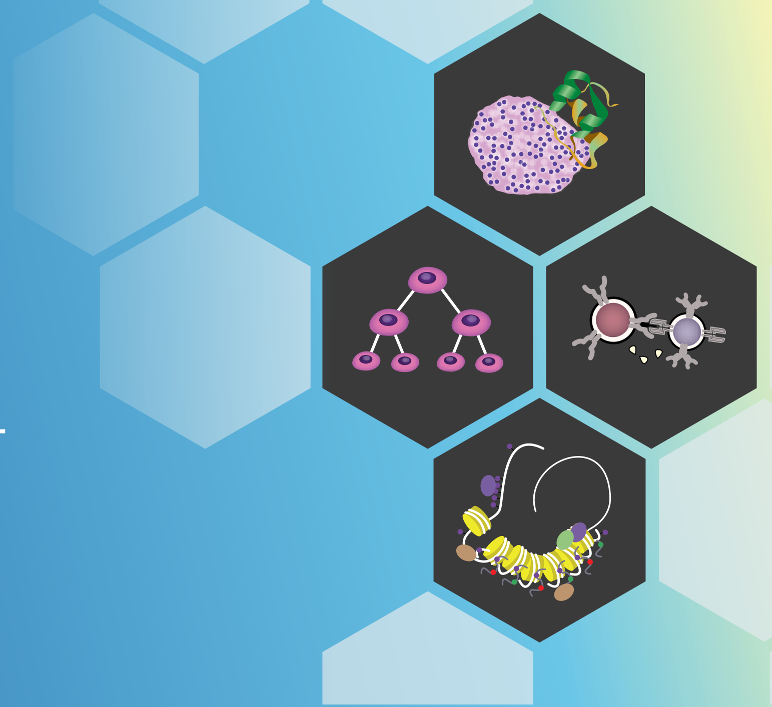

ABSTRACT BOOKLET

2024 RACHMIEL LEVINE-ARTHUR RIGGS

Diabetes Research Symposium

September 21 to 24, 2024

The Westin Pasadena, Pasadena, California

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2024 Rachmiel Levine-Arthur Riggs Diabetes Research Symposium ♦ September 21-24, 2024

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Beta cell-specific PAK1 enrichment ameliorates diet-induced glucose intolerance by promoting insulin biogenesis and minimizing beta cell apoptosis

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p21 (Cdc42/Rac1) activated Kinase 1 (PAK1) is depleted in type 2 diabetic human islets compared to non-diabetic (ND) human islets, and acute PAK1 restoration to type 2 diabetic (T2D) human islets can restore insulin secretory function *ex vivo*. We hypothesized that beta cell specific PAK1 enrichment *in vivo* can mitigate high-fat diet-induced glucose intolerance by increasing the functional beta cell mass.

T2D or ND human islets expressing exogenous PAK1 specifically in beta cells were used for bulk RNA-sequencing (RNA-seq). Human EndoC-βH1 cells overexpressing myc-tagged PAK1 were used for chromatin immunoprecipitation (ChIP) and ChIP-sequencing (ChIP-seq). The novel doxycycline-inducible beta cell specific PAK1 expressing (iβPAK1-Tg) mice were fed a 45% high-fat diet (HFD) pre-induction for 3 weeks and for a further 3 weeks ± doxycycline-induction. These HFD-fed mice were evaluated for IPGTT, IPITT, 6 h fasting plasma insulin and blood glucose, body composition, islet insulin content, and apoptosis.

Beta cell specific PAK1 enrichment in T2D human islets resulted in decreased beta cell apoptosis and increased insulin content. RNA-seq showed an upregulation of insulin (*INS*) gene transcription by PAK1. Using clonal human beta cells, we found that PAK1 protein was localized in the cytoplasm and the nucleus. ChIP studies revealed that nuclear PAK1 enhanced PDX1 and NEUROD1 binding to the *INS* promoter in a glucose-responsive manner. Importantly, the iβPAK1-Tg mice when challenged with a HFD and doxycycline-induction displayed enhanced glucose tolerance, increased islet insulin content, and reduced beta cell apoptosis compared to iβPAK1-Tg mice without doxycycline-induction.

PAK1 plays an unforeseen and beneficial role in beta cells by promoting insulin biogenesis via enhancing the expression of *PDX1*, *NEUROD1*, and *INS*, along with anti-apoptotic effects, that culminate in increased insulin content and beta cell mass *in vivo,* and ameliorate diet-induced glucose intolerance.

BET signaling and FXR signaling orchestrate to protect β cells

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In both type 1 and type 2 diabetes (T1D/T2D), insulin-producing β-cells undergo dysfunction due to inflammation, leading to impaired glucose responsiveness, dedifferentiation, and cell loss. Though bile acids (BAs) are known to influence T1D/T2D progression, any therapeutic relevance remains elusive. In this study, we characterized the dysregulation of BA profiling and farnesoid X receptor (FXR) signaling in progressive T1D and T2D mouse models. We identified the physiological protein-protein interaction between FXR and the bromodomain-containing protein 4 (BRD4) as regulatory network, that protect against β cell dysfunction. We show that FXR activation by Fexaramine (Fex) together with BRD4 inhibition by JQ1 synergistically suppressed IL-1β-induced inflammation, and also improved β cell identity and insulin secretion in db/db mice and human β cells. Additionally, dual inhibition of the BD2 domain of BET combined with FXR activation markedly improved β cell survival in human T1D and T2D models derived from human pluripotent stem cell (hPSC)-based islet-like organoids (HILOs). Collectively, our findings illustrate the bile acid-bromodomain axis's role in transcriptional regulation and highlight the promise of FXR agonists and BET inhibitors in countering β cell dysfunction in diabetes.

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DOC2b prevents cytokine-induced CXCL10 expression via attenuating IKKβ-NF-κB p65 and STAT-1 signaling in human islets

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Abstract
The reduction of β-cell dysfunction and demise hold promise for therapeutic intervention for type 1 diabetes. Previously, we reported the beneficial role of SNARE-regulatory protein DOC2b in preventing cytokine-induced β-cell stress and apoptosis. However, the underlying mechanism remains unknown. Our global DOC2b$^{+/-}$ knockout mouse islets showed increased chemokine ligand CXCL10 protein levels vs. wild-type control post-multiple-low-dose streptozotocin challenge. Thus, we hypothesized that DOC2b enrichment prevents cytokine-induced β-cell apoptosis via attenuating CXCL10 signaling. We used bulk RNA-seq, qPCR, proteomics, biochemical studies, and confocal microscopy using DOC2b-enriched or -depleted primary islets (human and mouse), as well as proinflammatory cytokine-treated clonal β-cells. DOC2b-depleted clonal β-cells and primary mouse islets had elevated cytokine-induced CXCL10 mRNA expression. By contrast, DOC2b enrichment resulted in marked attenuation of cytokine-induced CXCL10 mRNA and protein levels in human islets from donors without diabetes, and clonal β-cells. In β-cells, cytokine-induced CXCL10 expression is known to be regulated via upstream NF-κB p65 and STAT-1 signaling. Further, RNAseq analysis of human islets from donors with type 2 diabetes revealed a significant decline in NF-κB and inflammation pathways in DOC2b enriched vs. control islets. Intriguingly, we revealed DOC2b:NF-κB p65 and DOC2b:STAT-1 complexes in cytokine-stressed clonal β-cells via co-immunoprecipitation. DOC2b-enriched primary human islets showed a significant reduction in activated and total IKKβ, STAT-1, total NF-κB p65, and cytokine-stressed primary human islets showed increased total IκBβ protein. Additionally, DOC2b-enriched cytokine-stressed clonal β-cells harbor reduced STAT-1 levels in the nucleus. These data support a model in which DOC2b protects β-cells from cytokine stress and apoptosis via attenuation of IKKβ-NF-κB p65, STAT-1 and CXCL10 signaling.

