and are key targets for causal therapies for diabetes and obesity. A deeper understanding of endocrine lineage formation (endocrinogenesis) in these organs is essential to elucidate disease mechanisms and develop improved therapies. Here, we performed cross-species (mouse-human) and cross-system (in vivo-in vitro) comparisons of pancreatic endocrine progenitors (EPs), complemented by cross-organ (pancreas-intestine) analyses, using single-cell transcriptomics and epigenomics integrated with bulk proteomics. We uncovered shared and distinct gene regulatory networks (GRNs) and cell-cell communication patterns during lineage allocation across species, systems, and organs. Additionally, we identified cardiometabolic risk-associated genes restricted to embryonic pancreatic EPs but largely absent in the adult pancreas, implicating developmental origins of disease. Furthermore, our integrated, multi-source EP profiling resolved the transcriptional programs underlying off-target enterochromaffin cell formation during in vitro differentiation toward human islet cells. Finally, we revealed conserved, dynamic shifts in programs governing the cell cycle, mRNA translation, and cytoskeletal organization during endocrinogenesis. Collectively, this multi-source EP profiling establishes a resource that charts the molecular landscape of endocrinogenesis and informs therapies for metabolic disease.

P.19 Exploring the Mechanisms of Human Beta Cell Replication and Differentiation in Insulinomas

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ORAL COMMUNICATION Keywords: *insulinoma, beta cell replication, beta cell differentiation*

All types of diabetes result from insufficient numbers of insulin-producing beta cells. We previously showed that transcriptomic and genomic profiling on insulinomas - rare and benign human pancreatic adenomas - provides a roadmap for inducing human beta cell replication.

Interestingly, we observed epigenetic misregulation in majority of the insulinomas we surveyed. Therefore, we took a deeper dive into insulinoma epigenetics. We performed bulk ATAC-seq and H3K27Ac ChIPseq experiments on FACS-sorted human beta cells and compared these to human insulinomas. More recently, we also performed single cell multiome analyses on human insulinomas and correlated the results with published multiome datasets on non-diabetic human islets. Our bulk analyses suggested distinct patterns of transcriptional networks in beta cells vs insulinomas. Integration of accessible chromatin regions with the enhancer regions in the genome yielded three separate clusters: 1- Unique insulinoma enhancers with an open chromatin signature; 2- Unique beta cell enhancers with an open chromatin signature; and 3Enhancers with an open chromatin signature shared between insulinomas and beta cells. Interestingly, motif enrichment analysis indicated that AP-1 family members were significantly enriched in unique beta cell enhancers. In addition, we found a significant downregulation in the expression of some of the AP-1 family members. To confirm that downregulation of AP-1 family members in insulinomas was occurring in beta cells, we turned to single cell methodologies. Indeed, AP-1 family member expression was significantly diminished in beta cells from insulinomas. Finally, we performed validation experiments where we knocked-down some of the AP-1 family members in cadaveric human islets and validated that they are important regulators of beta cell replication and differentiation. Collectively, these studies indicate that insulinoma beta cells have altered epigenetic signatures compared to cadaveric human beta cells which is also reflected in the downstream differences in expression of genes that control cell cycle and beta cell function. Most importantly, these studies identify AP-1 family as an important regulator of human beta cell replication and differentiation. Finally, our results demonstrate that insulinomas continue to serve as a model to identify potential targets for novel therapies to induce human beta cell regeneration.

P.20 Comparison of Expression Regulation Modes of Glucagon Gene in Primary and Secondary Pancreatic Alpha Cells in Mice

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POSTER Keywords: *glucagon, alpha cells, transcriptome, development*

BACKGROUND & OBJECTIVES

Pancreatic alpha cells counteract hypoglycemia through glucagon secretion. However, their function is impaired in diabetes. As alpha cells play a crucial role in glycemic regulation, understanding the molecular mechanisms governing the establishment and maintenance of alpha cell identity and proper glucagon production is essential. In mice, the first glucagon-producing cells emerge in the pancreatic bud as early as embryonic day (E) 9.5 and are referred to as primary alpha cells. Secondary alpha cells are generated during the secondary transition, mainly between E13.5 and E14.5. Previous knockout studies of key progenitor and alpha cell genes have shown loss of glucagon production in secondary alpha cells, while its expression remains preserved in primary alpha cells1.

METHODS

We evaluated potential transcriptional regulators of glucagon in primary and secondary alpha cells. A conditional Isl1 knockout mouse model was generated (Isl1loxP/loxP;Neurod1Cre/+; Isl1CKO), targeting endocrine progenitors where ISL1 is critical for secondary alpha cell differentiation. Consistent with previous studies, Isl1CKO mice retained glucagon production in primary alpha cells, whereas loss of glucagon-positive cells became evident from E13.5. We performed single cell transcriptomics (scRNA-seq) of key embryonic stages of alpha cell differentiation and combined transcriptomic data with MEME suite motif analysis of the Gcg gene and its nearby regulatory regions. Subsequently, we constructed transcription factor regulatory networks for Gcg using NetREM analysis.

RESULTS

ScRNA-seq revealed a continuous transcriptomic shift from primary to secondary alpha cells. We identified candidate glucagon regulators in primary alpha cells that may act redundantly or cooperatively to induce Gcg expression. Gata4 and Ebf3 emerged as strong candidates in the primary alpha cell regulatory mode, whereas secondary alpha cell regulation appeared to depend on Pou6f2 and Isl1. Conclusions & Implications Regulation of glucagon gene expression is highly cell type- specific. Although the precise role of early glucagon-positive cells in pancreatic development remains unclear, presence of these cells is highly conserved. Understanding distinct regulatory mechanisms of glucagon expression may provide new strategies for alpha cell regeneration and diabetes therapy.

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P.21 A targeted epi-drug screen identifies HDAC6 inhibition as an enhancer of beta cell function and immune evasion

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ORAL COMMUNICATION Keywords: *Beta cell; epi-drug; HDAC; HLA; diabetes.*

BACKGROUND AND OBJECTIVES

The destruction or dysfunction of pancreatic beta cells is central to the aetiology and pathogenesis of type 1 and type 2 diabetes (T1D/T2D), respectively. Beta cells display inherent vulnerability to cytotoxic T lymphocytes vs other islet cells, and human genetic approaches have indicated that beta cell dysfunction is the key deleterious event in T2D. We and others have demonstrated the importance of epigenetic pathways, as drivers of beta cell functional maturation, function and survival. We therefore screened for epigenetic molecules with potentially beneficial effects on beta cell adaptation using a library of ‘epi’-drugs.

METHODS

We used a library of epigenetic-modifying compounds ('epi'-drugs) coupled with high-throughput imaging at single cell resolution in human beta cells. We employed functional readouts of HLA surface expression (crucial for T cell recognition) in response to inflammatory cytokines, and insulin content, as readouts of T1D and T2D, respectively. We validated enriched epi-loci from our screen using CRISPR-Cas9-mediated genetic deletion in human beta cells.

RESULTS

Our epi-drug screen uncovered a novel role for a cytosolic histone deacetylase (HDAC) 6 whose inhibition increased insulin content and release in immature human beta cells, primary islets and stem cell-derived islet clusters. RNA-seq analysis following HDAC6 inhibition revealed an enrichment for immune response pathways, increased expression of survival genes, as well as increased beta cell identity markers and diminished expression of 'disallowed' genes. HDAC6 inhibition attenuated STAT3 activity and reduced beta cell surface expression of HLA class I proteins in response to pro-inflammatory cytokines, the latter a major hallmark of T1D. We also interrogated a common point mutation in human populations at a highly conserved residue (R832H) in the HDAC6 gene that results in diminished expression of HDAC6 protein.
Conclusions and implications: Overall, our findings demonstrate that HDAC6 inhibition in human beta cells may have therapeutic potential to 1) prevent/slow the autoimmune attack early in T1D; 2) prolong the function of beta cells during pre(type 2) diabetes.

P.22 Postnatal NEUROG3 expression reflects δ-cell identity rather than progenitor reactivation

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POSTER Keywords: *NEUROGENIN3 β-cell regeneration models Somatostatin δ-cells*

BACKGROUND & OBJECTIVES. Pancreas development is orchestrated by many transcription factors with dynamic expression patterns, transitioning from broad early activity to cell- and stage-specific roles. Neurogenin3 (NEUROG3) is indispensable for endocrine pancreas development, and its absence results in diabetes. This well-established developmental role, together with the lack of robust detection in the adult pancreas, resulted in the prevailing view that NEUROG3 is restricted to embryogenesis. Consequently, its detection in postnatal β-cell regeneration models has often been interpreted as evidence of developmental program reactivation. However, emerging evidence suggests that this interpretation may be overly simplistic. RNA sequencing of sorted murine islet cells revealed enriched Neurog3 expression in somatostatinexpressing δ-cells compared to α- and β-cells, prompting us to reassess regeneration models and investigate its role in δ-cell identity and function. METHODS. We generated Sst-Cre; R26-YFP; Neurog3flox/flox (δ-Neurog3-KO) mice, enabling δ-cell-specific YFP expression and Neurog3 gene ablation. δ-Neurog3-KO mice and their Cre-negative control

littermates were subjected to partial duct ligation (PDL), streptozotocin-induced diabetes, or diphtheria toxin–mediated β-cell ablation. Gene expression was assessed by RTqPCR. δ-cell function was evaluated in vivo and in vitro, δ-cell identity was examined by bulk RNA sequencing of sorted YFP$^+$ cells, and NEUROG3 occupancy was assessed using DamID. RESULTS. PDL induced exocrine loss and relative enrichment of endocrine markers. Diabetic islets displayed reduced Ins1 transcripts and elevated Gcg and Sst transcripts. Neurog3 expression increased in the PDL pancreas and diabetic islets from control mice but was markedly attenuated in δ-Neurog3-KO mice. Loss of Neurog3 reduced glucose-stimulated somatostatin secretion without affecting whole-body glucose homeostasis. Transcriptomic analysis revealed downregulation of cholesterol metabolism pathways and upregulation of vesicle trafficking processes, with over half of the differentially expressed genes directly bound by NEUROG3. CONCLUSION. Postnatal Neurog3 expression in pancreas and β-cell injury models primarily reflects δ-cell-specific expression rather than reactivation of a progenitor program. NEUROG3 plays a modest, context-dependent role in maintaining aspects of δ-cell physiology. This work challenges the prevailing view of NEUROG3 as a strictly developmental factor and highlights the importance of cell type–resolved analyses when interpreting transcriptional changes in regenerative settings.

P.24 The role of 5-hydroxymethylcytosine and TET enzymes in regulating beta-cell function: a novel mechanism in diabetes pathogenesis.

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POSTER Keywords: *beta-cells, hydroxymethylation, 5hmC.*

BACKGROUND & OBJECTIVES

Type 2 diabetes (T2D), affecting over 425 million people worldwide, is driven primarily by pancreatic β-cell failure. While epigenetic regulation is critical for β-cell function, the specific contributions of cytosine methylation (5mC) and hydroxymethylation (5hmC) remain poorly understood. Conventional bisulfite-based sequencing conflates these marks despite their distinct and often opposing regulatory roles. This study aims to delineate the specific functions of 5mC and 5hmC in β-cell maturation, gene regulation, and T2D susceptibility.

METHODS

To independently interrogate these marks, we will utilize Nanopore sequencing to generate base-resolution 5mC and 5hmC maps from HPAP-sorted pancreatic cell types of non-diabetic and T2D donors across a broad age range. These maps will be integrated with chromatin-state and gene expression data to define regulatory impacts and test T2D-associated genetic variants. Concurrently, we will employ a genetic mouse model with β-cell-specific conditional ablation of the DNA demethylation enzymes TET2 and TET3 to establish a causal role for 5hmC in β-cell maturation, identity, and adaptation to metabolic stress.

RESULTS

Preliminary Nanopore data demonstrate that 5hmC is highly enriched at active islet enhancers. In human β-cells, 5hmC constitutes approximately 50% of all cytosine modifications—a level comparable to brain cells and drastically higher than most cell types (typically <5%). Crucially, 5hmC levels are reduced at the NKX2.2 locus, a key β-cell transcription factor, in T2D donors compared to non-diabetic controls. Furthermore, immunostaining reveals an age-associated increase in β-cell 5hmC levels in both mice and humans, supporting a conserved role in postnatal maturation and maintenance. Conclusions & Implications: These findings suggest 5hmC is a critical, independent regulator of β-cell identity. By establishing a direct causal relationship between 5hmC loss and β-cell failure, this project will deliver a comprehensive islet-cell 5hmC atlas and define mechanisms linking genetic variants to epigenetic dysregulation. Resolving these fundamental knowledge gaps will provide a vital foundation for developing novel, epigenetic-directed therapeutic strategies for the prevention and treatment of T2D.

P.25 Exploring miRNA heterogeneity within pancreatic β-cell subpopulations to improve β-cell therapies

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POSTER Keywords: *β-cell heterogeneity, microRNAs, single-cell transcriptomics, stem cell–derived islets, RNAscope*

BACKGROUND AND OBJECTIVES

Pancreatic β-cells within islets are functionally and molecularly heterogeneous. Single-cell RNA sequencing (scRNA-seq) has identified distinct β-cell subpopulations with unique transcriptional profiles, which are altered during diabetes progression and differ from those in stem cell–derived islets (SC-islets). These differences may contribute to disease susceptibility and/or reduced SC-islet functionality compared to mature islets. The contribution of microRNAs (miRNAs) to β-cell heterogeneity remains unexplored, as miRNAs are undetectable in standard scRNA-seq protocols. MiRNAs are negative regulators of gene expression essential for β-cell development, survival and function. Given recent findings showing heterogeneous miRNA expression and activity in other cell types, we hypothesize that miRNAs contribute to the molecular and functional heterogeneity of β-cells. We aim to apply cutting-edge molecular and imaging techniques to explore miRNA expression and targeting across mature SC-islets, human islets and pancreatic tissue in health and diabetes.

METHODS

We performed bulk small RNA-seq on primary human islets and glucose-responsive SC-islets and single-cell small RNA sequencing (scSMALL-seq) on FACS-sorted cells from human and mouse islets. RNAscope was performed on FFPE SCislets and human and mouse pancreatic tissue sections to simultaneously image mRNA, miRNA and proteins.

RESULTS

Bulk small RNA-seq detected ~400 islet miRNAs, with > 100 differentially expressed in SC-islets compared to primary human islets (padj <0.05, fold change >2-fold). Preliminary scSMALL-seq experiments enabled detection of 35-45 miRNAs per cell, including well-characterised β-cell miR-375-3p, miR-200c-3p and miR-7-5p. MiRNA reads comprised only ~1% of total reads in single cells, compared to 10-20% in 100 cell samples and up to 80% in bulk RNA-seq. RNAscope enabled detection of miR-125b, Pdx1 mRNA and INS/GCG protein in SC-islets and in human pancreatic sections, providing proof-of-principle that miRNAs can be robustly detected alongside mRNA/protein in situ. Conclusions and implications: These findings highlight differences in miRNA expression profiles between SC-islets and primary human islets, likely reflecting differences in cellular composition. We established scSMALL-seq and RNAscope as complementary approaches to investigate miRNA expression at single-cell and spatial resolution, enabling ongoing studies addressing whether miRNAs contribute to β-cell heterogeneity in health and diabetes.

P.26 Decoding the extracellular “Instruction Code” governing human β-cell differentiation and reproducibility.

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POSTER Keywords: *stem cell-derived β-cells (SC-β-cells), extracellular matrix (ECM), scRNA-seq*

BACKGROUND & OBJECTIVES

Pancreatic β-cells are the sole source of insulin, and their loss or dysfunction leads to diabetes. Human pluripotent stem cellderived β-cells (SC-β-cells) provide a powerful model to study human pancreatic development and offer promising strategy for cell replacement therapy. Despite recent advances, hPSCs cannot yet be reliably differentiated into fully functional β-cells with sufficient yield and reproducibility. Increasing evidence points to the extracellular environment (ECM and associated secreted factors) as a dynamic regulator of β-cell lineage progression and differentiation fidelity.

METHODS

hPSC models (HUES8, H1) engineered with CRISPR/Cas9 knockouts or doxycycline-inducible overexpression systems for SPOCK2, alongside treatment with recombinant factors (SPOCK2, MMP2) to probe ECM-mediated signaling. Directed β-cell differentiation performed using established 2D and 3D protocols. Cell identity and lineage progression assessed by immunofluorescence, flow cytometry, and Western blotting, with MMP2 activity measured by zymography. Functional maturity was evaluated by GSIS. Transcriptomic profiling (bulk and scRNA-seq; 10x Genomics, Illumina) enabled trajectory, pathway analyses, and downstream target identification. For in vivo validation, SC-β-cells were transplanted under the kidney capsule of SCID-Beige mice.

RESULTS

We identified SPOCK2, an ECM proteoglycan, as a negative regulator of immature β-cell proliferation. SPOCK2 KO SC-β-cells exhibited enhanced proliferation while maintaining elevated expression of maturation markers (INS, NKX6-1, PCSK1) and significantly improved GSIS. Mechanistically, SPOCK2 KOs showed increased MMP2 expression and activity, activating the βintegrin–FAK–c-JUN signaling axis. MMP2 promoted both short- and long-term expansion of SC-β-cells and enhanced GSIS in vitro and in vivo, comparable to human islets. SPOCK2 also functions as a stage-specific developmental regulator. Its loss accelerates progression from PPs to endocrine and β-cell stage. scRNA-seq trajectory analysis revealed faster advancement along β-cell differentiation paths, indicating altered timing of lineage transitions. Transcriptomic analyses identified additional SPOCK2-dependent ECM-associated factors, which regulate endocrine specification and progenitor progression, revealing a broader ECM-driven regulatory network. Conclusions & Implications Collectively, our findings establish the ECM as a critical regulator of β-cell proliferation, differentiation, and function. We define a molecular mechanism governing SC-β-cell expansion and maturation and identify an extracellular signaling environment that can be leveraged to enhance the generation of functional β-cells for therapeutic applications.

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P.27 Using tandem mass tagged proteomics to understand epigenetic repression of the HK1 gene in β-cells

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POSTER Keywords: *Hexokinase 1 (HK1) regulatory repression, congenital hyperinsulinism, liquid chromatography-mass spectrometry,*

chromatin remodelling, epigenetic repression

BACKGROUND & OBJECTIVES

Non-coding variants in a regulatory region of the Hexokinase 1 (HK1) gene have been identified as a cause for congenital hyperinsulinism[1, 2]. This region represses HK1 expression in pancreatic β-cells and its disruption leads to inappropriate insulin secretion. The locus contains putative motifs for NKX2, FOX and NFAT transcription factor families, suggesting they may mediate formation of a repressive complex that alters the epigenetic state of the HK1 gene. Understanding the interactions regulating this repressive complex will be key to discerning how variants in this region lead to hyperinsulinism.

METHODS

To study the composition of the repressive complex at the HK1 regulatory region, we used biotinylated oligonucleotides to capture nuclear proteins from EndoC-βH1 cells that bound the wild-type HK1 regulatory sequence. Biotin/streptavidin-captured proteins were digested, labelled by tandem mass tagging and identified by liquid chromatography-mass spectrometry. Additionally, separate oligonucleotides with disruptions to the FOX, NKX2 and NFAT motifs (referred to as FOXMOT-, NKX2MOT- and NFATMOT- respectively) were used to study the function of these transcription factors in controlling the composition of the repressive complex.

RESULTS

As expected, disruption of the FOX and NKX motifs resulted in fewer peptides of FOXA2 or NKX2-2/4 coprecipitation with the FOXMOT- and NKX2MOT- oligonucleotides respectively. However, although no NFAT family members were detected, 26 other transcription factors were uniquely reduced in abundance with NFATMOT- oligonucleotide. One of these, ZBTB11, partially matched the NFAT motif, however further validation is required. Notably, fewer members of the chromatin remodelling complexes, NuRD (e.g., CHD4, MTA2) and SWI/SNF (e.g., SMARCC1) were present with NKX2 or NFAT motif disruption. NKX2 disruption also resulted in reduced detection of proteins involved in DNA methylation and the Polycomb Repressive Complex (e.g., DNMT1 and CBX8). Disrupting the FOX motif had no effect on the abundance of chromatinmodifying or DNA methylation enzymes. Conclusions & Implications: Disrupting transcription factor motifs in the HK1 regulatory region alters the recruitment of transcription factors and chromatin-modifying repressive complexes. These results begin to reveal the combinatorial logic of transcription factor binding and provide new insights into how regulatory variants contribute to hyperinsulinism.

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P.28 Exploring stage-specific chromatin accessibility throughout early pancreatic development

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ORAL COMMUNICATION Keywords: *chromatin accessibility, stem cell, differentiation*

BACKGROUND AND OBJECTIVES

Pancreatic development is controlled by transcriptional networks that drive lineage specification. Chromatin accessibility plays a central role in controlling temporal transcriptional factor binding and to enact lineage. While canonical expression markers are widely used to define pancreatic differentiation stages, it remains unclear whether additional stage-specific genomic markers – particularly those delineated by chromatin accessibility – can provide further characterisation. Here, we combined in situ marker analysis with long-read chromatin stencilling to characterise genome-wide chromatin accessibility dynamics throughout pancreatic differentiation.

METHODS

H1 embryonic stem cells were differentiated to definitive endoderm (DE), proximal gut tube (PGT) and early pancreatic progenitors (PP). Nuclei were isolated at each stage, and stage identity was validated by flow cytometry using established markers at DE and PP stages. Chromatin stencilling was performed by incubating nuclei with Hia5 methyltransferase, which modifies accessible adenines with N6-methyladenine (6mA). Labelled chromatin was sequenced using Oxford Nanopore technology to achieve 30-40X genome coverage. Accessible regulatory regions were identified using the FIRE (Fiber-seq Inferred Regulatory Elements) pipeline.

RESULTS

Differentiation efficiency was high, with >90% cells at DE stage co-expressing CXCR4 and CD117, and ~80% of PP cells coexpressing PP markers NKX6.1 and PDX1. Chromatin accessibility profiling identified 9 gene promoter classes. These included ES-specific (614 genes), two DE-specific (536 and 1429 genes), PGT-specific (503 genes), and two PP-specific (1288 and 337 genes) clusters. CXCR4 exhibited DE-specific accessibility, whereas CD117/KIT remained accessible across all stages. PDX1 and NKX6.1 displayed PP-specific accessibility. Enrichment analysis revealed that DE-specific promoters were associated with FGF signalling and Vitamin C metabolism, and enriched for polycomb-marked ectodermal lineage genes. Many developmental endoderm regulators exhibited accessibility across multiple stages. PGT-specific promoters were enriched for SMAD-related genes, indicating competence to respond to BMP, and liver-specific promoter enrichment, suggesting alternative lineage potential post-PGT. PP-specific promoters were enriched in pancreatic precursor genes, consistent with lineage commitment. Conclusions and Implications: These findings demonstrate that promoter chromatin accessibility reflects not only current cell identity, but also developmental competence and alternative lineage potential. This provides a complementary information layer to gene expression and offers valuable insights for refining and optimising pancreatic differentiation protocols.

P.29 Investigating haplotype specific regulation of FOXA2 by chromatin stencilling

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POSTER Keywords: *Chromatin accessibility Congenital hyperinsulinism Nanopore sequencing Chromatin stencilling*

BACKGROUND AND OBJECTIVES

The correct dosage of FOXA2 is important for the correct development and differentiation of beta cells and their ultimate function. The haploinsufficient loss of FOXA2 or a recently identified FOXA2 control region comprising multiple regulatory elements, causes congenital hyperinsulinism [1]. How the loss of this control region results in hyperinsulinism is not currently understood, nor whether there is haplotype-specific regulation of FOXA2 in normal healthy beta cells. To gain insight into both questions, we employ new chromatin stencilling technology to understand haplotypespecific regulation of FOXA2 in the EndoC-βH1 cells.

METHODS

We profiled targeted haplotype-resolved base-pair level chromatin accessibility by Oxford Nanopore chromatin stencilling. This involves isolating EndoC- βH1 nuclei and employing an exogenous methyltransferase to convert accessible adenines to 6mA. We read these out at high depth (>300x) by adaptive sampling over a locus spanning the control region and FOXA2 gene. We phased long-reads between haplotypes and scored differential 6mA methylation between haplotypes, indicative of differential FOXA2 binding. We also employ RNA-seq using variants within FOXA2 to score allele-specific expression.

RESULTS

While the vast majority of FOXA2 regulation shows no haplotype differences, we identify 35 allele-specific 6mA methylation sites ($p < 0.01$), of which 6 span 5 previously identified islet regulatory regions. We scored these against transcription factor (TF) motifs and identified FOX, PAX, HNF4A, SOX, and IRX families with potential haplotype differential footprints. We found no evidence for this leading to FOXA2 allele specific expression. However, we find evidence of allele specific expression for adjacent and in stream LINC00261 that has been described to promote the expression of FOXA2, and vice versa [2]. We find FOXA2 exons spliced with LINC00261 exons, indicating that FOXA2 transcription can run-on to incorporate LINC00261. Conclusions and Implications: In conclusion, although the majority of FOXA2 regulation appears to be haplotype symmetric, it is clear that there are distinct hotspots that show allele-specific chromatin accessibility in the EndoC-BH1 beta cell model. This reveals new layers of FOXA2 regulation and lays the groundwork for investigating haploinsufficient loss of FOXA2 or its control region in congenital hyperinsulinism.

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P.30 Prioritisation of Regulatory de novo Variants in Monogenic Diabetes and Congenital Hyperinsulinism Using Developmental Epigenomic Profiling

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POSTER Keywords: *De novo, NDM, Hyperinsulinism, Non-coding, Variants*

BACKGROUND & OBJECTIVES

While coding variants explain most cases of neonatal diabetes (NDM) and a proportion of HI, a substantial fraction of patients remain genetically unresolved, suggesting an important role for non-coding regulatory variation. Previous approaches have identified disease-associated loci through regional clustering of variants; however, these are not explicitly informed by developmental regulatory biology. We aimed to develop a strategy to prioritise de novo variants within potential regulatory regions in unsolved NDM and HI cases using epigenomic profiling.

METHODS

We analysed whole genome sequencing data from 197 proband-parent trios with HI and NDM (69 permanent NDM, 12 transient NDM, 116 HI), including 33 genetically solved cases, and identified de novo variants using denovocnn. Candidate regulatory regions were defined as chromatin accessibility (ATAC-seq) peaks that are dynamically active during differentiation from human embryonic stem cells to pancreatic progenitors. These regions were grouped based on similarity of their accessibility patterns across developmental stages, capturing elements likely to be co-regulated. De novo variants were intersected with these regulatory regions and annotated according to their assigned cluster, prioritising those with nearby genes exhibiting similar accessibility dynamics.

RESULTS

We identified 34,231 disease-specific de novo variants. Of these, 540 candidate variants in unsolved HI and 350 in unsolved NDM patients resided within regions active during pancreatic differentiation. Four regulatory regions contained variants in multiple unrelated patients with the same phenotype. The strongest signal mapped to the HK1 locus, consistent with previous variant clustering-based discoveries and supporting the validity of our approach. Two additional candidate regions were identified within introns of NYAP2 and ARHGEF3, which may act as regulatory elements for more distal genes. Clustering based on shared developmental accessibility refined the non-recurrent variants to a smaller set with higher predicted functional relevance, including several variants in proximity to NDM genes. Additionally, six recurrent variants were identified outside ATAC-seq peaks but within 1 Mb of known NDM or HI genes. Conclusions & Implications We present a prioritisation strategy for non-coding regulatory de novo variants in monogenic diabetes and HI, complementing existing clustering approaches. These findings identify novel candidate loci for future functional validation and diagnostic application.

P.31 Resolving haplotype specific regulation across pancreatic differentiation and beta cell function

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ORAL COMMUNICATION

Keywords: *Pancreatic differentiation, chromatin accessibility, chromatin stencilling, allele specific regulation*

BACKGROUND

Understanding the gene regulatory basis of cell fate decisions during pancreatic differentiation and function in remains a fundamental challenge. This is especially acute in disease where we lack tools to interpret non-coding regulatory variants. To date, short-read technologies mapping transcription factor binding, chromatin accessibility, and histone modifications have made significant contributions to our understanding. Yet they have limitations: they cannot phase regulatory activity with genotype and DNA state. Here we employ chromatin stencilling (known as Fiber-seq) to build the highest resolution regulatory map of pancreatic differentiation and beta-cells to date. This technology provides a base-pair resolution haplotype-phased chromatin accessibility map that can distinguish regulatory regions harbouring transcription factor footprints from accessible linker and nucleosomal DNA.

METHODS

We employ chromatin stencilling across pancreatic differentiation from embryonic stem cells to pancreatic progenitors and in EndoC-BH1 cells. Briefly, we incubate nuclei with Hia5 methyltransferase to convert accessible adenines to 6mA. We read out with Oxford Nanopore sequencing and analyse data using an in-house pipeline.

RESULTS

Over differentiation to pancreatic progenitors we identify >684k regulatory regions, with >300k present at two stages; notably, this corresponds to 206k and 103k regions in a comparable ATAC-seq dataset. This discrepancy is best explained by small regulatory regions (<200 bp) which are poorly captured by ATAC-seq: chromatin stencilling: 365k peaks < 200bp; ATAC-seq: 18.8k (5.2%) peaks < 200bp. Example given in accompanying figure. Resolving accessible chromatin to haplotypes, we find >8.2k regions with haplotype-specific regulation, enriched at alleles exhibiting haplotype-specific 5mCpG DNA methylation (e.g. at S2/PGT stage OR>41.7, p<10-300). These regions are also enriched at loci bound by CTCF, PDX1, and SOX9 in pancreatic progenitors. In EndoC-BH1 cells, we resolve a type 2 diabetes risk variant in an NKX2-2 binding site that leads to allele-specific DNA methylation and chromatin accessibility.

CONCLUSIONS

We present a high-resolution regulatory map of pancreatic differentiation and beta-cell function. Beyond identifying regulatory regions, this resource integrates DNA methylation, transcription factor footprints, and chromatin state at singlemolecule resolution. We anticipate this dataset will enable functional studies into gene regulation and aid in the interpretation of non-coding variants in pancreatic islet disease.

P.32 Single-cell quantification of β-cell secretory capacity in Type 2 Diabetes

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POSTER

A major determinant of disease progression in type 2 diabetes (T2D) is deteriorating insulin secretion from the pancreatic βcells. However, the molecular changes that underlie β-cell dysfunction in T2D are still elusive. Recent single-cell studies have revealed transcriptional heterogeneity among β-cells, highlighting the need to investigate the relationship between β-cell molecular heterogeneity and functional decline in T2D. A limitation to current approaches is the difficulty in establishing functional profiles for different subtypes of β-cells. Here we perform fluorescent labelling of individual β-cells according to their secretory capacity and combine it with single-cell RNA-sequencing in the same cell. Our approach is based on the use of fluorescent tracers of endocytic membrane retrieval, which we show can be used as a proxy for insulin granule fusion and secretion. We use this methodology to characterize β-cell states associated with higher secretory function in nondiabetic donors and to identify potential mediators of β-cell failure during progression to T2D. By investigating islets from mouse models with increasing levels of β-cell dysfunction, as well as human islets from donors with and without T2D, we identified genes and pathways associated to decreased β-cell secretory capacity in both mouse and human β-cells. Some of these genes are known regulators of insulin exocytosis and mitochondrial function and are now being validated through mechanistic studies. Overall, our approach sheds light on the relationship between molecular heterogeneity and secretory capacity in β-cells and offers potential avenues for the development of new treatments targeting β-cell secretory failure.

P.33 Single-cell multiomic profiling of human islets reveals beta-cell-specific responses to inflammatory cues in a co-culture model of early insulitis

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POSTER Keywords: *T1D, single-cell, regulatory genomics*

BACKGROUND & OBJECTIVES

Type 1 diabetes (T1D) is a complex autoimmune condition developing from a combination of genetic factors and environmental triggers, leading to a progressive loss of beta-cell mass and development of insulitis over years before clinical diagnosis. The precise contribution of beta cells to their own demise and the factors that render them more susceptible to autoimmune destruction remain unclear.

METHODS

To model early insulitis, human pancreatic islets (HI) were co-cultured with activated $CD4^+$ T lymphocytes for 24 and 72 hours using a transwell system, allowing the exchange of soluble factors while preventing direct cell–cell contacts. Control samples consisted of islets cultured alone. Single-cell multiome profiling was performed on three HI donors per condition (12 experiments), enabling simultaneous assessment of the transcriptome (snRNA-seq) and chromatin accessibility (snATACseq) at single-cell resolution.

RESULTS

Our single-cell experiments captured 20,021 high-quality cells, with alpha (40%) and beta cells (35%) representing the major cell types, and smaller proportions of delta (6%), gamma (<1%), acinar (4%), ductal (11%), and stellate cells (2%). We identified differentially expressed genes (DEGs) and differentially accessible regions (DARs) in response to the co-culture model. Changes in chromatin accessibility and gene expression were strongly correlated, indicating a coordinated epigenetic and transcriptional response to the inflammatory stimulus. Beta cells displayed the highest number of DEGs and particularly DARs among endocrine cell types, supporting an increased responsiveness of beta cells to a pro-inflammatory environment. Importantly, our co-culture model recapitulated changes observed in beta cells from T1D patients, at both the transcriptional and chromatin accessibility levels, suggesting that this in vitro system captures relevant early disease mechanisms that remain active after diagnosis. Finally, we reconstructed beta-cell regulatory networks in response to the co-culture and identified candidate regulatory elements and genes potentially affected by T1D-associated SNPs from GWAS. Conclusions & Implications Our findings demonstrate that inflammatory cues are sufficient to induce coordinated transcriptional and epigenetic changes in human islet cells, with beta cells showing a heightened response. This integrative single-cell multiomic approach provides a valuable framework to dissect early events in T1D pathogenesis and to link genetic risk variants to functional regulatory mechanisms.

