BEST ABSTRACTS SESSION I

Abstract 1: Strategies for preventing the formation of cytotoxic IAPP amyloid in human stem cell-derived islets

Anja Ivis[1], Jing Cen[1], Svitlana Vasylovska[1], Gunilla Westermark[1], Joey Lau[1].
[1]Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden

Introduction: Transplantation of human stem cell-derived islets (SC-islets), as an alternative to human islets, is showing great promise and has the potential to overcome obstacles such as a shortage of human islet donors and insufficient graft survival. However, also in SC-islets, the formation of IAPP amyloid and beta cell death contributes to deteriorating graft function. The BRICHOS domain of integral membrane protein 2B (Bri2) expressed in the beta cells possesses anti-amyloid chaperone activity and has been shown to prevent amyloid formation. Thus, this study focuses on evaluating the amyloid formation in SC-islets *in vitro* under metabolic stress, and assessing if overexpression of Bri2 BRICHOS in SC-islets could improve beta cell function.

Method: In this study, we generated SC-islets from human embryonic stem cells (H1) according to a previously published protocol (Balboa et al., 2022). SC-islets were exposed to 5.5 mM glucose concentration or metabolic stress conditions, including high levels of glucose (20 mM), palmitate (1.5 mM), or a combination of both for 14 days to explore the accelerated amyloid formation *in vitro*. Bri2 BRICHOS overexpression in SC-islets was performed using adenovirus containing the sequence of the BRICHOS domain Bri2 (Ad-BRICHOS). SC-islets transduced with Ad-BRICHOS were then cultured under normal (5.5 mM) or high (20 mM) concentrations of glucose for seven days. After exposure, the amount of formed amyloid was determined by staining with the luminescent conjugated oligothiophene pFTAA. The exogenously introduced Bri2 BRICHOS expression was confirmed by immunostaining. Beta cell function in SC-islets was assessed by static glucose-stimulated insulin secretion (GSIS) and insulin content. The mRNA expression profile of interested genes, such as IAPP, was evaluated by qRT-PCR.

Results: Amyloid was formed in *in vitro* cultured SC-islets exposed to metabolic stress. Overexpression of the Bri2 BRICHOS domain in SC-islets was successfully achieved, which reduced the amyloid formation under high levels of glucose exposure. Compared with the SC-islets under normal glucose treatment, the stimulation index calculated from GSIS and the insulin content were decreased in the SC-islets exposed to high glucose concentration. The destroyed beta cell function could be improved by Ad-BRICHOS overexpression in SC-islets. The mRNA expression of IAPP was induced by high glucose stimulation but without a simultaneous increase in Bri2 BRICHOS overexpression.

Conclusion: The use of SC-islets as a model for studying amyloid formation mechanisms and the role of IAPP in diabetes is a promising avenue for future research. The potential of Bri2 BRICHOS overexpression to inhibit amyloid formation in SC-islets is a significant finding, suggesting it could be a viable strategy for protecting SC-islets from amyloid-related beta cell death post-transplantation.

Abstract 2: Development of a biovascular pancreas to deliver stem cell-derived islets as a therapy for type 1 diabetes

Hong Qian[1], Kaleb Naegeli[1], Mehmet Kural[1], Sachiko Imaichi[1], Laura Niklason[1].

[1]Humacyte Global, Inc., Durham, NC, United States

Introduction: Islet transplantation offers immense therapeutic potential for type 1 diabetes. However, traditional transplantation sites for pancreatic islets such as the portal vein and subcutaneous space are hypoxic environments that contribute to failure of the transplanted graft. To solve this mass transfer problem, we are generating a biovascular pancreas (BVP): an acellular tissue engineered vascular graft coated on the outside with stem cell-derived pancreatic islets within a biological hydrogel (Fig1A-B). When connected as an arteriovenous graft, islets are kept within ~1mm of arterial blood flow through the lumen of the graft, which previous work indicates can support islet survival[1]. Subsequent vascularization of the graft[2] can provide a robust source of oxygen to support islet viability and function. Previous proof-of-concept in rat models demonstrated feasibility of this graft[1]. In this study, we characterize development of stem cell-derived islets and initiate BVP proof-of-concept in nonhuman primate models.