Friend or Foe: Unveiling CD318 appearance on CD56+ cell and accompanied physiological alteration

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CD318 interacts with CD6 on T cells, suppressing their activity. Blocking the CD6-CD318 axis increases T cell activity, highlighting CD318 inhibitory role in immune regulation. Our research focuses on CD318+ CD56+ cells, demonstrating a gradual increase in CD318 expression when healthy donor PBMCs are cultured with IL-2. Only IL-2 and IL-15 induce CD318 on CD56+ cells, while other common γc receptor cytokines do not. Furthermore, CD318+CD56+ cells exhibited enhanced cytotoxicity, CD107a expression, and granzyme B production, indicating a more activated and cytotoxic state compared to CD318-CD56+ cells. We also observed a lower PD-1 and LAG-3 expression in CD318+CD56+ cells compared to CD318-CD56+ cells. Despite blocking PD-1 checkpoint, CD318-CD56+ cells, which showed higher PD-1 expression, still exhibited lower cytotoxicity compared to CD318+CD56+ cells. This suggests that CD318+ cells possess intrinsic biological differences that contribute to their enhanced cytotoxicity, independent of PD-1 blockade. Finally, we investigated the role of STAT5, a downstream molecule in the IL-2 receptor pathway, in relation to CD318 induction. Our findings indicate that STAT5 inhibitors do not inhibit CD318 expression on CD56+ cells. However, we are continuing to explore the involvement of other downstream pathways of IL-2-mediated signaling in CD318 induction on CD56+ cells. The role of IL-2 in immune regulation of autoimmune diseases like Type 1 Diabetes is critical, and abnormal IL-2 levels might trigger the disease conditions. Our research shows that immune cells can express CD318 after IL-2 stimulation, unlike cancer cells that express it spontaneously. This suggests CD318 might enhance cytotoxicity and influence immune regulation. The discovery of CD318 on natural CD56+ cells and its correlation with IL-2 suggests new treatment avenues. Our study identifies CD318 as a potential therapeutic target for autoimmune and chronic inflammatory diseases.

NUDC regulates cell cycle and cell identity-related genes in human β cells

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Abstract
The global incidence of insulin-dependent type 1 diabetes (T1D) has been steadily increasing over the past decades. Although insulin injection therapy prolongs the life expectancy of T1D patients, it often fails to maintain glycemic homeostasis, leading to hypoglycemic and hyperglycemic episodes that can ultimately result in mortality. Human pluripotent stem cells (hPSCs) offer an alternative to cadaveric human islets, providing a potentially unlimited supply of insulin-producing cells due to their pluripotency and self-renewal capacity. Despite this potential, hPSC-derived insulin-producing cells face significant scaling challenges due to technical difficulties and high production costs. Regulation of human β cell proliferation is attractive strategy to increase the scalability of hPSC-derived insulin-producing cells. However, the current understanding of the machinery governing human β cells proliferation and fate determination is limited, hindering efforts to scale up the production of functional human β-cells. Quiescent human β cells, which are long-lived and rarely undergo proliferation, exhibit higher identity and insulin secretion. Proliferative β-cells, however, are temporarily immature and dedifferentiated. To understand the mechanisms behind human β cell proliferation and identity, we utilized CRISPR-CAS9 knockout screening. We identified nuclear distribution C, dynein complex regulator (NUDC) as a key gene in the transition of these quiescent β cells to more proliferative immature β cells. NUDC expression correlates with proliferating cells during human β cell differentiation. In our study using EndoC-βH1 cells, we discovered that NUDC regulates cell cycle-related gene expression and M-phase transition in human β cells. However, NUDC has a negative impact on insulin secretion and its related gene expression. NUDC is predominantly localized in the cytoplasm, especially around the perinuclear region of EndoC-βH1 cells, but it becomes evenly distributed in proliferating cells. We propose that NUDC in the peripheral nucleus, potentially influencing gene expression to modulate human β cell proliferation and identity.

Acknowledgments: This work was supported by grants from CTSI-UCLA awards (UL1TR001881) and Beatson Foundation (2022-006). E.Y., is supported by JDRF Career Development Award (5-CDA-2022-1178-A-N). J.C., and H.P., are supported by the CIRM-training grant (EDUC4-12837).

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Poster Abstracts
2024 Rachmiel Levine-Arthur Riggs Diabetes Research Symposium ♦ September 21-24, 2024

Extracellular vesicles produced by proinflammatory cytokine-treated human ductal cells increase HLA class I expression in EndoC-βH1 beta cells

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Abstract
Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of beta cells in the pancreas. While the role of autoimmunity targeting beta cells is well-studied in T1D pathogenesis, the involvement of ductal cells, an exocrine cell type in the pancreas, remains unexplored. Considering the role of extracellular vesicles (EVs) in cell-cell communication, we aimed to characterize and evaluate the impact of ductal EVs on beta cells. Human ductal cells were isolated from cadaveric donors without apparent diseases and cultured in a 3D suspension culture. Graded doses of proinflammatory cytokines that simulate the T1D microenvironment were added to human ductal cells for 48 hours. We found that a combination of 25 IU/mL IL-1β, 250 IU/mL TNF-α, and 250 IU/mL IFN-γ was sufficient to increase cytokine stress marker *NOS2* by quantitative RT-PCR analysis, but live cell numbers remained unchanged by trypan blue exclusion analysis. Ductal EVs were isolated from control and cytokine-treated ductal cells using size exclusion chromatography. EV-enriched fractions were identified by the presence of EV markers and the absence of non-EV markers using Dot blot. EV enrichment was further confirmed by transmission electron microscopy and nanoparticle tracking analysis. Mass spectrometry analysis of ductal EVs revealed that cytokine treatment resulted in an upregulation of several protein cargos involved in inflammation and cellular signaling pathways. Addition of cytokine-treated ductal EVs to EndoC-βH1 cells, a human beta cell line, for 48 hours resulted in an increased expression of HLA class I molecules, a feature associated with T1D pathogenesis. Together, our results suggest that primary human ductal cells can respond to T1D-mimicking proinflammatory cytokines and secret EVs that impact beta cells and may implicate ductal cells in the pathogenesis of T1D.

Pancreatic β-cells Package Double C2-like Domain Beta Protein into Extracellular Vesicles via Tandem C2 Domains

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Abstract: Double C2-like domain beta (DOC2B) protein is critical for glucose-stimulated insulin secretion in β-cells. Recent-onset type 1 diabetes (T1D) individuals (vs. non-diabetic) show reduced DOC2B in residual β-cells. Reduced DOC2B levels are also detected in platelets of T1D individuals. To advance DOC2B for early T1D detection, when therapeutic strategies are more effective, it is critical to understand the mechanism by which DOC2B is released from the β-cells into the blood. Here, we show DOC2B's presence within membrane-delimited nanoparticles-extracellular vesicles (EVs) derived from human plasma, primary human islets (non-diabetic), and rat β-cells. Compared to two other cell types that regulate glucose homeostasis and functionally require DOC2B (human neuronal-like SH-SY5Y and rat L6-GLUT4myc myotubes), clonal β-cells (human EndoC- βH1, rat INS-1 832/13, and mouse MIN6 β-cells) produced significantly more EVs. DOC2B levels in EVs (over whole cell lysates) were higher in β-cells compared to myotubes; neuronal-like cells did not release appreciable DOC2B. Mechanistically, we show that DOC2B is localized to the EV lumen; the tandem C2 domains were sufficient to confer sorting to EVs. Because these EVs appear to contribute to the DOC2B secretome in blood, they may be a promising modality for monitoring changes to β-cells in a T1D setting.