P.34 Inflammation-induced chromatin remodeling and transcription factor networks reveal β cell regulatory vulnerabilities in T1D

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ORAL COMMUNICATION

BACKGROUND

T1D is an autoimmune disease in which β-cell destruction leads to insulin deficiency and hyperglycemia. Early insulitis perturbs β-cell function through immune cell infiltration and cytokine secretion. However, it remains unclear how inflammatory signals reshape β-cell transcription and chromatin, and how T1D genetic risk variants influence these responses.

METHODS

We modeled early T1D in-vitro by co-culturing human β cells or islets with activated primary $CD4^+$ T cells in a transwell system. Time-course profiling included scRNA-seq and scATAC-seq, followed by bulk RNA-seq, ATAC-seq, H3K27ac CUT&Tag, and ChIP-seq for key inflammatory and islet-specific transcription factors including IRF1, NKX6.1 and MAFB. We next conducted a massively parallel reporter assay (MPRA) to assess the functional impact of GWAS-defined T1D associated variants on β cell responses to activated $CD4^+$ T cells.

RESULTS

We generated high-resolution regulatory maps of human β cells exposed to inflammatory cues mimicking early T1D. By integrating single-cell and bulk transcriptomic and epigenomic data, we defined dynamic changes in chromatin activity linked to changes in gene expression. In-vitro expression responses mirrored those observed in islets isolated from early stages T1D patients. We classified β-cell lymphocytes-responsive regulatory elements as “potentiated” (pre-accessible gaining H3K27ac), “neo” (initially inactive gaining accessibility and acetylation) and “reduced” (losing accessibility/H3K27ac). Sequence composition analysis and scATAC-seq-inferred TF activity revealed loss of tissue-specific TFs at reduced elements, whereas induced sites (potentiated and neo) were enriched for inflammatory and β-cell TFs. Synergy analysis and ChIP-seq showed ~40% of induced elements co-bound by NKX6.1, MAFB, and IRF1, orchestrating the strongest transcription at lymphocyte-responsive sites. We next conducted an MPRA system to systematically detect T1D associated variants interfering with β-cell regulatory functions implicated in inflammatory responses. We identify dozens of functional T1D functional variants and leverage these data to identify gene targets and pathways underlying β-cell vulnerability during early stages of T1D.

CONCLUSIONS

Our work defines how inflammatory signals reshape β-cell chromatin via cooperative TF networks, driving a shift toward an inflammatory-responsive state. Moreover, our data reveal how

genetic risk variants interfere with β-cell inflammatory responses, uncovering vulnerable pathways and gene targets involved in the development of T1D.

P.35 Characterization of CD4+ T lymphocytes in co-culture with human beta cells

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POSTER

BACKGROUND & OBJECTIVES

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by immune-mediated destruction of insulin-producing β-cells. During early insulitis, inflammation and cytokine-mediated crosstalk between immune cells and β-cells contribute to β-cell dysfunction and death. Despite progress in understanding T1D pathogenesis, the precise mechanisms triggering and sustaining autoimmunity remain unclear, limiting the development of effective therapies.

METHODS

An in vitro transwell co-culture system was established using human EndoC-βH1 cells and primary CD4⁺ T lymphocytes from four donors to model early insulitis. Bulk RNA sequencing was performed at 6h, 24h, and 72h following T cell activation to capture dynamic transcriptional changes in both cell types. Additionally, a multiplex immunoassay was used to simultaneously detect chemokines and cytokines in the co-culture media, aiming to capture secreted proteins involved in cell-to-cell crosstalk.

RESULTS

Compared to activated CD4⁺ T cells alone, co-cultured lymphocytes exhibited a defined set of differentially expressed genes. Gene set enrichment analysis (GSEA) revealed significant pathway-level changes, indicating a shift toward a functionally active and responsive state. Cell–cell communication analysis identified key signaling pathways active at 72 hours, including MK, LT, CCL, TNF, GALECTIN, and WNT signaling, associated with inflammation, chemotaxis, and cell-cell adhesion processes. Integration of transcriptomic data with a multiplex immunoassay profile of the co-culture media revealed a highly diverse cytokine milieu, dominated by IFNγ but also including numerous other signaling molecules, many not previously linked to insulitis. These factors were released by both lymphocytes and β-cells, highlighting a bidirectional communication between the two cell types.

CONCLUSIONS

Our data delve in the early stage of inflammation and allow an in-depth characterization of the early “dialogue” established between β cells and lymphocytes. We provide a multi-layered characterization of early interactions between $CD4^+$ T lymphocytes and β-cells. Importantly, the data support a bidirectional signaling network involving β-cell-derived factors that influence lymphocyte gene expression. These findings uncover structured ligand–receptor interactions that may contribute to early immune–β-cell crosstalk in T1D.

P.36 Epigenetic regulatory mechanisms governing the progenitor potential of pancreatic ductal cells

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ORAL COMMUNICATION

Diabetes mellitus remains one of the major causes of public health concern worldwide, with over 500 million affected people and rising every year. Its high impact on the patient’s wellbeing as well as limited advances in its diagnosis and treatment have consolidated it among the leading causes of death in the last years. Among strategies guiding current research efforts for the development of better treatments, β-cell replacement therapies stand out for their potential as long-term or even definite cures for this disease. In recent years, the possibility of employing embryonic/induced stem cells as well as other adult pancreatic cell types as a source of insulin-secreting cells in multiple differentiation models has attracted a lot of attention, although an efficient method for the de novo generation of β-cells remains elusive. At the same time, the role of pancreatic ductal cells as facultative progenitors in the adult has been the subject of controversy in the last decades, as there is conflicting evidence regarding the extent of their regenerative capabilities. In our previous work, we characterized the heterogeneity of the pancreatic ductal tree and confirmed the potential of adult ductal cells to differentiate towards the endocrine lineage including insulin-producing cells- in organoid models. Moreover, some identified ductal subpopulations with unequal distributions through the ductal tree presented better endocrine differentiation capabilities than others. Our results suggested such heterogeneity might be caused by cell identity features beyond transcriptional differences. In the present work, we seek to elucidate the regulatory mechanisms behind the acquisition of progenitor capabilities by ductal cells in organoid models through profiling the transcriptional and chromatin-accessibility profiles of ductal cells upon culture. For this purpose, we have also screened a large number of epigenetic modulators for their capability to increase the efficiency of ductal-to-β differentiation in in vitro murine ductal organoid models, finding some capable of inducing significant increases in insulin expression and testing their effects on pancreatic islet formation in in vivo zebrafish embryo models.

P.37 Single cell multiome profiling of human healthy pancreas reveals novel ductal populations associated with pancreatic cancer prognosis and classification

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ORAL COMMUNICATION Keywords: *human pancreas; pancreatic ducts; ductal heterogeneity; single-nucleus multiomics; pancreatic development;*

Murine studies have demonstrated significant heterogeneity within the pancreatic ductal epithelium; however, the regulatory landscape of human ducts remains insufficiently understood. Moreover, the human pancreas is a complex multicellular ecosystem in which epithelial–mesenchymal communication influences tissue organization. However, a comprehensive framework integrating ductal diversity with regulatory and intercellular signaling programs is lacking. In this study, we conducted single-nucleus multimodal profiling (snRNA-seq and snATAC-seq) of healthy human pancreatic samples from 8 cadaveric donors, enriching ductal fractions to capture rare epithelial populations across the ductal tree. Our integrated analysis identified previously unrecognized ductal subpopulations characterized by distinct transcriptional and chromatin accessibility profiles, as well as previously described ones including conserved Ly6D$^+$ basal-like, interferonresponsive, EMT, and mucinous states, paralleling our murine ductal atlas. Gene regulatory network inference revealed divergent transcription factor programs that distinguish small and large duct identities in the pancreas. Additionally, multimodal characterization of mesenchymal populations has uncovered heterogeneity within stromal tissue and predicted ligand–receptor interactions linking specific ductal states to mesenchymal clusters, suggesting regulatory crosstalk underlying cellular specialization. Comparative analyses with fetal pancreas datasets highlighted the developmental relationships among ductal populations, while integration with pancreatic ductal adenocarcinoma (PDAC) datasets revealed the selective enrichment of defined ductal signatures associated with tumor subtypes and patient prognosis. This multimodal atlas enhances our understanding of human pancreatic epithelial-mesenchymal interactions and contributes to deepening our knowledge of the role of ductal cells in exocrine pathogenesis.

P.38 High-throughput functional assays and DNA sequence-to-function models to prioritize functional variants in causal non-coding variant discovery studies

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POSTER Keywords: *Human islet cis-regulatory elements, Rare variant prioritization, Massively parallel reporter assay, AlphaGenome*

Over 50% of patients clinically diagnosed with MODY (Maturity Onset Diabetes of the Young) remain genetically unexplained. The non-coding genome, harbouring key tissue-specific regulatory elements implicated in polygenic diabetes1, remains underrepresented in studies of monogenic forms. To discover new regulatory regions harbouring causal mutations, we have established two cohorts of lean patients with early-onset diabetes and suspected monogenic aetiology yet negative for mutations in clinical gene panels. We screened islet regulatory elements (n=916 index cases) or performed whole-genome sequencing (n=482 index cases), along with matched controls, to conduct burden and aggregation case-control studies. A major challenge in capturing a statistically significant burden of rare variants in such studies lies in the fact that even rare non-coding variants are mostly functionally neutral and are poorly scored by traditional in silico tools. To support the identification of regulatory regions harbouring disruptive mutations, we are developing a functionally guided framework that combines SuRE2 (Survey of Regulatory Elements), a massively parallel reporter assay, and AlphaGenome3, a deep learning model, to prioritise likely deleterious rare variants in islet cis-regulatory elements. We performed a large-scale screen of regions showing SuRE enhancer activity in ENDOCbH3 β-cells amongst >10000 elements (15 Mb). We further tested functional effects of selected rare variants and performed saturation mutagenesis across key β-cell CREs for benchmarking. To assess the performance of in silico predictions, we analysed the same variants with AlphaGenome. Our results show that SuRE robustly discriminated active from inactive regulatory elements. Saturation mutagenesis of selected regions further identified functionally disruptive variants. For example, HNF1A promoter revealed high-impact variants concentrated at an HNF4A binding site that has been shown to harbour causal mutations. AlphaGenome predictions showed variable performance across regions, with strong significant correlations in 10/25 regions relative to SuRE. Our work shows that SuRE MPRA assay and AlphaGenome can be integrated to help highlight variants with high impact on the regulatory function of pancreatic islet cis-regulatory elements. Finally, we developed SuRE-FIRE, a scalable method to prioritise rare non-coding variants and improve the burden test power in Mendelian diseases.

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P.39 An atlas of human pancreatic islet promoters and transcripts revealed by PPEARL (Promoter Paired-End Analysis of RNA Landscapes)

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POSTER Keywords: • *Pancreatic islets* • *Transcriptome annotation* • *Alternative promoters* • *Diabetes genetics*

Pancreatic islets play a central role in diabetes mellitus, yet have been underrepresented in human genome annotation efforts. High-quality transcriptome annotations are essential for interpreting genetic variation, designing targeted perturbation studies, and analysing single-cell transcriptomic data. We aimed to comprehensively annotate human islet transcripts and active promoters, addressing a critical gap in reference databases such as GENCODE1 , FANTOM, and GTEx. We implemented PPEARL (Promoter Paired-End Analysis of RNA Landscapes), a multiplatform strategy integrating Cap Analysis of Gene Expression (CAGE) to map transcription start sites at single-base resolution 2 , paired-end CAGE sequencing to directly link transcription start sites to cognate transcripts, PacBio long-read sequencing to resolve full-length isoforms, and Illumina short-read sequences from 193 human islet donors for de novo transcript assembly (Figure 1). Cell-type-specific transcript start site usage was estimated using single-cell VASA-seq data. We assembled 376,342 transcript models across 22,111 genes, of which only 12% are present in GENCODE v49, despite consistent expression across islet samples. We uncovered >1,000 currently unannotated promoters linked them to proteincoding genes, including numerous examples that constitute the major origin of transcription for genes with established roles in islet function and diabetes, among them tens of G-protein-coupledreceptor genes. We further identified previously unrecognised non-coding RNA genes and unannotated protein-coding exons in diabetes-relevant genes. Using these refined annotations, we resolved cell subtype-specific usage of alternative promoters in alpha and beta cells. These findings demonstrate that the human transcriptional landscape remains incompletely annotated, and that pancreatic islets harbour extensive promoter and isoform diversity not captured in current references. This resource supports genetic, functional, and translational studies of pancreatic islet biology and diabetes, with direct implications for variant interpretation in Mendelian and polygenic forms of diabetes.

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P.136 Analytical framework for confounder-robust burden testing of non-coding regulatory regions harbouring causal monogenic diabetes mutations

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POSTER Keywords: • *Rare variants* • *Non-coding regions* • *MODY* • *Monogenic* • *Population structure*

Many patients with suspected monogenic diabetes show negative diagnostic findings for maturity onset diabetes of the young (MODY) genes. Some such patients may harbor causal variants in previously unrecognized coding and noncoding genomic regions. We sequenced >1300 index patients with early-onset, non-obese diabetes, negative MODY diagnostic panels, and absence of type 1 diabetes criteria. Two case-control studies were assembled: DD-WGS, analyzed by whole genome sequencing; and DD-TS, analyzed by targeted sequencing of islet regulatory regions. This design enables collapsing tests to identify genomic regions harboring excess ultrarare variants in cases, although such analyses are challenged by confounders such as population stratification. Here we describe a strategy that mitigates the impact of potential confounders, whilst preserving adequate power to discover causal noncoding variants. We annotated beta-cell regulatory regions using single cell and bulk islet epigenomic datasets, and split CREs into 400-bp sliding windows. Genetic variants were categorized according to in-silico annotation tiers and minor allele frequency. Noncoding regions were scored using Regenie's burden test "max" algorithm under a dominant inheritance model (1). We used the Cochran-Mantel-Haenszel (CMH) test to jointly analyze both cohorts (2). Evidence across Tiers and MAFs was combined using ACAT (3). Significance threshold was defined using the minP method to account for correlation between test statistics of neighbouring regions and a parametric Monte Carlo simulation using 25 principal components to account for potential unknown confounders. Despite the selection criteria, ~4% cases harbored pathogenic HNF1A, HNF4A, GCK mutations, providing cases to benchmark analytical pipelines. Validations were sought in young-onset non-obese patients in UK Biobank. The CMH-ACAT method yielded a high signal-to-noise ratio based on known MODY genes, with significant regions showing high concordance across cohorts, tiers and MAF levels. The minP method combined with the parametric Monte-Carlo simulation allowed us to obtain a significance threshold when test statistics are correlated and cohorts are multi-center.

CONCLUSIONS

The CMH-ACAT workflow with a significance level defined via simulation by the minP and parametric bootstrap is a promising method to circumvent potential confounding and ascertainment bias in studies aimed at discovering regions harbouring disease-associated burden of rare/ultrarare variants.

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THEMATIC AREA 03

HUMAN ISLET MODELLING 03

18 abstracts in this area

P.40 Chaperone mediated autophagy regulates NGN3 stability and enhances stem cell-derived beta cell generation

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POSTER Keywords: *endocrine progenitor autophagy chaperone iPSC*

BACKGROUND AND OBJECTIVES

NGN3 is the master regulator of endocrine pancreas development, and both the timing and amplitude of its expression are critical for effective beta cell formation. Even modest changes in NGN3 stability can markedly alter endocrine output. We previously1 identified an interaction between NGN3 and HSPA8, a central component of chaperone mediated autophagy (CMA), which targets proteins bearing KFERQ like motifs for lysosomal degradation. We therefore investigate whether CMA plays a role in NGN3 turnover and whether pharmacological modulation of this pathway can enhance beta cell generation from human induced pluripotent stem cells.

METHODS

To dissect the mechanisms regulating NGN3 stability, we pharmacologically inhibited lysosomal function, HSPA8-mediated CMA, and macroautophagy and checked the impact on NGN3 stability and interactive properties. We used selective protein kinase C (PKC) inhibitors to explore KFERQ phosphorylation dependent targeting of NGN3 to CMA. These compounds were applied during the defined NGN3 expression window during iPSC to beta cell differentiation. NGN3 protein levels, endocrine progenitor numbers, and beta cell output were quantified by immunofluorescence and lineage marker analysis.

RESULTS

In cells expressing exogenous NGN3, HSPA8 interacted with and promoted NGN3 destabilisation, supporting a functional link between NGN3 and CMA. Lysosomal inhibition increased NGN3 abundance and enhanced its association with HSPA8, consistent with active lysosomal targeting. Specific PKC inhibitors markedly stabilised NGN3 in HEK293, implicating an atypical PKC isoform in phosphorylation dependent engagement of CMA. In differentiating iPSCs, inhibition at multiple levels of the CMA pathway, including PKC activity, HSPA8 function, and lysosomal degradation, consistently stabilised endogenous NGN3 expression and significantly increased beta cell yield. Inhibition of macroautophagy similarly expanded the NGN3+ progenitor pool and enhanced beta cell production, suggesting coordinated lysosomal control of endocrine specification. Conclusions and Implications These findings position CMA as a previously unrecognised regulator of NGN3 stability and a critical checkpoint in human endocrine differentiation. Transiently limiting lysosomal degradation stabilises NGN3 and translates into a measurable increase in beta cell generation. Targeting CMA and related

lysosomal pathways therefore represents a rational strategy to improve the efficiency and robustness of stem cell derived beta cell production.

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P.41 Deciphering the regulatory role of a long non-coding RNA in pancreatic endocrine development and diabetes

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ORAL COMMUNICATION Keywords: *ONECUT1 regulation; long non-coding RNA (lncRNA); pancreatic endocrine development; Human embryonic stem*

cells (hESCs); in vitro pancreatic differentiation Gene regulatory networks play a vital role in pancreatic development and function, yet the pathomechanisms associated with the non-coding genome are only beginning to emerge. Our recent work demonstrated that mutations in the ONECUT1 gene cause a new neonatal diabetes syndrome in homozygous patients, whereas heterozygous carriers develop early-onset diabetes1. Stage-specific characterization of in vitro differentiated human embryonic stem cells (hESCs) further revealed that ONECUT1 plays a critical role in transcriptional regulation during pancreatic progenitor formation2. Nonetheless, the complex regulation of ONECUT1 expression remains only partly understood3. While investigating the ONECUT1 locus, we identified a variant most strongly associated with T2D (rs2440374), located within the lncRNA RP11-209K10.2 (lncRNA-RP11) approximately 11 kbp from ONECUT1. The GTEx Portal shows decreased lncRNA-RP11 expression in the pancreas in the presence of the T2D-associated allele, and this SNP disrupts a binding motif for the transcription factor NKX2.2, which is essential for endocrine pancreas development. These findings led us to hypothesize a regulatory role for this non-coding region. Following deletion of the lncRNA-RP11 gene and in vitro differentiation, pancreatic progenitor (PP) formation was not impaired, but stem-cell-derived islets generated from these progenitors exhibited increased alpha-cell number and function; however, no significant loss of ONECUT1 expression was detected. Interestingly, our omics data revealed transcription factor binding peaks in the promoter region of lncRNA-RP11. Since promoters of lncRNAs can also act as enhancer elements regulating neighboring genes, we anticipated that the lncRNA-RP11 promoter may regulate ONECUT1. Luciferase enhancer reporter assays demonstrated that the promoter region exhibits enhancer activity, which is further increased by ONECUT1 or NKX2.2 binding, supporting its function as an enhancer during pancreatic development. To achieve a more refined analysis, we deleted the lncRNA-RP11 promoter region. Although PP formation is not disturbed, expression of markers ONECUT1, NKX6.1 and PDX1 is significantly increased suggesting repressive function of this region. These hESCs are currently being differentiated into pancreatic endocrine cells to assess the impact on lineage specification and beta-cell function. Together, this characterization will provide insights into the role of the lncRNA-RP11 locus in endocrine development and

pathophysiology, thereby advancing our understanding of diabetes-related transcriptional regulation.

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P.42 Mapping the MAFA interactome in sc-islets

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POSTER Keywords: *MAFA, sc-islets, proteomics, RNA processing, diabetes*

BACKGROUND & OBJECTIVES

Stem cell–derived islets (sc-islets) represent a promising cell replacement therapy for diabetes; however, they do not fully recapitulate the functional and transcriptional maturity of primary human islets. MAFA is a key transcription factor that maintains mature pancreatic β-cell function by regulating glucose-stimulated insulin gene transcription. The S64F MAFA variant, a rare gain-of-function mutation, blocks phosphorylation-dependent degradation of MAFA, causing protein stabilization and dysregulated insulin transcription. This causes inappropriate βcell activity leading to diabetes (mainly in males) or insulinomatosis (mainly in females).1 However, its effects on MAFAassociated protein networks remain poorly defined. Our objective is to systematically map MAFA-associated protein networks and define how WT and S64F MAFA differentially shape the β-cell interactome to uncover novel regulatory mechanisms and therapeutic entry points.

METHODS

We performed immunoprecipitation of doxycycline-inducible WT or S64F MAFA in sc-islets followed by quantitative proteomic analysis using LC–MS/MS.2 Differentially associated proteins were identified and analyzed using pathway enrichment approaches to define functional interaction networks.

RESULTS

WT MAFA preferentially associated with proteins involved in mitochondrial gene expression and translation, as well as amino acid metabolic and biosynthetic processes (FDR < 0.05), with additional enrichment of RNA-processing pathways. It is consistent with its role in supporting β-cell metabolic competence. In contrast, the S64F variant showed reduced enrichment of these metabolic pathways and instead displayed strong associations with RNA-processing networks, including mRNA splicing via the spliceosome, ribonucleoprotein complex assembly, and mRNA export and transport (FDR < 0.05). These findings indicate a relative shift in the MAFA-associated interactome, from a predominantly metabolicsupportive network in WT cells toward increased engagement with RNA-processing and post-transcriptional regulatory pathways in S64F-expressing cells.

CONCLUSION

We propose that the S64F mutation functionally redirects MAFA from its canonical role in maintaining β-cell metabolic gene programs towards increased interactions with RNA-processing machinery. This rewiring may alter RNA splicing and mRNA processing, thereby disrupting the expression of genes required for β-cell metabolic competence. Such changes may promote transcriptional dysregulation and cellular stress, ultimately contributing to loss of β-cell identity and function.

P.43 MAST1 dynamics in iPSC-derived pancreatic progenitors

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POSTER Keywords: *Beta cell development, MAST1, USP7*

Human induced pluripotent stem cells (iPSCs) provide a powerful platform to model human pancreatic development and generate functional beta cells for diabetes therapy. Despite substantial advances in differentiation protocols, the protein regulatory networks that govern the transition from pancreatic progenitors to mature beta cells remain incompletely understood. A deeper understanding of these mechanisms is essential to improve differentiation efficiency and cell functionality. We previously identified the deubiquitinase USP7 as a critical regulator of pancreatic progenitor to beta cell differentiation, highlighting the importance of ubiquitin mediated protein homeostasis in this process. Here, using co immunoprecipitation coupled with quantitative proteomics, we identify Microtubule associated serine threonine kinase 1 (MAST1) as a novel USP7 interacting protein. While MAST1 has been implicated in neuronal differentiation, its role in pancreatic development has not been explored. In this study, we dissect the USP7 MAST1 regulatory axis and investigate how USP7 controls MAST1 protein dynamics. Using HEK293T cells, we establish the molecular interaction between USP7 and MAST1 and define how USP7 modulates MAST1 stability. We then extend these findings to human iPSC derived pancreatic organoids, where we examine the functional consequences of altering MAST1 stability during differentiation. Specifically, we assess how USP7 dependent stabilisation or destabilisation of MAST1 influences the progression from pancreatic progenitors to beta-like cells. Together, our findings uncover a previously unrecognised regulatory pathway linking deubiquitination to kinase signalling during human pancreatic differentiation. This work provides new mechanistic insight into protein homeostasis in beta cell development and offers a potential avenue to enhance stem cell-based strategies for diabetes modelling and cell replacement therapy.

P.44 Identification of RXRG as a novel regulator of adult human beta cell function

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ORAL COMMUNICATION

BACKGROUND & OBJECTIVES

Pancreatic β cells acquire their terminal maturation only after birth, guided by signalresponsive transcription factors (TFs) that become active postnatally. The identity and activity of these age-dependent TFs in human β cells remain only partially understood, largely due to differences with mice. Here, we aimed to identify and functionally characterize novel TFs specifically involved in adult human β cell maturation.

METHODS

We integrated publicly available single-cell and bulk RNA-seq datasets from prenatal and adult human islets. We prioritized TFs specifically expressed in β cells compared to α cells that are upregulated in adult primary β cells relative to stem cell-derived (SC-)β cells. This analysis identified retinoid X receptor γ (RXRG) as a candidate regulator of human β cell maturation that is not expressed in mouse β cells. To investigate the role of RXRG in human islets, we performed lentiviralmediated RXRG knockdown (KD) in primary human islet cells reaggregated into pseudoislets. We assessed β cell function by measuring glucose-stimulated insulin secretion (GSIS) in vitro and performing an intraperitoneal glucose tolerance test (IPGTT) following transplantation into immunocompromised mice. We are currently performing β cell RNA-seq and wholeislet proteomics to identify RXRG downstream targets.

RESULTS

RXRG KD pseudoislets showed impaired GSIS and reduced expression of β cell maturation markers, including MAFA and INS, but no change in total insulin content in vitro. Transplantation of RXRG KD pseudoislets into mice resulted in blunted human insulin secretion and elevated blood glucose compared to controls, confirming that RXRG is required for β cell function. Ongoing transcriptomic and proteomic studies are defining the downstream targets of RXRG in adult human β cells, which are enriched for pathways such as calcium signaling, regulation of cell cycle and response to retinoic acid. Conclusions & Implications: Our findings identify RXRG as a novel transcriptional regulator required for adult human β cell maturation and function. These results provide new insight into species-specific regulatory mechanism and may impact the therapeutic possibilities for Type 1 and Type 2 Diabetic patients.

P.45 The transcription factor SIM1 directs pancreatic endocrine development

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POSTER Keywords: *SC-islet differentiation, transcription factors, pancreatic development*

BACKGROUND AND OBJECTIVES

SIM1 is a bHLH-PAS transcription factor expressed in hypothalamus, where it contributes to neuronal development [1]. Heterozygous SIM1 mutations in humans and mice are associated with severe, early-onset obesity [2]. Through bioinformatic and transcriptomic analyses, we have identified SIM1 expression within adult pancreatic islets, and in the developing pancreas. Despite this expression pattern, the role of SIM1 within pancreatic development remains to be elucidated.

METHODS

The Akerman lab is now able to produce stem cell-islets (SC-islets) that can partially recapitulate the functional and metabolic landscape of endogenous islet cells, providing a valuable resource for modeling pancreatic development in vitro. We aimed to utilise this model to explore the role of SIM1 within beta cell differentiation. To do this, we generated SIM1 knockout stem cells using CRISPR-Cas9 and differentiated SIM1+/+ and SIM1-/- stem cells to SC-islet stage. We conducted transcriptomic and functional analyses on SIM1-/- and SIM1+/+ SC-islets throughout differentiation to understand the phenotype of SIM1 deficiency on pancreatic development.

RESULTS

Our results suggest that SIM1 loss of function impairs endocrine pancreas development. Within SIM1-/- SC-islets, we observed a reduction in expression of INS-GFP, as well as key regulators of the endocrine cell fate, ISL1 and MAFB. Transcriptomic analyses display altered cell-type distribution between SIM1-/- and SIM1+/+ SC-islets, and an altered transcriptional landscape within SIM1-/- beta cells, highlighting the role of SIM1 in beta cell differentiation and maturation. Furthermore, calcium imaging displays blunted glucose-stimulated insulin secretion in SIM1-/- SC-islets, suggesting functional deficiencies accompany the transcriptomic differences observed between wild-type and SIM1-/- SC-islets. Conclusions and Implications Taken together, our results reveal that SIM1 plays an essential role in pancreatic beta cell development and maturation, identifying SIM1 as a key regulator of endocrine pancreatic development. The manipulation of SIM1 within stem cell to beta cell differentiation protocols could therefore provide a strategy to increase beta cell numbers within SC-islets.

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P.46 Beta cell maturation is accelerated in vascularized SC-islets derived exclusively from hPSCs

Yi-Ting Lee

POSTER

Human pluripotent stem cell (hPSC)-derived pancreatic islets (SC-islets) could overcome the limited supply of donor islets for diabetes therapy. Incomplete β-cell maturation and the persistence of irrelevant cell types are important current limitations of SC-islets. Islet vascular cells comprise endothelial cells and pericytes and are essential for endocrine specification and βcell functionality. Vascular cells generate the extracellular matrix of the islet and secrete signalling molecules thus promoting β-cell functionality and survival. Despite their importance, their timely inclusion in differentiating SC-islets has not been explored and this work addresses this important gap. We have developed robust procedures for the generation of very highly enriched pancreatic progenitors and essentially pure pericytes and endothelial cells. To maximize the yield in PDX1+/SOX9+/NKX6.1+ PP cells, we introduced changes in both early and PP specification steps. Single-cell RNA Seq confirmed a highly synchronous and efficient differentiation process. To generate vascular cells, we adapted a protocol that takes advantage of their common developmental origin. The resulting pericyte and endothelial progenitors are separated by magnetic-activated cell sorting and further differentiated into pure pericytes and endothelial cells. These procedures are applicable to different hPSC lines and allow the independent derivation and cryopreservation of PPs and vascular cells from the same hPSC line. To closely mimic islet development we clustered together endothelial cells, pericytes and pancreatic progenitors, before proceeding with the final differentiation stages. We established the optimal starting ratio of pancreatic progenitors : pericytes : endothelial cells, the initial amount of vascular medium and the rate of its progressive reduction during the last differentiation stages. Gene expression and immunofluorescence analyses showed that this approach allowed the maintenance of both types of vascular cells in what we named vascularized SC-islets (vSC-islets). Strikingly, vSC-islets responded much more robustly to glucose and incretins indicating that VCs accelerated the maturation of β-cells in the clusters. The establishment of vascularized SC-islets will allow the analysis of the integrated development of human vascular and pancreatic endocrine cells, the implication of vascular cells in β-cell dysfunction, and the potential of vSC-islets in advanced diabetes cell therapies.

P.47 Type 2 diabetes-associated ZnT8/SLC30A8 variants induce proteomic changes associated with lowered apoptosis in human embryonic stem cell–derived islets

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ORAL COMMUNICATION Keywords: *Stem cell islet, Type 2 Diabetes, Zinc, Zinc transporter, SLC30A8*

BACKGROUND

Loss-of-function mutations in SLC30A8, encoding the zinc ion (Zn2+) transporter ZnT8 in pancreatic beta cells, protect against apoptosis induced by Zn2+ depletion, a process associated with type 2 diabetes (T2D)1,2. The mechanisms underlying this protection remain unclear. We investigate the impact of intracellular Zn2+ depletion on the survival and function of human stem cell-derived islets and the effect of the protective R138X variant.

METHODS

Human embryonic stem cells INS(GFP/w) (MEL1) and CRISPR/Cas9-derived R138X lines were differentiated into stem cell islets1. Intracellular Zn2+-depletion was induced using N,N,N′,N′-tetrakis(2-pyridylmethyl)-1,2-ethanediamine (TPEN, 1 µM, 48h). Apoptosis was assessed by TUNEL staining and protein expression by immunofluorescence. Glucose-stimulated Ca2+ signaling was measured using Cal590 and insulin secretion by time-resolved fluorescence. Bulk mRNA sequencing and LC-MS/MS proteomics were performed.

RESULTS

Zn2+ depletion increased apoptosis in wild-type (WT) islets (p=0.0068), whereas R138X islets were protected (p<0.02). R138X+/- islets showed a mild increase in GCG+ cells (p = 0.0481), R138X+/+ islets exhibited increased NKX6.1+ cells (p=0.0017), without affecting INS+/GCG+ or INS+/NKX6.1+ populations. These changes were reversed under Zn2+-depleted conditions, indicating Zn2+-dependent remodeling of endocrine cell composition. Transcriptomic and proteomic analyses revealed upregulation of pathways related to vesicle trafficking, secretion, Ca2+ signaling and mitochondrial metabolism, consistent with enhanced glucose-stimulated insulin secretion in R138X+/+ islets under Zn2+-depleted conditions. Conversely, pathways linked to extracellular matrix remodeling, metal-handling, apoptosis and cellular stress were downregulated. R138X islets displayed lowered Ca2+ signaling, decreased area under the curve (p<0.0001) and oscillation amplitude (p=0.0002), but increased frequency (p<0.0001) compared to WT islets. These differences were reversed by TPEN. TPENtreated WT islets showed decreased AUC (p=0.0168) and amplitude (p<0.0012), indicating beta cell dysfunction.