Method: To demonstrate function of our stem cell-derived (SC) islets, 2000 IEQ were delivered under the left kidney capsule of STZ-induced diabetic immunodeficient (NSG) mice. We subsequently monitored blood glucose and serum C-peptide levels over the course of 13 weeks. To establish loading densities of islets within the graft, we created a dual bioreactor system around BVPs to maintain an extracellular pO_2 of 40mmHg (hypoxic) and an intralumenal pO_2 of 90mmHg, which mirrors the pO_2 of arterial bloodflow. Finally, to further develop this concept in vivo, we transplanted BVP containing allogenic islets in an aorta-aorta configuration in cynomolgus macaques and monitored the graft for islet survival for up to 3 months.

Results: We are able to generate BVP grafts with consistent distribution of islets within the biological hydrogel (Fig 1C). In diabetic mouse studies, 2000 IEQ of SC islets restored euglycemia after 9 weeks of implantation (Fig 2A), and detected human C-peptide levels in the serum increased steadily over the course of the experiment, demonstrating graft maturation and function. In our hypoxia bioreactor system, islets within the BVP survived at significantly higher rates compared to islets in hypoxia conditions similar to those of the hepatic portal vein. SC islets also survived at an effective loading dose of ≥300k IEQ/BVP graft, establishing an initial optimal loading dose of SC islets that would still allow for efficient mass transfer. In NHP models, islets in the BVP survived (Fig 2B), developed vasculature, and expressed islet markers, demonstrating that the BVP supported islet engraftment.

Conclusion: An acellular tissue engineered vessel loaded with islets embedded within a biological hydrogel and implanted in arterial circulation can support the survival and engraftment of islets. This BVP construct creates a vascular niche to support pancreatic islets and is a potential therapeutic for Type 1 Diabetes.

Portions of this work were collaboratively supported through an Industry Discovery and Development Partnership with Breakthrough T1D.. The authors of this study own stock or stock options in Humacyte Global, Inc..

References:

[1] Han EX, Wang J, Kural M, et al. Development of a Bioartificial Vascular Pancreas. Journal of Tissue Engineering. 2021;12. doi:10.1177/20417314211027714

[2] Robert D. Kirkton et al. Bioengineered human acellular vessels recellularize and evolve into living blood vessels after human implantation.Sci. Transl. Med.11,eaau6934(2019).DOI:10.1126/scitranslmed.aau6934

Abstract 3: Modeling the effects of early hyperinsulinemia due to the R1420H SUR1 mutation using isogenic iPSC derived pancreatic Islets