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LGR4 is essential for maintaining β-cell homeostasis through suppression of RANK

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Loss of functional insulin-producing β-cells and inability to regenerate them play a key role in the onset of both Type 1 and Type 2 diabetes. Therefore, understanding what is required to maintain healthy β-cells and how to enhance their regeneration and survival is crucial in treating this disease. GPCRs (G protein-coupled receptors) comprise the largest group of receptors regulating key cellular processes in all tissues. They also are therapeutic targets for multiple diseases, including diabetes. LGR4 (Leucine-rich repeat-containing G protein-coupled receptor 4) is the fourth most abundant GPCR in human islets expressed in pancreatic β-cells. However, how LGR4 regulates β-cell homeostasis is not known. Our study shows that LGR4 is critical for maintaining β-cell health and its expression is modulated by stressors. *In vitro* knockdown of *Lgr4* in rat insulinoma INS1 cells and mouse islets decreases β-cell proliferation and increases cell death. Overexpression of LGR4 significantly protects β-cells against cytokine-induced cell death in INS1 cells, mouse and human islets. Mechanistically, LGR4 protects β-cells by suppressing Receptor Activator of Nuclear Factor Kappa B (NFκB) (RANK) Tumor necrosis factor receptor-associated factor 6 (TRAF6) interaction and activation of NFκB. *In vivo*, β-cell-specific *Lgr4*-conditional knockout (cko) in young adult mice does not affect glucose homeostasis but significantly increases β-cell death and reduces β-cell proliferation, the latter only in female mice, compared to wild-type controls. Male *Lgr4*cko mice exposed to high fat diet (HFD) and multiple low dose streptozotocin (MLDS) treatment had significantly reduced β-cell proliferation and increased death, compared to wild-type mice. Upon aging, female but not male, *Lgr4*cko mice exhibit significantly impaired glucose tolerance and decreased plasma insulin; however, both male and female *Lgr4*cko mice have reduced β-cell proliferation and survival compared to WT littermates.

Our findings highlight the importance of LGR4-RANK stoichiometry for maintaining normal β-cell homeostasis under basal and stress-induced conditions.

STX4 the latest addition to skeletal muscle mitochondrial structure, function and quality control

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The plasticity of mitochondria is essential for the viability of skeletal muscle function, metabolism and integrity. Key mitochondrial quality control (MQC) processes of biogenesis, fission and fusion dynamics and mitochondrial turnover by mitophagy ensure regulation of healthy mitochondrial content that preserves cellular homeostasis. Thus, dysfunctions in these MQC processes are associated with metabolic conditions such as peripheral insulin resistance which raises risk for the development of prediabetes and its progression into type 2 diabetes. Recently, we discovered that Syntaxin 4 (STX4), traditionally a cell surface transmembrane SNARE protein required for glucose uptake, was also associated with the outer mitochondrial membrane (OMM) in mouse skeletal muscle. Furthermore, we demonstrated that inducible STX4 enrichment in skeletal muscle-specific STX4 overexpressing (skmSTX4-iOE) male mice reversed HFD-induced fragmented mitochondrial phenotype and reduced activation of mitochondrial fission protein Drp1, suggesting a novel role in MQC regarding fission/fusion dynamics. However, it remains to be determined whether mitochondrial STX4 is required for mitochondrial function and if it is essential for additional key MQC processes such as biogenesis and mitochondrial turnover by mitophagy. Using a doxycycline-inducible skeletal muscle-specific STX4-knockout (skmSTX4-iKO) mouse model that inherently develops insulin resistance, our data suggests that STX4 is essential for mitochondrial structure and function. Furthermore, *in vitro* studies of STX4 depletion via siRNA transfection in clonal skeletal muscle cells revealed dysfunction in oxidative phosphorylation system complexes IV, II and I. Interestingly, STX4 depletion revealed reduced mitochondrial turnover by mitophagy via pH sensitive mt-Keima fluorescence protein and reduction in key mitochondrial biogenesis genes (*PGC1-α* and *NRF1*), suggesting an unknown association with mitochondrial biogenesis and mitophagy. Thus, we propose a model whereby STX4 is essential for maintenance of mitochondrial structure, function and MQC in skeletal muscle.

Impacts of blood exchange on age-related liver adiposity and vascular complications in diabetes

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Diabetes complications and aging are intricately linked, as chronic hyperglycemia leads to vascular damage and inflammation, accelerating the aging process and worsening age-related health complications. Understanding the role of circulating factors in the blood that are believed to exacerbate the decline in organ function and tissue repair mechanisms seen in aging potentiates the development of targeted therapies to mitigate the dual impacts of aging and diabetes. To this end, we have employed a multi-mouse, syringe pump-based system for depleting plasma in aged mice, and examined the aged, fatty, and fibrotic liver for adiposity and fibrosis. Remarkably, a single isochronic or heterochronic blood exchange significantly decreased liver adiposity to levels comparable to those observed in young mice and showed overall improvement of aged liver metrics. Further, triple heterochronic blood exchanges have improved tissue blood perfusion in aged mice. These results have motivated additional pilot testing using a hind limb ischemia model of diabetes vascular complications, and in preventing or delaying onset of type I diabetes in NOD mice through therapeutic blood exchanges in NOD mice.

Loss of hepatic Mig6 prevents acetaminophen-induced toxicity in male mice.

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Epidermal growth factor receptor (EGFR) signaling is activated in response to liver damage in an effort to initiate cellular repair and function. However, the robustness and durability of this signaling pathway is compromised by feedback inhibition of the adaptor protein mitogen inducible gene 6 (Mig6/Gene33/Errfi1). We previously established that loss of hepatic Mig6 in mice improves insulin resistance and liver metabolism when challenged by a variety of diabetogenic and pro-oxidative stressors, such as diet-induced-obesity and aging. To further understand the mechanisms underlying the oxidative protection conferred by Mig6 hepatic ablation, fasting male mice were challenged with an overdose of acetaminophen (APAP), which acutely creates oxidative stress and is the leading cause of acute liver failure (ALF). Following APAP administration, liver damage was dramatically decreased in mice lacking Mig6 in hepatocytes (LKO) compared to control littermates, as observed by a reduction of hepatic necrotic area and periportal lesion size, correlating with a ~50% decrease in serum alanine and aspartate transaminases. In addition, liver endoplasmic reticulum stress markers such as C/EBP-homologous protein (CHOP), the phosphorylation of eukaryotic initiator Factor 2α (eIF2α), and stress-activated protein kinase/Jun amino-terminal kinase (JNK), as well as the expression of inflammatory markers such as *Itgam* or *Tnfα* were markedly reduced in LKO mice. Importantly, basal glutathione levels and its depletion to detoxify APAP were not different in LKO vs. control mice, indicating that APAP bioactivation was unaffected. Moreover, 24 h after the APAP-administration we observed enhanced liver recovery in LKO mice compared to control mice, determined by glutathione and glycogen resynthesis. Thus, preventing feedback inhibition of EGFR by deleting Mig6 limits toxicity through a mechanism downstream of APAP metabolism. Therefore, Mig6/Errfi1 could be a novel therapeutic target for diseases such as type 2 diabetes and ALF from acetaminophen overdose.