CONCLUSION

Intracellular Zn2+ depletion compromises stem cell islet identity and function, while the SLC30A8 R138X variant protects beta cells from these effects. These findings highlight the therapeutic potential of targeting ZnT8 in T2D and support its relevance for improving cell-based therapies.

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P.48 β-Cell Obligation in α-Cell Glucagon Response to Low Glucose

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ORAL COMMUNICATION Keywords: *Type 1 Diabetes, Alpha-cells, Beta-cells, Glucagon, Hypoglycemia*

BACKGROUND & OBJECTIVES

Pancreatic α-cells safeguard against hypoglycemia by secreting glucagon in response to low glucose. In Type 1 diabetes (T1D), this counterregulatory response is severely impaired, contributing to life-threatening hypoglycemia. Although α-cell dysfunction is well documented, it remains unclear whether this reflects intrinsic defects or disruption of intra-islet signaling. Here, we tested the hypothesis that α-cell counterregulation is not cell-autonomous but depends on β-cell input within the islet microenvironment.

METHODS

Human pseudoislets were generated from FACS-purified α- and β-cells from non-diabetic donors, enabling controlled reconstruction of defined islet cellular compositions. Glucagon secretion was measured following a shift from basal (5.6 mM) to hypoglycemic (3 mM) glucose in monotypic α-, mixed αβ-, and polytypic pseudoislets (containing all islet cell types). In parallel, transgenic mice enabling inducible, cell type–specific ablation of islet cells (RIP-DTR, SST-DTR and PPY-DTR) were used to generate α-only, β-cell–deficient, and partially β-cell–reconstituted islets with minimal residual β-cells. Counterregulatory glucagon responses were assessed in vivo under controlled hypoglycemic conditions.

RESULTS

Monotypic α-pseudoislets failed to mount appropriate glucagon responses to low glucose despite preserved responsiveness to non-glucose stimuli, demonstrating a selective defect in glucose-dependent secretion. Reintroduction of βcells fully restored α-cell counterregulatory responses, recapitulating physiological regulation. In vivo, β-cell ablation abolished glucagon responses to hypoglycemia across multiple islet configurations, whereas preservation of even a minimal β-cell population (~1–5 cells per islet) was sufficient to maintain robust counterregulation. Across both human and mouse models, loss of β-cells specifically accounted for the failure of hypoglycemia-induced glucagon release, while broader dysregulation of glucose-dependent secretion was also observed in isolated α-cells. Conclusions & Implications: These findings demonstrate that hypoglycemia-induced glucagon secretion is not an intrinsic property of α-cells but an emergent

function of the islet network requiring β-cell input. β-cell loss alone is sufficient to drive counterregulatory failure, identifying disrupted α–β cell communication, rather than intrinsic α-cell dysfunction, as the primary mechanism underlying impaired glucagon secretion in T1D. This work reframes hypoglycemia risk as a failure of islet network integrity and highlights preservation or restoration of intra-islet β-cell signaling as a key therapeutic target for improving glycemic stability.

P.49 Multiplexed Analysis of Donor Variation in Human β-Cell Proliferation and Function Using a Scalable, Physiologically Relevant, and High-Precision Platform

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POSTER Keywords: *proliferation, human islet model, spheroids, physiological models*

Limitations in the translatability of cell line and animal models have fueled a longstanding question in the field: can adult human β-cells proliferate? Primary human islets present unique experimental challenges for proliferation studies: intact islets suffer a rapid loss in viability and function, while dispersed cells dedifferentiate and hyperproliferate. Previously, we developed a novel, high-throughput-compatible methodology to assess changes in β-cell proliferation in reaggregated primary human islet microtissues (hIsMTs). Islet MTs represent a uniform and functionally robust in vitro primary islet model that maintains native-like endocrine composition and cell-cell interactions, which are critical for physiological functional and proliferative responses. Notably, their long-term stability (>28 days) uniquely facilitates assessment of β-cell proliferation and resulting changes in β-cell mass over time. We leveraged our platform's multiplexed high content confocal microscopy and functional endpoints to evaluate the effects of high glucose and harmine treatments on β-cells in more than 20 human islet donors. Staining of DAPI (nuclear marker), NKX6.1 (β-cell marker), and EdU (proliferation marker) and custom CellPathFinder pipeline analyses yielded precise β-cell detection across various stress conditions, enabling the identification of rare β-cell proliferation events. Our approach identified low to no human β-cell proliferation under basal conditions (0-0.2%) as previously reported, a moderate induction following culture in high glucose (8 mM), and a significant enhancement with 4-day harmine treatment (up to 1.3%). Proliferative capacity varied by donor, exhibiting significant negative correlation (p=0.03) with donor age and a trend in positive correlation with β-cell fraction. Furthermore, while harmine treatment induced more non-β-cell than β-cell proliferation, it nevertheless increased glucose-stimulated insulin secretion and fold induction of insulin secretion. Lastly, low-input transcriptomic analysis from just 3 hIsMTs per sample revealed significant upregulation of proliferation-associated transcripts with harmine treatment. In summary, using a standardized 3D primary human islet platform, we identified trends in proliferative capacity of human βcells correlated with donor characteristics such as age. Our

hIsMT platform's physiological relevance, extended culture window, and moderate-throughput capacity enables the robust study of novel drug candidates, as well as the fundamental processes leading to β-cell proliferation.

P.50 Lipidomic and proteomic analyses of human primary and stem cell-derived islets reveals differences in lipid composition and fuel-use pathways

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POSTER Keywords: *Stem cell islets; lipidomics; metabolism*

BACKGROUND & OBJECTIVES

Human embryonic stem cell (hESC)-derived islet clusters lack functional maturity and have a diminished insulin secretion response to glucose, yet a robust insulin secretion response to lipids. Endogenous lipid metabolism and lipid-signaling pathways, however, remain poorly characterized within hESC-derived islets. Thus, we aim to characterize the role of endogenous lipids in hESC-derived islet function (or lack thereof) across stages of maturation in comparison to primary human islets.

METHODS

We utilized lipidomics and proteomics to investigate differences in lipid composition and pathway enrichment to elucidate putative mechanistic drivers for functional differences between hESC-derived islet clusters and islets from primary human donors.

RESULTS

Lipidomics identified 403 lipid features common to both cell types from a total of 416 identified in primary human islets and 781 in hESC-derived islet clusters. Further, hESC-derived islet clusters showed elevated abundance of triacylglycerols and sterols relative to the total lipid pool. Specifically, more mature clusters had the greatest relative accumulation (28.9%) compared to younger (16.4%) clusters and primary human islets (2.4%). Fittingly, hESC-derived islet proteomics revealed enrichment for proteins related to cholesterol biosynthesis, perhaps indicative of lipid sequestration. Proteins related to mitochondrial fatty acid oxidation were significantly altered between hESC-derived islet clusters and human donor islets that may highlight an impairment in mitochondrial fuel use and switching in the clusters. Ongoing work assessing metabolic flux in mitochondria characterizes functional differences that may underlie differences in insulin secretion responses. Conclusions & Implications: Consistent disparities across omics modalities offer insights of a sub-optimal phenotype of hESCderived clusters compared to primary islets and can identify tangible targets to modulate for improved function.

Together, characterization and manipulation of these lipid-signaling pathways could be utilized to improve maturation and insulin secretion function, ultimately refining these cells for clinical application.

P.51 Alpha cell impact on stem cell-derived pseudoislet function

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POSTER Keywords: *pseudoislet, alpha cell, beta cell, preproglucagon, stem cell*

BACKGROUND AND OBJECTIVES

Islets are endocrine cell clusters which release hormones to regulate glycemia and nutrient uptake. Islets contain five endocrine cell types, of which alpha and beta cells comprise ~90% of islet mass. Cell type ratios vary between species, individuals, and healthy and diabetic states. As these cells communicate via physical and hormonal interactions, variations in these ratios are likely critical to islet function. Our objective was to determine how islet alpha cell proportion influences insulin secretion.

METHODS

We used stem cell-derived alpha and beta cells with genetically encoded insulin and glucagon reporters to make pseudoislets with controlled sizes (500, 1500, 3000, 6000, and 9000 cells) and cell ratios (beta/alpha; 100/0, 80/20, 60/40, and 40/60) to study their impact on insulin secretion. We tested glucose stimulated insulin secretion (GSIS) under standard conditions and with norepinephrine added. Standard in vitro testing focuses solely on the effects of glucose; however, many stimuli affect both alpha and beta cells. Norepinephrine stimulates glucagon but not insulin secretion. We have also developed and used preproglucagon knockout dual reporter cell lines (GCGKO) to study the loss of preproglucagon on islet function without removing alpha cells.

RESULTS

Under standard conditions, pseudoislets with 6000 cells and 20% alpha cells had the greatest stimulation index (SI: high glucose / low glucose insulin release). With norepinephrine, SI increased when alpha cells were added, with a trend for increasing SI with increasing alpha cell ratio (1.82 (beta/alpha 100/0), 2.24 (80/20), 2.38 (60/40), and 2.57 (40/60)). GCGKOpseudoislets responded similarly to glucose alone but released significantly more insulin in response to glucagon and Exendin-4 (a GLP1R agonist) stimulation in high glucose media compared to controls with functional preproglucagon. Conclusions and Implications: Alpha cells improve pseudoislet function, but the ideal ratio differs when norepinephrine is added. Furthermore, pseudoislets containing 6000 cells have greater SIs than other sizes tested. These findings suggest future in vitro studies on alpha cell incorporation into pseudoislets, should test in vivo relevant stimuli beyond glucose alone and use 6000 cell pseudoislets for improved responses. Lastly, preproglucagon absence seems to increase sensitivity to glucagon and Exendin-4.

P.52 Increased proportion and maturation of alpha cells improve the functionality of stem cell-derived islets

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ORAL COMMUNICATION Keywords: *SC-islets, transcriptomic analysis, alpha cells*

BACKGROUND & OBJECTIVES

The global rise in diabetes underscores the need for innovative therapeutic strategies that extend beyond glucose-lowering approaches to restore and replace functional pancreatic islets. Although stem cell–derived islets (SC-islets) have advanced considerably, they remain transcriptionally and metabolically distinct from primary human islets. Notably, glucagonproducing alpha cells are under-characterized, and their contribution to islet maturation and function remains poorly understood. This study aims to elucidate the role of alpha cells in SC-islet maturation and functionality.

METHODS

Single-cell RNA sequencing (scRNA-seq) datasets from fetal alpha and beta cells were compared with SC-islet cells across endocrine maturation. A dual-reporter stem cell line expressing insulin (GFP) and glucagon (mScarlet) was used to isolate beta and alpha cells via fluorescence-activated cell sorting (FACS). Sorted cells were reaggregated into pseudo-islets at defined proportions and maturation stages. Functional assessment included glucose-stimulated responses and mitochondrial activity measurements.

RESULTS

Pearson correlation analysis was used to compare the developmental trajectory of fetal alpha cells to SC-alpha cells and fetal beta cells and SC-beta cells. This analysis revealed a lower correlation between fetal (10-18 weeks post conception) and SC-islets for alpha cells compared to beta cells, indicating reduced maturation of alpha cells during differentiation. Consistently, monohormonal alpha cells emerged later than beta cells in stage 7 of SC-islet differentiation (n = 2–4). Reaggregated pseudo-islets containing 70% beta cells and 30% alpha cells exhibited enhanced glucose and KCl responsiveness compared to 100% beta aggregates (n = 1). Furthermore, incorporation of more mature alpha cells, which respond to hypoglycemia and adrenaline, into pseudo-islets improved glucose responsiveness and increased oxygen consumption in immature beta cells (n = 1). These findings highlight a functional role for alpha cell maturity in islet performance. Conclusions & Implications: These findings indicate that alpha cells are transcriptionally less mature in our standard SC-islet differentiation protocol and are likely underappreciated contributors to islet functionality. Importantly, mature alpha cells enhance beta cell performance, suggesting that optimizing alpha cell maturation could significantly improve SC-islet general function. Refinement of differentiation strategies to promote alpha cell maturation may enable the generation of more physiologically relevant islets for therapeutic applications.

P.53 AAV-KP1 facilitates high efficiency transduction in reaggregated human islets with minimal functional impact

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POSTER Keywords: *Adeno-associated virus; Human islet microtissues; Gene knockdown; pancreatic β-cells*

Challenges to genetic manipulation of primary human islets include limited vector penetration into the islet core, tropism, and potential vector-induced impairment of β-cell function. In this study we identified adeno-associated virus (AAV) capsids that achieve high transduction and transgene expression in human islet microtissues (hIsMTs) while preserving β-cell function. We furthermore validated the lead candidate vector for sustaining gene knockdown under standard and disease conditions. hIsMTs of uniform size and homogeneous endocrine composition were generated from dispersed primary human islet cells and transduced during reaggregation with a panel of natural and engineered capsids encoding GFP. Transduction efficiency, transgene expression and cell counts were quantified using 3D high-content confocal imaging. β-cell function and islet viability were assessed by glucose-stimulated insulin secretion and quantification of intracellular insulin and intracellular ATP content, respectively. Knockdown efficiency of the long non-coding RNA MEG3 was tested with AAV-KP1 under standard and pro-inflammatory conditions. Finally, we evaluated the functional impact of AAV-KP1 encoding a non-targeting shRNA in hIsMTs generated from nine human donors. While most capsids achieved high transduction, AAV-KP1, AAV2.7m8, AAV2.NN, and AAV6 displayed high transduced cell fractions with robust GFP expression, without reducing total or β-cell numbers. Notably, these AAVs significantly outperformed the commonly used AAV8. AAV-KP1 was of particular interest based on its dose-independent performance, demonstrating robust transduction efficiency and transgene expression across low and high MOIs. Furthermore, AAV-KP1mediated delivery of shRNA achieved up to 91% knockdown of MEG3. Functional validation across hIsMTs generated from nine human donors showed that AAV-KP1 transduction was well-tolerated, with only a minor decrease in stimulated insulin secretion (-11.8%) and no effect on the stimulation index, insulin storage, or ATP levels of hIsMTs. AAV-KP1 supports high-efficiency gene delivery and shRNA-mediated knockdown in 3D human islet microtissues with preserved viability and minimal impact on β-cell secretory function. This platform provides a practical tool for systematic and sustained knockdown or overexpression studies in primary human islets and a benchmark for the study of AAV vectors for diabetes research and therapeutics development.

P.54 ETVs direct hPSC fate by regulating biophysical properties.

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POSTER Keywords: *ETV transcription factors, Human pluripotent stem cells (hPSCs), Cell differentiation, Biophysical properties, Cell fate*

regulation

BACKGROUND & OBJECTIVES

Pancreatic endocrine development depends on branching morphogenesis, a process in which progenitor cells delaminate and reorganize to form the islets of Langerhans. This morphogenetic remodeling requires tight coordination between transcriptional networks and cytoskeletal dynamics.

METHODS

Here, we use human pluripotent stem cells (hPSCs) and two morphogenesis models, gastruloids and directed pancreatic differentiation, to identify ETS Variant Transcription Factors (ETVs) as key regulators of biophysical properties and lineage specification.

RESULTS

Genetic ablation of ETV1, or combined loss of ETV1/ETV4/ETV5, enhances cell-cell and cell-ECM adhesion, resulting in aberrant multilineage differentiation, disrupted germ layer organization, loss of ectoderm, and overgrowth of extraembryonic cells in gastruloids. In pancreatic differentiation, ETV1 deletion abolishes pancreatic progenitor formation. Single-cell RNA sequencing and follow-up assays reveal dysregulated mechanotransduction via the PI3K/AKT pathway and enrichment of genes associated with cytoskeletal organization. Conclusions & Implications: These findings suggest that ETV1 regulates key morphogenetic events during endocrine progenitor specification by modulating cell adhesion and cytoskeletal remodeling.

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P.55 Inducible gene expression platforms for pancreatic endocrine differentiation

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POSTER Keywords: *Stem cell-derived islets, inducible gene expression, lentivirus, CRISPR activation*

BACKGROUND & OBJECTIVES

Protocols to differentiate pluripotent stem cell to pancreatic islets are widely used for diabetes modeling and the development of islet replacement therapy. However, dissecting gene function during differentiation remains challenging as many genes act in a stage- and dose-specific manner. Existing approaches are limited by transgene silencing during differentiation and by toxicity resulting from non-physiological overexpression. To address these limitations, we developed two independent tools that enable (i) temporal-controlled gene activation or (ii) cell type-specific and dosagecontrolled overexpression of the gene of interest.

METHODS

The first tool enables gene activation during differentiation and is based on a stem cell line harboring a dCas9VP192 transcriptional activator integrated into a safe harbor locus. The activator protein is fused to a destabilizing domain, which is degraded under normal conditions but stabilized upon trimethoprim addition, allowing temporal stabilization and reduction of toxicity. Constitutive GFP expression enables tracking of construct expression, while WPRE and UCOE elements enable stable gene expression and reduce transcriptional silencing. The second tool is a lentiviral-based system in which the gene of interest is driven by a doxycycline-inducible Tet-on system under control of the human insulin promoter. This design restricts expression to beta cells and allows precise dosage control through titration of doxycycline concentration and viral multiplicity of infection.

RESULTS

We successfully showed that the CRISPR activation system is transcribed during differentiation to endocrine progenitors and enables effective gene activation at different stages. The second approach showed that we can generate high titer lentiviruses and efficiently transduce SC-islets. Gene expression was shown to be beta cell specific and induced only upon doxycycline addition. Importantly, expression levels could be fine-tuned by adjusting viral dose and doxycycline concentration. Conclusions & Implications: We present two independent platforms for controlled gene expression during pancreatic endocrine differentiation. One enables temporal controlled CRISPR-based gene activation during differentiation which opens possibilities for high-throughput CRISPR screens, while the other allows dose-controlled and beta cell specific gene expression in SC-islets. These two orthogonal systems provide new opportunities to dissect stage- and dose-dependent gene function to advance our understanding of beta cell development and function in health and disease.

P.56 Modelling HNF4A-MODY1 using SC-derived islets

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POSTER Keywords: *HNF4a, MODY1, SC-islets, ECM*

Bsckground & objectives Maturity-onset diabetes of the young 1 (MODY1) is a rare monogenic form of diabetes caused by heterozygous mutations in the transcription factor–encoding gene HNF4A. The disease is characterized by impaired insulin secretion and altered metabolic regulation. Standard treatment relies on sulfonylureas, which carry a risk of hypoglycemia. However, limited availability of patient material and the incomplete understanding of disease mechanisms have hindered the development of improved therapeutic strategies. To address this gap in knowledge, we generated HNF4A-deficient human embryonic stem cell (SC) lines and assessed their cell type composition and functional impairments after differentiation into SC-islets.

METHODS

To model the effect of the HNF4A R141X mutation on pancreatic development and function, ESC lines carrying the R141X mutation were created using CRISPR-Cas9. Upon differentiation into SC-islets, endocrine cell populations were quantified and functional responses to established stimulation paradigms were assessed. In addition, bulk proteomics was performed to characterize molecular changes of the generated SC- islets.

RESULTS

R141X islets exhibited reduced INS+/NKX6.1+ β-like and SST+ δ-like cell fractions, while NKX6.1+ and GCG+ α-like cell populations remained stable. Functionally, heterozygous R141X SC-islets displayed slightly increased basal hypersecretion of C-peptide and largely preserved secretory response to high glucose, but reduced response to Exendin-4 compared to the control. Proteomic analysis revealed downregulation of multiple ECM components, lipid metabolism, several members of the SERPIN family and hormones SST and GHRL in HNF4A islets. Conversely, several presynaptic and Ca2+-dependent proteins, as well as the maturity marker IAPP were upregulated. Conclusions & Implications Our findings provide novel insights into the pathophysiology of MODY1 by identifying a developmental defect of endocrine cell types and altered islet composition as well as secretory impairments, specifically in response to Exendin-4. Together, the results underline the critical role of HNF4α in islet development and function, and suggest the need for deeper investigation into the regulatory networks underlying MODY1. More in-depth analysis of the involved gene regulatory networks, as well as the molecular changes underlying the secretion effect are required to fully understand the effect of HNF4A-MODY on islet architecture and function.

P.57 Synthetic Peptide Growth Factors featuring KGF Alternative Peptide

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POSTER Keywords: *#regenerative medicine #cell therapy #growthfactor #KGF #FGF7*

Keratinocyte Growth Factor (KGF, also known as FGF7) is a member of the FGF family that binds to FGFR2b and promotes the proliferation and differentiation of various endoderm-derived cells, including pancreatic islet cells, alveolar epithelial cells, and hepatocytes. In particular, it plays a key role in inducing the differentiation of pluripotent stem cells into islet-like cells, making it a vital factor in regenerative approaches for diabetes. However, similar to the other FGFs, KGF suffers from poor stability at 37˚C, rapidly losing activity and requiring frequent medium exchanges or high-dose usage, posing significant operational and cost challenges. To address this limitation, we developed a stable peptide-based alternative to KGF with equivalent biological activity. Using PeptiDream's Peptide Discovery Platform System (PDPS), we identified cyclic peptides that bind to human FGFR2b. Agonistic activity toward FGFR2b was evaluated by Extracellular signal-regulated kinase (ERK) phosphorylation and proliferation assays using FGFR2b-overexpressing BaF3 cells (pro-B cell line). Flow cytometric analysis of transcription factors and cell proliferation markers was performed to evaluate the differentiation efficiency of iPSCs into pancreatic betacells treated with PG-012 or KGF. We identified PG-012, which was chemically linked to form dimeric structures designed to mimic KGF's agonistic activity. Peptide sequences and linkers were optimized to generate the lead compound PG-012. PG-012 activated ERK signaling downstream of FGFR2b and promoted the proliferation of FGFR2b-expressing BaF3 cells. In contrast to recombinant KGF, PG-012 maintained full activity after prolonged incubation at 37˚C, demonstrating superior thermal stability. PG-012 also supported the differentiation of iPSCs into islet beta-cells at levels comparable to KGF. Peptigrowth's PG-012 is a promising KGF alternative peptide with high stability and KGF-equivalent activity. It can reduce medium exchange frequency and reagent use, offering practical advantages for stem cell research and regenerative medicine.

THEMATIC AREA 04

ISLET DYSFUNCTION BEYOND T1D & T2D

14 abstracts in this area

P.58 Role of Stac2 adaptor protein on pancreatic β-cell excitability and insulin release

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POSTER Keywords: *Electrophysiology Ion channels Insulin secretion*

Laura E. Häfele1, Ryoichi S. Taguchi1, Stefanie M. Geisler1, Petronel Tuluc1 Stac2 adaptor proteins show a broad tissue expression pattern including endocrine cells. Overexpression studies in heterologous systems have demonstrated that Stac2 inhibits L-type voltage-gated calcium channels calcium-dependent inactivation. Since single cell RNAseq analysis shows that Stac2 is expressed in mouse pancreatic β-cells, we hypothesized that the genetic deletion of the endogenous Stac2 will increase L-type calcium channel inactivation causing impaired β-cell excitability and insulin release. However, voltage clamp experiments show that Stac2 genetic ablation does not affect whole-cell calcium influx biophysical properties. Nevertheless, the voltage gated sodium current density is significantly smaller in Stac2-/- β-cells compared to control while the kinetics properties are similar. Current clamp recordings in β-cells still part of the intact islet show that compared to WT the Stac2-/- β-cells have higher action potential thresholds, plateau potentials and inter-train potentials in a glucose-dependent manner. Calcium imaging of isolated islets shows that Stac2 deletion decreases the basal fluorescence but increases calcium transient amplitude when stimulated with 7.5 mM glucose. Consequently, Stac2-/- islets secrete more insulin compared to controls when stimulated with 7.5 mM glucose, difference that is lost in higher glucose levels. Together our data show that Stac2 modulates pancreatic β-cell and islets excitability, calcium transients and insulin release in a glucose-dependent manner. Current experiments are on the way to identify the mechanisms responsible for altered ion channel conductances, membrane excitability, and vesicle exocytosis. 1 Leopold-Franzens University of Innsbruck

P.59 A focal intrinsically disordered region in the PDX1 C-terminus exerts critical roles during pancreas organogenesis

Maureen Gannon[1], Doris Stoffers[2], Scott Soleimanpour[3], Ondine Cleaver[4], Hassane Mchaourab[5], Muhammad Shamoon[3], Jie Zhu[3], Neha Ahuja[4], Thomas Mehaffey[5], Tianqi Wu[5], Courtney Campbell[3], Anthony Wokasch[5], Jennifer Fuhr[1]

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ORAL COMMUNICATION Keywords: *PDX1, pancreas organogenesis, beta cell*

Mutations in the transcription factor PDX1 contribute to both maturity-onset diabetes of the young type 4 (MODY4) and type 2 diabetes (T2D). Despite the identification of multiple disease-associated variants within the PDX1 C-terminus, the functional contribution of this domain remains poorly defined in vivo. Deletion of a predicted C-terminal focal intrinsically disordered region (IDR) spanning amino acids 207-223 (Pdx1Δ207) causes moderate defects in PDX1 nuclear localization as well as major deficits in binding at the target genes Ins1, Ins2, and Nkx6.1, MIN6 beta cells. Pdx1Δ207 homozygous embryos displayed pancreatic developmental defects as early as embryonic day (E)11.5, leading to severe pancreas hypoplasia by late gestation. Pdx1Δ207 homozygotes exhibited reductions in ductal lumen volumes within the dorsal and ventral pancreatic buds, leading to abnormal ductal morphology and disrupted epithelial morphogenesis, including impaired branching and epithelial cysts. Endocrine development was also impacted in these embryos, resulting in reduced NGN3+ endocrine progenitors, decreased endocrine cell numbers, and impaired beta-cell proliferation and maturation. These findings establish the functional importance of a predicted PDX1 C-terminal focal IDR in pancreas and beta-cell development, with implications for diabetes pathogenesis.

P.60 First Deep Intronic Variant in EIF2AK3 Causing Wolcott–Rallison Syndrome through Aberrant Splicing

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ORAL COMMUNICATION Keywords: *Wolcott–Rallison syndrome, early-onset diabetes, EIF2AK3, pseudoexon, deep intronic variant*

Abstract

BACKGROUND AND OBJECTIVES

Deep intronic variants can disrupt splicing causing monogenic disease, but they are not systematically picked through genetic testing. The aim of this study was to assess the contribution of deep intronic variants to Wolcott–Rallison syndrome (WRS), a recessive disorder characterized by neonatal diabetes and progressive multisystem disease caused by biallelic variants in EIF2AK3.

METHODS

We identified 116 individuals referred to the Exeter genomics laboratory for genetic testing who had diabetes diagnosed <2 years and at least one additional feature consistent with WRS: hepatic dysfunction, skeletal abnormalities, or developmental delay. All known genetic causes of early-onset diabetes, including coding EIF2AK3 variants, had been excluded, and genome sequencing data was available for intronic analysis. We searched for rare (<0.001% gnomAD

frequency) intronic EIF2AK3 variants, including homozygous and compound heterozygous (intronic/intronic or intronic/coding). Candidate variants were assessed using SpliceAI, and those predicted to disrupt splicing were functionally evaluated using a minigene splicing assay. Minigenes were designed with the variantcontaining intron flanked by its surrounding exons and ~180 bp of adjacent intronic sequence, cloned into a pSPL3 vector, and expressed in HEK293 cells. Splicing outcomes were analysed by RT-PCR and Sanger sequencing.

RESULTS

We identified rare biallelic EIF2AK3 variants in four individuals, including two affected siblings. Only one variant, c.1651–180G>T, detected in the two siblings, was predicted to affect splicing (spliceAI donor gain score 1.00). The siblings, born to consanguineous parents, developed diabetes in early infancy (21 weeks and 1 year) and were also diagnosed with liver dysfunction, skeletal abnormalities, developmental delay, and thyroid dysfunction, consistent with WRS. The minigene assay demonstrated that the c.1651–180G>T variant creates a novel donor splice site resulting in inclusion of a 79-nucleotide pseudoexon within intron 9, leading to a frameshift predicted to result in nonsense-mediated decay. This result enabled the variant to be classified as pathogenic. Conclusions and Implications We report the first deep intronic variant in EIF2AK3 causing WRS through aberrant splicing, expanding the mutational spectrum beyond coding regions. This highlights the importance of systematic intronic analysis and functional validation to resolve unsolved cases.

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P.61 A de novo KCNMA1 variant is a cause of syndromic neonatal diabetes

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ORAL COMMUNICATION Keywords: *Neonatal diabetes, Genome sequencing, De novo, KCNMA1*

BACKGROUND & OBJECTIVES

Uncovering novel genetic causes of monogenic diabetes provides insights into beta-cell biology. Neonatal diabetes (NDM) is a monogenic form of diabetes diagnosed before 6 months with over 40 known aetiological genes. A significant proportion of NDM cases remain genetically unsolved, including many individuals with extra-pancreatic features suggestive of a genetic syndrome. Our aim was to identify novel causes of syndromic NDM using a trio genome sequencing approach.

METHODS

We performed genome sequencing on 26 proband-unaffected parents trios. All probands were diagnosed with NDM and at least one of the following features: deafness, developmental delay,

epilepsy, neurological complications, muscle weakness, skeletal malformations, or liver abnormalities. We used denovoCNN to select high-quality, de novo variants. Rare (GnomAD frequency <0.001) variants resulting in a premature termination codon, and missense variants predicted deleterious in silico by REVEL and AlphaMissense were prioritised for further analysis.

RESULTS

Our strategy prioritised 5 de novo variants (2 stop-gain, 3 missense), each in a different gene (FBXO11, NBAS, TPST1, PDX1, and KCNMA1), in 4 different individuals. Only the KCNMA1 variant had been previously reported as pathogenic. We identified the KCNMA1 p.(Gly375Arg) variant in an individual diagnosed with diabetes at 3 days. She also had bilateral hydronephrosis, clubfoot and malabsorption. The patient was insulin treated from diagnosis until her decease aged 18 months. The KCNMA1 p.(Gly375Arg) variant has previously been reported in multiple individuals with Liang-Wang syndrome, including 2 with NDM. KCNMA1 encodes the alpha subunit of the large-conductance Calcium- and Voltage-activated Potassium channel (BKC). BKCs are widely expressed and play a critical role in regulating cell excitability. Previous in vitro studies showed that the KCNMA1 p.(Gly375Arg) variant causes loss of BKC activity. Whilst BKCs are expressed in beta-cells, their function in insulin secretion is currently unclear. Conclusion & implications The identification of the KCNMA1 p.(Gly375Arg) variant in a third NDM case confirms this variant as a cause of NDM. Further studies are needed to determine if other KCNMA1 loss-of-function variants cause NDM. These results highlight a role for BKCs in insulin secretion, highlighting the need for further investigation of their role in beta-cells.

P.62 Collagen glycation drives amylin aggregation and islet toxicity

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POSTER Keywords: *Amyloid, Advanced glycated end products, Islet dysfunction, Diabetes*

BACKGROUND AND OBJECTIVES

Amylin is a 37 amino acid peptide hormone co-secreted with insulin to regulate glycaemic levels. Under hyperglycaemic conditions, insulin and amylin expression increases to counter elevated glucose. Amylin is a well-known amyloidogenic peptide that aggregates under overexpression and forms toxic amyloid deposits in pancreatic tissue, leading to dysfunction and death of beta cells. Several factors contribute to amylin aggregation, including protein misfolding, liquid–liquid phase separation and interaction with cell-surface proteins such as collagen and β2-microglobulin. Protein glycation has recently emerged as a key driver of faster amylin aggregation. Glycation is a Maillard reaction between glucose and amine side chains of lysine and arginine, leading to advanced glycation end products (AGEs). However, the regulation of aggregation rate and molecular interactions between glycation and amyloid remains poorly understood. Here, we demonstrate that glycated collagen increases the rate of amylin

aggregation upon co-incubation, proportional to the extent of glycation, and enhances amyloid toxicity in beta cells.

OBJECTIVES

1. Investigate interactions between glycated collagen and amylin in amyloid formation 2. Study the impact of combined glycation and amyloid stress on beta cells 3. Design peptide-based drugs targeting these interactions and test efficacy in diabetic rodent models

RESULTS

Glycation accumulates in slow turnover proteins; hence collagen was selected. Collagen was glycated using Methylglyoxal (MGO). The aggregation rate of amylin increased with glycated collagen, with highly glycated collagen showing the fastest aggregation. 15N-1H HSQC NMR revealed that glycated collagen produced larger chemical shift perturbations (CSPs) and broader residue interaction than native collagen. Residues R11, H18, G24, L27, and Y37 showed strongest responses. Native collagen showed weaker and localized effects. Glycated collagen also caused stronger reduction in signal intensities, particularly in R11–H18 and L27–N35 regions. Live/dead assay showed that cells treated with amylin + glycated collagen had the highest cell death compared to individual treatments. Conclusion and implications NMR results show glycated collagen interacts with structurally critical regions of amylin, enhancing aggregation. Glycated collagen also increases amylin toxicity in beta cells. Understanding this interplay could explain progression from pre-diabetic to diabetic state, highlighting glycation–amyloid interaction as a key contributing factor.

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P.63 Multi-Omics of decremental β-cell mass and insulin receptor blockade reveals β-cell loss-specific systemic programs and BAT whitening

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ORAL COMMUNICATION Keywords: *β-cell loss, BAT whitening, plasma multi-omics, Lipidomics*

BACKGROUND & OBJECTIVES

Significant β-cell loss is a hallmark of advanced T1D and T2D, yet the degree of loss that triggers the stages of systemic metabolic failure remains poorly understood. Moreover, no biomarkers have been clinically implemented to detect β-cell loss reliably, partly because distinguishing its consequences from those of insulin signaling deficiency is challenging. To address this, we conducted a systems-level analysis of metabolic and molecular remodeling following graded β-cell ablation or insulin receptor blockade.