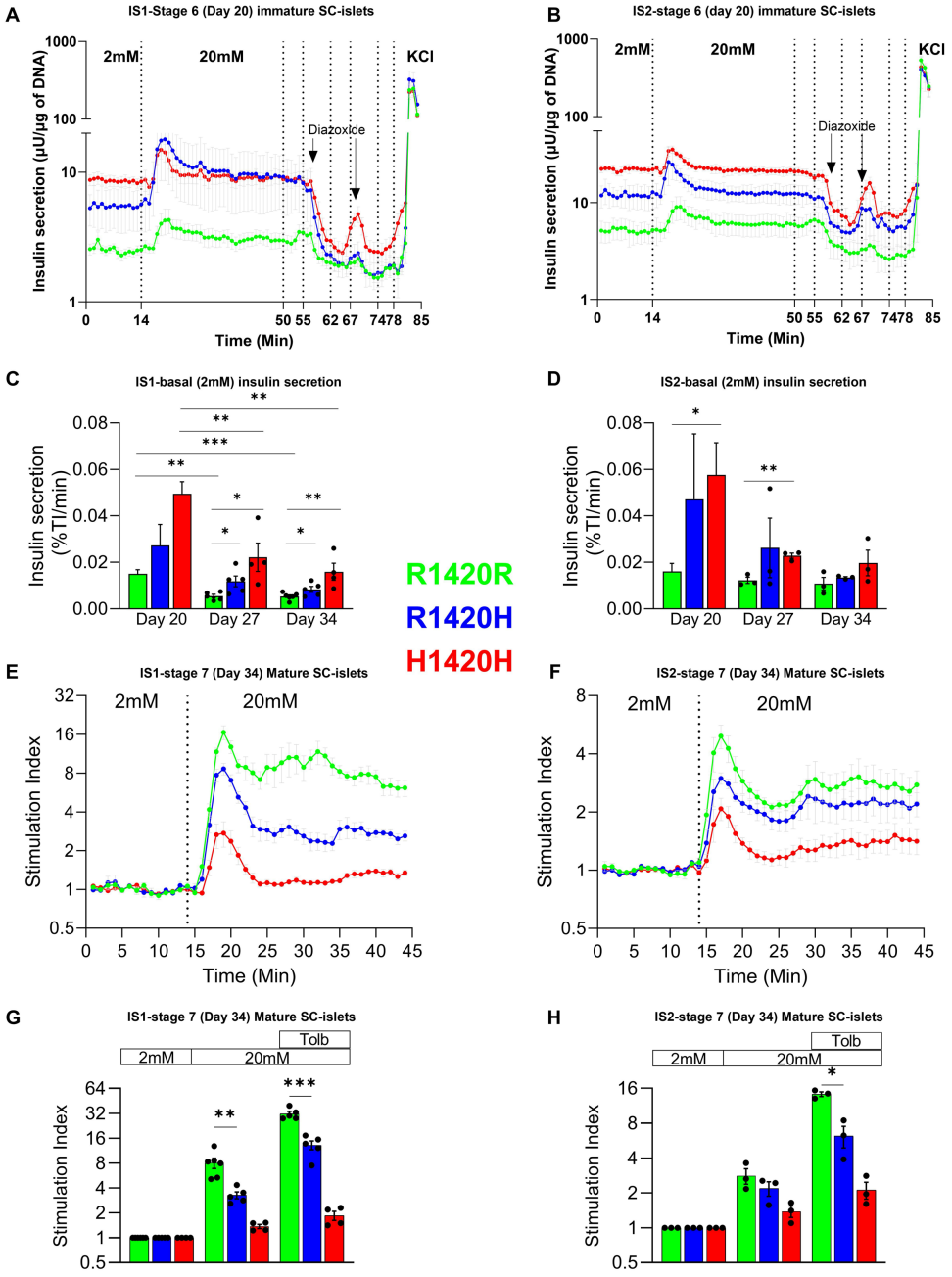
Anup Nair[1], Katiya Barkho[1], Koushik Ponnanna Cheranda Ravi[1], Michael Traurig[1], Jeff Sutherland[1], Divya Anup[1], Clifton Bogardus[1], Leslie Baier[1].

[1]Phoenix Epidemilogy and Clinical Research Branch, NIDDK/NIH, Phoenix, AZ, United States

Introduction: A previously reported R1420H mutation in SUR1 doubles the risk for type 2 diabetes (T2D) in heterozygous carriers who, on average, had a 7-year earlier onset of T2D despite being leaner. This suggests that dysregulation of insulin secretory function, rather than obesity induced insulin resistance, was the primary contributor to their T2D. One homozygous H1420H carrier was identified, and this individual had hyperinsulinemic hypoglycemia during infancy (HHI). Using CRISPR edited induced pluripotent stem cells (iPSCs), we created models for the R1420H K_{ATP}-channel mutation (K_{ATP}HI) to study its effects on insulin secretion and genome-wide expression patterns.