Human pancreatic α-cell heterogeneity and trajectory inference analyses of human islets uncover SMOC1 as a β-cell dedifferentiation gene

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Type 2 diabetes (T2D) involves a significant transition from β-cell to α-cell-like phenotypes, a key process in disease progression. To explore this β-to-α-cell shift, we employed paired donor single-cell and nucleus RNA sequencing (scRNA/snRNA-seq), coupled with advanced analytical methods such as partition-based graph abstraction (PAGA), RNA velocity, cell trajectory inference, and gene commonality analysis. Our investigation of α-β cell fate switching in islets from non-diabetic adults identified five distinct GCG-expressing α-cell subclusters (α1 to α4, AB) with diverse transcriptomes. Trajectory inference revealed a bifurcated potential from AB cells toward both α- and β-cell fates, with ten genes, including SMOC1, PLCE1, and ZNF331, emerging as key regulators in the α-cell trajectory.

Notably, the T2D dataset from HIRN-HPAP displayed afo unique β-to-α trajectory indicative of de-differentiation, with a significant increase in SMOC1 expression in T2D compared to near-zero levels in non-diabetic samples. To validate these findings, sequential experiments with EndoC-βH1 cells and human islets were conducted, showing that SMOC1 was markedly expressed in T2D β-cells, but not in non-diabetic ones. SMOC1 expression in both non-diabetic human islets and EndoC- βH1 cells reduced insulin secretion and β-cell signature gene expression, while enhancing a T2D β-cell-like phenotype and cumulative T2D scores. Transmission electron microscopy revealed that SMOC1 reduced mature insulin granules but increased immature ones in human pancreatic β-cells. Additionally, as a calcium-binding protein, soluble SMOC1 significantly reduced cAMP content in EndoC- βH1 cells when treated with secretagogues such as glucose, exendin4, and forskolin.

Our findings elucidate the transcriptional transitions among islet cell subpopulations, establish a gene signature for β- to α-cell conversion, and identify SMOC1 as a pivotal gene in T2D β-cells. These insights advance our understanding of islet cell dynamics in T2D and may inform future therapeutic strategies to preserve β-cell function.

Elucidating the role of reactive metabolic by-products as predictors and drivers of type 2 diabetes

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More than 35% of adults in the US have prediabetes, the precursor to type 2 diabetes (T2D). Understanding the mechanisms that drive this transition is critical to developing biomarkers and clinical tools to slow or potentially stop progression to T2D. A potential driver of this progression is metabolic alterations associated with prediabetes; however, the mechanisms by which these alterations drive disease are not well-defined. Methylglyoxal (MG) is a reactive metabolic byproduct that induces covalent adducts on DNA, RNA, and protein associated with mutations and reduced stability. We propose that MG-adducts may play a role in disease progression. A unique feature of MG-adducts is that they not only induce a physiological effect but are also biomarkers of diabetes. However, there is a ***gap in knowledge*** regarding the role of MG-adducts in driving the transition from prediabetes to T2D. My ***central hypothesis*** is that MG and MG-adducts induce beta cell dysfunction by causing genomic instability and can be used as biomarkers of the progression from prediabetes to T2D. Our lab has developed novel analytical tools to quantitatively measure MG and MG-adducts from cells, tissue, urine, and blood.

Results: Our preliminary data shows that hyperglycemia increases accumulation of MG-adducts in INS-1 832/13 rat insulinoma cells and in patients with both prediabetes and T2D compared to nondiabetic individuals. MG and MG-adduct treatments may decrease glucose stimulated insulin secretion. Interestingly, hyperglycemia and MG may increase double strand DNA damage in INS-1 832/13 cells.

Conclusions: This work provides the first evidence that MG and MG-adducts drive beta cell dysfunction through genomic instability and are associated with the transition from prediabetes to T2D.

Enhancing β-cell survival and modulating immune response in type 1 diabetes through harmine and exendin-4 combination therapy

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Type 1 diabetes (T1D) results from the loss of immune tolerance and the destruction of functional β-cells. Therefore, therapeutic approaches for T1D should focus on the preservation and regeneration of β-cells while restoring immune tolerance. Administration of harmine (H) plus exendin-4 (E) has been shown to markedly induce human β-cell expansion *in vitro* and *in vivo*. However, whether H+E can further enhance human β-cell survival, diminish β-cell immunogenicity, or reprogram autoimmunity in the context of T1D is unknown. Here, we utilized single-cell RNA sequencing (scRNA-seq), CyTOF, and other conventional methods to test the effects of H+E on human β-cells under cytokine and ER stress, and on human PBMC with sub-optimal T cell activation.

First, we found that H+E significantly reduced both thapsigargin (ER stress)- and cytokine-induced human β-cell apoptosis, an effect not observed with either drug alone. Second, scRNA-seq analysis of human islets treated for 6 hours with cytokines and H+E showed that the combination reduced IL1 β-, TNFα-, and IFNγ-mediated signaling in β-cells. Further analysis revealed that genes in the intrinsic and extrinsic apoptotic pathways were upregulated by cytokines and normalized by H+E. Additionally, the expression of CXCL9-11, HLA-ABC, and IRF1 and 2 was upregulated by cytokines and normalized by H+E in human β-cells, whereas HLA-E expression was increased by H+E. Validation of these observations by qPCR, ELISA, and immunostaining in human islets showed that the expression of ER stress markers (*CHOP, GRP78, sXBP1, IRE1, ATF6*), NO and CXCL10 levels, proinsulin/insulin ratio, HLA-ABC expression, and proteasome subunits 8-10 were significantly attenuated by H+E treatment. Additionally, CyTOF analysis of human PBMC treated with a low dose of αCD3/αCD28 showed that H+E accelerated T cell exhaustion with increased expression of EOMES, TIGIT, TOX, and PD1, further reducing perforin in cytotoxic CD8+ T cells. Surprisingly, H+E treatment also increased the proportion of immune suppressive Tregs by 15% compared to non-H+E treatment.

Collectively, these results indicate that harmine plus exendin-4 enhances β-cell survival through the regulation of inflammation and pro-apoptotic genes and decreases markers of immunogenicity. Moreover, this combination enhances suppressive immunity by reprogramming T cell activation and exhaustion. These findings highlight the therapeutic potential of this drug combination for the treatment of T1D.

Modeling the effects of early hyperinsulinemia due to the R1420H SUR1 mutation using isogenic iPSC-derived pancreatic islets

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SUR1 complexes with KIR6.2 to form the pancreatic-beta cell K_{ATP}-channel critical for appropriate regulation of insulin secretion. We previously reported a R1420H mutation in SUR1 carried by 3% of an Indigenous community from Arizona and identified one homozygous carrier (1420HH) who had hyperinsulinemic hypoglycemia during infancy (HHI). Heterozygous (1420RH) carriers do not develop HHI but have higher birthweight and a 2-fold risk of developing T2D. We used an iPSC-derived pancreatic-islet (SC-islet) based system to 1) recapitulate hyperinsulinemia caused by this K_{ATP}-channel mutation, 2) model the change in insulin secretory response in 1420RH and 1420HH SC-islets during islet maturation and 3) identify the developmental and transcriptomic consequences of basal hyperinsulinemia. Combining gene editing with disease modeling, we show that 1420HH immature SC-islets (mimic fetal islets) have higher insulin secretion in basal condition whereas upon maturation (resemble adult islets), the 1420RH and 1420HH SC-islets have significantly lower insulin secretory response to high glucose despite having a similar proportion of SC-β cells. K_{ATP}-channel activator diazoxide can prevent, to an extent, the hyperinsulinemia from immature 1420HH SC-islets, however, the K_{ATP}-channel blocker tolbutamide only elicited either a diminished or no insulin secretory response from mature 1420RH and 1420HH SC-islets. Assessing the insulin secretory response in 1420RH SC-islets to various glucose concentrations (5mM – 25mM) suggested a lower glycolytic flux and we were able to demonstrate that the GCK activator dorzagliatin could restore insulin secretory response in 1420RH SC-islets. Finally, single-cell RNA sequencing was used to catalog gene expression differences in SC-β cells and SC-α cells due to this K_{ATP}-channel mutation. We identified dysregulated genes related to glucose metabolism, Ca^{+} signaling/binding and immediate early response. In summary, we show that SC-islets are a valid tool for studying T2D mutations and lay the framework for the discovery/use of appropriate therapeutic agents for subjects with the R1420H SUR1 mutation.