METHODS

Using a transgenic mouse model (RIP-DTR), we induced graded β-cell ablation (~50%, ~85%, ~95%, ~99%) and compared outcomes to mice treated with an insulin receptor antagonist (S961). Ten days post-treatment, we performed plasma quadriomics (metabolomics, lipidomics, proteomics, and miRNA-seq), bulk RNA-seq of ten peripheral organs, and lipidomics of interscapular BAT.

RESULTS

We defined β-cell loss thresholds altering key diabetic parameters (insulin deficiency, hyperglucagonemia, glucose intolerance). Pancreatic atrophy and BAT whitening emerged as β-cell ablation-specific phenotypes at ~85% β-cell loss. BAT removal specifically blunted ablation-induced α-cell proliferation. We identified 120+ plasma molecules reacting exclusively to β-cell ablation — most at ~99% loss, but ~12 responding across all ablation levels — and 150+ altered by both treatments, representing core mediators of systemic insulin action. Bulk RNA-seq revealed that BAT whitening reflects a βcell ablation-specific collapse of mitochondrial function and lipid droplet fusion, without fibrosis or WAT gene upregulation. Among 80+ ablation-specific BAT lipid markers, two phospholipids show concentration drops that precede transcriptomic changes, closely mirror β-cell mass reduction, and may serve as early sensors of β-cell loss. Conclusions & Implications Our study consolidates two decades of rodent research on β-cell mass reduction and advances understanding of diabetic stage transitions. β-cell loss-specific molecular programs — including circulating proteins, miRNAs, lipids, and metabolites — provide a foundation for high-precision therapeutics and diagnostics. The coordinated molecular hierarchy governing BAT whitening suggests BAT functions as both sensor and effector of β-cell mass. Since BAT mass peaks in infancy and no study has analyzed BAT in children with T1D, these findings highlight BAT whitening as a potentially important factor in T1D early disease pathogenesis.

P.64 Divergent Glycaemic Outcomes in KATP-Induced Diabetes Reflect Differential β-cell and Entero-Islet Adaptation

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ORAL COMMUNICATION Keywords: *KATP, monogenic, therapy, metabolism, identity*

BACKGROUND

KATP-channel gain-of-function (GOF) mutations are the most common cause of neonatal diabetes. These mutations suppressed β-cell excitability and impair insulin secretion. Individuals with KATP-GOF mutations exhibit variable clinical outcomes, but the mechanisms underlying this heterogeneity remain unclear. This study aimed to define the adaptive responses that drive progression toward distinct outcomes.

METHODS

KATP-GOF expression was induced in β-cells from adult mice via tamoxifen. Blood glucose was monitored longitudinally. KATPGOF mice were stratified as Resistant (<13.9mmol/L), Compensated (13.9–19.4mmol/L), and Severe (>19.4mmol/L), alongside littermate Controls. Metabolic phenotyping included OGTT, insulin secretion, ITT, plasma C-peptide, glucagon, incretins, and inflammatory markers. Pancreatic immunostaining, protein/gene expression and calcium dynamics was also analyzed.

RESULTS

Despite carrying the same mutation, mice exhibited distinct glycemic trajectories: 10% remained resistant, 7% became compensated, and 83% progressed to severe diabetes. During OGTT, severe mice showed profound glucose intolerance, resistant mice maintained near-normal glucose clearance, and compensated mice demonstrated intermediate glucose intolerance despite insulin levels comparable to controls. ITTs revealed preserved insulin sensitivity in resistant mice, with progressively impaired responses in compensated and severe mice. C-peptide levels were preserved in resistant mice, partially reduced in compensated, and markedly decreased in severe mice. This was accompanied by stepwise increases in glucagon, GIP/GLP-1, reflecting entero-islet axis dysfunction. IL-6 levels peaked in the compensated group, suggesting a transient inflammation, while TNFα remained stable. At the islet level, resistant mice exhibited preserved β-cell identity and α-cell expansion, while compensated mice showed early β-cell dysfunction, with altered gene expression and calciumdynamics, and severe mice had loss of β-cell identity/insulin content. Conclusions-Implications KATP-GOF-induced β-cell dysfunction drives divergent glycaemic trajectories. Resistance is characterized by preserved β-cell identity, insulin secretion-sensitivity, and adaptive α-cell remodeling, whereas compensated mice exhibit early metabolic stress, disrupted Ca^{2+}-dynamics, and transient inflammation despite maintained insulin levels. Severe diabetes reflects loss of β-cell identity-insulin content, insulin resistance, and ineffective hormonal compensation despite elevated glucagon and incretins. These findings define progressive β-cell failure and islet-entero axis dysfunction as key determinants of disease progression, highlighting preservation of β-cell identity and inter-organ signaling as therapeutic strategies.

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P.65 miR-125a-p overexpression affects beta cell morphogenesis and enhances insulin secretion following high-glucose preconditioning

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POSTER Keywords: *miRNA-125a-5p, beta cells, insulin secreation, mechanotransduction, beta cell morphology*

BACKGROUND AND AIMS

MicroRNAs are critical regulators of post-transcriptional gene expression and play central roles in pathways controlling glucose homeostasis. MiR-125a-5p has been associated with the regulation of apoptosis, proliferation, and inflammatory pathways across multiple cell types; however, its contribution to pancreatic β-cell function and structural plasticity remains poorly understood. This study aimed to provide new insights into the functional and molecular effects of miR-125a-5p overexpression in beta cells.

MATERIALS AND METHODS

INS-1E cells with lentiviral-induced miR-125a-5p overexpression (OE) were compared with nontargeting control (NTC) cells. An integrated approach combining quantitative proteomics, glucose-stimulated insulin secretion (GSIS) assays, RT-qPCR, cell viability assessment, and immunofluorescence confocal imaging was used to evaluate functional and structural changes. Human islet preparations were analyzed for miR-125a-5p mRNA expression.

RESULTS

MiR-125a-5p expression was elevated by approximately 3-fold in OE cells. Proteomic profiling identified 90 differentially expressed proteins, highlighting pathways related to cellular organization, adhesion, and metabolic regulation. Consistent with these findings, imaging analyses demonstrated a changed cellular morphology and increased abundance of p120ctn, actin, and RhoA, supporting a role for miR-125a-5p in cytoskeletal and cell adhesion remodeling While no functional differences were observed under basal glucose conditions (5 mM), miR-125a-5p overexpression significantly enhanced GSIS following high-glucose preconditioning (20 mM, 48 hours), indicating a context-dependent effect. Gene expression analysis revealed a selective upregulation of Gcg, with no significant changes in Pdx1, Ins1, or Ins2 under basal glucose conditions. Notably, in human islet preparations, miR-125a-5p levels positively correlated with miR-200a-5p, -200c-3p, -99a-3p. -99b-3p abundance ($r>0.6$, $p<0.001$, donors=78) suggesting the relevance of miRNA interrelationship.

CONCLUSIONS

Collectively, these findings suggest that miR-125a-5p modulates beta-cell responsiveness under metabolically challenging conditions and may contribute to the regulation of insulin secretion capacity. The associated changes in cytoskeletal and adhesion-related pathways point toward a link between beta-cell mechanotransduction and functional adaptation. Ongoing studies are aimed at further delineating the mechanistic basis of these effects and their relevance in murine and human islet physiology.

P.66 Sustained dexamethasone exposure induces insulin resistance in mice, increases proliferation and DNA damage in beta-cells and alters their function

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ORAL COMMUNICATION Keywords: *Dexamethasone, insulin resistance, DNA damage, proliferation*

Cancer survivors have an increased risk of developing Type 2 diabetes suggesting that chemotherapy may exert off-target effects to islets. We have recently shown that cisplatin—a platinum-based chemotherapy agent—has profound effects on insulin secretion in vitro and in vivo in mice1. Patients undergoing platinum-based chemotherapy are commonly prescribed a glucocorticoid, typically dexamethasone, to prevent nausea. We hypothesize that exposure to dexamethasone, which can induce peripheral insulin resistance, could worsen the impact of cisplatin on mouse islets. The goal of this study was to establish a 2-week continuous dexamethasone delivery protocol that induces insulin resistance and to determine its effects on β-cell function. In our first cohort, mice were exposed to dexamethasone via drinking water at a target dose of 1 mg/kg/day. In our second cohort, subcutaneous dexamethasone pellets (1 mg or 10 mg), designed for continuous delivery over 2 weeks, were implanted into mice. Body weight, fasting blood glucose, water consumption and insulin sensitivity were assessed throughout the study. Islets were isolated at the end for perifusion assays and flow cytometry. In both cohorts, dexamethasone had no impact on body weight, inconsistent effects on fasting blood glucose levels, but significantly increased water consumption. Exposure to dexamethasone also induced gastroparesis. Importantly, male and female mice exposed to dexamethasone exhibited marked insulin resistance 1-week post-exposure and had increased fasting plasma insulin levels. Islets isolated from dexamethasone-exposed mice had an earlier first-phase glucose-stimulated insulin peak and altered KCl-stimulated insulin secretion dynamics. Dexamethasone treatment led to an increased percentage of insulin+Ki67+ cells in both cohorts, suggesting enhanced β-cell proliferation. Additionally, the percentage of insulin+γH2A.X+ cells, indicative of DNA damage, was increased in islets from dexamethasone-exposed mice. The impact of dexamethasone on β-cell insulin secretion, proliferation, and DNA damage suggests that glucocorticoid exposure may increase the vulnerability of islets to cisplatin-induced damage. Nonetheless, the dexamethasone exposure protocol requires further refinements to avoid adverse reactions such as gastroparesis.

Elucidating the crosstalk between dexamethasone and cisplatin on islets could inform cancer treatment decisions and improve understanding of chemotherapy-associated metabolic risk.

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P.67 Deciphering the Role of Ferroptosis in Glucocorticoid-Induced Insulin Secretion Impairment

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ORAL COMMUNICATION Keywords: *Glucocorticoid, Ferroptosis, Insulin secretion, Iatrogenic Diabetes,*

BACKGROUND & OBJECTIVES

Glucocorticoids are a significant iatrogenic factor in the development of diabetes, partly because they impair insulin secretion from pancreatic β cells. Evidence increasingly indicates that ferroptosis contributes to ß-cell dysfunction and the onset of diabetes. Ferroptosis induces cellular damage via iron-dependent phospholipid peroxidation. This damage mainly results from decreased expression of SLC7A11, a transporter for glutathione (GSH) precursors, and lower levels of Glutathione Peroxidase 4 (GPx4), an enzyme that detoxifies phospholipid peroxides using GSH as a cofactor. Nonetheless, the role of the ferroptosis pathway in mediating glucocorticoid effects remains unknown. This study aims to investigate the mechanisms by which ferroptosis contributes to glucocorticoid-induced impairment of insulin secretion.

METHODS

Human or mouse pancreatic islets and insulin-secreting cell lines (INS832/13 and MIN6) were incubated for 24 hours with either 200 nM dexamethasone (Dex) or 1 µM prednisolone (Pre), with or without 500 nM ferroptosis inhibitor Liproxstatin-1 (Lip-1). Protein and RNA levels were measured by western blot and qPCR. Mitochondrial activity was assessed by oxygen consumption rate (OCR) with Seahorse. Lipid peroxidation was evaluated with Mitochondrial Lipid Peroxide Live Cell Ferroptosis Indicator, and cell death was measured using MTT and SYTOX Green dye. Insulin levels were determined by ELISA.

RESULTS

The expression levels of SLC7A11 and GPx4 proteins were impaired in human islets exposed to Dex and Pre. While Gpx4 mRNA levels remained unchanged, Slc7A11 expression was significantly reduced. Similar effects were observed in three independent single-cell transcriptomics datasets (GSE225901 & GSE167250). 17 Additional ferroptosis gene markers were identified in these data. The basal and glucose-induced mitochondrial OCR was significantly impaired in mouse islets exposed to Dex. This correlates with an increase in mitochondrial phospholipid peroxidation. Interestingly, 24 hours of Dex treatment does not induce cell death, whereas 48 hours of treatment does, suggesting that the changes observed are not

due to cell death. Interestingly, Lip-1 prevents glucocorticoid-induced impairment of insulin secretion. Conclusions & Implications: This study uncovers the role of the SLC7A11/GSH/GPx4 axis in glucocorticoid-induced impairment of insulin secretion. It explores the potential role of ferroptosis in the etiology of diabetes and as a novel therapeutic target.

P.68 Sugar-coated habits: hidden impacts on pancreatic β-cells function

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POSTER Keywords: *pre-diabetes NOD mice glucolipotoxicity insulin secretion*

BACKGROUND & OBJECTIVES

Persistent glucose consumption disrupts the metabolic equilibrium, placing significant pressure on the insulin-secreting capacity of pancreatic β-cells. Although these cells demonstrate remarkable plasticity in adjusting to increased insulin demand, diabetes can eventually overwhelm these adaptive responses. We aimed to delineate the mechanisms of β-cell stress adaptation in an inflammation-prone experimental model of T1D, following sustained glucose exposure. Methodology: The experimental approach employed the use of 4 weeks old female non-obese diabetic (NOD) mice, which were administered high-glucose water (HGW) or standard water (NW) over various durations (3, 4, 24 weeks). Following acute HGW exposure, pancreatic tissues were evaluated using fluorescence microscopy, while isolated islets were subjected to a comprehensive transcriptomic profiling. Furthermore, serum samples were collected to evaluate systemic lipid profiles, specifically targeting triglyceride and total cholesterol levels. Results: Long-term exposure to HGW (24 weeks) postponed the onset of diabetes and significantly lowered its overall incidence, with no impact on glucose control after acute exposure. Following immunofluorescence analysis, we observed a shift in secretory capacity, characterized by insulin accumulation, independent of proinsulin amounts. Alongside these cellular changes, biochemical assessment of serum triglycerides and total cholesterol revealed an additional systemic metabolic stress. Transcriptomic profiles of islets from NOD mice with acute HGW exposure showed alterations in metabolic pathways compared to the control group. Gene Ontology (GO) enrichment analysis identified potential markers involved in mitochondrial lipid stress management. These findings suggested that the adaptive mechanism that pancreatic β-cells adopt is non-proteotoxic and it may function independently of the canonical unfolded protein response (UPR). Conclusion: Pancreatic β-cells are capable to develop an adaptation mechanism that enables them to maintain insulin production despite the metabolic stress. This research was funded by the Romanian Academy and by the Romanian Ministry of Research, Innovation, and Digitization, PNRR program, CF 197-2022/PNRR-III-C9-2022-I8 (contract number 760059/23.05.2023); the grant PED47/2025 (ReGenTACol, PN-IV-P7-7.1-PED-2024-1926; and the grant RO-NO-2019-0544; contract number 21/2020 BETAUPREG/the NO Grants 2014-2021.

P.69 Impact of Non-Coding Mutations in Insulinoma Pathogenesis

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POSTER Keywords: *Insulinoma, Non-coding mutations, β-cell identity, Functional screening, Single-cell multiomics*

BACKGROUND

Insulinomas are rare, insulin-secreting neuroendocrine tumors characterized by a low mutation burden, though approximately 30% harbor a recurrent YY1 hotspot mutation. Recent bulk profiling suggests that non-coding mutations disrupting regulatory elements may drive β-cell transformation. However, bulk analyses obscure tumor heterogeneity and the specific impact of non-coding mutations. This study aims to uncover the distinct regulatory mechanisms and mutational events driving insulinoma pathogenesis by integrating single-cell multiomic analyses, functional validation of candidate regulators, and high-throughput screening of non-coding mutations.

METHODS

We integrated whole-genome sequencing (WGS) of 13 insulinoma samples with published WGS and whole-exome sequencing to obtain a large dataset (n = 40) of paired tumor-normal samples. Somatic variants mapping to ChIP-seq defined regulatory elements were selected, and their regulatory activity was assessed using a Massively Parallel Reporter Assay (MPRA) in EndoC cells. While most of these variants are non-recurrent at the sequence level, they frequently converge on shared regulatory regions. To assess their impact in a physiological context, MPRA results were integrated with bulk RNAsequencing, single-cell multiomic data, and histone ChIP-seq data from human insulinomas.

RESULTS

Integration of WGS data and regulatory maps generated by ChIP-seq revealed an overrepresentation of mutations at H3K27ac enriched sites. Although the genomic distribution of the mutations may be driven by the tissue-specific chromatin landscape of the tumoral cell of origin, this finding presents an opportunity to explore how somatic mutations in insulinoma may impact β-cell tissue-specific regulatory functions as well as pathways involved in neoplastic processes. We performed an MPRA in human β-cells to test 1,645 somatic mutations mapped to regulatory elements, identifying functional variants targeting genes involved in insulin secretion, glucose sensing machinery, and cell cycle regulation. Furthermore, integration with bulk RNA-seq and single-cell multiomic data is being utilized to determine the extent to which these candidate genes are actively dysregulated in primary human insulinomas.

CONCLUSIONS

This study aims to define how heterogeneous non-coding mutations converge to drive insulinoma pathogenesis through shared gene networks. We unravel novel regulatory mechanisms that

govern β-cell identity and regulate insulin secretion, providing relevant insights into potential therapeutic targets for both neuroendocrine tumors and diabetes.

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P.70 Alterations of pancreatic α-cell function and identity in gestational diabetes mellitus.

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POSTER Keywords: *Gestational diabetes; Islets; Pancreatic α-cells; Pregnancy.*

BACKGROUND AND OBJECTIVES

Pregnancy is a period of significant metabolic changes in the mother to adapt to the demanding requirements and ensure the supply of nutrients to the fetus. An insufficient adaptive response of the endocrine pancreas can lead to gestational diabetes mellitus (GDM). GDM is one of the most common metabolic disorders during pregnancy, with significant consequences for both the mother and the fetus. Furthermore, it predisposes mothers and their children to type 2 diabetes (T2D). Despite the importance of this pathology, the cellular and molecular adaptations involved are not yet fully understood. Given the key role of α-cells in glucose homeostasis and T2D, we analyzed their function in GDM.

METHODS

We have previously developed a GDM animal model based on high-fat-diet (HFD) treatment, which recapitulates the main characteristics of human GDM (Boronat-Belda et al. 2026). Four groups of mice were analyzed: control pregnant (CT-P), HFD pregnant (HFD-P), control non-pregnant (CT-P), HFD non-pregnant (HFD-NP).

RESULTS

At gestational days GD14-15, we found fasting hypoglucagonemia in the CT-P and HFD-P groups compared to CTNP controls, as well as lower glucagon levels during a glucose tolerance test. Remarkably, lower plasma glucagon levels were found in HFD-P in both fed and fasting states at GD14-15 when compared with CT-P mice. Similar results were found at GD9, while these differences disappeared at GD18, indicating dynamic temporal patterns. Furthermore, the glucagon response of the HFD-P group to hypoglycemia was found to be lower during an insulin tolerance test. The study of voltagedependent K+ currents in isolated α-cells showed no significant changes at this level. Conversely, PCR analysis revealed dysregulated expression of glucagon, Arx, and Foxo1 in isolated islets from HFD-P mice compared to CT-P. However,

further analysis by immunocytochemistry demonstrated a decreased proportion of Arx+Gcg+ cells in the islets of HFD-P mice.

CONCLUSIONS

Our data indicate that pancreatic α-cell function, identity and glucagon release are impaired in GDM. The Ca2+ signaling and secretion experiments we are currently conducting will allow us to understand in greater depth this altered αcell responses in GDM.

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P.71 Gabapentin increases pancreatic islet and β-cell excitability

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POSTER Keywords: *Gabapentinoids, Voltage-Gated Calcium Channels, α2δ-1, Hypoglycaemia*

Pancreatic β-cells glucose-induced excitability and insulin vesicle exocytosis is controlled by the activation of the high voltage-gated calcium channels (CaVs). Genetic deletion of the CaV α2δ-1 auxiliary subunit reduced β-cell calcium influx causing impaired excitability, insulin release, and diabetes. The anticonvulsant and analgesic gabapentinoids bind to the $\alpha_2\delta$-1 subunit reducing CaV membrane incorporation. Therefore, gabapentinoid use should reduce β-cell function causing elevated blood glucose levels. Nevertheless, clinical reports paradoxically associate gabapentinoid treatment with an increased incidence of hypoglycaemia. Here, we investigated how gabapentin (GBP) modulates Ca^{2+} signaling and electrical activity in intact mouse pancreatic islets and β-cells. Ca^{2+} imaging revealed that GBP-treated islets exhibit heightened glucose-induced activity with ~50% of islets showing spontaneous Ca^{2+} transients in 5 mM glucose where untreated islets remain electrically silent. In 10 mM glucose GBP-treated islet displayed continuous Ca^{2+} transients in ~66% of islets while control islets displayed only oscillatory Ca^{2+} transients. Current-clamp recordings in β-cell part of the intact islet confirm that GBP treatment increases β-cell glucose-induced excitability both in 5 mM and 10 mM extracellular glucose. However, voltage-clamp recordings revealed that neither whole-cell and L-type Ca2+ current densities nor total Kv or KATP K^+ currents were altered by GBP treatment. In contrast, voltage-gated Na^+ (NaV) current amplitude was significantly reduced but exhibited slower inactivation kinetics. Since β-cell glucose-induced excitability is highly dependent on islet paracrine modulation, we are currently investigating if altered somatostatin-secreting δ-cells or glucagon-secreting α-cell excitability following GBP treatment contributes to increased pancreatic islet and β-cell excitability and insulin release.

THEMATIC AREA 05

ISLET TREATMENT STRATEGIES

31 abstracts in this area

P.72 Inhibition of fatty acid oxidation in islet macrophages enhances β cell proliferation

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ORAL COMMUNICATION Keywords: *Cpt1a, islet macrophages, β cell proliferation, Clusterin, lipotoxicity*

BACKGROUND & OBJECTIVES

In Type 2 Diabetes Mellitus (T2DM), loss of functional β cell mass is driven by chronic lipid overload, which induces a proinflammatory phenotype in islet macrophages (IMs) in a fatty acid oxidation (FAO)-dependent manner [1,2]. Carnitine Palmitoyltransferase 1A (Cpt1a) is the rate-limiting enzyme of FAO. This study investigates whether myeloid-specific deletion of Cpt1a preserves β cell mass and function under lipotoxic stress.

METHODS

Myeloid-specific Cpt1a knockout (Cpt1a MKO) mice were generated and fed a high-fat diet (HFD) for 16 weeks. Metabolic phenotyping (GTT, ITT), islet morphology, and β cell proliferation ($Ki67^+$/$insulin^+$ and EdU^+/$insulin^+$) were assessed. Bone marrow-derived macrophages (BMDMs) from WT and Cpt1a MKO mice were treated with palmitic acid (PA), and conditioned medium was applied to MIN6 cells for proliferation assays. Proteomic profiling, Seahorse analysis, and mitochondrial mass measurements were performed.

RESULTS

Cpt1a MKO mice fed a HFD exhibited significantly improved glucose tolerance compared to HFD-fed WT controls (GTT AUC: 1625.9 ± 74.0 vs. 1907.5 ± 160.2, $p < 0.01$). This was accompanied by enhanced β cell proliferation, as demonstrated by increased percentages of $Ki67^+$/$insulin^+$ (0.875 ± 0.221% vs. 0.597 ± 0.197%, $p < 0.05$) and EdU^+/$insulin^+$ (2.204 ± 0.669% vs. 1.500 ± 0.412%, $p < 0.05$). In vitro, conditioned medium from PA-treated Cpt1a-deficient BMDMs significantly promoted MIN6 cell proliferation compared to WT BMDM-conditioned medium (104.8 ± 2.8% vs. 96.4 ± 3.4% of WT+BSA control, $p < 0.001$). Proteomic analysis identified Clusterin as a key secreted factor, which was suppressed in PA-treated WT BMDMs but restored in Cpt1a-deficient BMDMs. Neutralizing Clusterin abolished the pro-proliferative effect. Mechanistically, Cpt1a deletion preserved mitochondrial mass and ATP production under lipotoxic stress, preventing AMPK hyperactivation and thereby promoting Clusterin secretion. Conclusions & Implications Myeloid-specific Cpt1a deletion alleviates HFD-induced glucose intolerance by promoting β cell proliferation, mediated through preservation of mitochondrial health and upregulation of Clusterin. These findings identify Cpt1a in islet macrophages as a potential therapeutic target for preserving β cell mass in T2DM.

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P.73 GLP1-E2 Therapy Delays Autoimmune Diabetes in Late-stage Prediabetic NOD Mice and Potentiates Low-dose Anti-CD3 Therapy for Enhanced Disease Protection

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ORAL COMMUNICATION Keywords: *Anti-CD3 Combination therapy GLP1-E2 Non-obese diabetic Type 1 diabetes*

Anti-CD3 delays progression to stage 3 T1D in high-risk individuals by modulating autoimmunity. Nevertheless, responses remain variable and transient, with therapy providing only indirect beta-cell protection. We investigated whether GLP1-E2, a beta-cell-targeted compound that enhances beta-cell survival and function, could potentiate anti-CD3 in preventing autoimmune diabetes in NOD mice. We hypothesised that co-targeting immune dysregulation and beta-cell fragility would provide complementary and potentially synergistic benefits, resulting in more durable protection than either monotherapy. Female late-stage prediabetic NOD mice were randomised into four groups: untreated controls, aCD3-monotherapy, GLP1E2-monotherapy, and combination therapy. Anti-CD3 was administered intravenously (2.5 µg/day for five consecutive days), while GLP1-E2 was given subcutaneously (100 nmol kg-1day-1 for 18 weeks). Mice were monitored for diabetes onset. The pancreas was analysed by spatial transcriptomics and immunostaining to assess immune infiltration, beta-cell integrity, and pathway alterations. At 30 weeks of age, diabetes incidence was 77% in untreated controls, 66% in mono-aCD3-treated mice, and 61% in monoGLP1-E2-treated mice. Combination therapy significantly reduced incidence to 38% and delayed disease onset by 6 weeks, with sustained protection persisting for 5 weeks after treatment cessation. GLP1-E2-monotherapy reduced islet immune cell infiltration to a similar extent as aCD3-monotherapy and combination therapy, without affecting peripheral lymphocyte counts. Spatial transcriptomics showed increased gene responses linked to beta-cell stress, dedifferentiation, ‘disallowed’ genes, antigen presentation, and inflammation during disease progression. These processes were attenuated by mono and combination therapies, with anti-CD3 mostly restoring beta-cell identity and GLP1-E2 reducing beta-cell stress and immunogenicity. Staining for CD81 and TUNEL in 17-week-old-treated mice revealed levels comparable to 12-week-old normoglycaemic NOD mice, while being increased in 17-week-old untreated mice. This reduced beta-cell dedifferentiation and death was associated with improved beta-cell protection and better preservation of beta-cell mass at 26.5 weeks compared with new-onset (diabetic) mice. Anti-CD3- or GLP1-E2-monotherapy delayed diabetes onset and preserved beta-cell mass in NOD mice, while the combination provided superior protection.

Simultaneously targeting immunodysregulation and beta-cell vulnerability highlights the potential of combination therapy to enhance and prolong immunotherapeutic efficacy in T1D.

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P.74 Pancreas-derived Mesenchymal Stromal Cells Enhance Subcutaneous Vascularization and Neonatal Porcine Islet Engraftment: Cytokine Licensing Further Increases Vascularization but Impairs Engraftment

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ORAL COMMUNICATION Keywords: *Islet transplantation; Mesenchymal stromal cells (MSCs); subcutaneous niche; vascularization; neonatal porcine islets*

(NPIs).

BACKGROUND AND OBJECTIVES

Islet transplantation can restore glycaemic control and reduce hypoglycaemic burden in type 1 diabetes, but poor engraftment limits long-term graft survival. The subcutaneous (SC) space is an attractive transplant site: accessible, minimally invasive, and retrievable, a key safety feature for emerging islet sources such as stem cell-derived and xenogeneic islets. However, the SC space is poorly vascularized, compromising islet survival1. Mesenchymal stromal cells (MSCs) promote angiogenesis through paracrine signalling, and cytokine pre-conditioning (licensing) further augments this potential2. Herein, we evaluated whether unlicensed or licensed pancreatic MSCs improve SC vascularization and support neonatal porcine islet (NPI) engraftment and diabetes reversal.

METHODS

Human pancreatic MSCs were unmodified or licensed for 24h with TNFα, IL1β, and IFNγ (10 ng/mL). PLGA+gelatin (PLGA+G) scaffolds were implanted SC as controls or coated with 5 million unlicensed (PGM) or licensed (PGSM) MSCs. Vascularization was assessed 2 weeks post-implantation via FITC-lectin injection. Streptozotocin-diabetic immunodeficient mice then received 3000 NPIs into the pre-vascularized niche. Efficacy outcomes included vascular metrics and diabetes reversal.

RESULTS

Licensing upregulated MSC expression of CXCL1/5/6/8, FGF2, and VEGF (all $p<0.01$). PGM and PGSM increased vessel area/mm² (PGM $p=0.0138$; PGSM $p<0.0001$) and vessel number/mm² (PGM $p=0.0011$; PGSM $p<0.0001$) vs PLGA+G, with PGSM exceeding PGM across all metrics.

Lectin (PGM p=0.0029; PGSM p<0.0001), CD31 (PGM p<0.0001; PGSM p<0.0001), and SMA positive cells (PGM p<0.0001; PGSM p<0.0001) confirmed denser, mature vasculature with licensing. Despite enhanced vascularization, only PGM improved diabetes reversal (10/12 vs 4/12 in control; p=0.0139) and reduced reversal time (14 vs 22 weeks; p=0.0177), whereas PGSM did not improve reversal compared to control (p>0.05). Conclusions and Implications: Pancreatic MSCs enhanced SC vascularization, yet only unlicensed MSCs improved NPI engraftment and diabetes reversal, indicating further work is needed to translate vascular gains into functional improvements. Establishing a well-vascularized, retrievable SC niche remains particularly relevant for next-generation islet sources requiring graft retrievability for safe clinical translation. Future work will characterize the licensed MSC secretome and assess immune cell recruitment within the SC space to identify and mitigate factors impairing engraftment while preserving pro-angiogenic benefits.

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P.75 Selective TYK2 vs JAK1/2 Inhibition Differentially Tunes the Inflammatory Transcriptome and Protects Human β-cells from CTL mediated killing

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ORAL COMMUNICATION Keywords: *microtissue, baricitinib, tyk2, co-culture, T1D*

In type 1 diabetes (T1D) β-cells co-orchestrate their demise by increasing visibility to the immune system through overexpression of HLA class I (HLA-ABC) and CXCL10. This islet-immune "dialogue" is driven by interferon (IFN)-activated JAK/TYK2 signaling, making the JAK1/2 inhibitor baricitinib and the selective TYK2 inhibitor deucravacitinib promising therapeutic candidates. However, a head-to-head comparison in a physiologically relevant, intact human islet system is lacking. We utilized a 3D biomimetic platform of human islet microtissues (MTs) exposed to T1D-relevant cytokine cocktails (IFN-α, IFN-γ, TNF-α, IL-1β) or co-cultured with patient-derived, preproinsulin-specific cytotoxic T-lymphocytes (CTLs) and evaluated the dose-dependent effects of baricitinib (JAK1/2i) and deucravacitinib (TYK2i) using 3D quantitative imaging, glucose stimulated insulin secretion (GSIS), low-input bulk RNA sequencing and Luminex secretome analysis. Both inhibitors reduced cytokine-induced HLA-ABC and PD-L1 expression (p<0.001). Surprisingly, TYK2i remained effective even in the absence of IFN-α (IFN-γ + TNF-α + IL-1β condition), suggesting a broader role for TYK2 in islet inflammatory signaling than previously recognized. RNAseq confirmed that both inhibitors reversed T1D-like gene signatures, including expression of CXCL10, NLRC5 and TXNIP (FDR<0.001). However,

JAK1/2i and TYK2i exhibited distinct transcriptomic tuning: JAK1/2i exerted a broader impact, suppressing MHC Class I alongside STAT1 and the CIITA-driven MHC Class II machinery (FDR<0.001). In contrast, TYK2i achieved a more targeted modulation, selectively reducing MHC Class I without significant effects on STAT1 transcripts or global MHC Class II expression. The inhibitors' effects translated also to functional protection in CTL co-cultures: JAK1/2i and TYK2i preserved both β-cell count (87% and 80%, respectively, vs 6% survival in control), and stimulation index (9.8-fold and 11.6-fold vs 1.1-fold in control) (p<0.001). Furthermore, pharmacological inhibition correlated with an altered secretome, specifically the suppression of CXCL10 (JAK1/2i: -99%, TYK2i: -81%) and CTL effector molecules like granzyme B (both -84%) and IFN- γ (JAK1/2i: -93%, TYK2i: -83%) (p<0.001). In conclusion, both baricitinib and deucravacitinib protect β-cells from immune injury by somewhat differential tuning of immune visibility and simultaneously desensitizing islets to inflammatory stress. Furthermore, our findings suggest a broader than expected role for TYK2 in islet-immune crosstalk.

P.76 Neuropeptide Y4 receptor activation delays autoimmune diabetes by reprogramming β-cell stress and immune tolerance.