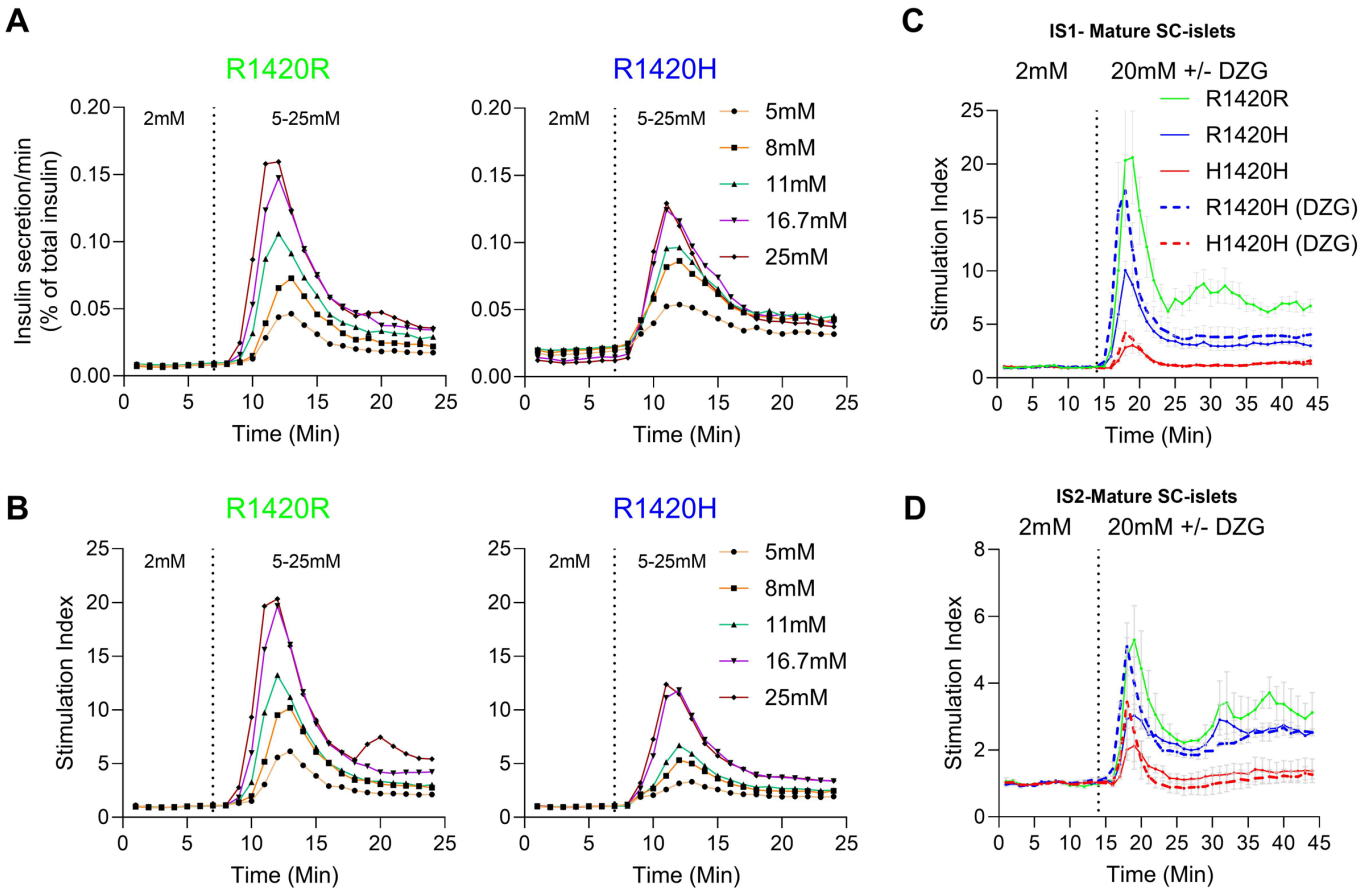
Methods: iPSCs from 2 different individuals, one R1420R (IS1) and one R1420H carrier (IS2), were selected for CRISPR/Cas9 editing to generate two sets of isogenic cell lines homozygous (H1420H) and heterozygous (R1420H) for the risk allele and homozygous (R1420R) for the normal allele. These isogenic iPSCs were differentiated into stem cell islets (SC-islets) using a 7-stage differentiation protocol to characterize functional and transcriptomic differences at various stages of differentiation.