Novel metabolomic profiles of elevated blood pressure remission five years after bariatric surgery among adolescents

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Objective: We performed feature selections on plasma metabolite profiles and tested the hypothesis that identified plasma metabolite profiles can predict long-term elevated blood pressure (EBP) changes better than known risk factors in a cohort of adolescents with severe obesity undergoing bariatric surgery.

Methods: Plasma metabolite profiles at baseline were assessed in adolescents with severe obesity who received bariatric surgery in the prospective Teen-Longitudinal Assessment of Bariatric Surgery study (Teen-LABS, n = 135), and in a parallel cohort of predominantly Hispanic adolescents (n = 79). EBP status was assessed baseline (pre-operatively) and 5 years post-surgery. EBP was defined as SBP ≥ 120 or DBP ≥ 80 or taking anti-hypertensive medication. We used elastic net regression with a stability selection procedure to identify metabolites. The top ten features were selected based on their frequency of selection. We compared the prediction performance of models of identified metabolites with known risk factors to the known risk factors only models in both cohorts. We included age, race, sex, parents' annual income, SBP, DBP, and BMI at baseline as known risk factors.

Results: Ten metabolites were predictive of EBP changes at five years in Teen-LABS. The model of identified metabolites with known risk factors (Area under curve (AUC): 0.76; 95%CI: [0.760.82]) predicted EBP changes significantly better than the known risk factor only model (AUC: 0.43; 95%CI: [0.43-0.50], p < 0.001). When we applied the same analytical workflow in the parallel prospective cohort without interventions, we also found identified metabolites with known risk factors (AUC: 0.75; 95%CI: [0.70-0.81]) performed significantly better than the models without the metabolites (AUC: 0.46; 95%CI: [0.46-0.51], p < 0.001).

Conclusions: We identified 10 metabolites to be associated with changes in blood pressure following bariatric surgery and when applied to a model inclusive of known risk factors has excellent predictive prognostic value.

Perinatal nicotine exposure on pancreatic development and adult-onset diabetes

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Abstract
Cigarette smoking is a well-recognized risk factor for type 2 diabetes, and may result in islet β cell damage and impaired insulin secretion. According to the Centers for Disease Control and Prevention, as of 2021, 11.5% of the US adult population smokes cigarettes and smokers are 30% to 40% more likely to develop diabetes than people who do not smoke. Additionally, children whose mothers smoked during pregnancy were up to four times more likely to develop adult-onset diabetes. E-cigarettes have emerged as a “healthier” alternative to conventional smoking, yet, nicotine from these sources can disrupt metabolic processes in the developing fetal organs, possibly leading to metabolic diseases in the offspring later in life. However, the maternal inheritance of nicotine effects to the offspring and their underlying mechanisms remain elusive.

To investigate the effects of perinatal e-cigarette exposure on the pancreatic development of the fetus and its association to the development adult-onset diabetes, we exposed C57BL/6J female mice to nicotine aerosols using an e-cigarette exposure chamber one week before conception through weaning. Offspring from nicotine-exposed dams sustained a lower body weight and intraperitoneal glucose tolerance test (i.p.GTT) showed a significant dysregulation of glucose tolerance in offspring from nicotine-exposed dams at 8 weeks of age. We identified a set of differentially expressed genes (DEGs) in the hypothalamus related to nicotine addiction, and a set of DEGs in pancreatic islets and white adipose tissues, suggestive of prediabetes in the offspring from nicotine-exposed dams. Immunohistological analysis of pancreatic islets shows that offspring from nicotine-exposed dams have larger islets than controls, in line with the pancreatic compensatory mechanisms seen in prediabetes. Our ongoing research aims to elucidate the transgenerational effects of nicotine exposure on metabolic dysfunction in offspring, focusing on how nicotine-induced dysregulation affects inter-organ communication.

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Dynamic changes in Enhancer-promoter connectome and Chromatin 3D architecture under diabetic conditions in human macrophages

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In diabetes, the aberrant activation of monocytes and macrophages contributes to increased expression of cytokines/chemokines associated with chronic inflammation and complications. Epigenetic mechanisms, including enhancer-promoter interactions and chromatin 3D re-organization, play significant roles in gene expression, but the role of such long-range epigenomic mechanisms in diabetes-induced macrophage activation and inflammatory gene expression remains unclear. We treated human macrophages differentiated from $CD14^+$ monocytes (from healthy volunteers) with HG+TNF-α that mimics diabetes milieu and determined 3-D epigenome and transcriptome maps using ChIP-seq and HiChIP (which evaluates 3D chromatin conformation) with H3K27ac antibody (active enhancer mark), ATAC-seq and RNA-seq versus normal glucose. Our results from integrative analyses of these high dimension datasets showed that HT altered transcriptional programs, and extensively remodeled the chromatin architecture and enhancer looping to regulate inflammatory pathways in macrophages similar to Type 2 diabetes. HiChIP/ChIP-seq identified differentially regulated chromatin loops between enhancers and promoters in HT-treated macrophages. Integrated Omics data analysis demonstrated a significant correlation between the upregulation of inflammatory genes with increased chromatin access and enhancer-promoter loops, while these interactions were reduced at downregulated cell cycle genes. HT also disrupted boundaries of topologically associating domains at inflammatory genes. Integrated analysis of transcription factor (TF) motifs and STRING protein interactions database identified TF subnetworks involving interaction between enhancer-bound TFs (pioneer TF function and signal-dependent activation) with TFs at inflammatory gene promoters. Using ChIP and 3C assays, we validated H3K27ac enrichment and novel enhancer-promoter interactions mediating the upregulation of inflammatory genes *CCL2* and *IRAK2*. We also found that HT-induced enhancers harbored SNPs linked with Type 2 diabetes. These findings demonstrate that diabetic conditions reprogram the genome-wide enhancer-promoter connectome and dysregulate the 3D architecture in macrophages to drive inflammatory phenotypes. Further understanding of these 3D epigenetic mechanisms can lead to novel therapies for chronic inflammatory diabetes complications.

Human pluripotent stem cells derived extracellular vesicles for diabetic complications and beta cell proliferation

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Extracellular vesicles (EVs) are a group of nano-sized, membrane enclosed particles, secreted by a wide range of host cells. Evolved as mediators of cell-cell communication underlying developmental and cellular processes, EV cargos typically contain ranges of gene products (mRNA, miRNA, proteins), signaling molecules (ligands, receptors), and metabolites which reflect their cell of origin. Recent studies show that EVs derived from multipotent stem cells have therapeutic potential, including anti-inflammatory and anti-aging activities. Previously we uncovered that human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) produce EVs that are about 70-120nm in size and express canonical EV cell-surface markers (CD63 and CD81). These EVs carry pluripotency factors (e.g., OCT4, SOX2, NANOG, KLF4 - OSKM) and miRNAs (miR-302 family), and prior studies show that brief transgenic overexpression of OSKM increases murine lifespan, and is both cardioprotective, and improves beta cell function *in vivo*. In the first portion of this report, we provide an update on our studies demonstrating human pluri-EVs and hPSCs-derived cardiomyocytes are protective from high glucose induced hypertrophy and cardiac toxicity. Secondly, we explore a potential role for pluri-EVs in human beta cells proliferation and islets homeostasis under diabetic conditions. Our central hypothesis is that beta cell interactions with human pluri-EVs will significantly improve beta cell proliferation and viability, both *in vitro* (EndoC-BH1 and primary islets) and *in vivo.* Therefore, we 1) describe our efforts to scale up xeno-free and fully defined human pluripotent stem cell EVs and purification for *in vivo* applications, and 2) provide an update on current experiments and results, which explore hPSC-EV mediated beta cell rejuvenation.