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ORAL COMMUNICATION Keywords: *Type 1 Diabetes, β-cell resilience, NPY4R.*

BACKGROUND

Type 1 diabetes (T1D) is caused by immune-mediated destruction of pancreatic β-cells. Current disease-modifying therapies mainly target immune cells and do not directly enhancing β-cell resilience. We investigated whether selective activation of the neuropeptide Y4 receptor (Y4R) with K22, a PEGylated Y4R-selective agonist, can protect β-cells and modulate the islet immune microenvironment.

METHODS

Y4R expression was mapped in mouse and human islets by cell sorting/qPCR, RNAscope, and fluorescent K22-TAMRA ligandcompetition imaging. β-cell viability, apoptosis, function, and proliferation were assessed under inflammatory, cytotoxic, lipotoxic, and ER stress conditions. Bulk RNA-seq profiled K22-induced transcriptional programmes. Chemokine release, immune cell migration, invasion, and Foxp3 preservation were assessed in co-culture systems. In vivo efficacy was tested in an NY8.3→Rag1$^{-/-}$ NOD adoptive transfer model.

RESULTS

Npy4r was enriched in CD45⁻ islet cells (4.32 × 10^{-6} ± 3.33 × 10^{-6} vs 18S), with RNAscope puncta confined to insulin-positive β-cells; K22–TAMRA robustly labelled human (72.46 ± 12.20%) and mouse (43.66 ± 5.63%) β-cells in a Y4R-selective manner. K22 reduced basal and stress-induced caspase-3/7 activity by ~40–70%, halved cleaved caspase-3⁺ β-cells, preserved glucose-stimulated insulin secretion, and increased Ki67⁺ β-cells to 5.13 ± 1.05%, comparable to Exendin-4. RNA-seq revealed reinforced β-cell identity (Ins1/2, Pdx1, Ucn3) with KEAP1–NFE2L2-linked antioxidant and proteostasis programmes and suppression of EIF2-dependent translational stress pathways. K22 lowered CXCL10 (20.69 ± 0.92 vs 84.71 ± 3.78 pg/mL) and other chemokines, attenuated cytokine-driven CD8⁺/CD4+ T-cell and macrophage recruitment, preserved Foxp3⁺ signatures, and delayed diabetes onset in NY8.3→Rag1⁻/⁻ NOD mice ($p<0.037$).

CONCLUSION

Y4R agonism integrates direct β-cell stress adaptation with local suppression of inflammatory chemokine networks a dual mechanism that delays autoimmune diabetes in vivo. These findings position Y4R as a β-cell-centric therapeutic target that could complement existing immunotherapies and support islet-replacement strategies in T1D.

P.77 Enhancing Survival and Immune Evasion of Stem Cell-Derived Islets

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POSTER

The current standard care for Type 1 Diabetes (T1D) remains exogenous insulin administrations. While islet transplantation offers a route towards functional cure for severe cases, its widespread use is limited by donor islet availability. Stem cellderived islets (SC-islets) generated from induced pluripotent stem cells (iPSCs) represent a transformative and scalable alternative. However, emerging clinical trials using SC-islet transplantation have revealed a significant bottleneck, substantial post-transplant cell loss driven by cellular stress and immune attack. To overcome this, we sought to engineer next-generation SC-islets with enhanced survival and immune-evasive properties by targeted modulation of intrinsic gene expression. We utilized the biocompatible pABOL delivery system, which forms nanoscale complexes with RNA via electrostatic interactions. pABOL was formulated with RNA targeting genes in key pathways involved in pro-survival, antiapoptotic responses and immune regulation. This platform enabled efficient delivery in iPSCs and robust, measurable efficacy in SC-islets. Strikingly, pABOL-mediated gene modulation markedly improved SC-islet survival under inflammatory and immune stress conditions. In parallel, we are developing a doxycycline-inducible stable cell line to enable precise, ondemand control of gene expression. Together, this work advances a clinically relevant

strategy to enhance SC-islet graft survival by targeting cell-intrinsic vulnerabilities, bringing stem cell-based therapies for T1D closer to durable therapeutic impact.

P.78 The type 1 diabetes candidate gene EBI2 regulates virus-induced β-cell apoptosis through the MDA5-IRF7 pathway

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POSTER Keywords: *Apoptosis, candidate genes, β-cells, inflammation, type 1 diabetes*

BACKGROUND AND AIMS

The early stages of type 1 diabetes (T1D) are marked by islet inflammation modulated by genetic and environmental factors. GWAS have identified over 90 loci associated with T1D risk. Among them, the EBI2 gene has been shown to regulate an IRF7-driven inflammatory network (IDIN) in monocytes, potentially contributing to disease susceptibility1. EBI2 is also expressed in β-cells, but its function in these cells remains poorly understood. The present study aimed to evaluate whether EBI2 plays a role in β-cell responses to viral infections and to determine the mechanisms involved.

MATERIALS AND METHODS

INS-1E cells were transfected with small-interfering RNAs targeting EBI2 and then exposed to Poly(I:C), a mimic of viral dsRNA, or to coxsackievirus B5 (CVB5). Apoptosis was evaluated by DNA-binding dyes. mRNA and protein expression were determined by qPCR and western blot, respectively. Promoter activity was assessed by luciferase assay.

RESULTS

EBI2 knockdown (KD) induced apoptosis at baseline and further augmented poly(I:C)- and CVB5-induced apoptosis ($p<0.05$). Compared to control cells, EBI2-silenced cells exposed to poly(I:C) showed higher mRNA expression levels of IRF7 (5-fold; $p<0.05$), IFNβ (20-fold; $p<0.05$), CXCL10 (6-fold; $p<0.05$), and CCL5 (10-fold; $p<0.05$). Studies of promoter activity showed that EBI2 KD increased poly(I:C)-stimulated ISRE activity by nearly 10-fold, while IFNβ activity was 6- to 10-fold higher in EBI2-inhibited cells. After poly(I:C) exposure, EBI2/MDA5 double-KD diminished IFNβ activity secondary to EBI2 inhibition. Similarly, an EBI2/IRF7 double-KD showed that IRF7 was key to the increase in IFNβ activity in poly(I:C)-treated, EBI2-silenced cells. Finally, we observed that treatment with 7α,25-dihydroxycholesterol, an EBI2 agonist, reduced poly(I:C)induced apoptosis in INS-1E and EndoC-βH1 cells ($p<0.05$).

CONCLUSIONS

Our results indicate that EBI2 modulates β-cell antiviral and inflammatory responses via MDA5 and IRF7. As EBI2 regulates the IDIN pathway in monocytes and other immune cells, this newly discovered role of EBI2 in pancreatic βcells points to a role for this candidate gene in the deleterious dialogue between β-cells and immune cells in the pathogenesis of T1D.

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P.79 Exploring transplantation sites for pluripotent stem cell–derived islets in β-cell replacement therapy

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LUMC

ORAL COMMUNICATION Keywords: *Stem-cell derived islets, transplantation site*

β-cell replacement through transplantation of donor islets in the portal vein can restore glucose homeostasis in type 1 diabetes. However, this intervention is limited by the availability of donor organs, the need for immunosuppression and graft loss caused by the instant blood-mediated inflammatory reaction (IBMIR). Pluripotent stem cells (PSCs) offer a potential solution for donor scarcity by providing a scalable source of β-cells. However, the optimal transplantation site for PSCderived islets (SC-islets) remains undefined. In addition to IBMIR-related cell loss, the liver lacks graft traceability or retrievability. In this study, we established preclinical models to systematically compare transplantation sites for donor- or SC-islets in immunodeficient rodents. To compare extrahepatic sites to liver transplantations in humans, we developed a surgical approach for transplantation into the portal vein of normoglycemic, immunodeficient rats. We've successfully applied this technique using donor islets (n=5 donors), showing similar C-peptide secretion and cell survival in both liver-, and kidney transplants (542±377 vs. 790±404 pmol/L at 1 month). Additionally, streptozotocin-induced diabetic rats were transplanted with human donor islets (n=2 donors) resulting in a rapid reversal of hyperglycemia within one week. Next, we investigated the subcutaneous space as an accessible extrahepatic site, with minimal risk of (surgical) complications. Preliminary data show that donor islets (n=1) had poor survival in the subcutis of normoglycemic, immunodeficient mice (C-pep 98±48 pmol/L at 1 month). Survival was improved by implanting an angiocatheter 4-6 weeks before transplantation, where we aimed to trigger a foreign body response, creating a 'pocket' with neovascularization (Cpep 606±574 pmol/L at 1 month). In contrast, SC-islets successfully engrafted in the unmodified subcutis and demonstrated progressive maturation and function over 12 weeks. Notably, stimulated C-peptide levels were higher in unmodified compared to pre-vascularized subcutis (792±370 vs. 511±375 pmol/L at 12 weeks post-transplantation). These findings reveal distinct, site-dependent engraftment characteristics between donor islets and SC-islets, underscoring the importance of transplantation site selection for PSC-based therapies. Future work will directly compare site-specific SCislet engraftment, to guide translational site selection.

P.80 Transient ER stress unlocks beta cell proliferative capacity

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ORAL COMMUNICATION Keywords: *Beta cell proliferation - Endoplasmic reticulum stress - Glucose-dependency - Vascularisation - Diabetes*

BACKGROUND & OBJECTIVES

Regenerating endogenous pancreatic ß-cells is a potentially curative yet elusive strategy for diabetes therapy. Mimicking the microenvironment of the developing pancreas and leveraging vascular signals supporting pancreatic endocrinogenesis may promote ß-cell regeneration. We aimed to investigate whether recovery from experimental hypovascularisation of the endocrine pancreas could trigger ß-cell proliferation.

METHODS

A doxycycline (DOX)-inducible transgenic mouse model was used to induce intra-islet hypovascularisation. In this model, vascular endothelial growth factor (VEGF)-A signalling within pancreatic islets is antagonised via ß-cell-specific overexpression of a VEGF-A decoy receptor, sFLT1. Cessation of sFLT1 overexpression was achieved by DOX withdrawal. sFLT1 expression, vessel kinetics, and ß-cell proliferation following DOX administration and withdrawal were analysed using quantitative RT-PCR and immunostaining. Single-cell RNA-seq was used to investigate effects on the islet cell transcriptome and perform pathway enrichment analysis. RIP-rtTA;TetO-GFP mice were studied in parallel to assess cell cycle induction dependency on vessel manipulation. Additionally, in vitro experiments were conducted to further elucidate in vivo findings.

RESULTS

Serendipitously, we discovered that sFLT1 overexpression in ß-cells induces endoplasmic reticulum (ER) stress and activates proliferation-associated pathways. Upon cessation of sFLT1 overexpression, ER stress decreased and ß-cell proliferation was promoted independently of vessel recovery, as shown by cumulative BrdU labelling over 7 days (mean ± SEM vs control: 14.3 ± 1.3% vs 5.2 ± 0.6%) during the DOX withdrawal period. Transient GFP overexpression also induced ER stress and a subsequent reduction thereof resulted in increased ß-cell proliferation (mean ± SEM vs control: 7.2 ± 0.4% vs 5.1 ± 0.5%). Chemical, transient induction of ER stress in vitro by ER-stress-inducing compounds reproduced this ß-cell cycling response, as assessed by cumulative EdU labelling during a 3 day washout period (mean ± SEM vs control: 2.6 ± 0.4% vs 0.8 ± 0.2% for thapsigargin and 3.8 ± 0.9% vs 1.0 ± 0.2% for tunicamycin), which further increased under high-glucose conditions when islets were exposed to thapsigargin (mean ± SEM vs control: 9.0 ± 1.2% vs 2.0 ± 0.4%). Conclusions & Implications Our findings uncover a link between transgene (over)expression, ER stress, glucose and cell cycle activation in mouse ßcells.

P.81 Systems-Level Comparison of Inflammatory Signaling Inhibition in IFN-α–Exposed Human β-Cells

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ORAL COMMUNICATION Keywords: *IFN-α, β-cells, JAK/STAT signaling, JNK signaling, human beta cells*

BACKGROUND & OBJECTIVES

The pro-inflammatory cytokine interferon-α (IFN-α) plays a key role in T1D by impairing pancreatic β-cell function and inducing HLA class I overexpression, thereby increasing their killing by CD8+ T-cells, and pharmacological targeting of these pathways may reduce β-cell inflammation and preserve function. Several signaling pathways downstream of IFN-α, including JAK/TYK2/STAT and JNK, are under investigation as therapeutic targets, yet their relative impact on IFN-α-induced transcription remains unclear. We performed comparative analysis of inhibition of JAK1/2, TYK2, and JNK signaling in IFNα–exposed human β-cells to identify shared and pathway-specific responses.

METHODS

Human EndoC-βH1 cells were exposed to IFN-α for 24h in the presence/absence of inhibitors targeting JAK1/2 (baricitinib), TYK2 (deucravacitinib), or JNK (SP600125). Transcriptional responses were assessed using RNA sequencing (n=4), followed by determination of differential mRNA expression and pathway enrichment analyses. Transcriptome-wide overlap between conditions was evaluated using Rank–Rank Hypergeometric Overlap analysis. Selected genes and proteins were validated by qPCR and Western blotting. Pathway specificity was assessed using siRNA-mediated knockdown of JAK1, TYK2, or JNK. Key findings were validated in primary human islets.

RESULTS

IFN-α stimulation induced transcriptional activation of inflammatory and immune-related pathways in human β-cells, including upregulation of HLA class I, B2M, and CXCL10, together with the stress-associated genes ATF4, XBP1, TXNIP, and DDIT3 ($p<6.22E-14$, $p<0.00948$, $p<0.00485$, $p<2.91E-20$, respectively). Inhibition of JAK1/2 or TYK2 induced overlapping reductions in canonical interferon-stimulated gene expression and antigen presentation pathways, but TYK2 inhibition led to greater downregulation of antiviral response genes. In contrast, JNK inhibition had limited effects on canonical interferonstimulated genes but modulated subset of stress-related genes, including ATF4 and DDIT3. These findings were confirmed using pharmacological inhibitors or siRNAs in EndoC-βH1 cells and human islets. Conclusions & Implications This comparative transcriptomic analysis demonstrates that pharmacological targeting of IFN-α signaling pathways produces shared and pathway-specific transcriptional effects in human β-cells. Inhibition of JAK1/2 and TYK2 broadly suppresses inflammatory signaling programs, with differential effects on antiviral responses, while targeting JNK preferentially influences stress response pathways. These findings support selection of pathway-specific therapeutic strategies for T1D that may need to be used in combinations.

P.82 Glicentin, a lesser known proglucagon-derived peptide, stimulates insulin secretion and enhances both pancreatic beta-cell growth and survival.

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POSTER Keywords: *Glicentin, GLP-1, Oxyntomodulin, Insulin secretion, cAMP/Ca²⁺*

BACKGROUND

Processing of the proglucagon gene within enteroendocrine L-cells by prohormone convertase 1/3 (PC1/3) leads to the generation and secretion of glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), oxyntomodulin (OXM) and glicentin (GLIC). The biological action of GLP-1, GLP-2 and OXM are well-documented, but the function of GLIC remains unclear. Therefore, the current study aimed to elucidate the effects of GLIC on beta-cell function in direct comparison with GLP-1 and OXM. Methods: A comprehensive investigation of the impact of these three peptides on betacell function was undertaken using BRIN-BD11 beta-cells (n=8) and mouse islets (n=3-4). This included evaluation of glucose (5.6 and 16.7 mM) and concentration (10-12 – 10-6 M) dependent insulin secretory effects as well as PKA and PKC signalling pathway dependence, real-time assessment of islet cell cytosolic cAMP and Ca^{2+} levels, as well as effects on beta-cell turnover. Results: enhanced (3.5-4.4-fold; $p<0.05$-0.001) glucose-stimulated insulin secretion in BRIN-BD11 cells and primary murine islets. Insulin secretory actions correlated with elevations ($p<0.05$-0.01) of islet cell cAMP and Ca^{2+}, although to a lesser extent with GLIC than for GLP-1 and OXM. Accordingly, all peptides activated PKA-dependent beta-cell signalling pathways, with GLIC and OXM also employing PKC linked beta-cell conduits. GLP-1, OXM and GLIC (10-8 – 10-6 M) independently promoted beta-cell proliferation (1.26- 1.43-fold; $p<0.05$-0.001) and conferred significant beta-cell protection against cytokine-induced apoptosis ($p<0.05$-0.001). Conclusion: GLIC possesses similar bioactive profiles to GLP-1 and OXM in islet cells that includes amplification of insulin release as well as stimulation of beta-cell growth and survival, linked to a direct elevation of cAMP and Ca^{2+} concentrations. Whilst GLP-1 signals predominantly via PKA to augment beta-cell function, GLIC and OXM appear to activate complementary beta-cell signalling pathways. These results suggest that GLIC or related analogues may have therapeutic potential alongside GLP-1 and OXM.

P.83 Breaking molecular barriers to enhance the endocrine features of human fibroblasts-derived insulin-producing cells

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ORAL COMMUNICATION Keywords: *β-cell replacement Cell Reprogramming Transplantation*

Beta (β) cell replacement represents a leading strategy for curing type 1 diabetes. While donor islet transplantation has been implemented, donor scarcity and immune rejection limit its use. Stem cell-derived islets are an alternative undergoing clinical evaluation. However, their pluripotency-associated risks and high costs hinder broad clinical translation. To overcome these obstacles, we developed a direct reprogramming protocol to generate insulin-producing cells from human fibroblasts, enabling autologous transplantation and bypassing pluripotency. The induced (i)β-like cells express β-cell genes and secrete insulin, although their granules remain immature1. Bulk-RNAseq revealed sustained expression of REST, a disallowed gene silenced in mature β cells. REST encodes a transcriptional repressor that inhibits genes involved in insulin processing and secretory machinery. We aim to investigate whether REST inhibition can improve the endocrine features of the iβ-like cells. Human foreskin fibroblasts were converted towards iβ-like cells following the introduction of the transcription factors Neurog3, Pdx1, Mafa, Pax4 and Nkx2.21. REST knockdown (KD) was achieved by transfecting a REST-targeting siRNA. iβ-like cells underwent gene expression analysis by qPCR and bulk-RNseq, ultrastructural evaluation via transmission electron microscopy (TEM) and insulin secretion and content quantification by ELISA. REST KD led to a 57%±0.11% reduction in REST gene expression and a 65%±0.10% decrease in REST protein levels at the end of the protocol. This knockdown enhanced the upregulation of known REST targets already induced by reprogramming, including NEUROD1, PCSK1 and SNAP25. Importantly, REST KD also triggered de novo activation of genes involved in insulin granule biogenesis (CHGA) and trafficking (SYT4), and insulin processing (PCSK2). TEM revealed granules with a distinct halo surrounding an electrodense core, consistent with increased maturation. ELISA showed a twofold increase in both intracellular insulin content and insulin secretion, despite no significant changes in insulin gene expression. In conclusion, REST inhibition enhances the endocrine identity of iβ-like cells by promoting expression of secretion-related genes and insulin granule maturation. Further in vitro and in vivo characterization is ongoing to fully assess their functionality. Together, these results substantiate the feasibility of using direct reprogramming strategies to convert fibroblasts into functional insulin-producing cells.

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P.84 Disulfiram blocks gasdermin D-mediated pyroptosis in human islets

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POSTER Keywords: *Human islets Pyroptosis Inflammation Disulfiram Type 1 diabetes*

BACKGROUND & OBJECTIVES

Beta-cell loss in type 1 diabetes occurs alongside progressive islet inflammation, suggesting the involvement of inflammatory cell death pathways. Pyroptosis is a proinflammatory programmed cell death form mediated by gasdermin proteins such as gasdermin D (GSDMD) which upon proteolytic cleavage by caspases form membrane pores resulting in the release of proinflammatory factors such as interleukin (IL)-18. However, the contribution of pyroptosis to islet inflammation and beta-cell death in type 1 diabetes and its potential as a therapeutic target remain unknown. We recently found that proinflammatory cytokines increase the expression of multiple pyroptosis-related genes in human islets but are insufficient to induce GSDMD cleavage. However, cytokine exposure primes human islets for GSDMD cleavage and pyroptosis induction by the innate immune activators lipopolysaccharide (LPS) and nigericin. We presently investigated whether the FDA/EMA-approved drug disulfiram, recently reported to act as an inhibitor of GSDMD, can attenuate pyroptosis in cytokine-primed and LPS+nigericin-treated human islets.

METHODS

Isolated human islets from multiple non-diabetic donors were primed with proinflammatory cytokines (IL-1β, IFNγ, TNFα) followed by LPS+nigericin treatment to trigger pyroptosis in the presence or absence of disulfiram. Pyroptosis was assessed by immunoblotting of full-length and cleaved GSDMD, measurement of cytotoxicity and quantification of IL-18 release into the culture medium.

RESULTS

Cytokine priming of human islets followed by LPS+nigericin treatment induced cleavage of GSDMD accompanied by increased cytotoxicity and IL-18 release. Addition of disulfiram during the LPS+nigericin treatment consistently blocked GSDMD cleavage and reduced cytotoxicity and IL-18 release. Conclusions & Implications: These data support that cytokines prime human islets for pyroptotic cell death induction by innate immune activators and that disulfiram is a potent inhibitor of this process. Targeting GSDMD-mediated pyroptosis by disulfiram may therefore represent a novel strategy to dampen islet inflammation and beta-cell loss in type 1 diabetes.

P.85 β cell-targeted MANF overexpression reverses diabetes in MANF knockout mice, while MANF-deficiency in liver leads to hepatic ER stress

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POSTER Keywords: *Diabetes, MANF, ER stress, Beta cell protection and regeneration, Hepatic dysfunction.*

BACKGROUND & OBJECTIVES

Sustained endoplasmic reticulum (ER) and oxidative stress in pancreatic β cells and liver contribute to β cell dysfunction, impaired insulin secretion, and systemic metabolic dysregulation in diabetes. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER stress-regulating factor with protective and immunomodulatory roles. We previously showed that MANF knockout (Manf–/–) mice develop insulin-deficient diabetes after birth due to sustained ER stress and β cell loss. In humans, MANF-deficiency is associated with syndromic diabetes. We recently demonstrated that elevated MANF in β cells protects against streptozotocin-induced T1D in vivo by reducing islet β cell ER and oxidative stress, p53 targets and immune responses. Using triple-transgenic Manf–/– mice with β cell-specific MANF overexpression, we investigated whether MANF overexpression can reverse diabetes and elucidate the tissue-specific roles of MANF in β cell function and hepatic metabolism.

METHODS

Doxycycline-inducible β cell-specific INS-MANF mice were crossed with Manf+/– mice to generate INS-MANF::Manf–/– mice. Mechanistic investigations are being performed using RT-qPCR, Western blotting, immunocytochemistry, confocal imaging, assays for detecting reactive oxygen species and Oil Red O staining.

RESULTS

Prenatal β cell-specific MANF overexpression in INS-MANF::Manf–/– mice reversed diabetes and prolonged survival of Manf–/– mice. This was associated with restored β cell mass and increased proliferation. Glucose tolerance and insulin sensitivity were comparable to control mice at 6 weeks of age. Furthermore, β cell-specific overexpression restored serum insulin levels, reduced ER stress, and increased expression of β cell identity markers in islets, maintaining normoglycemia for at least 5 months. Despite sustained normoglycemia, prolonged liver MANF-deficiency caused increased hepatic ER stress and impaired insulin sensitivity at 5 months. Given the oxidative stress observed in β cells of Manf–/– mice, we will further investigate the protective effects of MANF in β cell oxidative stress and hepatic insulin metabolism. Conclusions & Implications: Prenatal β cell-targeted MANF overexpression reverses diabetes, restores β cell function and alleviates ER stress in MANF-deficient mice. Prolonged liver MANF deficiency promotes hepatic ER stress and systemic insulin resistance. These findings establish MANF as a key regulator of ER stress with therapeutic potential for β cell failure and hepatic metabolic dysfunction in diabetes.

P.86 Sodium tungstate enables vascularization for effective beta-cell replacement

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POSTER Keywords: *beta-cell replacement, transplantation, vascularization*

BACKGROUND AND OBJECTIVES

A major limitation of cell-based therapies for diabetes is delayed and insufficient graft vascularization, leading to poor cell survival and function post-transplantation. We previously showed that Ptp1b deficiency in transplanted islets increases early graft angiogenesis, improving its vascularization, function and survival1. Here, we aimed to evaluate whether sodium tungstate (NaW), an orally available PTP1B inhibitor with a favorable safety profile and no reported toxicity or adverse effects in a Phase I/II clinical trials, can pharmacologically reproduce these results.

METHODS

We assessed the effect of NaW on graft vascularization using multiple in vivo transplantation models, including human iBetalike cell spheroids generated via direct reprogramming2, stem cell–derived islets and, in a preliminary experiment, human islets in the anterior chamber of the eye. In parallel, in vitro studies were performed to evaluate direct effects of NaW on both endothelial and reprogrammed iBeta-cells and to elucidate the mechanisms of its action.

RESULTS

NaW robustly enhances early graft vascularization across all models in this study, which correlates with decreased cell death, improved overall graft survival and circulating human insulin. Mechanistically, we identified a dual effect: NaW increases VEGFA production in transplanted endocrine cells, but also enhances proliferation, migration and tube formation in host endothelial cells, specifically in VEGF-activated contexts. Conclusion and implications: Our study identifies NaW as a promising pharmacological strategy to enhance graft revascularization in beta-cell replacement therapies by acting on both donor-derived endocrine cells and host endothelial cells. This strategy may represent a clinically translatable approach to overcome a critical limitation in cell-based therapies for diabetes.

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P.87 Stabilizing the ChREBPα:14-3-3 Interaction with a Novel Molecular Glue Protects Human β-cells from Diabetogenic Stress

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ORAL COMMUNICATION Keywords: *Type 1 and type 2 Diabetes, Molecular glue, β-cell preservation, ChREBP*

BACKGROUND

Both Type 1 (T1D) and Type 2 (T2D) diabetes feature the progressive loss of functional β-cell mass. In healthy β-cells, 14-3-3 proteins sequester the transcription factor ChREBPα in the cytoplasm. Metabolic or inflammatory stress dissociates this complex, permitting ChREBPα nuclear translocation. This primes expression of the hyperactive ChREBPβ isoform, triggering a pathogenic feed-forward loop of sustained ChREBPβ expression causing β-cell dedifferentiation and apoptosis. In vivo, β-cell-specific ChREBPβ overexpression causes severe β-cell depletion and glucose intolerance, while targeted ChREBPβ deletion prevents glucolipotoxic death and dedifferentiation. We hypothesized pharmacologically stabilizing the ChREBPα:14-3-3 complex prevents harmful ChREBPβ induction, preserving β-cell mass.

METHODS

We developed Compound 43 (C43), a small-molecule "molecular-glue" stabilizing the ChREBPα:14-3-3 interaction. Efficacy was evaluated in primary human islets (N=3–8 donors/assay) and INS-1 cells (N=3–5 replicates). Cells were exposed to glucolipotoxicity (20 mM glucose + 500 µM palmitate) or inflammatory stress (TNFα 100 ng/ml; IL1β 10 ng/ml; IFNγ 100 ng/ml) to assess survival, gene expression, and function.

RESULTS

HPAP scRNA-seq reveals MLXIPL (ChREBP), its heterodimer MLX, and target genes in β-cells, are elevated in autoantibody-positive (AAB+), T1D, and T2D donors. Pathologic ChREBPβ upregulation drives β-cell failure: T2D donors exhibit increased ChREBPβ protein and higher ChREBPβ:ChREBPα mRNA ratios; normoglycemic NOD mice show early β-cell ChREBPβ before insulitis; db/db and high-fat diet (HFD) mice display prominent nuclear ChREBPβ; and primary human β-cells robustly induce ChREBPβ under stress. C43 selectively enhances the ChREBPα:14-3-3 interaction 60-fold (EC50: 3.9±0.2 µM). In human islets (4 donors), 10 µM C43 prevented stress-induced ChREBPα nuclear trafficking and ChREBPβ upregulation under glucolipotoxic and cytokine stress ($p<0.05$). Thereby reducing β-cell death to baseline, preserved identity markers (PDX1, insulin), and suppressed inflammatory signaling (TXNIP, NOS2, PDL-1, N=3–7;$p<0.01$). Functionally, C43 significantly improved glucose-stimulated insulin secretion (GSIS) during high glucose and cytokine toxicity (N=4;$p<0.01$).

CONCLUSIONS

Pathogenic ChREBPβ upregulation is a critical, shared therapeutic target in T1D and T2D. C43 stabilizes ChREBPα:14-3-3 interaction, sequestering ChREBPα to halt pathogenic ChREBPβ. This protects human β-cell identity and survival under stress yielding a translatable treatment for T1D and T2D.

P.88 Discovery of HIVEP2 as a New Regulator of β cell Susceptibility to Apoptosis and Inflammation in Type 1 Diabetes.

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POSTER

BACKGROUND

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the specific destruction of pancreatic insulin-producing β cells. Recent evidence indicates that β cells contribute to their own destruction in T1D. This includes impaired action of transcription factors (TFs) leading to dysregulated β cell gene expression in T1D proinflammatory environments (T1D-inflammation). Here, we aimed to identify and perturb novel β-cell TFs dysregulated in T1D and T1Dinflammation, relevant to ongoing β cell replacement efforts.

METHODS

Integrating publicly available scRNA-seq datasets from surviving β cells of T1D versus non-diabetic patients1 and from human islets exposed to a cytokine cocktail that mimics T1D-inflammation2, we identified the TF HIVEP2 as dysregulated in surviving β cells of T1D patients and in T1D-inflammation. Using CRISPR/Cas9 and lentishRNA approaches in primary human islets, we perturbed HIVEP2 expression (HIVEP2t). Following exposure to a T1D-inflammation cytokine cocktail2 (+3-Cyto) versus control conditions (-3-Cyto), we measured inflammation markers and apoptosis, using TUNEL. Following transplantation of HIVEP2t versus Controls into streptozocin T1D mice, we measured glucose for 10 days and collected grafts for immunostaining. We performed CUT&RUN using an anti-HIVEP2 antibody to identify HIVEP2 targets in human islets exposed to T1D-inflammation.

RESULTS

We found that HIVEP2 is upregulated in β cells of T1D patients and in human islets exposed to T1D-inflammation. Following exposure to +3-Cyto, loss of HIVEP2 in human islets led to reduced expression of pro-inflammatory markers, including CXCL10. In β cells, it also reduced TUNEL-positive marks. Following transplantation into STZ T1D mice, HIVEP2t reversed hyperglycemia. On graft sections, HIVEP2t β cells showed reduced TUNEL and cleaved Casp3 compared to controls. Using immunohistochemistry on human islets, we found that HIVEP2 increases its expression and nuclear translocation in β cells in T1D-inflammation. HIVEP2 CUT&RUN in human islets identified HIVEP2-regulated genomic regions in T1D inflammation, with associated targets enriched for interferon and apoptosis pathways.

CONCLUSIONS

Our findings identify HIVEP2 as a novel TF leading to death in T1D. Perturbation of its expression and of downstream targets could enhance the survival of β cells and improve therapies for individuals affected with T1D.

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P.89 Human Islet Endothelial Signatures Improve Vascularization and Transplantation of Islets with Stem Cell-Derived Arteries

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POSTER

BACKGROUND

The vasculature of pancreatic islets is essential for delivering nutrients and regulatory signals, and islet endothelial cell (EC) dysfunction is implicated in progression of diseases like type 2 diabetes. Upon transplantation of replacement islet cells, their re-vascularization is crucial for durable islet survival and function to treat type 1 diabetes. However, our understanding of islet vascular biology reflects a reliance on transcriptomics, and largely derives from cell line or non-human pancreatic studies.

METHODS

We analyzed SmartSeq2-RNAseq of islet vascular cells from 8 non-diabetic donors and validated arterial or venous markers by immunostaining human pancreas sections. Transcriptome analysis compared islet ECs with stem cell-derived arterial ECs (SC-arteries), and SC-veins. We generated vascularized islet organoids with SC-arteries and transplanted these cell mixtures in immunocompromised NSG mice, and measured serum human insulin during IPGTT. We confirmed persistence of transplanted SC-arteries with immunohistology and confocal microscopy of recovered grafts.

RESULTS

Analysis of islet ECs revealed enrichment for capillary transcripts, as well as arterial markers. We validated these markers by immunohistology, and found co-localization of capillary (e.g. PLVAP, COL15A1), and arterial (e.g. DLL4, GJA4), but not venous markers (e.g. NRP2, ACKR1, VCAM1) in native human pancreatic islet ECs. Comparison of transcriptomes showed that islet ECs shared more differentially expressed genes with SC-arteries (n=217), than with SC-veins (n=33). Thus, we developed a protocol for culture and stable integration of SC-arteries into vascularized islet organoids. After incorporation of SC-arteries, islet organoids transplanted into immunocompromised mice had improved islet function, including ~30% increased graft insulin content, compared to non-vascularized controls. Moreover, SC-arteries expressed collagen IV like native islet ECs, and anastamosed with host blood vessels.

CONCLUSIONS

Our studies of native human islet ECs revealed unexpected enrichment for arterial and capillary transcripts. Prior protocols to pre-vascularize islets for transplantation relied on human umbilical vein ECs or generic SC-ECs. We show that SC-arteries share more features with native islet ECs than SC-veins and that SC-arteries can improve islet transplantation. We further identified EC-associated cells, such as fibroblasts and pericytes, and transcriptomes from those should

guide efforts to generate SC-fibroblasts and SC-pericytes, to better reconstitute human islet vasculature.