Results: We show that stage 6 (day 20) R1420H and H1420H immature SC-islets have higher insulin secretion in basal condition (2mM glucose) which resembles the clinical phenotype of HHI (Figure 1A-B). The immature SC-islets respond to the K_{ATP}-channel activating therapeutic agent diazoxide albeit the response was lower in H1420H SC-islets.



Upon maturation (stage 7: days 27 and 34), the SC-islets show tighter regulation of insulin secretion in basal condition, but the K_{ATP}HI mutant SC-islets still had higher insulin secretion (Figure 1C-D). However, the insulin secretory response to high glucose (20mM, Figure 1E-F) and K_{ATP}-channel inhibitor tolbutamide (Tolb, Figure 1G-H) were significantly lower in R1420H day 34 mature SC-islets whereas no

response was seen in H1420H SC-islets despite having a similar proportion of SC-β-cells. The insulin secretory response to different glucose concentrations (5mM–25mM) for the mature R1420H SC-islets was suggestive of lower glycolytic flux (Figure 2A-B) and the R1420H SC-islets are responsive to the GCK activator therapeutic agent dorzagliatin which promotes glycolytic flux (DZG, Figure 2 C-D).



We used single-cell sequencing to catalog gene expression differences in SC-β-cells due to the K_{ATP}HI mutation which could explain the observed results. We identified dysregulated genes related to glucose metabolism (*GCK*, *GPI*, *TPI*, *GAPDH*, *G6PC2*), Ca+ signaling/binding (*S100* family genes) and immediate early response genes (*FOS*, *FOSB*, *JUN*, *JUNB*, *JUND*, *EGR1*).

Conclusion: We have modeled hyperinsulinemia in immature R1420H mutant SC-islets that switches to lower insulin secretory response after maturation and explains the T2D risk in carriers. This study lays the framework for the discovery of appropriate therapeutics for T2D in subjects with the R1420H SUR1 mutation.

NIDDK Intramual Funding.

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[1] Baier LJ, Muller YL, Remedi MS, Traurig M, Piaggi P, Wiessner G, Huang K, Stacy A, Kobes S, Krakoff J, Bennett PH, Nelson RG, Knowler WC, Hanson RL, Nichols CG, Bogardus C. ABCC8 R1420H Loss-of-Function Variant in a Southwest American Indian Community: Association With Increased Birth Weight and Doubled Risk of Type 2 Diabetes. *Diabetes*. 2015;64(12):4322-32.

Abstract 4: Human stem cell-derived beta-cells delivered with vasculogenic hydrogels reverse hyperglycemia in diabetic immunodeficient mice

Sophia Kioulaphides[1], Angelica Torres[1], Michael Hunckler[2], Nathaniel Hogrebe[3], Jeffrey Millman[3], Esma Yolcu[4], Haval Shirwan[4], Andres Garcia[2].

[1]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, United States; [2]Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, United States; [3]Medicine, Washington University in St. Louis, St. Louis, MO, United States; [4]Medicine, University of Missouri, Columbia, MO, United States

Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreas. The most promising therapy thus far is the transplantation of cadaveric islets, but this approach is severely limited by the lack of donors, poor engraftment, and the need for chronic immunosuppression [1]. To address the lack of insulin-producing cells, human stem cell-derived β (SC-β) cells have become a promising scalable source of insulin-producing cells [2, 3]. This study focuses on hydrogel delivery vehicles for to promote engraftment and function of SC-β cells. We first transplanted $5.5 \bullet 10^6$ SC-β cells to the gFP of streptozotocin (STZ)-induced diabetic SCID-beige mice, both with and without PEG-VEGF hydrogels. At 21 days post-transplant, normoglycemia was restored in both treatment groups and persisted for 90 days. At 6 weeks post-transplant, an intraperitoneal glucose tolerance test (IPGTT) confirmed that the grafts responded to glucose stimuli in real-time, and blood serum samples showed the presence of circulating C-peptide. To further understand why the SC-β cells could engraft in the gFP without a hydrogel carrier, a human VEGF ELISA confirmed that SC-β cells were releasing their own VEGF into the surrounding environment. These results motivate current dosing studies to determine the marginal graft that can correct diabetes, and whether the PEG-VEGF gel carrier can promote vascularization of the marginal graft and reverse hyperglycemia. These studies showed that when $3 \bullet 10^6$ SC-β cells were transplanted to the gFP, the PEG-VEGF hydrogel enhanced their engraftment and function across 100 days, and IPGTT and C-peptide ELISA tests further confirmed these results. Success and powering up of these experiments will be a step forward to future T1D correction studies incorporating immunomodulatory strategies in the omentum of immune competent and larger animal models.
NIH R01 DK128840. NIH R01 DK133702.