Deletion of Mitogen-inducible gene-6 (Mig6) in the liver alters glucose homeostasis and delays Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) progression

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is a spectrum of chronic liver diseases that can progress from benign steatosis to metabolic dysfunction-associated steatohepatitis (MASH) and cirrhosis. The strongest risk factor for progression of MASLD to MASH is insulin resistance. Previous work in our lab identified that the adaptor protein mitogen-inducible gene-6 (Mig6) is increased in obese mice and that deletion of Mig6 in the liver (LKO) improves glucose tolerance and insulin action. Thus, we sought to elucidate the extent to which loss of Mig6 modulates the progression of MASLD to MASH. We hypothesized that liver-specific loss of Mig6 improves whole-body glucose homeostasis and protects against MASLD-mediated liver damage. LKO mice and matched, control littermates (CON) were fed a MASH diet (40% fat, 40% carbohydrate, 2% cholesterol) or a low-fat-matched diet (LFD; 10% fat, 70% carbohydrate) for up to 40 weeks. Compared to the MASH-fed CON mice, MASH-fed LKO mice had lower body weights starting at 10-weeks and persisting throughout the study. Interestingly, whereas intraperitoneal glucose tolerance tests did not identify significant differences in glucose handling between the LFD and MASH fed mice, oral glucose tolerance was significantly improved in 20-week MASH-fed LKO mice, suggesting alterations in the incretin response in this MASH model. Bulk RNA-sequencing of these livers identified 1623 DEGs that were enriched in pathways related to wound healing and downregulated in pathways related to inflammation. Lastly, histological analysis revealed MASH-fed LKO mice had a preservation of hepatic lipid zonation, decreased F4/80 macrophage positive staining, and blunted levels of circulating alanine aminotransferase compared to MASH-fed CON, indicating less hepatic damage in LKO mice. These results suggest that liver-specific loss of Mig6 improves whole-body glucose handling, which in turn ameliorates hepatic damage during MASLD progression and reveals a new therapeutic area for the prevention and treatment of MASH.

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Donor adipocytes after hematopoietic stem cell transplantation and their effect on metabolic syndrome

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Introduction: Allogeneic stem cell transplantation (Allo-HSCT) is a curative treatment modality for many hematological malignant as well as nonmalignant conditions. However, there is a higher prevalence of metabolic syndrome i.e., 49% in Allo-HSCT recipients, a 2.2-fold increase compared with controls.

Primary Objective: In this study we explore the presence of donor-derived adipocytes in subcutaneous fat of Allo-HSCT survivors and their impact on metabolic syndrome.

Methods: Patients who were eighteen and older, and 2 years or more post Allo-HSCT were eligible for the study. Major exclusion criteria were the presence of diabetes before the Allo-HSCT, glucocorticoid therapy more than 10 mg prednisone daily and coagulation disorders. Patient and donor must have archived pretransplant DNA available for chimerism analysis. After consenting, patients were provided physical exams, including anthropometry, and graft-versus-host disease (GVHD) assessment. Body composition was measured by bioelectrical impedance. Clinical tests included fasting blood glucose, insulin levels for measurement of HOMA-IR, lipid panel, CRP and cytokine panel (archived for later analysis). Subcutaneous fat biopsies were performed by a small incision in the abdominal wall and excision of up to 10-gram subcutaneous adipose tissue. The adipose tissue was fractionated, RNA Expression was used to verify fractionation employing sentinel RNA markers specific for adipocytes and immune cells in the stromal endothelial cells. DNA chimerism was quantified by Power Plex 21. The proportion of donor adipocytes and immune cells in each fraction was assessed by donor/recipient chimerism levels of informative markers compared to archival pretransplant donor and recipient DNAs. WHO criteria were used for definition of metabolic syndrome. Percentages of donor whole adipose, donor adipocytes and donor cells in stromal vascular fraction were studies using the baseline measurement with samples at the initial visit.

Results: Sixteen patients enrolled, one patient had 3 times, and 2 patients had 2 times fat biopsies, each one year apart. The average time between ALLO-HSCT and the fat biopsy was 6.55 years (3-17 years). Among the 16 patients, 10 patients had metabolic syndrome, and 9 patients had GVHD. The patients who were diagnosed with metabolic syndrome had higher BMI 29 vs 23 kg/sqm, waist circumference (107.69 vs 93.47 cm), triglyceride (177.7 vs 141.3 mg/dl and insulin resistance measured by HOMA IR (5.6 vs 1.5), lower HDL cholesterol (43.1 vs 55.1 mg/dl), however both had similar systolic and diastolic blood pressure. The percentage of donor adipocytes was statistically higher in subjects with metabolic syndrome compared to those without (5.5% vs 1.6%, P value=0.03 two-sided T test with unequal variance). The percentages of donor cells in SVF were significantly different between patients with or without GVHD. The average percentage of donor cells in SVF is 50.3% and 19.0% (P value=0.01) for patients with and without GVHD, respectively.

Conclusion: Our study is the first to demonstrate difference in the donor adipocytes post Allo-HSCT and its association with metabolic syndrome. We demonstrate that Allo-HSCT is associated with increased donor adipocytes in subcutaneous fat tissue of recipients, whereas GVHD is associated with increased donor stromal cells in the recipient. Whether these donor adipocytes originated from hematopoietic or mesenchymal lineages and the role they play in metabolic syndrome needs further study.

Optical sensing with artificial intelligence for non-invasive glucose monitoring: A clinical trial

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Abstract

Background: Accurate glucose monitoring is crucial for diabetes management. We present a novel non-invasive device combining optical sensors with adaptive artificial intelligence (AI) for glucose measurement.

Methods: Blood glucose measured using both a standard enzymatic colorimetric method and the prototype device (MLS-Glycosa) within a 30-second window. The device utilizes multiple light sensors from fingers for data acquisition with AI algorithms for glucose measurement. Light sensor derived glucose data was imported into the AI program.

Patients: This study involves 805 participants (57.27% female; mean age 50.99 ± 15.97 years; 14.91% diagnosed with diabetes). Six hundred seven patients were used for training AI, and 168 for independent measurement by AI and paired with blood glucose level to examine its accuracy. Agreement between methods was assessed using modified Bland-Altman analysis and mean absolute relative difference (MARD). The study was approved by the Ministry of Health and Medical Education (Iran).

Results: Of 168 paired measurements (20% test), 88 had reference glucose levels ≤100 mg/dL, and 80 had levels >100 mg/dL. For levels ≤100 mg/dL, 89.8% of prototype measurements were within ±15 mg/dL or ±15% of reference values. For levels >100 mg/dL, 63.8% were within these limits. The overall MARD was 9.84 ± 8.33%. HbA_{1c} and estimated average glucose (EAG) were significantly associated with differences between methods ($p < 0.0199$). Participants with glucose levels >100 mg/dL had significantly higher HbA_{1c} and EAG levels ($p < 0.0001$).