P.90 Conditioned medium from activated macrophages induce GSDMD-mediated pyroptosis in human EndoC-βH5 cells

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POSTER Keywords: *β-cell death Pyroptosis Inflammation Innate immunity Type 1 diabetes*

BACKGROUND & OBJECTIVES

Pancreatic β-cell destruction is a defining feature of type 1 diabetes (T1D) and is driven by autoimmune-mediated inflammation within the islet microenvironment. While apoptosis has been considered the primary mode of β-cell death in T1D, its inherent anti-inflammatory nature contrasts with the progressive islet inflammation observed during disease development. This suggests that additional pro-inflammatory β-cell death pathways contribute to T1D pathogenesis. In addition to adaptive immune responses, islet-resident and infiltrating macrophages contribute to β-cell damage through the release of pro-inflammatory mediators. Our previous data suggests that pyroptosis, a highly inflammatory form of programmed cell death mediated by gasdermin D (GSDMD), may play a role in β-cell loss. However, it is currently unclear if and how macrophages promote pyroptotic signaling in human β-cells. This study therefore aimed to investigate whether macrophage activation induces pyroptotic signaling in human β-cells and to elucidate the underlying mechanisms.

METHODS

Human EndoC-βH5 cells were treated with pro-inflammatory cytokines or conditioned medium (CM) from lipopolysaccharide (LPS)-stimulated THP-1 macrophages. GSDMD cleavage, ATP secretion, and cell death were assessed, and the effects of pharmacological inhibition were evaluated.

RESULTS

Pro-inflammatory cytokines upregulated full-length GSDMD expression in EndoC-βH5 cells but did not trigger its activation by cleavage. Contrary, CM from LPS-stimulated THP-1 macrophages induced cleavage of GSDMD in Endo-CβH5 cells and cell death, indicating that the “secretome” from LPS-stimulated THP-1 cells includes activation factor(s) of pyroptotic signaling. LPS stimulation of THP-1 cells increased ATP secretion and cell death in a dose-dependent manner. Importantly, an inhibitor of GSDMD reduced ATP release from THP-1 cells and attenuated CM-induced GSDMD cleavage in EndoC-βH5 cells.

CONCLUSION

These findings suggest that macrophage activation can trigger human β-cell pyroptosis, potentially via proinflammatory cytokine- and ATP-mediated signaling. Further investigations of

islet cell pyroptosis in T1D-like settings are warranted and could open new avenues for therapeutic interventions aimed at preserving β-cell function by mitigating islet inflammation.

P.91 Enhancing hypoxia resilience in SC-derived beta cells for T1D therapy

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POSTER Keywords: *SC-islets, hypoxia, hypoxia-responsive elements (HRE), mitochondrial metabolism, transplantation*

Background & Objective: Stem cell-derived islets (SC-islets) face a severely hypoxic environment immediately after transplantation, resulting in graft losses exceeding 50% of the initial islet mass. As beta cells rely on oxidative phosphorylation for glucose-stimulated insulin secretion (GSIS), they are particularly vulnerable to oxygen deprivation. To address this, we use a genetic construct that relies on hypoxia-responsive elements (HRE) [1] to conditionally drive the expression of a sensor or a protective target gene exclusively under hypoxic conditions, with the aim of sustaining SC-islet survival until sufficient vascularization is established.

METHODS

Stable iPSC-lines expressing the HRE construct were generated via electroporation and subjected to 1% oxygen for 48h. Hypoxia-specific activation was assessed by bioluminescent sensor Antares and a FLAG-tagged gene. Additionally, WT SC-islets were subjected to 1% oxygen for 24h and characterized by Seahorse respirometry (OCR, ECAR) as well as qRTPCR for hypoxic markers.

RESULTS

Under hypoxia, engineered iPSC lines showed specific activation of the Antares sensor and detectable expression of the FLAG-tagged target gene, confirming conditional construct activity at stem cell level. WT SC-islets subjected to 1% oxygen for 24h showed significantly reduced OCR, elevated ECAR and significant upregulation of GLUT1, MCT4 and PDK1, indicating a metabolic shift from oxidative phosphorylation toward glycolysis.

CONCLUSION

The prononounced hypoxia-driven suppression of mitochondrial respiration and compensatory glycolytic shift highlight the need for targeted genetic intervention. These findings support the rationale for using HRE-driven expression of mitochondrial modulators to mitigate ROS accumulation and preserve the capacity for oxidative metabolism to protect SCislet function during the critical pre-vascularization window.

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P.92 Development of Inducible Hypoimmunogenic iPSC-Derived β-Cells for Immune-Evasive Cell Replacement Therapies in Type 1 Diabetes

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POSTER

Induced pluripotent stem cell (iPSC)-derived islets offer a scalable and renewable source of human β-like cells for cell replacement therapy and disease modelling in type 1 diabetes. Differentiation protocols attempt to mimic pancreatic development through staged exposure to defined factors. However, in addition to immune rejection, variability in efficiency and reproducibility across iPSC lines remains a major barrier, largely due to intrinsic differences in differentiation capacity. In this project, we optimised the widely use protocol described by Balboa et al. to the hiPSCi line Kute4, whose differentiation potential to beta cells is less efficient. Systematic modifications revealed that shortening the definitive endoderm stage improved progression to pancreatic progenitors, which in turn guaranteed the effective differentiation to islets. Optimised cultures exhibit glucose-responsive insulin secretion and a marked reduction in polyhormonal cells, indicating enhanced lineage fidelity and maturation. These findings demonstrate that stage-specific timing adjustments can overcome intrinsic resistance to differentiation in certain iPSC lines. Building on the optimised system, we designed an inducible gene regulation strategy to modulate immunogenicity. Specifically, we propose the doxycycline-dependent modulation of β2-microglobulin (β2M) and Class II Major Histocompatibility Complex Transactivator (CIITA), combined with controlled overexpression of the immunomodulatory protein CD47 to minimise the NK-mediated 'non-self' response. This combination of genes has been trialled in the past but has lacked a strategy to offer temporal control over immune modulation, reducing the risks associated with constitutive immune evasion. Engineered iPSCs will be differentiated with our optimised protocol and evaluated for function and immune interactions in vitro and in vivo. This platform has the potential to improve the safety and efficacy of stem cell-based therapies for type 1 diabetes and reduce the reliance on donor islets and systemic immunosuppression.

P.93 Generating immune stress-resistant β cells for cell replacement therapy

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POSTER Keywords: *SC islets, immune stress, CRISPR/Cas9, co-culture*

BACKGROUND

Type 1 diabetes (T1D) is an autoimmune disorder resulting in destruction of insulin-producing β cells. CD4+ T cells play a crucial role in the manifestation of autoimmunity as well as in graft

rejection, in the case of transplanted islets. Stem cellderived β (SC β) cells pave the way for restoration of glycaemic control in patients after transplantation of in vitro generated stem cell-derived islets (SC-islets), circumventing the shortage of cadaveric islets. However, recurrent autoimmunity still poses a threat to transplanted SC-islets. Collaborators in the Pasquali laboratory established a co-culture system using EndoC-βH1 cells / human islets and activated CD4+ T cells. Activating Transcription Factor 3 (ATF3) was identified as a possible target. It is involved in immune-related stress response and contains a single nucleotide polymorphism associated with T1D from Genome Wide Association Studies (GWAS). This study aims to generate SC-islets resistant to immune-related stress.

METHODS

Co-culture analysis was done using RNA- and ATAC-sequencing. CRISPR/Cas9 mediated deletion of ATF3 and insertion of an H2B-Venus was achieved in the previously published insulin C-peptide mCherry reporter iPSC line. Clones were genotyped using PCR and sequenced for mutations and correct integration. Pluripotency enrichment was performed using MACS sort based on TRA-1-60 and confirmed with flow cytometry. Subsequent differentiation to SC-islets followed Barsby et al. We tested the reporter signal with IFN-γ, IL-1β and TNF-α, using live imaging, flow cytometry or immunohistochemistry as readout.

RESULTS

To test whether ATF3-loss of function could have beneficial immunomodulatory effects, we generated an ATF3 knock out iPSC cell line. We differentiated these cells to SC-islets and confirmed reporter activation by stress stimuli. Mimicking the presence of CD4+ T cells in the established co-culture system, we tested concentrations of cytokines released by activated CD4+ T cells and assessed the viability of cells. Our results suggest that manipulation of ATF3 may influence β cell response to immune-related stress. This will be further investigated with the complete co-culture system.

CONCLUSION

By generating SC β cells resistant to immune-related stress, we could support the advancement in the field of β cell replacement therapy for T1D.

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P.95 RNA Editing Deficiency in Mouse Beta Cells Recapitulates Key Features of Early T1D Independently of Autoimmunity

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ORAL COMMUNICATION Keywords: *Type 1 diabetes; RNA editing; interferon response; insulitis; adaptive immunity*

BACKGROUND & OBJECTIVES

An antiviral type-I interferon (IFN-I) response to double stranded (ds)RNA is implicated in early stages of type-1 diabetes (T1D), but evidence for a causal viral infection is limited. Endogenous RNA species, potentially retroelements, represent an alternative source for dsRNA, normally edited by the ADAR enzyme to prevent aberrant antiviral responses. It was recently suggested that this process is disrupted in autoimmune and autoinflammatory conditions, including T1D. Consistently, knockout of ADAR in mouse beta cells (beta-AdarKO) induced a robust IFN-I response and islet inflammation (insulitis), beta cell loss of identity preceding their selective elimination and diabetes, thereby mimicking key features of human T1D. In this study we have characterized insulitis composition and assessed the importance of adaptive immunity in beta-AdarKO mice.

METHODS

We characterized insulitis composition using immunohistochemistry for cell-type specific and immune activation markers, and measured plasma insulin autoantibody levels using an ELISA-based assay. To assess the importance of adaptive immunity, we either induced T and B-cell deficiency by crossing with RAG1 knockout mice, or overactivated T cells using anti-PD1 checkpoint blockade.

RESULTS

Immunohistochemistry revealed islet inflammation dominated by myeloid cells, with T and scattered B-cells present. However, T-cells lacked the activation marker Granzyme B, and insulin autoantibodies were not detected in the plasma, suggesting that adaptive immune cells are bystanders in this model of diabetes. Consistently, T and B-cell deficiency did not affect insulitis development, diabetes incidence, beta cell loss of identity and selective beta-cell death. Surprisingly, PD-1 checkpoint blockade increased diabetes incidence. Conclusions & Implications: These findings challenge the current paradigm which poses T-cells as essential drivers of betacell damage in early-stage T1D. The increased diabetes incidence following PD-1 checkpoint blockade may represent a novel model for checkpoint diabetes and suggests that upon activation, T-cells might contribute to the disease. Future research will explore the role of innate immune cells in driving beta-cell damage and diabetes in this model, providing novel mechanistic insights into the early events which drive T1D.

P.96 Whole-organ 3D analysis of residual β-cell mass in a long-standing T1D pancreas reveals inverted proportions between extra-islet and islet-associated β-cells

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POSTER Keywords: *T1D, β-cells, Whole-organ, 3D-imaging*

BACKGROUND & OBJECTIVES

Residual β-cell function can positively affect diabetes regulation in T1D, but details of residual β-cell mass distribution in T1D is largely lacking. We implemented an optical 3D imaging pipeline to generate a first account of the 3D-spatial and volumetric distribution of the remaining β-cells throughout the volume of an entire human late onset T1D pancreas, at a microscopic resolution.

METHODS

Pancreata from diseased donors were mounted in agarose in a 3D printed matrix designed for generation of 2.8 mm thick tissue slabs with known spatial origin. This thickness allowed for full reagent penetration. The individual slabs were stained with antibodies and imaged by OPT and/or LSFM. The reconstructed 3D datasets were stitched back into 3D space to recreate the entire organ, much like a 3D jigsaw puzzle in which the position of each piece is known beforehand

RESULTS

As expected, β-cell mass was dramatically lower than in non-diabetic pancreas and the exocrine volume was greatly reduced as previously described. The pancreatic head displayed a morphology and size resembling the non-diabetic pancreas and displayed a 3 times higher β-cell density compared to the rest of the organ. However, only a fraction of these residual βcells were located within islet structures. Instead, the absolute majority were present as extra-islet β-cells, either as scattered individual cells or as punctated clusters of β-cells, spatially separated from all other endocrine cell-types. Strikingly, these extra-islet β-cells comprised the absolute majority of the residual β-cell mass in the T1D pancreas, completely inverting the proportional relationship between extra-islet and islet associated β-cells versus non-diabetic pancreas (n=4). Conclusions & Implications This 3D whole organ depiction of a long standing, late onset, T1D pancreas shows that individual β-cells may be preserved in a highly regionalized manner, and that further investigation of residual β-cells could give clues to developing potential strategies for β-cell preservation in T1D.

P.97 Anti-inceptor antibodies potentiate β-cell insulin signalling, function and survival in healthy and type 2 diabetes mice

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ORAL COMMUNICATION Keywords: *Type 2 diabetes; inceptor; humanized antibody; insulin receptor signalling; β-cell function; β-cell mass*

BACKGROUND

Type 2 diabetes (T2D) is characterized by insulin resistance and progressive β-cell loss and dysfunction. Insulin signalling is essential for β-cell survival, proliferation, and insulin secretion. In this context, the insulin inhibitory receptor (inceptor) has been identified as a novel regulator of insulin receptor signalling desensitization1, 2, making it a promising target for β-cell protection.

AIM

This study aimed to investigate the therapeutic potential of humanized anti-inceptor antibody (HAb) in enhancing β-cell function and preserving β-cell mass in physiological and T2D settings.

METHODS

We developed humanized anti-inceptor HAb targeting the native inceptor ectodomain. These antibodies were tested in vitro using engineered rat insulinoma cells E cells and primary mouse islets, and in vivo in hyperglycaemic clamp studies and a severe T2D mouse model (BKS-db/db) and a multiple low-dose streptozotocin (STZ)-induced diabetic mouse model. Pharmacokinetics, biodistribution, and glucose-dependent target engagement were characterized. Findings were further validated in human islets and human β-cells (EndoC-βH5 cells) to confirm clinical translational relevance.

RESULTS

Anti-inceptor HAb treatment potentiated glucose-stimulated insulin secretion (GSIS) across in vitro and ex vivo assays and during hyperglycaemic clamp studies in vivo, with a clear dose-response relationship. In healthy and diabetic (STZ-induced and BKS-db/db) mouse models, anti-inceptor HAbs showed pancreatic β-cell engagement, improved GSIS, increased β-cell proliferation, and preserved β-cell mass. In human islets and EndoC-βH5 cells, anti-inceptor HAbs potentiated GSIS and increased INSR signalling, as reflected by elevated protein kinase B phosphorylation and improved glucose responsiveness.

CONCLUSION

Pharmacological inceptor blockade using therapeutic antibodies improves β-cell function and preserves β-cell mass in healthy and T2D mouse models by sensitizing INSR signalling. These findings highlight the potential of inceptor as a target for T2D therapy, offering a novel approach to maintain glycaemic control.

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P.98 Re-introducing extracellular matrix to isolated islets and effects on islet function

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ORAL COMMUNICATION Keywords: *Extracellular matrix, islet transplantation*

Islet transplantation represents a promising therapy for diabetes but is hampered by poor islet graft function and survival. Loss of islet extracellular matrix (ECM) during the isolation process is thought to contribute to this. We investigated different approaches to introduce ECM to islets and the effects of this on function. Mouse and human islets were dispersed and reformed with mesenchymal stomal cells (MSCs; known to secrete ECM components) or mouse basement membrane matrix (BMM). Static insulin secretion assays were used to investigate reformed islet function, CalceinAM and ethidium homodimer staining was used to investigate islet cell death and immunofluorescent staining for ECM components was used to investigate ECM deposition in islets. Mouse islets reformed with MSCs showed increased laminin, fibronectin and collagen IV compared to islets reformed without MSCs and whole islets (Laminin: 4.3±0.4%(whole), 2.0±0.1%(reformed), 16.3±1.5%(MSC-reformed). Fibronectin: 1.1±0.1%(whole), 2.3±0.5%(reformed), 4.1±0.1%(MSC-reformed). Collagen-IV: 2.7±0.5%(whole), 2.2±0.3%(reformed), 8.7±0.6% (MSC-reformed), $p<0.05$). Laminin deposition was also increased in MSC-reformed human islets (Laminin: 11.9±1.8%(whole), 21.5±2.6%(reformed), 31.7±6.6%(MSC-reformed)). 20mM glucose-induced insulin secretion was increased in murine islets reformed with different ratios of MSCs (whole: 0.4±0.1ng/islet/hr, reformed: 0.8±0.1 ng/islet/hr, 1:1 islet:MSC reformed: 1.1±0.1 ng/islet/hr, 1:2 islet:MSC reformed: 1.3±0.1 ng/islet/hr, 1:4 islet:MSC reformed: 1.3±0.1 ng/islet/hr, $p<0.05$). Similarly, glucose-stimulated insulin secretion was also increased in MSC-reformed human islets compared to reformed islets but not whole islets (20mM glucose: 0.3±0.05ng/islet/hour (whole), 0.06±0.01ng/islet/hour (reformed), 0.1±0.02 ng/islet/hour (MSC-reformed)). Mouse islets reformed with mouse BMM showed increased laminin and collagen-IV (laminin: 4.5±0.6%(whole), 5.2±0.7%(reformed), 51.8±9.0%(BMM-reformed. Collagen IV: 1.7±0.5%(whole), 1.2±0.2%(reformed), 33.3±5%(BMM-reformed), $p<0.05$) and this was associated with an increase in glucose stimulated insulin secretion (20mM glucose insulin secretion: 0.3ng/islet/hour (reformed), 1.1.ng/islet/hour (BMM-reformed)) but no difference in islet cell death. Reforming islets with MSC or BMM increases islet ECM and is associated with improved insulin secretory function. These approaches may represent a promising way to improve function of islet grafts after transplantation.

P.99 The inguinal subcutaneous white adipose tissue enables rapid revascularisation and intravital imaging of engrafted islets in mouse.

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ORAL COMMUNICATION Keywords: *Graft revascularisation; inguinal subcutaneous white adipose tissue; intravital imaging; islet transplantation; stem*

cell-derived islets

BACKGROUND & OBJECTIVES

Stem cell-derived islets (SC-islets) offer a promising avenue for beta cell replacement therapy in diabetes. Successful clinical translation, however, requires a transplantation site that is safe, accessible, and supports long-term graft function. While the subcutaneous space is attractive, it often fails to support durable engraftment due to poor and delayed revascularisation. The inguinal subcutaneous white adipose tissue (ISWAT), perfused by epigastric blood vessels, represents a candidate alternative site1, however its revascularization dynamics and ability to support the engraftment of SC islets has not yet been studied. We hypothesised that ISWAT provides a superior subcutaneous niche for rapid graft revascularisation compared with conventional subcutaneous sites, enabling intravital imaging of graft survival, vascular integration, and functional engraftment.

METHODS

Primary mouse islets or human SC-islets were transplanted into ISWAT, under the kidney capsule, or into the dorsal subcutis of normoglycaemic or diabetic syngeneic immunocompetent or SCID-Beige immunodeficient mice, respectively. We compared graft survival, vascularisation, and metabolic function across the different sites. Intravital imaging through inguinal windows enabled longitudinal monitoring of graft revascularisation and was used to showcase experimental designs for dynamic in vivo analysis.

RESULTS

Intravital imaging, by stereo- and two-photon microscopy, revealed rapid and consistent vascular network formation and progressive vascular remodeling in ISWAT. Vessel density in ISWAT grafts was significantly higher than in the dorsal subcutis for both mouse islets and human SC-islets. Functionally, the transplantation of 500 mouse islets into ISWAT reversed diabetes, whereas 700 islets in the dorsal subcutis failed to do so. ISWAT grafts of 3000 SC-islets produced increasing levels of circulating human C-peptide from 12 weeks onwards, indicating in vivo functional maturation. Conclusions & implications ISWAT is a subcutaneous transplantation site that supports superior revascularisation, enhances graft survival, and enables intravital imaging of mouse islet and human SC-derived islet grafts in mice. This site provides a valuable preclinical platform for dynamic in vivo monitoring of graft-host interactions and holds translational potential for SC-islet therapy, outperforming conventional subcutaneous sites.

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P.100 Spatial transcriptomic studies investigating the beta-cell RNA landscape in human isolated islets and perioperative biopsies of living donors

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ORAL COMMUNICATION Keywords: *pancreatic beta-cell, isolated human islets, perioperative biopsy, spatial transcriptomics, GeoMx DSP*

INTRODUCTION

The use of human islets has a central role in the diabetes field. Isolated human islets have been instrumental in the investigation of islet biology, and islet transplantation has been recently approved for adults with type 1 diabetes complicated by severe hypoglycemic events. Human islets are isolated from brain-dead organ donors through a process that exposes islet cells to multiple stresses, including cold and warm ischemia, mechanical stress, starvation, and lack of neuronal and vascular signals. This process might have an impact on islet phenotype. In an attempt to improve our understanding of islet biology, we have performed spatial transcriptomics of alpha- and beta-cells from isolated islets cultured in CRML medium and perioperative biopsies collected from living donors.

METHODS

GeoMx DSP was used to perform the RNA profiling of endocrine cells in fresh frozen sections of biopsies and isolated human islets. The spatial profile of the whole transcriptome was performed in populations of cells identified by anti-insulin and antiglucagon antibodies. Isolated islets in culture were further analyzed by performing insulin release assays and quantitative RT-PCR.

RESULTS

Differential expression analysis showed that when compared to pancreatic biopsies, beta-cells of isolated islets presented upregulated expression of beta-cell-specific transcription factors and other genes associated with beta-cell function. Gene set enrichment analysis revealed that olfactory and GPCR receptors were enriched in the biopsies of living donors, while pathways associated with insulin signalling and secretion, cell metabolism and translation were enriched in isolated islets. Quantitative RT-PCR of isolated islets showed that expression of genes associated with beta-cell function and maturity was upregulated in isolated islets cultured in CRML medium.

CONCLUSIONS

These preliminary data reveal that the phenotype of beta-cells from isolated human islets is quite distinct from that of perioperative biopsies, which merits further investigations in order to increase our understanding of beta-cell biology.

P.101 Delineating cellular niches and β-cell maturation during development and regeneration in zebrafish

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ORAL COMMUNICATION Keywords: *beta-cell, development, regeneration, maturation, zebrafish*

BACKGROUND & OBJECTIVES

Zebrafish have significant plasticity and regenerative capacity of insulin-producing β-cells, and to fully explore these features precise genetic editing approaches and tools are needed.

METHODS

Here, we introduce an expanded knock-in strategy using CRISPR-Cas9, PCR amplification to yield dsDNA with 5' end protection as the knock-in template, and gRNA targeting sites upstream of the STOP codon in the last exon. This method readily results in knock-in at the expense of a short truncation of the C-terminus of the endogenous gene product, circumventing the need of a gRNA targets spanning over the STOP codon region of the targeted locus. We successfully used this expanded knock-in strategy to generate zebrafish lines with dual cell labelling and lineage tracing functions, targeting the loci hand2 to label fibroblasts, and nkx6.2 to monitor β-cell maturity.

RESULTS

Using these newly generated lines, we found that hand2+ fibroblasts are heterogeneous in the pancreas and can form an important niche factor lining the luminal ducts. In the pancreatic islet, we found that nkx6.2 labels the functional proportion of β-cells, and that the knock-in line can be used for instant visualization of β-cell maturity. Following β-cell ablation the regeneration of insulin-producing cells appears in different cellular states, out of which those originating from differentiation appeared as mature nkx6.2+ β-cells, whereas those originating from endocrine-cell reprogramming appeared immature and remained bihormonal.

CONCLUSIONS

Our expanded 3' end knock-in method is widely applicable to various cells, tissues and models, and the generated zebrafish lines are powerful tools for delineating pancreatic niches, plasticity, and β-cell heterogeneity. The findings increase our understanding of β-cell development and regeneration and may be leveraged in translational models of diabetes.

P.103 A novel autophagy-activating compound improves glucose tolerance via an mTORC1-independent mechanism in pancreatic beta cells

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POSTER Keywords: *Autophagy, β-cell function, mTOR-independent pathway, Type 2 diabetes, High-throughput screening*

BACKGROUND & OBJECTIVES

Autophagy is an essential protein degradation system for maintaining cellular homeostasis, and its impairment in pancreatic beta cells contributes to the pathogenesis of type 2 diabetes (T2D). We hypothesized that activating autophagy in diabetic beta cells would mitigate their dysfunction and aimed to identify small molecules that increase autophagic flux.

METHODS

We performed high-throughput screening of 9,600-compound library using MIN6 cells expressing a pHluorin-LC3-mCherry reporter to assess autophagic flux based on the pHluorin/mCherry ratio. Hit compounds were prioritized through counterscreening in autophagy-deficient MIN6 cells and dose–response analyses, followed by mechanistic studies. The lead compound was subsequently evaluated in vivo using pHluorin-LC3-mCherry reporter mice and diabetic model mice.

RESULTS

Our primary screening identified 108 compounds that increased autophagic flux in MIN6 cells. Our counter-screening with the autophagy-deficient MIN6 cells and secondary screening to evaluate dose-responses narrowed down the candidates to four. Based on its structure and physiological aspects, we focused on T-068677, which upregulated autophagic flux in MIN6 cells under lipotoxic conditions. A proteomic analysis confirmed that T-068677 significantly degrades a representative autophagic substrate, p62. Interestingly, T-068677 did not inhibit mTORC1 activity, in contrast to the potent autophagy activator Torin-1. T-068677 increased autophagic flux in pancreatic islets in pHluorin-LC3-mCherry-expressing mice, whereas no detectable changes were observed in the liver. Finally, T-068677 treatment significantly improved glucose tolerance in db/db mice (AUC in IPGTT, one-way ANOVA, $p < 0.0001$), with both the 0.2 mg/kg and 1 mg/kg groups showing significant reductions versus control (Dunnett's test, $p = 0.0002$ and $p < 0.0001$, respectively; Figure). Furthermore, the insulin-toglucose AUC ratio was significantly increased in both treatment groups compared with control (one-way ANOVA, $p < 0.0001$), with significant differences in the 0.2 mg/kg and 1 mg/kg groups (Dunnett's test, $p = 0.0391$ and $p < 0.0001$, respectively). Conclusions & Implications These findings suggest that T-068677 improves glucose homeostasis by increasing autophagic flux via an mTORC1independent mechanism and enhancing insulin secretion. T-068677 may represent a promising therapeutic candidate for type 2 diabetes.

P.104 NKX6.1 mRNA copy number as an actionable biomarker to improve prediction of islet graft potency and clinical outcomes

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ORAL COMMUNICATION Keywords: *Type 1 diabetes, Islet transplantation, biomarker, primary graft function, islet treatment*

Background & Objectives. Clinical outcomes after islet transplantation for type 1 diabetes remain heterogeneous1,2, highlighting the need for robust and actionable biomarkers of graft potency. Current release criteria, including islet mass, purity, and viability, poorly predict graft function and long-term outcomes. We hypothesized that NKX6.1, a key β-cell transcription factor controlling identity and function, could serve as a predictive biomarker of islet graft performance3. Methods. NKX6.1 mRNA copy number was quantified by digital PCR in 114 clinical islet preparations. Graft potency was assessed in a standardized in vivo immunodeficient mouse bioassay. Clinical outcomes were analyzed in 34 recipients with type 1 diabetes, with primary graft function (PGF) assessed 1 month after transplantation (BETA-2 score), and long-term outcomes including graft survival and insulin independence. RNA-seq and ChIP-seq were performed in human islets Direct evidence studies included lentiviral shRNA-mediated NKX6.1 knockdown and pharmacological induction with silymarin. Results. NKX6.1 mRNA copy number strongly correlated with in vivo graft function in mice ($r=0.47$, $p<0.001$), independently of islet mass, purity, and viability, whereas islet mass alone was not predictive. In clinical cohorts, higher NKX6.1 expression was significantly associated with improved PGF at 1 month ($p=0.007$), with confirmation in an independent cohort ($p=0.028$). Higher NKX6.1 expression was also independently associated with improved long-term graft survival ($p=0.008$) and sustained insulin independence over 10 years ($p=0.018$). Transcriptomic analyses showed enrichment of β-cell identity and metabolic pathways in NKX6.1-high preparations, with marked downregulation of inflammatory and stress-related pathways (Figure 1A). ChIP-seq showed increased NKX6.1 occupancy at regulatory regions of key β-cell genes, supporting direct transcriptional regulation (Figure 1B). Functionally, NKX6.1 knockdown impaired insulin secretion and graft function ($p=0.037$), whereas silymarin-induced upregulation significantly improved in vivo graft performance ($p=0.028$). Conclusions & Implications. NKX6.1 mRNA copy number is a robust and actionable biomarker of islet graft potency. Digital PCR quantification could improve graft selection and patient stratification. NKX6.1 modulation, such as silymarin treatment, represents a promising strategy to enhance graft function pre-transplantation. These findings may also support quality control strategies for stem cell–derived islets, where assessment and modulation of β-cell identity and functional maturity remain critical.

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THEMATIC AREA 06

METABOLISM IN ISLET CELLS

30 abstracts in this area

P.105 TRPM7 Kinase Is Required for Islet Cell Integrity and Hormonal Regulation

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ORAL COMMUNICATION

OBJECTIVES

Although obesity is a major risk factor for diabetes, many individuals remain normoglycemic due to robust compensatory responses in pancreatic islets. Targeting pathways that enhance pancreatic islet cell compensation could offer new therapeutic strategies. The transient receptor potential cation channel subfamily M member 7 (TRPM7) protein is a chanzyme consisting of a divalent cation-selective channel linked to a protein kinase domain. The kinase moiety of TRPM7 plays an essential role in various cellular processes, including proliferation, cell growth, and exocytosis. While TRPM7 is abundantly expressed in human and mouse islets, its role in glucose regulation and insulin and glucagon secretion remains unclear. This study explores the role of TRPM7 kinase in pancreatic islet cells to assess its potential as a therapeutic target for diabetes. Material and methods: The metabolic phenotype of Trpm7 kinase–dead mice (Trpm7R/R) under chow or high-fat diets were investigated. Islets were isolated from these mice, and β- and α-cell function were assessed.

RESULTS

TRPM7 kinase inactivation via single point mutation in the active site of the kinase leads to impaired glucose tolerance and reduced insulin and glucagon secretion in mice. Notably, Trpm7R/R mice under the obesogenic diet exhibited severely impaired glucose tolerance and hyperglycemia. Ex vivo analysis of islets isolated from Trpm7R/R mice revealed a significant reduction in insulin and glucagon content, as well as decreased islet cell mass and proliferation compared to control littermates. RNA-seq analysis of islets isolated from high-fat diet–fedTrpm7R/R and control islets revealed significant downregulation of key genes involved in insulin and glucagon biosynthesis, including Ins1, Ins2, Gcg, Pdx1, Mafa and Mafb, in the absence of functional TRPM7 kinase. Thus, we attribute the detrimental glucoregulatory effects of TRPM7 kinase deficiency to altered transcriptional regulation in pancreatic islet cells.

CONCLUSION

In obesity, insulin resistance elicits compensatory adaptations within pancreatic islets. Our findings identify TRPM7 kinase as a key regulator of islet function, supporting both β- and α-cell dynamics and preserving glucose homeostasis under metabolic stress induced by an obesogenic diet.

P.106 IL-36α signalling emerges as a cell-specific inflammatory pathway within pancreatic islets in diabetes

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ORAL COMMUNICATION

BACKGROUND AND AIMS

Inflammatory signalling within pancreatic islets contributes to dysfunction during diabetes development. Members of the IL-36 cytokine family regulate immune responses in peripheral tissues, but their role in pancreatic islet biology remains unknown. This study aimed to assess plasma concentrations of IL-36α across murine models of metabolic dysfunction and to determine whether IL-36α signalling is active within pancreatic islets. We further sought to define the cellular distribution of the IL-36 receptor and characterise transcriptional responses to IL-36α stimulation.

MATERIALS AND METHODS

Plasma IL-36α levels were measured by ELISA in high-fat diet–fed mice, multiple low-dose streptozotocin (STZ)-treated mice, and diabetic NOD mice. IL-36 receptor (Il1rl2) expression was assessed using analysis of publicly available bulk RNA sequencing datasets, single-cell RNA sequencing, and immunofluorescence staining of isolated mouse islets. To investigate functional responses, mouse and human pancreatic islets were treated with recombinant IL-36α at 100 pg/ml and changes in gene expression were evaluated by qRT-PCR.

RESULTS

Plasma IL-36α concentrations were increased across all diabetes-relevant mouse models examined ($p<0.05$), indicating activation of this pathway in both metabolic and autoimmune contexts. Transcriptomic analyses demonstrated Il1rl2 expression within pancreatic islets, with single-cell and immunofluorescence analyses showing predominant localisation to glucagon-positive α-cells and minimal expression in insulin-positive β-cells. Analysis of NOD islet transcriptomic datasets further revealed increased Il1rl2 expression with disease progression ($p<0.001$), and receptor expression was also elevated in islets isolated from STZ-treated mice ($p<0.01$). IL-36α stimulation induced a distinct transcriptional response in mouse and human isolated islets, characterised by increased expression of pro-inflammatory mediators, Il6 and Cd11b, and TNFA ($p<0.05$), respectively.