References:

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[2] Pagliuca F. Generation of functional human pancreatic beta cells in vitro. *Cell*. 2014. 159: 428-39.
[3] Hogrebe J. Generation of insulin-producing pancreatic beta cells from multiple human stem cell lines. *Nature Protocols*. 2021. 16: 4109-43.

Abstract 5: SC-beta cell MafA expression: for better or worse

Veronica Cochrane[1,5], Marianne Aniag[1,5], Jeeyeon Cha[2], Roland Stein[3], Audrey Parent[1], Matthias Hebrok[4,5].

[1]Diabetes Center, UCSF, San Francisco, CA, United States; [2]Medical Center, Division of Diabetes, Endocrinology and Metabolism, Vanderbilt University, Nashville, TN, United States; [3]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, United States; [4]Center for Organoid Systems, Technical University Munich, Munich, Germany; [5]Institute for Diabetes and Organoid Technology, Helmholtz, Munich, Germany

Introduction

Stem-cell derived beta cells (SC-beta cells) are invaluable tools for diabetes research, both for their use in disease modeling as well as their potential as a limitless resource for cell replacement therapy. Although significant progress has been made in generating SC-beta cells that are glucose-responsive, SC-beta cells continue to have divergent metabolic and transcriptomic profiles compared to primary human beta cells. A key beta cell transcription factor that is regularly reported to be expressed at relatively low levels in SC-beta cells is MafA. In humans, MafA expression levels correspond with beta cell functional maturation. Low MafA expression is associated with functionally immature juvenile (<9yo) beta cells, whereas functionally mature adult (>9yo) beta cells have high MafA expression (Fig. 1A). However, a naturally occurring mutant variant MafAS64F with enhanced protein stability has been shown to be detrimental to beta cell function and may cause senescence, suggesting that MafA activity must be balanced (Fig. 1B).