Conclusion: The MLS-Glycosa device demonstrated reasonable accuracy in non-invasive glucose monitoring, particularly for levels ≤100 mg/dL. Performance correlated with HbA_{1c} and EAG values, suggesting the influence of overall glycemic control on device accuracy. While promising, further refinement is needed to improve accuracy at higher and lower glucose levels. This technology represents a significant step towards painless, convenient glucose monitoring for individuals with diabetes.

Protein carbonylation in NOD mice is a combination of extra and intracellular generation of reactive oxygen species

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Abstract
Background: Islet inflammation contributes significantly to the dysfunction and eventual death of β-cells in type 1 diabetes. Pro-inflammatory immune cells release cytokines and reactive oxygen species (ROS) which contribute to increased oxidative stress, including the production of compounds such as 4-hydroxynonenal (4HNE), a reactive lipid aldehyde that is a major product of lipid peroxidation. Pancreatic β-cells are especially susceptible to oxidative stress due to their lack of key antioxidant enzymes. When produced, 4HNE and other lipid aldehydes covalently modify nucleophilic amino acid sidechains, an irreversible damage process termed protein carbonylation. We hypothesize that many proteins in β-cells, including key proteins involved in insulin exocytosis, are susceptible to carbonylation by reactive lipid aldehydes, which arise from cytokine-induced inflammatory pathways.

Methods: This study aims to observe the effects of these compounds on insulin secreting cells and to compare them with pancreatic islets isolated from pre-diabetic NOD mice. We used a mass spectrometry-based proteomic approach to identify proteins that are carbonylated in each model system, and we performed bioinformatics analysis to identify cellular pathways that are uniquely impacted in each system.

Results: Treatment of cultured cells with 4HNE resulted in suppression of insulin secretion within minutes. From LC-MS analysis, carbonylation of over 1000 proteins significantly increased in 4HNE-treated versus control cells. Similarly, treatment with cytokines for 72h also significantly impaired insulin secretion and increased carbonylation of over 800 proteins. In islets from 10-week-old prediabetic NOD mice, carbonylation of 1692 proteins was significantly increased compared to WT mice. Gene ontology analysis indicates that overlapping but distinct sets of cellular pathways are targets of protein carbonylation depending on whether the aldehyde source is exogenous (4HNE treatment) or endogenous (cytokine treatment). The observed pattern of protein carbonylation in prediabetic NOD islets is a combination of both the exogenous and endogenous cell culture models, suggesting that pre-T1D islets experience both intracellular and extracellular sources of ROS.

Conclusion: These data are the first to globally assess and compare protein carbonylation in β-cell culture and in an early animal model of type I diabetes and identify specific cellular pathways that are impacted.

Towards 3D printed encapsulation devices using novel, synthetic polymers

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Since the Edmonton protocol, cell transplantation promises to replace lost or damaged cells, such as the β cells of people with Type I diabetes; however, transplanted primary or stem cell-derived cells are usually rejected without immune suppression. Novel encapsulation technologies can protect cells without immune suppression, while allowing for the metabolic exchange of nutrients and therapeutic molecules. Our chemically crosslinked hydrogels can be bioprinted into high surface area non-immunogenic constructs. These non-degradable hydrogel matrices serve as biocompatible scaffolds that encapsulate cells, offering protection from the host immune system while allowing for the diffusion of nutrients and oxygen essential for cell survival and function. The bioprinted product is a stable yet flexible device that can be minimally invasively transplanted. Host immune cells do not penetrate the hydrogels *in vitro* or *in vivo*, preventing direct cell-cell contact between the transplanted islets and the host cells. Additional coatings reduced IgG in-diffusion fourfold, showing the ability to protect the graft from the humoral immune responds while smaller molecules can diffuse freely. Polymer modifications increased cell viability of encapsulated human induced pluripotent stem cells, and reduced immunogenicity *in vivo*. The shape factor greatly influenced the host response to the implants with 3D printed lattices facilitating host integration and vascularization while retaining integrity. The hydrogels demonstrate immune protection of xenogenic cell transplants into immuno-competent animals without immunosuppression. Currently, we are refining hydrogel compositions for improved immune protection from soluble proteins and implant vascularization. By harnessing the regenerative potential of cells and the unique properties of hydrogels, this novel polymer platform holds great promise for restoring function after injury or disease.

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Poster Abstracts
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FXYD2 marks and regulates maturity of β cells via ion channel-mediated signal transduction

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Abstract
Human pancreatic islets are the key regulators of development, and whole-body lipid and glucose homeostasis. Dysfunction of islet hormone secretion and/or loss of β cell mass triggers the pathogenesis of diabetes. Human induced pluripotent stem cells (hiPSCs) provide a potential alternative source to cadaveric human pancreatic islets for replacement therapy in diabetes. However, islets generated from hiPSCs *in vitro* often exhibit heterogeneous immature phenotypes such as aberrant gene expression and inappropriate insulin secretion in response to glucose. This heterogeneity and functional immaturity of hiPSC-derived islet-like organoids (HILOs) presents a challenge to the consistency and universality of these therapeutic applications. Here we show that FXYD Domain Containing Ion Transport Regulator 2 (FXYD2) marks and regulates functional maturation and it's heterogeneity by regulating the β cell transcriptome necessary for glucose-stimulated insulin secretion (GSIS). FXYD2 is restrictedly expressed in primary human β cells and δ cells, which is downregulated in human pluripotent stem cell (hPSC)-derived human β-like cells and δ-like cells. Mechanistically, we found that FXYD2 physically interacts with SRC to regulate SRC-TEAD1 signaling to modulate β cell transcriptome and GSIS function. We show that $FXYD2^{High}$ HILOs exhibit a higher GSIS activity than $FXYD2^{Low}$ HILOs. In addition, transplantation of $FXYD2^{High}$ HILOs significantly improves glucose regulation in streptozotocin (STZ)-induced diabetic immune deficient mice (NOD-SCID), compared to $FXYD2^{Low}$ HILOs. These results suggest that FXYD2 marks and regulates β cell maturation via channel-sensing signal transduction and it can be used as a selection marker for functional heterogeneity of stem cell derived human islet organoids.

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Induction of mixed chimerism and trans-differentiation of pathogenic T cells into Treg cells in unconditioned neonatal NOD mice

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Induction of mixed chimerism by injection of bone marrow (BM) cells from another strain of mice in non-autoimmune neonatal mice was reported 70 years ago by Nobel Laurate P. B. Medawar for establishing organ transplantation immune tolerance. However, induction of mixed chimerism with bone marrow only in autoimmune neonatal mice has not been reported so far. Autoimmune non-obese diabetic (NOD) mice remain the best murine model reflecting pathogenesis of human type 1 diabetes (T1D), in which autoimmune lymphocytes (i.e., T cells) destroy insulin-producing β cells. Recently, we observed that although BM alone induced mixed chimerism in unconditioned non-autoimmune neonatal BLAB/c mice, BM with additional $CD4^+$ T-depleted spleen cells containing $CD8^+$ T and NK cells were required to induce mixed chimerism in unconditioned neonatal wild-type NOD, transgenic BDC2.5 NOD, and Rag-$1^{-/-}$ BDC2.5 NOD mice.