CONCLUSION

These findings indicate that IL-36 signalling operates within pancreatic islets and raise the possibility that α-cells contribute to intra-islet inflammatory signalling during metabolic disease. This work provides a framework for investigating how cytokine signalling may influence islet function in diabetes.

P.107 In Vivo and Ex Vivo β-Cell Function Align Only in Diabetes and Reflect Intrinsic Islet Secretory Capacity

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ORAL COMMUNICATION Keywords: *human islets, glucose-stimulated insulin secretion, in vivo β-cell function, non-glucose dependent insulin secretion,*

glucose sensitivity.

BACKGROUND & OBJECTIVES

In vivo insulin secretion reflects integrated pancreatic islet function within a multi-organ network, whereas ex vivo stimulation of isolated human islets assesses intrinsic β-cell secretory capacity. The correspondence between these measures in humans remains uncertain.

METHODS

We studied 63 individuals undergoing partial pancreatectomy who underwent a preoperative oral glucose tolerance test with model-based assessment of β-cell function. During surgery, pancreatic tissues were collected for ex vivo islet isolation and glucose stimulation at low glucose (3.3 mM), high glucose (16.7 mM) and glucose plus arginine (3.3 mM + 20 mM arginine). Ex vivo function was quantified as the stimulation index (SI) and associated with in vivo indices of insulin secretion.

RESULTS

SI was reduced in islets from diabetic subjects compared with non-diabetic subjects (0.78 ± 0.23 vs 1.04 ± 0.49, $p < 0.001$), while insulin response to arginine was preserved in diabetic subjects (1.92 ± 1.40), with no significant differences compared with non-diabetic subjects (1.88 ± 1.52, $p = 0.93$). In non-diabetic individuals, no significant associations were detected between SI and indices of β-cell function in vivo, including AUC C-peptide ($\rho=-0.18$, $p=0.65$), total insulin secretion rate ($\rho=-0.08$, $p=0.85$) and glucose sensitivity ($\rho=-0.03$, $p=0.98$). In contrast, in diabetes, SI was positively associated with measures of glucose-stimulated insulin secretion, such as AUC C-peptide ($\rho=0.67$, $p=0.02$), total insulin secretion rate ($\rho=0.62$, $p=0.02$) and glucose sensitivity ($\rho=0.63$, $p=0.02$). In diabetic group, SI was negatively associated with the potentiation factor ratio (PFR1) ($\rho=-0.54$, $p=0.05$), a measure of non-glucose–dependent (time-varying) amplification of insulin secretion. In multivariable analysis, a composite index of intrinsic glucose-dependent β-cell function remained independently associated with SI after adjustment for PFR1 (standardized $\beta = 0.69$, 95% CI 0.32–1.07, $p = 0.002$).

CONCLUSIONS

These findings show that systemic β-cell function aligns with intrinsic islet secretory capacity only in diabetes. This suggests that loss of network regulation unmasks intrinsic islet defects, allowing ex vivo islet function to better reflect in vivo insulin secretion. These results provide rationale for a more informed use of isolated human islets, considering differences between diabetic and non-diabetic donors.

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P.108 Iron deficiency induces maturation-dependent loss of pancreatic β-cells.

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ORAL COMMUNICATION Keywords: *Iron metabolism, beta cell maturation*

BACKGROUND AND OBJECTIVES

Pancreatic β-cells maintain glucose homeostasis through glucose-stimulated insulin secretion, a process driven by mitochondrial ATP production and dependent on iron-containing components of the electron transport chain. Notably, the transferrin receptor (TFRC), which mediates cellular iron uptake, is highly expressed on β-cells compared to neighboring αand ∂-cells. While iron overload is cytotoxic, the role of iron sufficiency in β-cell development, function, and survival remains poorly understood.

METHODS

Genetic iron deficiency was modeled using β-cell-specific conditional (Ins1-Cre;Tfrcfl/fl or β-Tfrc-KO) and inducible (Ins1CreERT;Tfrcfl/fl or β-Tfrc-iKO) Tfrc knockout mice. In parallel, α- and ∂-cell-specific Tfrc knockout models (Gcg-Cre; Tfrcfl/fl and Sst-Cre; Tfrcfl/fl) were generated. Iron availability was also chemically modulated using deferoxamine (DFO) in neonatal and adult mouse and human islets, EndoC-βH1 cells, and human induced pluripotent stem cell (iPSC)-derived β-cells.

RESULTS

β-cell-specific Tfrc deletion (β-Tfrc-KO) resulted in progressive β-cell loss and diabetes onset from 5 weeks of age. Iron restriction in β-cells impaired their oxidative metabolism, triggered compensatory glycolysis, and reduced β-cell survival via increased p53-mediated cell death. This phenotype could be rescued by iron supplementation1. Inducible Tfrc deletion in neonatal β-cells (β-Tfrc-iKO, postnatal day 5) caused hyperglycemia and impaired glucose tolerance, whereas deletion in adult β-cells (β-Tfrc-iKO, 10 weeks) had no effect. In addition, Tfrc loss in α- and ∂-cells did not affect their function or survival1. Chemical iron depletion increased TFRC expression in mouse and human islets, EndoC-βH1, and iPSC-derived β-cells. Iron depletion during the endocrine precursor cell stage (stage 5) of iPSC differentiation toward stem cell-

derived islets induced significant cell death, whereas resistance to iron deprivation increased at more advanced stages of differentiation1. Conclusions and implications Our results establish iron as a critical regulator of β-cell maturation, with β-cells demonstrating a pronounced stagedependent sensitivity to iron availability. TFRC-mediated iron import is essential for β-cell survival and function during development but becomes largely dispensable in mature cells. These findings highlight a key link between iron metabolism and β-cell developmental biology and suggest strategies to enhance the functional maturation of stem cell-derived β-cells.

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P.109 New Aspects of Beta Cell Physiology Revealed by mTORC1 Signaling

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ORAL COMMUNICATION

Keywords: *Insulin secretion, Feedback inhibition, mTORC1, Functional heterogeneity, Division of labor*

BACKGROUND AND OBJECTIVES

Pancreatic beta-cells are critical for maintaining glucose homeostasis; however, the intrinsic mechanisms by which they monitor and limit their secretory workload to prevent cellular exhaustion remain poorly defined. This study characterizes mTORC1 signaling as a dynamic sensor of beta-cell activity and elucidates its role in the feedback regulation of insulin secretion. By examining how beta-cells manage metabolic demand, the research aims to identify signaling pathways that balance high-capacity output with long-term cellular viability.

METHODS

mTORC1 activity was quantified using phospho-S6 (pS6) as a marker in human and mouse islets stimulated with various nutrients and secretagogues, assessed via flow cytometry. To clarify downstream mechanisms, fluorescence-activated cell sorting (FACS) and immunostaining were combined with phosphoproteomics and RNA sequencing of sorted beta-cell subsets. Additionally, beta-cell activation and in vivo spatial architecture were evaluated following glucose challenges to map individual cell responses within the intact islet.

RESULTS

The findings indicate that acute pharmacological inhibition of mTORC1 during glucose stimulation enhances insulin release, suggesting that mTORC1 functions as an intrinsic feedback regulator that restrains secretion. Phosphoproteomic profiling demonstrates that mTORC1 modulates the phosphorylation of proteins involved in actin remodeling and vesicle trafficking, particularly within the RhoA-GTPase pathway. Mechanistically, mTORC1 promotes RhoA activation and F-actin polymerization, which limits vesicle movement and reduces the second phase of insulin secretion. This glucose-mTORC1RhoA signaling axis constitutes an autonomous feedback loop that constrains insulin exocytosis and maintains metabolic balance. Additionally, transcriptomic profiling of activated beta-cells reveals a functional continuum based

on activation thresholds. Low-threshold cells prioritize stress resilience for sustained secretion, whereas high-threshold cells specialize in high-capacity protein synthesis and insulin secretion to manage elevated glucose peaks. Conclusions and Implications Analysis of mTORC1 dynamics identified two core mechanisms by which beta-cells control their workload. First, beta-cells utilize an autonomous mTORC1-RhoA-dependent feedback loop to self-regulate and constrain exocytosis. Second, they employ a division of labor through a spatially organized hierarchy of cells with distinct activation states. This organized recruitment optimizes total islet output while preventing individual cellular over-exertion, offering critical insight into how beta-cells resist failure under metabolic stress.

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P.110 Limited oxygen availability increases the vulnerability of pancreatic beta cells to nutrient excess

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POSTER Keywords: *Glucolipotoxicity Mitochondria Bioenergetics β-cells Hypoxia*

Background – Pancreatic beta cell dysfunction caused by excessive levels of glucose and fatty acids contributes to development of obesity-related type 2 diabetes. The mechanism behind this glucolipotoxicity remains incompletely understood, but restricted mitochondrial β-oxidation likely diverts non-esterified free fatty acids into anabolic pathways that impair β-cell function1. Glucose-stimulated insulin secretion demands much oxygen and beta cells indeed experience transient periods of hypoxia during which hypoxia-inducible-factor-1α (HIF-1α) is stabilised2. HIF-1α-mediated hypoxic responses generally include increased anaerobic glycolysis to compensate for decreased oxidative ATP synthesis3. Here we explore the bioenergetic response of beta cells to limited oxygen availability in different nutritional contexts. Methods – INS-1E insulinoma cells were cultured under a range of oxygen atmospheres for 18 hours at various glucose concentrations with or without BSA-conjugated palmitate. Oxygen diffusion path length was set by medium volume. Bioenergetic behaviour was assessed by extracellular flux analysis, protein expression by Western blotting analysis and mitochondrial content with MitoTracker Green. Results – We found that limited oxygen availability decreases the rate of mitochondrial ATP supply without a glycolytic compensatory increase. This bioenergetic phenotype coincides with HIF-1α stabilisation, but the expression of its key metabolic targets (lactate dehydrogenase and pyruvate dehydrogenase kinase 1) is not affected. Mitochondrial content appears unaffected too. Consistent with the disallowed nature of the Ldha gene in beta cells, lactate dehydrogenase protein was not detected at any oxygen tension. Palmitate exposure dampens both mitochondrial and glycolytic ATP supply rates, effects that are exacerbated by limited oxygen availability. Even mildly hypoxic conditions lower the free palmitate concentration at which ATP supply decreases. Conclusion – Our findings suggest an unusual response of pancreatic beta cells to hypoxia in so much that decreased oxidative energy metabolism is not compensated by anaerobic glycolysis.

This bioenergetic response appears to increase the cells' vulnerability to nutrient excess. We are currently investigating the physiological relevance of the apparent control of oxygen over the bioenergetic behaviour of cells that face transient hypoxia as part of their insulin secretory function. Furthermore, we are exploring whether incompletely understood mechanistic aspects of beta cell glucolipotoxicity may be explained by such oxygen control.

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P.111 Glucose-Driven Oxidative Signaling Reshapes the β-Cell Proteome in Human Islets

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POSTER Keywords: *human EndoC-βH5, redox signaling, glucose, ROS, redox proteomics*

Glucose metabolism in pancreatic β-cells triggers production of reactive oxygen species (ROS) that are important signaling molecules essential for proper physiological functions and insulin secretion. The ROS-mediated redox signaling is facilitated by reversible cysteine oxidation, however, the specific mechanisms and downstream protein targets remain poorly defined. In addition, excessive ROS production leads to oxidative stress, a hallmark of Type 2 Diabetes. The aim of this study was to characterize the glucose-induced redox proteome in human pancreatic β-cells and to identify key redox-sensitive proteins and pathways that govern insulin secretion. We applied iodoTMT-based quantitative redox proteomics to human EndoC-βH5 cells stimulated with glucose. This approach enables selective labeling and multiplexed quantification of reversibly oxidized cysteine residues. We developed a robust and reproducible protocol for redox sample preparation optimized for β-cell analysis. To evaluate evolutionary conservation of redox signaling, we performed a cross-species comparison of reversibly oxidized cysteines between human and mouse βcells. We demonstrate that glucose promotes ROS production and induces widespread reversible cysteine oxidation in human pancreatic β-cells. Our optimized protocol yielded reproducible and robust datasets, identifying 156 peptides with significantly enriched reversible cysteine oxidation following glucose stimulation. Proteins of key glucose metabolic pathways, including glycolysis, the Krebs cycle, and protein synthesis in the endoplasmic reticulum, showed increased cysteine oxidation upon glucose stimulation. Cross-species comparison revealed a subset of conserved reversibly oxidized cysteines in glucose metabolic proteins shared between human and mouse β-cells, alongside species-specific oxidation events, pointing to both universal and divergent redox regulatory mechanisms. Reversible cysteine oxidation emerges as a key post-translational modification in glucose-induced metabolism in human βcells. This work reconstructs the complex redox signaling network governing insulin release and provides critical new understanding of how ROS modulate β-cell function. The

identification of both conserved and species-specific redox targets offers novel therapeutic avenues to prevent or reverse β-cell dysfunction in diabetes.

P.112 Dynamic Remodeling of the Intra-Islet Vasculature During Short-Term Culture: Consequences for Beta Cell Function and Graft Revascularization

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POSTER Keywords: *graft revascularization, intra-islet endothelium, endothelial-beta cell crosstalk, T1DM*

The transplantation of isolated pancreatic islets represents a minimally invasive approach to beta-cell replacement that offers the potential to restore physiological glucose homeostasis in type 1 diabetes. The intra-islet endothelium critically regulates beta cell function, angiocrine signaling, and post-transplant revascularization. Despite its importance, the fate of the islet vascular network during short-term ex vivo culture, a required step for clinical transplantation, remains poorly understood. We therefore aimed to characterize the structural and transcriptional dynamics of the intra-islet endothelium during culture and to determine their functional consequences for beta cell physiology. Pancreatic islets from Tie2-GFP mice were cultured for up to five days. Intra-islet capillary architecture was assessed longitudinally by three-dimensional confocal microscopy. Vascular identity and signaling capacity were evaluated through transcriptomic profiling of endothelial and pericyte-associated genes. Mitochondrial oxidative function and glucosestimulated insulin secretion were measured in parallel to monitor islet metabolic fitness throughout the culture period. Three-dimensional confocal imaging revealed progressive capillary fragmentation and spatial disorganization over five days, independent of islet size, consistent with active endothelial remodeling rather than passive cell loss. Gene expression analysis demonstrated partial preservation of vascular identity and a late re-expression of endothelial support markers at day five, indicating residual vascular plasticity under culture conditions. Despite this endothelial deterioration, mitochondrial oxidative capacity remained intact. Glucose-stimulated insulin secretion was nonetheless attenuated, driven primarily by elevated basal insulin release, pointing to impaired endothelial–beta cell functional coupling rather than intrinsic beta cell metabolic failure. Together, these findings establish that the intra-islet endothelium undergoes dynamic yet selective remodeling during shortterm culture with direct consequences for beta cell secretory regulation. Importantly, the dissociation between preserved mitochondrial function and impaired secretion highlights endothelial–beta cell crosstalk as a key determinant of islet quality that is not captured by standard metabolic assessments. Targeting endothelial stability and paracrine signaling during ex vivo culture, therefore, represents a rational strategy to improve islet preparation protocols, promote efficient posttransplant revascularization, and enhance long-term transplantation outcomes.

P.113 M6PR modulates insulin secretion and incretin responses by protecting β-cell lysosomal function

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ORAL COMMUNICATION Keywords: *M6PR, lysosomes, β-cells, islets, diabetes*

BACKGROUND & OBJECTIVES

Emerging evidence implicates lysosomal dysfunction in β-cell failure in type 2 diabetes (T2D), yet its link to insulin secretion remains unclear. The cation-dependent mannose-6-phosphate receptor (M6PR) mediates lysosomal hydrolases delivery, but its role in β-cells remains unknown. We previously identified M6PR as a target of miR-125b, a glucose/AMPK-regulated microRNA whose overexpression impairs insulin secretion and lysosomal function. We hypothesise that M6PR regulates β-cell function by controlling lysosomal-dependent processes, thereby influencing insulin secretion.

METHODS

M6PR expression was measured by RT-qPCR and Western blot in islets and β-cell lines. Lysosomal proteolytic function, autophagy, insulin secretion, cAMP production and receptor internalization were assessed by fluorescence assays following siRNA/shRNA-mediated M6PR knockdown. In vivo relevance was evaluated using β-cell-specific M6pr knockout (M6prβKO, M6PRfl/fl, Ins-Cre) mice.

RESULTS

Islets from high-sugar, high-fat diet (HSHFD)-fed mice contained elevated miR-125b and reduced M6PR (1.42-0.72fold, $p<0.05$) with a strong negative correlation ($r=-0.655$, $p<0.001$). In INS-1(832/3) cells, M6PR knockdown reduced insulin secretion in response to glucose (GSIS), GIP and GLP1 (0.75-0.63-0.53-fold, $p<0.05$), agonist-stimulated GIPR and GLP1R internalization (0.66-0.76-fold, $p<0.05$-0.01) and cAMP production (0.84-0.58-fold, $p<0.05$), lysosomal proteolytic capacity (0.69-fold, $p<0.01$) and autophagosome maturation ($p<0.05$), and mTORC1 signaling. Reduced GSIS and cAMP responses to GIP upon M6PR knockdown were confirmed in human islets. M6prβKO male mice fed a HSHFD, but not chow, developed glucose intolerance and impaired insulin secretion. Isolated M6prβKO islets showed decreased GSIS (0.45-fold, $p<0.01$) and insulin content whilst, paradoxically, secreted more insulin in response to GLP1 and GIP (2-fold, $p<0.05$-0.1) despite reduced cAMP responses (0.67-0.42-fold, $p<0.05$). Additionally, M6prβKO islets displayed reduced lysosomal activity (0.79-fold, $p<0.05$) and accumulation of abnormal lysosomes. RNA-seq data revealed dysregulation of genes involved in lysosomal, clathrin-mediated endocytic and vesicle-trafficking, and mitochondrial oxidative phosphorylation pathways. Conclusions & Implications: Diet-induced obesity and hyperglycaemia reduce M6PR expression, likely through miR-125b. M6PR is essential for lysosomal hydrolase trafficking and degradative function, including autophagy, in β-cells. These alterations likely underlie defective

secretory and signaling responses to glucose and incretins in β-cells lacking M6PR, with potential implications for T2D pathogenesis.

P.114 Metabolic Control of RNA Editing as a Driver of Early β-Cell Inflammation in Type 1 Diabetes

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ORAL COMMUNICATION Keywords: *Type 1 Diabetes, mTORC1, ADAR1 Phosphorylation, RNA Editing, Metabolic Stress*

BACKGROUND AND OBJECTIVES

Aberrant type I interferon (IFN-I) signaling is a hallmark of early-stage Type 1 Diabetes (T1D), yet the initiating molecular triggers remain poorly defined. Normally, the RNA-editing enzyme ADAR1 prevents endogenous double-stranded RNA from triggering spontaneous IFN-I responses. While ADAR1 inactivation is known to cause β-cell destruction1, T1D lacks inherent ADAR1 mutations, pointing to an unknown metabolic or environmental driver of its inhibition. We hypothesize that the central metabolic kinase, mTORC1, serves as this missing link. By acting as a rapid sensor of metabolic workload2, we propose that mTORC1 acutely phosphorylates ADAR1 to suppress its catalytic function, disrupting β-cell RNA homeostasis and priming the cell for innate immune activation.

METHODS

Human islets exposed to varying glucose concentrations (± rapamycin) were analyzed via phosphoproteomics and RNA-sequencing (Alu Editing Index). To isolate the functional impact of the identified phosphorylation event, we engineered phospho-deficient and phospho-mimetic ADAR1 mutants in a human β-cell line. Wild-type (WT) and mutant cells were cultured under varying metabolic conditions. Catalytic RNA editing was subsequently evaluated using a known endogenous target transcript.

RESULTS

Phosphoproteomics of human islets revealed that high glucose triggers mTORC1-dependent ADAR1 phosphorylation at a specific serine residue. Corresponding RNA-seq data showed that this mTORC1 activation globally reduces A-to-I RNA editing. In WT human β-cells, high glucose decreased the editing of a targeted transcript, an effect completely reversed by the mTORC1 inhibitor, rapamycin. Crucially, mutating this serine residue abolished this metabolic control. A phospho-deficient mutant maintained consistently high editing regardless of glucose levels, whereas a phosphomimetic mutant kept editing permanently suppressed. This conclusively proves mTORC1 suppresses ADAR1 activity through single-site phosphorylation. Conclusion & Implications: These findings establish a novel mTORC1-ADAR1 axis where metabolic stress acutely regulates β-cell RNA homeostasis. By demonstrating that mTORC1-dependent phosphorylation acts as a direct inhibitory switch on ADAR1 activity, we uncover a mechanistic link between early metabolic perturbation and defective RNA editing. This axis represents a

potentially targetable node to preserve β-cell function and prevent the initial triggers of autoimmune activation in T1D.

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P.115 Investigating the role of iron-sulfur cluster transport protein “BOLA3” in the beta cell metabolism and function.

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ORAL COMMUNICATION Keywords: *sc-beta cells, diabetes, metabolism, ferroptosis, cell therapy*

BACKGROUND

BOLA3 is a mitochondrial iron-sulfur (Fe-S)-cluster protein that is involved in delivering Fe-S-clusters, crucial cellular cofactors, to key metabolic mitochondrial enzymes. This study aims to elucidate the metabolic pathways influenced by BOLA3 and explore its effects on key cellular processes, including mitochondrial function, redox regulation and ferroptosis. Ferroptosis is an iron-dependent form of cell death driven by harmful lipid peroxides. While iron overload is associated with risk of type 2 diabetes (T2D), how “Ferroptosis” contributes to the beta cell dysfunction and T2D remains poorly understood. Additionally, we examine the connection between BOLA3 and beta cell maturation, ultimately evaluating the therapeutic potential of BOLA3 as a regulator of beta cell metabolism in T2D.

METHODS

We generated a tissue-specific, conditional Bola3 knockout mouse model; developed a doxycyline-inducible Bola3 knockout and overexpression system in the human stem cell derived (SC)-beta cells. The phenotype was characterized by glucose-stimulated insulin secrretion (GSIS), mitochondrial stress test, flow-cytometric asssesment of beta cell identity markers, proteomics and electron microscopy. Ferroptatic stress was evaluated through changes in the labile iron, GSH, 4HNE, TFR-levels.

RESULTS

In this study, we demonstrated that Fe-GSH axis plays an important role for beta cell development and function. Loss of BOLA3 expression in both mouse and human beta cells perturbs this homeostatic axis and leads to increased iron levels and glutathione depletion, which is followed by an increase in lipid peroxidation, eventually compromising the beta cell integrity and oxidative metabolism. Short-term ectopic expression of mitochondrial BOLA3 enhanced the GSIS-response and mitochondrial respiration in the beta cells, however sustained BOLA3 expression impaired the beta cell health, by reducing cell viability and respiration capacity, likely due to iron insufficiency.

CONCLUSION

Our findings shed light on the importance of a previously understudied ferroptosis/iron-gluothione axis in the beta cell development and highlight BOLA3 as a key determinant of functional and metabolic maturation of the beta cells. Precise regulation of BOLA3 expression sustains the balance between iron defficiency and overload and conveys resilience against iron-induced ferroptotic stress in the beta cells. Modulation of BOLA3 expression could provide benefits for SCderived beta cells intended for cell therapy.

P.116 Calorie restriction modulates beta cell IP3R activity to regulate calcium homeostasis and cell network connectivity

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ORAL COMMUNICATION Keywords: *Calorie restriction, Pancreas tissue slice, Calcium dynamics, cAMP signaling, ER calcium homeostasis*

BACKGROUND & OBJECTIVES

Calorie restriction (CR) promotes beta cell longevity by regulating cell identity, organelle and protein homeostasis, and metabolism pathways. Furthermore, CR mitochondria are denser and have an elevated potential to generate ATP. Due to increased peripheral insulin sensitivity, CR mice have reduced insulin secretion. In this study, we investigate changes in calcium dynamics in beta cells in pancreatic tissue slices as effects of changed mitochondrial metabolism causing changes in insulin release, hypothesizing changed [cAMP] baseline as main factor.

METHODS

We used acute pancreatic tissue slices prepared from ad-libitum (AL) or CR mice loaded with a low-affinity calcium indicator and recorded cytosolic calcium with fast confocal imaging. We exposed these slices to increasing and decreasing glucose concentrations, testing for differences in patterns of activation, maintenance, and inactivation. cAMP baseline was tested by applying epinephrine, while we simulated elevated [cAMP] by applying forskolin. ER calcium capacity was investigated using acetylcholine and cyclopiazonic acid (CPA) preventing uptake of calcium into ER. We applied our semi-automatic pipeline to detect thousands of individual beta cells followed by identification of individual calcium spiking events. Hormone secretion was measured using insulin ELISA assays.

RESULTS

CR beta cells have fast short-amplitude calcium oscillations correlating with relatively disconnected beta cell networks across the islet. While CR beta cells showed higher baseline calcium activity, the activation time does not differ between both groups. However, CR beta cells

stayed active longer during post-stimulatory glucose depletion. Acetylcholine stimulation lead to faster IP3R-driven calcium oscillations linked to higher cytosolic [cAMP] protect beta cells against acute ER depletion. SERCA inhibition shows higher buffer capacity in CR beta cells. Surprisingly, insulin measurements showed no difference in secretion between AL and CR slices.

CONCLUSION

This study shows that CR might not only influence beta cells on a morphological but also functional level. We provide evidence that [cAMP] in islets of CR mice is higher than in AL mice. Furthermore, CR mice control and maintain beta cell functionality with higher speed and precision. We show that this is due to better calcium homeostasis in the ER, but indifferent insulin release.

P.117 A fatty acid / ROS level switch determines endocrine specification and β-cell functionality

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POSTER

Diabetes modeling and cell therapies rely on hPS cell-derived β-cells. Growing evidence suggests that a precisely regulated differentiation of pancreatic progenitors (PPs) into the endocrine lineage is crucial for subsequent β-cell functionality. We have shown that the mitochondrial Aldehyde dehydrogenase 1b1 (Aldh1b1) is expressed in mouse PPs, but not in differentiated cells. Aldh1b1 regulates the timing of PP differentiation and is necessary for β-cell functionality. Aldh1b1 null PPs differentiate earlier and Aldh1b1 null adult mice develop β-cell dysfunction and age-related hyperglycemia. We hypothesized that Aldh1b1 acts as a metabolic regulator linking the self-renewal and differentiation of the PPs with the functionality of their progeny. Aldehyde derivatization of wild type and Aldh1b1 null progenitors revealed that Aldh1b1 acts as a key facilitator of fatty acid oxidation by detoxifying aliphatic aldehydes which are byproducts of the process. In its absence, ROS levels increase and trigger downregulation of glycolysis and collapse of oxidative phosphorylation. Changes in the metabolism can be reflected on chromatin accessibility and transcription of PPs. Epi-CyTOF experiments together with ATAC-seq confirmed reduced chromatin accessibility and transcriptomic changes that affect endocrine development via the disruption of the ERK signaling cascade. Treatment of Aldh1b1 null embryos in utero with N-acetylcysteine reversed the βcell dysfunction phenotype indicating that ROS levels are the key mediator of these effects. To link these early PP deficiencies with β-cell function, we performed the same multilevel analyses on postnatal β-cells. Results revealed the amplification of chromatin and gene expression defects related to β-cell function and metabolism. In summary, fatty acid oxidation is a key metabolic pathway in pancreatic progenitors and Aldh1b1 enables this central role by detoxifying aliphatic aldehydes and maintaining low ROS levels. Timely downregulation of Aldh1b1 may be the physiological trigger for endocrine differentiation. Deciphering the metabolic programming and molecular mechanisms that govern

pancreatic progenitor differentiation and β-cell maturation will contribute to achieving full maturation of hPS cellderived β-cells for diabetes modelling and cell therapy.

P.118 Resistance training alters cytokine and metabolomic profile contributing to improved beta-cell function in mice islets

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POSTER Keywords: *exercise, cross-talk, metabolism*

BACKGROUND AND OBJECTIVES

Resistance training improves pancreatic beta-cell function and survival in healthy and type 1 diabetic mice. These effects are mediated by exercise-induced factors released in the bloodstream. Here, we analyzed the serum of resistance-trained mice to identify molecules potentially involved in these adaptations. Methods: Male C57BL/6 mice were divided into two groups: control (CON) and resistance training (RT). RT mice performed one exercise session/day (8 climbs at four different loads), 5 days/week, for 10 weeks. Blood samples were collected to obtain serum, which was used for: in vitro experiments, metabolomic analysis by nuclear magnetic resonance, and cytokine array analysis. For in vitro experiments, pancreatic islets from C57BL/6 mice were incubated for 24h with medium containing 10% of serum from CON or RT mice. Insulin secretion was then assessed. Data are mean±SEM, P≤0.05 (Student's t-test or One-Way ANOVA/Tukey's post-hoc test). Results: Pancreatic islets treated with the serum from resistance-trained mice secreted more insulin than islets treated with serum from CON mice (Stimulation index [high/low insulin]: CON 2.933±0.49, RT 3.717±0.63). Next, we analyzed the serum by cytokine array and metabolomic analysis. Regarding cytokines, 24 were upregulated, and 27 were downregulated in RT serum compared to CON (Only cytokines with |log2 fold change| ≥ 0.38 were considered). The cytokine profile of resistance-trained mice showed elevation of molecules associated with hematopoietic, immune-regulatory, and angiogenic pathways alongside reductions in inflammatory and immune-activating mediators such as chemokines (e.g., KC/CXCL1, LIX/CXCL5, and IL-1α/TREM-1). Metabolomic analysis revealed reduced concentration of acetate, citrate, isobutyrate, isoleucine, leucine, phenylalanine, trimethylamine, and tyrosine in the serum of RT mice (p≤0.05). These alterations reflect systemic metabolic adaptations to long-term resistance training, characterized by enhanced mitochondrial efficiency, increased amino acid uptake by skeletal muscle, reduced proteolysis, and altered gut microbiota-host metabolic crosstalk. Conclusions: The reduction in specific amino acids, TCA-related metabolites, and those derived from gut microbiota induced by resistance exercise changes the cytokine profile, resulting in reduced systemic inflammation and immunometabolic adaptation. Together, these alterations may contribute to a favorable environment for improving pancreatic beta-cell function.

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P.119 Beyond glucose tolerance: Wisp1 controls adipose tissue inflammation and β-cell remodelling in obesity

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ORAL COMMUNICATION Keywords: *inflammation, plasticity, obesity, β-cell, adipose*

BACKGROUND & OBJECTIVES

WNT1-inducible signaling pathway protein 1 (Wisp1/CCN4) is a matricellular protein elevated in the circulation during early postnatal development in mice, and in children as compared to adults. We previously showed that exogenous Wisp1 stimulates β-cell proliferation, suggesting a role in β-cell mass regulation. Besides, Wisp1 has also been identified as an adipokine associated with inflammation and insulin resistance in obesity, but its contribution to whole-body glucose metabolism and type 2 diabetes remains unclear. Our study addresses this gap by investigating these relationships in a preclinical mouse model.

METHODS

We used a systemic Wisp1 knockout (WispKO) mouse model to examine Wisp1's role in adult glucose homeostasis under normal conditions and obesity-related metabolic stress. For diet-induced obesity, 6-week-old male and female mice were fed a high-fat (HF) diet for 20 weeks, followed by metabolic testing, morphometric analysis, plasma measurements, and gene-expression profiling.

RESULTS

Under physiological conditions, Wisp1 deficiency did not affect glucose tolerance, insulin secretion, or pancreatic endocrine cell mass in either sex. However, WispKO male, but not female, mice on a HF diet exhibited enhanced insulin sensitivity, accompanied by lower insulin levels, and a marked reduction in adipose tissue inflammation, as evidenced by diminished macrophage infiltration and attenuated expression of proinflammatory cytokines, such as Tnf, Itagx and Ccl2, in the visceral fat pad. Additionally, levels of the monocyte chemoattractant protein Ccl2 were similarly reduced in the circulation of WispKO male mice. Despite the improved

insulin sensitivity, WispKO mice failed to exhibit the robust β-cell mass expansion induced by HF diet in wild type mice, aligning with lower macrophage infiltration in islets. Conclusions & Implications: Our study highlights the complex interplay between inflammation and β-cell plasticity in obesity. Wisp1 plays a sexdependent role in modulating visceral adipose tissue inflammation and the β-cell adaptive response to obesity. Our data suggest that improved insulin sensitivity in WispKO mice occurs likely due to mitigated adipose tissue inflammation, without changes in glucose tolerance. Overall, Wisp1 primarily regulates local inflammatory responses in adipose tissue, which then indirectly shape how pancreatic islets adapt to metabolic stress.