Full and haplo-MHC-mismatched mixed chimerism totally prevented insulitis and T1D development in all types of NOD mice. Particularly, while control Rag-$1^{-/-}$BDC2.5 developed lethal T1D ~25 days after birth, neonatal induction of mixed chimerism totally prevented insulitis and T1D. The mixed chimeras showed no signs of graft-versus-host disease (GVHD). The mixed chimerism augmented thymic Treg generation, pathogenic BDC2.5 T trans-differentiation into peripherally induced pTreg cells, formation of tertiary lymphoid structure (TLS) with dominant pTreg cells in the pancreas, and expansion of mature host-type fibroblastic reticular cells (FRCs) in the pancreatic lymph node and pancreas that was reported to augment autoreactive T cell tolerance. Therefore, induction of full or haplo-mismatched mixed chimerism in unconditioned neonatal NOD mice prevents T1D by re-establishing central and peripheral immune tolerance. Induction of mixed chimerism with non-toxic regimen at neonatal stage may represent a novel approach for preventing autoimmune diseases such as T1D and may also serve as a platform for gene therapy also.

Canonical WNT signaling is required for mammary adipocyte dedifferentiation during lactation

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Abstract
In the mammary gland during pregnancy and lactation, adipocytes undergo dedifferentiation into Pdgfrα^{+}, adipocyte progenitor-like cells (1). The mechanisms governing mammary adipocyte dedifferentiation remain unclear. Mammary adipocyte RNA sequencing showed that transcription factors related to Wnt signaling were upregulated during pregnancy. We then established a trans-well assay and showed that co-culturing with lactating mammary gland tissue caused dedifferentiation of *in vitro* differentiated adipocytes, mimicking the *in vivo* remodeling of adipocytes. Adipocytes in other fat depots do not undergo de-differentiation during pregnancy and lactation. However, in the *in vitro* setting, lactating mammary gland-caused adipocyte dedifferentiation is not depot- or sex-specific, indicating a paracrine regulation of the mammary gland on the surrounding mammary adipocytes. Adding WNT transfer inhibitors or β-catenin target inhibitors inhibits adipocyte dedifferentiation. The TCF/Lef:H2B-GFP reporter mouse strain also showed that Wnt/β-catenin signaling was activated during adipocyte dedifferentiation. Parathyroid hormone-related protein (PTHrP), a hormone secreted by lactating mammary epithelium, is critical for inducing adipocyte dedifferentiation by promoting WNT secretion from the mammary gland. This tightly controlled, site- and stage-specific physiological remodeling of adipocytes offers the potential for future region- and stage-specific control of adipose tissue mass.

(1) Wang, Q. A. *et al.* Reversible De-differentiation of Mature White Adipocytes into Preadipocyte-like Precursors during Lactation. *Cell Metabolism* 28, 282-288.e283, doi:https://doi.org/10.1016/j.cmet.2018.05.022 (2018).

The gut-fat crosstalk in metabolic regulation through microbiome

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Current treatments are still limited in their ability to mitigate obesity and its complications. Obesity develops due to an imbalance between nutrient intake and energy expenditure; therefore, there is an urgent need to better understand the mechanisms governing nutrition and energy balance, with the long-term goal of developing novel strategies to treat obesity and associated metabolic diseases. Our previous study uncovered a novel link between intestinal AMPK activation and brown adipose tissue (BAT) thermogenic regulation through modulating the anti-microbial peptides (AMPs)-controlled gut microbiota and their metabolites. We also identified the new AMPK-mediated mechanism of intestine-BAT communication that may partially underlie the effects of metformin in metabolic diseases. Our current study discovered that another nutrient sensor mTOR crosstalk with white adipose tissue (WAT) browning regulates glucose homeostasis and energy metabolism. We observed significantly enhanced WAT browning and glucose homeostasis in intestinal epithelium-specific mTOR knockout (mTOR-IKO) mice compared to $mTOR^{fl/fl}$ mice. Moreover, the gut microbiota profile of mTOR-IKO mice was markedly shifted and we observed enhanced WAT browning and glucose homeostasis in WT mice after fecal microbiota transplantation from mTOR-IKO mice. Furthermore, expression of various AMPs, including REG3 and RELMb were significantly lower in the intestines of mTOR-IKO mice. These results indicate that intestinal mTOR remotely controls WAT browning through a mTOR–AMPs–microbiome–WAT axis. Determining the role of intestinal AMPK/mTOR-AMPs pathways in energy and metabolism will help fill the gap in our current understanding of how the intestine serves as an important site of AMPK/mTOR action in metabolic regulation.

Alteration of bile acid conjugation by gut microbiota mediates VSG-induced fat loss

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Abstract
Bariatric surgery, such as vertical sleeve gastrectomy (VSG), is known to efficiently reduce adiposity and lead to type 2 diabetes (T2D) remission. However, the molecular mechanism underlying VSG-induced fat loss is still elusive. Previous studies have addressed the roles of altered circulating bile acids (BAs) in metabolic benefits after surgery. In this study, we observed that the circulating and cecal conjugated bile acid (CBA) levels were dramatically increased after VSG, accompanied with enhanced adipocyte lipolysis, energy expenditure, sympathetic innervation, and reduced inflammation in epididymal white adipose tissue (eWAT) of mice. Therefore, we hypothesized that the increased CBAs could mediate the VSG-induced energy expenditure and fat loss. To determine the metabolic effects of CBAs, we used a germ-free (GF) mouse model in which CBAs can be modulated. We demonstrated that low CBA levels led to increased adiposity, glucose intolerance and inflammation, and decreased adipocyte lipolysis and fatty acid β-oxidation in eWAT. The results were opposite when the CBA levels were increased. Further mechanism studies revealed that CBAs prevented intestinal permeability, reduced circulating LPS, and alleviated adipose tissue inflammation, which eventually lowered the catecholamines degradation and accelerated WAT lipolysis and energy expenditure. This study highlighted that targeting gut microbiota to manipulate the BA conjugation may be a potential therapeutical approach to mimic bariatric surgery for obesity and diabetes treatment.

Key words: VSG, adipose tissue, lipolysis, gut microbiota, conjugated bile acids, bile acid receptors

High-throughput screening identifies three molecules that impact adult human pancreatic ductal cell survival and activity

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Type 1 Diabetes (T1D) results in the destruction of glucose-sensitive, insulin-secreting beta cells in the pancreas, and only the transplantation of isolated islets has yielded insulin independence in T1D patients. One potential source of beta-like cells is adult human pancreatic ductal progenitor-like cells, which can both self-renew and differentiate towards endocrine cells *ex vivo* in Matrigel-containing culture. However, Matrigel is a mouse sarcoma cell-derived product enriched for extracellular matrix and other xenogeneic proteins, and a Matrigel-free and chemically defined culture system is thus critically needed for future clinical application. Here, our study aims to identify FDA-approved compounds that promote the survival and proliferation of the adult human ductal progenitor-like cells. Islet-depleted human pancreatic exocrine tissues were dissociated into single-cell suspension, cryopreserved, thawed, and plated into a defined 3D suspension (spheroid) culture in the absence of Matrigel. In this culture, ductal progenitor-like cells and ductal non-progenitor cells survive, but acinar cells do not. Next, two chemical libraries containing over 3,000 unique compounds were added. Using CellTiter Glo-3D, an indicator of intracellular ATP, 92 compounds were identified as hits. Validation using four donor samples then identified three lead compounds: Crenolanib, AT13148, and JNK-IN-7. Morphological and gene expression analysis indicated that each lead compound increased markers associated with cell proliferation and metabolism. However, only Crenolanib, but not AT13148 and JNK-IN-7, increased cell numbers. Crenolanib primarily inhibits PDGFRα, PDGFRβ, and FLT3, and single-cell RNA-sequencing data showed that only FLT3 is expressed by human pancreatic ductal cells. In human hematopoietic stem and early progenitor cells, FLT3 signaling is required for development and self-renewal, and FLT3 expression decreases during the maturation process in most hematopoietic lineages. Therefore, our future efforts will test whether modulation of FLT3 signaling controls adult human ductal progenitor-like cell activity both *in vitro* and *in vivo*.