P.120 From Stress to Adaptation: β-Cell Resilience Following Partial Ablation and Metabolic Challenge

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POSTER Keywords: *β-cell adaptation, insulin secretion, metabolic stress*

Background & Objectives. Diabetes is a widespread metabolic disorder marked by progressive β-cell dysfunction and loss, highlighting the importance of understanding how the remaining β-cells respond to metabolic challenges to preserve glucose homeostasis. Our aim was to examine how reduced β-cell mass influences early metabolic and molecular adaptations to diet-induced stress. Methods. To address this, we used the NSG RIP-DTR mouse model, in which the human diphtheria toxin (DT) receptor is expressed under the rat insulin promoter in an immunodeficient background. Upon 50% β-cells ablation by DT administration, mice were subjected to a high-fat diet (60% kcal from fat) combined with high-glucose drinking water (20%) for 4 weeks. Physiologic parameters were assessed at 2 and 4 weeks, alongside transcriptomic profiling of isolated pancreatic islets. Results. DT-treated mice exposed to the obesogenic diet initially displayed impaired glucose tolerance and decreased insulin staining in the islets at 2 weeks, indicative of early metabolic stress. Temporary loss of function could be observed by decreased nuclear Pdx1 expression in the insulin-positive cells. However, by 4 weeks, insulin levels normalized and glucose handling improved, suggesting a degree of metabolic adaptation. Gene expression analysis demonstrated dynamic changes over time, with early enrichment of pathways related to β-cell identity and stress responses, followed by a shift toward adaptive processes, particularly those involved in protein folding and maintenance of glucose homeostasis. Conclusions & Implications. Overall, these results underscore the remarkable plasticity of β-cells and their ability to adapt to combined metabolic and cellular stress. Understanding these compensatory mechanisms may provide new insights for preserving β-cell function and improving therapeutic strategies in diabetes. This research was funded by the Romanian Academy and by the Romanian Ministry of Research, Innovation, and Digitization, PNRR program, CF 197-2022/PNRR-III-C9-2022-I8 (contract number 760059/23.05.2023); the grant PED47/2025 (ReGenTACol, PN-IV-P7-7.1-PED-2024-1926; and the grant RO-NO-2019-0544; contract number 21/2020 BETAUPREG/the NO Grants 2014-2021.

P.121 Impact of aging and obesity on pancreatic islet function

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ORAL COMMUNICATION Keywords: *Insulin secretion, aging, high-fat diet, transcriptomics, mice*

BACKGROUND AND OBJECTIVES

Obesity and aging are major risk factors for the development of type 2 diabetes. Both conditions induce insulin resistance, thereby increasing the functional demand on pancreatic β-cells. High-fat diet (HFD) feeding is known to trigger adaptive responses within islets to meet this elevated insulin demand; however, it remains unclear whether these adaptive mechanisms are age-dependent. This study investigated whether HFD-induced metabolic and transcriptomic responses in islets differ between young adult and aged mice.

METHODS

Young adult (3-month-old) and aged (18-month-old) male C57Bl/6J mice were fed either a low-fat diet or a HFD for 3 months. Glucose tolerance and islet secretory function were assessed, and transcriptomic profiling was performed on isolated islets to evaluate age- and diet-related changes in gene expression.

RESULTS

Aged mice exhibited a greater susceptibility to HFD-induced insulin resistance than adult mice. Although HFD impaired glucose tolerance in both age groups, plasma insulin concentrations were markedly higher in aged HFD mice. Proinsulin levels increased exclusively in aged HFD mice, suggesting elevated β-cell workload and potential secretory stress with aging. Transcriptomic analysis revealed that adult islets mounted a substantially stronger transcriptional response to HFD than aged islets. Pathway enrichment analysis identified enhanced activation of cell-cycle pathways and protein-processing in ER pathways in adult HFD islets, whereas these responses were markedly attenuated in aged islets. HFD also induced cytokine-interaction pathways in adult islets; in contrast, aging alone increased cytokine-related pathways in islets from aged mice, and HFD did not further elevate these pathways. Notably, HFD selectively reduced expression of ion channel–related genes in adult islets, whereas aged islets already exhibit reduced expression of these genes, with no additional effect of HFD. Conclusions and implications: Aged C57Bl/6J mice are more vulnerable to HFD-induced insulin resistance and exhibit increased insulin and proinsulin secretion to maintain glucose homeostasis. However, their islets display markedly blunted transcriptional adaptability to metabolic stress, suggesting a shift toward greater reliance on post-transcriptional or post-translational mechanisms to sustain insulin secretion with aging.

P.122 Mitochondrial glutamate pathways mediate sex-specific and age-dependent metabolic rewiring of the ß-cells under a diabetogenic diet

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ORAL COMMUNICATION Keywords: *Type 2 Diabetes Aging Metabolism*

Mitochondrial glutamate dehydrogenase (GDH) amplifies insulin secretion via glutamate formation in ß-cells and, while dispensable for glucose homeostasis under standard conditions, contributes to diet-induced obesity. Its role in glucose intolerance and prediabetes development remains unknown. Here, we characterized islet metabolic rewiring under prediabetes induced by a high-calorie (HC) diet in female and male mice across ages, with and without ß-cell GDH. Male and female mice aged 1, 3, 12, and 24 months, both ß-cell-specific GDH knockout (ßGlud1-/-) and age-matched control, were fed a standard (ST) diet, or HC for 2 months for groups sacrificed at 3 and 12 months prior to sacrifice (n=5–11 per group). Weekly bodyweight, random glycemia, glucose (GTT), and insulin (ITT) tolerance tests were performed prior to sacrifice. Pancreata were collected and cryosectioned for in-situ spatial metabolic enzyme activity using the Nitro-Blue Tetrazolium assay (GDH: glutamate pathways; GAPDH: glycolysis; LDH: anaerobiosis; SDH: mitochondrial activity). In ST-fed mice, aging did not alter glucose homeostasis in either controls or ßGlud1-/- mice, with both sexes maintaining glucose tolerance through 24 months. HC diet at 3 months likewise had no effect on glucose tolerance, though islet anaerobic glycolysis was elevated in young control males versus ST counterparts, but not in ßGlud1-/- mice. At 12 months, HC diet induced glucose intolerance in both control and ßGlud1-/- mice without affecting insulin sensitivity. 12-month-old control males, not females, gained fat mass and bodyweight. This was accompanied by upregulation of islet glutamate, glycolytic, and anaerobic pathways. In contrast, ßGlud1-/- males were resistant to HC diet-induced adipogenesis, with islet metabolic pathways preserved except for the genetically absent GDH activity, implicating ß-cell GDH and aging as synergistic drivers of the HC diet phenotype in control males. Female mice were protected against HC diet effects regardless of age. Aging alone does not significantly alter islet metabolism or glucose homeostasis. However, HC diet triggers a glycolytic shift in male islets preceding phenotypic changes, while females remain protected against the combined effects of aging and HC diet, revealing sex-dimorphism. The islet metabolic rewiring and weight gain observed in HC-fed males are driven by ß-cell GDH.

P.123 Chronic bisphenol-A increases glucose-stimulated insulin secretion, whilst impairing mitochondrial efficiency in INS-1 (832/13) pancreatic β-cells.

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POSTER Keywords: *BPA, mitochondria, insulin, β-cells, INS-1.*

BACKGROUND & OBJECTIVES

Bisphenol A (BPA) is a widely used plasticiser commonly found in food and drink packaging. Increased exposure to BPA is associated with an increased incidence of type 2 diabetes (T2D) in humans. However, underlying molecular mechanisms remain unclear. Here, we investigated whether chronic exposure to BPA impacted cytotoxicity, glucose-stimulated insulin secretion (GSIS) and mitochondrial oxygen consumption rate (OCR) in a pancreatic β-cell line.

METHODS

For all experiments, rat INS-1 (832/13) β-cells were cultured ± BPA (10nM) for 48h. 0.1% DMSO was used as the vehicle control. GSIS was measured in response to 30 min stimulation with 20mM glucose and determined by ELISA (Mercodia). OCR was assessed using a Seahorse extracellular flux analyser. Cytotoxicity was measured using a CellTox™ Green assay (Promega). Data are presented as mean±SEM.

RESULTS

Cytotoxicity did not differ between BPA-treated and control cells. However, BPA-treated cells increased GSIS, in comparison to control cells (BPA=0.61±0.05 vs Control=0.30±0.07 ng/mL µg protein, $p<0.001$, n=3). BPA-treated cells also displayed decreased ATP-linked OCR (BPA = 58.43±1.41 vs Control=71.61±1.049, contribution to total OCR %, $p<0.0001$, n=8), but no change in mitochondrial leak, in comparison to control (BPA = 46.98±1.62 vs Control=49.51±0.99, contribution to total OCR %, $p=0.69$, n=8). Conclusion & Implications: These data demonstrate that BPA significantly increases GSIS while impairing mitochondrial efficiency in pancreatic β-cells. Increased insulin secretion and reduced ATP-linked respiration are aligned with early signs of β-cell stress, commonly observed within T2D development. Hence, these findings suggest that BPA exposure may contribute to early T2D-related β-cell dysfunction.

P.124 Loss of keratin 18 leads to diminished pancreatic islet mass and disrupted β-cell GLUT2 targeting

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ORAL COMMUNICATION Keywords: *Cytoskeletal keratin networks Islet mass Insulin secretion glucose homeostasis GLUT2*

Background/Ojectives: Pancreatic β-cells in the islets of Langerhans control the glucose stimulated insulin secretion (GSIS) and blood glucose homeostasis. Epithelial keratin (K) filaments have distinguished structural and stress-protecting cell functions, as shown in multiple animal models, cell lines, and further demonstrated by the keratinopathies described in humans. In human β-cells, simple epithelial type I K18 and type II keratin K8 pair together producing functional keratin filaments, whereas in mice, both K8 and K7 type II keratins pair with K18. In this study we hypothesize that K18, as the only type I keratin in islet cells, has a central role in β-cell function. Methods and Results: We utilized the K18 knock-out (K18–/–) mouse model to examine the impact of total keratin loss on pancreatic islet histomorphology, β-cell function and maintenance of glucose homeostasis. Glucose and insulin tolerance tests revealed that K18–/– mice exhibit improved glucose tolerance, with no difference in insulin sensitivity compared K18 wild-type (K18+/+) controls. Basal serum glucose and serum insulin levels were unchanged. Histopathological analysis demonstrated a significant reduction in both the number and size of pancreatic islets in K18–/– mice, resulting in a 2-fold decrease in islet mass. Despite this, total pancreatic insulin content relative to pancreas mass was comparable to K18+/+ mice. Notably, an increased presence of red blood cells within K18–/– islets was observed, suggesting potential alterations in islet vascularity or capillary dilation. Preliminary ex vivo GSIS analysis suggests that K18–/– islets may exhibit a delayed insulin secretory response compared to K18+/+. Consistent with this, immunofluorescence analysis indicates reduced plasma membrane localisation of glucose transporter 2 (GLUT2) in K18–/– β-cells.

CONCLUSIONS

Taken together, these findings identify cytoskeletal K18 as a novel and important regulator of islet structure and β-cell function. Despite reduced islet mass and altered GLUT2 localisation, K18–/– mice maintain normal basal serum and pancreatic insulin levels, and exhibit improved glucose tolerance, suggesting the presence of compensatory mechanisms that preserve metabolic homeostasis.

P.125 Extracellular vesicle microRNAs regulate β-cell proliferation during obesity progression

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ORAL COMMUNICATION Keywords: *- Extracellular vesicles - Interorgan crosstalk - microRNAs - Beta-cell proliferation - Obesity*

BACKGROUND & OBJECTIVES

Type 2 diabetes (T2D) involves progressive pancreatic β-cell dysfunction driven by obesity and insulin resistance. Glucose homeostasis depends on interorgan communication, recognized to be mediated by circulating extracellular vesicles (EVs) carrying bioactive molecules such as microRNAs (miRNAs). Although EVs are emerging as regulators of metabolic adaptation, their impact on β-cell fate during obesity progression remains unclear. This study aimed to determine how circulating EVs influence β-cell proliferation and survival during early and prolonged exposure to obesogenic diets.

METHODS

Mice were fed a high-fat diet (HFD, 45% lipids) for 2 or 12 weeks, or a high-fat, high-cholesterol diet (HFHC, 60% lipids, 0.5% cholesterol). Plasma EVs were isolated and characterized for functional assays and small RNA sequencing. β-cell proliferation and apoptosis were assessed in dissociated islet cells (DICs) using Ki67 immunostaining and TUNEL assays, respectively.

RESULTS

EVs from mice fed an HFD for 2 weeks induced a twofold increase in β-cell proliferation when administered in vivo to control mice. This effect was reproduced in DIC cultures treated with 2-week-HFD EVs. In contrast, EVs from mice subjected to prolonged HFD no longer promoted proliferation. Moreover, EVs isolated from HFHC-fed mice showed marked detrimental effects, reducing β-cell proliferation by 50% and increasing apoptosis threefold. Small RNA sequencing identified 21 differentially expressed EV-miRNAs through diet conditions. Among them, 14 miRNAs were downregulated under prolonged HFD or HFHC feeding, including miR-484, while 7 miRNAs were upregulated early during HFD exposure and remained elevated at later stages. Combined inhibition of three of these upregulated miRNAs (miR-122-5p, miR-194-5p, miR-192-5p) abolished the proliferative effect induced by early HFD EVs, identifying this miRNA set as key mediators of adaptive β-cell expansion. Conclusions & Implications Circulating EVs undergo a functional transition during obesity progression, promoting adaptive βcell expansion at early stages but contributing to β-cell loss under advanced obesogenic conditions. EV-associated miRNAs emerge as key mediators of this process and may represent biomarkers and therapeutic targets to preserve β-cell mass and delay T2D progression. Funded by the EFSD/BI Programme and ISCIII (grant PI20/00658, cofunded by EU).

P.126 Chromogranins and secretogranins in the pathogenesis of T1D

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POSTER Keywords: *T1D pathology, insulin granule proteins, beta cell dysfunction*

Type 1 diabetes (T1D) is a complex and heterogeneous disease characterized by the immune-mediated destruction of pancreatic beta cells. This destruction might be linked to beta cell stress, which can lead to abnormal protein processing and accumulation, potentially triggering immune responses. While most of the research has focused on proinsulin processing to insulin, other molecules within the insulin granules, specifically chromogranins, secretogranin (known as granins) and islet amyloid polypeptide (IAPP), could undergo similar processing errors and be targeted by the immune system. Granins are essential for granule assembly, insulin sorting and condensation. Our study analyzed the expression levels of several granins and IAPP in pancreatic tissue from organ donors, including individuals with and without T1D and double autoantibodypositive (dAAb+) using different microscopy platforms, from whole-slide to confocal imaging. At the islet level, granin expression was detected in both beta and alpha cells and was comparable across all donor groups, irrespective of disease stage. In contrast, high-resolution confocal imaging revealed a decrease in chromogranin A (CHGA) intensity in individual beta cells of dAAb+ and T1D donors, while insulin intensity was not different between the donor groups. These findings suggest that changes in granule composition occur early and persist throughout disease development. Overall, our results identify alterations in granin expression as a potential marker of beta cell dysfunction and could sever as early indicators of T1D.

P.127 Human stem cell-derived islet function and survival is improved by a mesenchymal stromal cell secretory peptide cocktail

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ORAL COMMUNICATION Keywords: *Stem cell derived islet, MSC, islet metabolism*

BACKGROUND

Human islet transplantation can improve blood glucose control in type 1 diabetes, but islet availability is ratelimiting. We have generated glucose-responsive stem cell-derived islets (SC-islets) using the seven stage “Helsinki” differentiation protocol, but they are not as functionally mature as primary human islets. We have previously shown that mesenchymal stromal cell (MSC) secretory products protect human islets from apoptosis and improve their function. Here we assessed the effects of a cocktail of MSC secretory molecules on SC-islet survival, mitochondrial function and insulin secretion.

METHODS

Mature SC-islets from four independent differentiations were treated with 5nM annexin A1, 10nM stromal cellderived factor-1 and 10nM complement component C3 (‘cocktail’) for 72hrs. Cytokine-

induced apoptosis was assessed by measuring caspase 3/7 activities, oxygen consumption rate (OCR) was measured by Seahorse mitochondrial assay and 20mM glucose-stimulated insulin secretion (GSIS) and dynamic insulin secretion were quantified by radioimmunoassay.

RESULTS

Cocktail pre-treatment protected SC-islets against cytokine-induced apoptosis (basal: 100±3.2%; cytokines: 426.8±30.5%; cytokines + cocktail: 285.3±27.3%, n=4, p<0.0001). While cocktail pretreatment did not significantly increase glucose-stimulated OCR (ctrl: 143.9±12.0 pmol/min; cocktail:177.1±14.4, n=4, p=0.08), or maximal respiration (ctrl: 137.2±9.0 pmol/min; cocktail: 156.1±9.7 p>0.1), it did significantly increase ATP-coupled respiration (ctrl: 80.1±8.0 pmol/min; cocktail: 103.6±7.7, p<0.05). Cocktail pretreatment significantly increased dynamic insulin secretion from stage 7-week 2 SC-islets (2mM glucose AUC, ctrl: 12.9±0.8, cocktail: 19.1±2.2, p<0.01; 7mM glucose AUC, ctrl: 25.6±1.6, cocktail: 34.5±3.6, p<0.01; 15mM glucose AUC, ctrl: 58.6±7.1, cocktail: 73.0±8.6, p<0.05). GSIS was also significantly increased in stage 7-week 6-8 islets in static incubations (ctrl, 2mM glucose: 0.15±0.02 ng/islet/hour; 20mM glucose: 0.23±0.02; cocktail, 2mM glucose: 0.25±0.02; 20mM glucose: 0.40±0.02, p<0.0001).

CONCLUSION

Pre-treating SC-islets with an MSC secretory peptide cocktail reduces apoptosis, increases ATP-coupled respiration and enhances glucose-induced insulin secretion. Refinement of this approach may lead to more functionally mature SC-islets for transplantation, which more closely resemble the gold standard human islets.

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P.128 Single-Cell Analysis of HLA-I Heterogeneity in Type 1 Diabetes: A Lesson in Beta Cell Survival?

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ORAL COMMUNICATION Keywords: *HLA-I, beta cells, T1D*

BACKGROUND

The upregulation of both classical and non-classical HLA class I (HLA-I) molecules in the insulin-containing islets of those diagnosed with type I diabetes has been previously reported. However, the expression of HLA-I molecules in differentially sized endocrine objects (EOs) in type I diabetes has not been studied.

AIM

Here, we explore the expression of classical (HLA-A and HLA-B) and non-classical (HLA-E) in the pancreatic beta cells of people with and without type I diabetes.

METHODS

Pancreas tissue sections from EADB and nPOD collections were staining against HLA-A, HLA-B, HLA-E and insulin using multiplex immunofluorescence. Whole slide scans were analysed using the Indica HALO software.

RESULTS

In alignment with previous findings, the expression of HLA-A,-B and -E is minimal in the islets of individuals without diabetes whereas it is reported to be upregulated in insulin-containing islets and diminished in insulin-deficient islets of those diagnosed with type 1 diabetes. HLA-A and HLA-B were present predominately in beta cells, and HLA-E was predominantly localised to alpha cells. HLA-A expression is membranous in comparison to that of HLA-B and HLA-E which appears predominately cytoplasmic. The expression of certain HLA-I molecules appears to be lobular, and it is reported that insulin containing islets express HLA-I molecules in a varied way, meaning that some islets express more HLA-A whereas others express more HLA-B.

CONCLUSIONS

This provides an insight into the distribution and expression of classical and non-classical HLA-I molecules. We suggest that the HLA-I isoform expression balance may influence the extent and pace of beta cell demise during autoimmune attack and that the remaining insulin-containing islets may be in a different stage of the disease progression. This knowledge could help inform strategies to protect native islets and stem cell-derived islets in the future.

P.129 Ellagitannin-Rich Chestnut Extract Modulates Early Metabolic Dysfunction and Beta Cell Activity in Western Diet–Fed Male Mice

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POSTER Keywords: *Metabolic syndrome Western diet Ellagitannins Pancreatic beta cells Insulin resistance*

BACKGROUND & OBJECTIVES

Metabolic syndrome (MetS) is a growing disorder linked to obesity, insulin resistance, and increased risk of type 2 diabetes and cardiovascular disease. Diets high in fats and sugars, such as the Western diet (WD), induce early metabolic and pancreatic dysfunction, promoting MetS progression [1]. Chestnut-derived ellagitannins interact with digestive enzymes like α-amylase, α-glucosidase, and lipase, reduce carbohydrate and lipid absorption, and modulate insulin signaling and pancreatic beta-cell function, suggesting a multifaceted protective mechanism [2,3]. This study investigated whether chestnut-derived ellagitannins (FT50) supplementation can prevent or ameliorate WD-induced early metabolic and pancreatic alterations in male C57BL/6J mice.

METHODS

A total of 21 male C57BL/6J mice were fed a WD or WD supplemented with FT50 for 12 weeks. Body weight, adiposity, glycemia, glucose tolerance, and insulin resistance were monitored

longitudinally to assess the development of WD-induced metabolic alterations. Fasting glucose, insulin, and HOMA-IR were determined to estimate insulin sensitivity. Pancreatic islet function was evaluated ex vivo using Ca^{2+} imaging, enabling quantification of beta cell activation dynamics, plateau-phase activity, and islet network coordination.

RESULTS

WD feeding induced pronounced weight gain, increased adiposity, hyperglycemia, and glucose intolerance, accompanied by changes consistent with impaired insulin action and compensatory hyperinsulinemia. FT50 supplementation attenuated weight gain, fat accumulation, and organ hypertrophy, while improving glucose homeostasis and insulin sensitivity–related parameters. These systemic effects suggest a partial protection against WD-induced metabolic disturbances. At the islet level, beta cells from FT50-treated mice exhibited higher activation thresholds, prolonged activation delays, and reduced plateau-phase Ca^{2+} activity, while maintaining coordinated islet network function. Collectively, these functional adaptations are consistent with reduced beta cell workload and an attenuation of early metabolic stress. Conclusions & Implications: FT50 supplementation modulates early WD-induced metabolic alterations and beta cell functional dynamics in response to metabolic stress in male mice. Although limited to early-stage changes, these findings suggest that chestnut-derived ellagitannins may represent a potentially promising bioactive dietary intervention to attenuate early diet-induced metabolic dysfunction and preserve pancreatic beta cell function.

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P.130 Dietary Fat Source Modulates Insulin and IL-1β Expression in Pancreatic β-Cells Under High-Fat, High-Sucrose Conditions

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POSTER Keywords: *β-cell, IL-1β, high fat diet*

BACKGROUND

Chronic elevation of circulating glucose and free fatty acids induces glucolipotoxicity, a key contributor to pancreatic β-cell dysfunction through inflammatory and metabolic stress pathways. This process is strongly implicated in the pathogenesis of insulin resistance and type 2 diabetes. Dietary fat composition plays a critical role in modulating these effects. This study aimed to investigate the differential impact of a fish-oil-based high-fat, high-sucrose diet (Fish-HFDS) versus a cocoa-butter-based high-fat, high-sucrose diet (Cocoa-HFDS) on glucose homeostasis, insulin secretion, and inflammatory signaling, specifically interleukin-1β (IL-1β), in pancreatic β-cells of mice.

METHODS

Male C57BL/6 mice (n=30) were randomly allocated into three dietary groups: chow, Fish-HFDS, or Cocoa-HFDS, and maintained on these diets for 22 weeks. Metabolic assessments included intraperitoneal glucose tolerance tests (IP-GTT) and insulin tolerance tests (IP-ITT), performed before and after the dietary intervention. Plasma insulin concentrations were quantified using a Milliplex multiplex assay. Pancreatic tissues were collected for immunohistochemistry to quantify insulinand IL-1β-positive-areas.

RESULTS

Mice fed the Fish-HFDS exhibited significantly improved metabolic profiles compared to the Cocoa-HFDS group, including lower fasting blood glucose concentrations and reduced area under the curve (AUC) for both IP-GTT and IP-ITT, indicating enhanced glucose tolerance and insulin sensitivity. Additionally, plasma insulin levels were significantly elevated in the Fish-HFDS group. Immunohistochemical analysis revealed increased insulin staining intensity in pancreatic islets of Fish-HFDS-fed mice relative to Cocoa-HFDS-fed mice, suggesting preserved β-cell function. In contrast, IL-1β expression was markedly higher in the Cocoa-HFDS group compared to both Fish-HFDS and chow groups, indicating heightened inflammatory signaling associated with cocoa butter-based fat intake.

CONCLUSIONS

Cocoa-HFDS induces pronounced metabolic and inflammatory stress, characterized by increased IL-1β expression and impaired insulin production in pancreatic β-cells. In contrast, Fish-HFDS exerts protective effects by improving glucose homeostasis, enhancing insulin secretion, and attenuating inflammatory responses. These findings underscore the importance of dietary fat composition in modulating glucolipotoxicity and suggest that fish oil may mitigate sucrose-induced metabolic dysfunction while preserving pancreatic β-cell integrity and function.

P.131 CD74 upregulation in β-cells as a destruction signal associated with type 1 diabetes progression

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ORAL COMMUNICATION

Keywords: *β-cell destruction, T-cell infiltration, inflammation, HLA-I hyperexpression, Type 1 diabetes.*

CD74 is a protein primarily known for its role in antigen presentation, regulating the transport of HLA-II molecules. It also acts as a transcriptional activator of pro-inflammatory pathways. Through single-cell genomics, we have discovered that CD74 is heterogeneously upregulated in β-cells with high levels of NF-kB signaling in zebrafish and exhibits elevated expression in β-cells of NOD mice during insulitis. To explore the role of CD74 in type 1 diabetes (T1D) pathogenesis, we have generated transgenic zebrafish with β-cell specific expression of CD74. Strikingly, CD74 expression in β-cells led to their complete destruction in juvenile fish, the onset of hyperglycemia and islet-infiltration by T-cells. Zebrafish recovered from hyperglycemia by forming hybrid β/δ-

cells, which silenced CD74 expression and were thus, spared from further destruction. Based on these findings, we analyzed CD74 protein expression in human pancreas from organ donors, including individuals with T1D, controls without T1D, and multiple autoantibody-positive donors (mAAb+). Donors were divided by age into children (3,5 to 10 years old) and adults (19 to 30 years old) and disease duration was shorter than 5 years. Our results indicate that increased CD74 expression in β-cells is specific to prediabetic and diabetic stages of T1D, being significantly higher in children compared to adults with T1D. CD74 expression was also increased in the peri-islet area of some islets, making a “halo effect”, which might point to inflammation in the surrounding acinar cells. Across donors with T1D and mAAb+ individuals, increased CD74 expression was positively associated with HLA-I hyperexpression, a hallmark of T1D. Furthermore, islets with CD74+ β-cells showed increased T-cell infiltration. Thus, through the joint analysis of CD74 across species, we uncover a key role for CD74 in mediating the aberrant interaction between β-cells and immune cells. Our findings show that CD74 is upregulated in β-cells, is associated to the main hallmarks of the disease, and its expression is significantly higher in children with T1D, making it a potential therapeutic target that deserves further investigation.

P.132 Chronic exposure to DEHP increases glucose-stimulated insulin but impairs mitochondrial efficiency, in INS1 (832/13) pancreatic β-cells.

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POSTER Keywords: *Metabolism, mitochondria, phthalates, pollution, beta-cell*

BACKGROUND AND OBJECTIVES

Di(2-ethylhexyl)phthalate (DEHP) is a widely used plasticiser commonly found in food and drink packaging. Increased exposure to DEHP is associated with an increased risk of insulin resistance, hyperglycaemia, obesity and type 2 diabetes (T2D) in humans. However, the underlying molecular mechanisms by which this occurs, and whether DEHP is directly detrimental to pancreatic β-cell function, remains unclear. In the current study, we investigated if chronic exposure to DEHP impacted cytotoxicity, glucose-stimulated insulin secretion (GSIS) and mitochondrial function in INS-1 (832/13) β-cells.

METHODS

For all experiments, rat INS-1 (832/13) β-cells were cultured ± DEHP (1μM) for 48hr. 0.1% DMSO was used as the vehicle control. GSIS was measured in response to 30 min stimulation with 20mM glucose and determined by ELISA (Mercodia). Cellular Oxygen Consumption Rate (OCR) was used to assess mitochondrial function and was measured using a Seahorse extracellular flux analyser (Agilent). Cytotoxicity was measured using a CellTox™ Green assay (Promega). Data are presented as mean±SEM.

RESULTS

Cytotoxicity did not differ between DEHP-treated and control cells. However, DEHP-treated cells displayed increased GSIS, in comparison to control cells (DEHP = 1.73±0.20 vs Control = 1.11±0.04 ng/ml µg protein, p<0.01, n=3). DEHP exposure also reduced basal OCR (DEHP = 10.89±0.69 vs Control = 16.61±0.29 pmol O2/min/µg protein, p<0.001, n=8), decreased ATP-linked OCR (DEHP = 6.53±0.45 vs Control = 11.15±0.21 pmol O2/min/µg protein, p<0.001, n=8) and coupling efficiency (DEHP = 59.97±0.64% vs Control = 67.19±1.3%, p<0.05, n=8). Conclusions and Implications: Chronic exposure to DEHP increased GSIS while impairing mitochondrial efficiency, in INS-1 (832/13) β-cells. Although these results seem contradictory, both hyperinsulinemia and impaired mitochondrial metabolism are early markers of β-cell failure in the development of T2D. Hence, these findings suggest that exposure to environmental pollutants, like DEHP, may drive T2D progression via β-cell dysfunction.

P.133 Intrinsic Metabolism and Islet Architecture Dictate Beta Cell-Selective Interferon Responses to Endogenous dsRNA

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ORAL COMMUNICATION Keywords: *interferon response, islet inflammation, beta cell vs. alpha cell sensitivity*

BACKGROUND

Type 1 diabetes (T1D) onset is marked by a type I interferon (IFN-I) response and innate inflammation in pancreatic islets, yet the molecular drivers of these pathogenic events remain unclear. Genetic associations with IFIH1, TYK2, PTPN2, and STAT4 implicate double-stranded RNA (dsRNA) sensing and IFN signaling in T1D etiology and support a role for viral infection in disease onset. However, despite decades of research, no causal virus has been identified. We have proposed an alternative model in which, under conditions of impaired RNA editing, endogenous rather than viral transcripts form dsRNA structures that are sensed by MDA5/IFIH1, thereby activating IFN-I and inflammatory responses. Recent genetic evidence linking reduced RNA editing to T1D further supports this hypothesis.

RESULTS

Consistent with this model, we show that ADAR deficiency in mouse and human beta cells induces dsRNA accumulation, triggering an IFIH1-dependent IFN-I response, islet inflammation, beta-cell dysfunction and death, and diabetes, recapitulating hallmarks of early-stage T1D. We further demonstrate that glucose metabolism amplifies the dsRNAinduced IFN-I response in beta-AdarKO islets through a calcium-dependent mechanism, and that enforced hyperglycemia in beta-AdarKO mice exacerbates insulitis, revealing a feedforward loop between beta-cell workload and islet inflammation. In contrast, impaired RNA editing in mouse alpha cells does not induce IFN-I or inflammatory responses and does not affect cell function or survival. We

hypothesize that this divergence reflects intrinsic metabolic differences and distinct islet architecture: in ad libitum-fed mice, beta cells remain continuously metabolically active, whereas alpha cells are relatively quiescent. In addition, beta cells are organized in clusters that promote intercellular communication, while alpha cells are more sparsely distributed at the islet periphery. By manipulating metabolic activity and spatial organization in dsRNAaccumulating alpha and beta cells, we identify cell-autonomous metabolic activity and paracrine communication as key determinants of the IFN-I response. Implications: These findings establish metabolism as a central modifier of dsRNA sensing and IFN-I responses in pancreatic islets, provide a mechanistic framework for the differential sensitivity of alpha and beta cells to impaired RNA editing, and define fundamental principles linking metabolic state, islet architecture, and innate immune activation in early T1D.

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P.134 Constitutive HIF-2α activation induces beta cell dedifferentiation and diabetes

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ORAL COMMUNICATION Keywords: *beta cell dedifferentiation, hypoxia, HIF-2α*

BACKGROUND & OBJECTIVES

Hypoxia-inducible factors (HIFs) are central regulators of cellular adaptation to low oxygen and control key processes including metabolism, angiogenesis, and cell identity. Activation of HIF-2α has been reported in pancreatic beta cells under diabetic conditions, suggesting a potential causal role in beta cell dysfunction. However, whether sustained HIF-2α activation directly alters beta cell identity and glucose homeostasis in vivo remains unclear. This study aimed to determine the impact of constitutive HIF-2α stabilization in beta cells on metabolic control and beta cell identity.

METHODS

Mice with beta cell–specific stabilization of HIF-2α were generated using the Cre/Lox system (Ins-Cre;HIF2dPA). Heterozygous and homozygous mice were characterized by glucose tolerance tests, in vivo insulin secretion analyses, and histological and immunohistochemical evaluation of pancreatic islets. Transcriptomic profiling of isolated islets was performed using microarray analysis to assess global changes in gene expression associated with HIF-2α overactivation.

RESULTS

Homozygous Ins-Cre;HIF2dPA mice developed overt diabetes by two months of age, associated with a marked reduction in insulin production. Histological analysis revealed pronounced islet

hypervascularization. Notably, beta cells exhibited an almost complete loss of insulin expression together with reduced expression of mature beta cell markers, including Pdx1 and Glut2. This was accompanied by induction of markers associated with immature or dedifferentiated states, such as Sox9 and Aldh1a3. Transcriptomic analysis identified significant alterations in genes involved in glycolysis, angiogenesis, and pathways critical for beta cell function and identity. Heterozygous mice displayed an intermediate phenotype characterized by glucose intolerance without overt diabetes and a milder reduction in mature beta cell markers. Conclusions & Implications: Sustained activation of HIF-2α in pancreatic beta cells is sufficient to drive loss of beta cell identity and impaired insulin production. These findings suggest that aberrant hypoxia signaling may contribute directly to beta cell failure in diabetes.

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