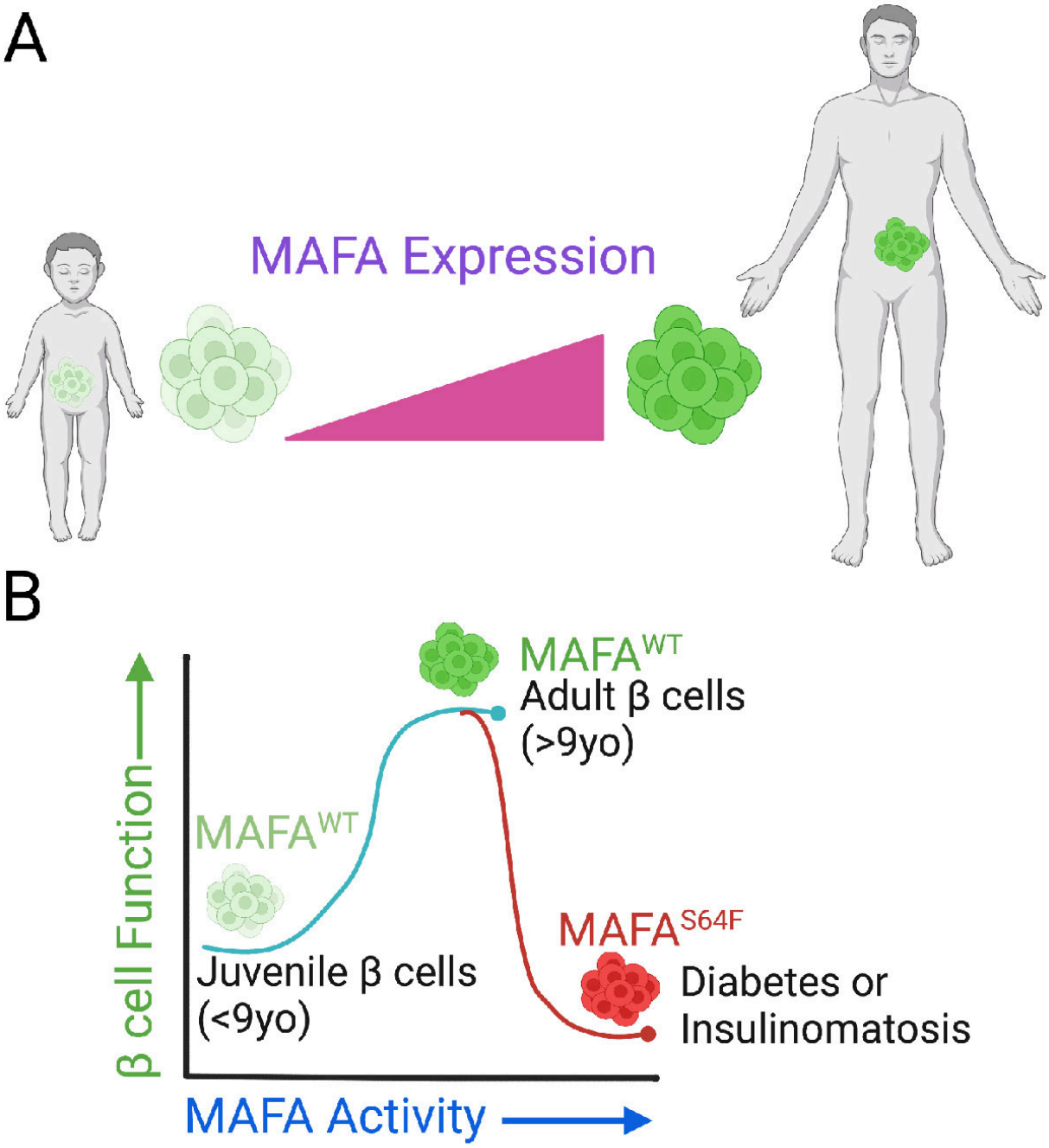


Figure 1. MafA expression and activity in human beta cells. A) Relative MafA expression in juvenile and adult beta cells. B) Effect of MafA activity on beta cell function.

Method

Here we use a hESC line modified with a doxycycline-inducible overexpression cassette to examine the impact of MafAWT and MafAS64F on SC-beta cell insulin secretory capacity and metabolic activity (Fig. 2A). MafA overexpression was induced for a duration of 3, 7, or 14 days (Fig. 2B).

Results and Conclusion

Overexpression of MafAWT progressively enhanced SC-beta cell glucose-stimulated insulin secretion (GSIS) throughout the time course, suggesting that increased MafA levels are a critical determinant of SC-beta cell function (Fig. 2C). In striking contrast, MafAS64F only transiently improved SC-beta cell GSIS function before rendering the cells non-functional (Fig. 2D). Notably, MafAWT (14d OE) and MafAS64F (3d OE) SC-beta cells with enhanced GSIS capacity exhibited greater metabolic activity whereas, MafAS64F SC-beta cells that were non-functional (14d OE) had greatly diminished metabolic capacity, indicating that modified glucose metabolism underlies MafA-induced functional changes (Fig. 2E, F). Ongoing metabolic studies and transcriptomic analyses are expected to provide novel insights as to the mechanisms underlying beta cell functional maturation and how dysregulation of these mechanisms contribute to a loss of beta cell function.

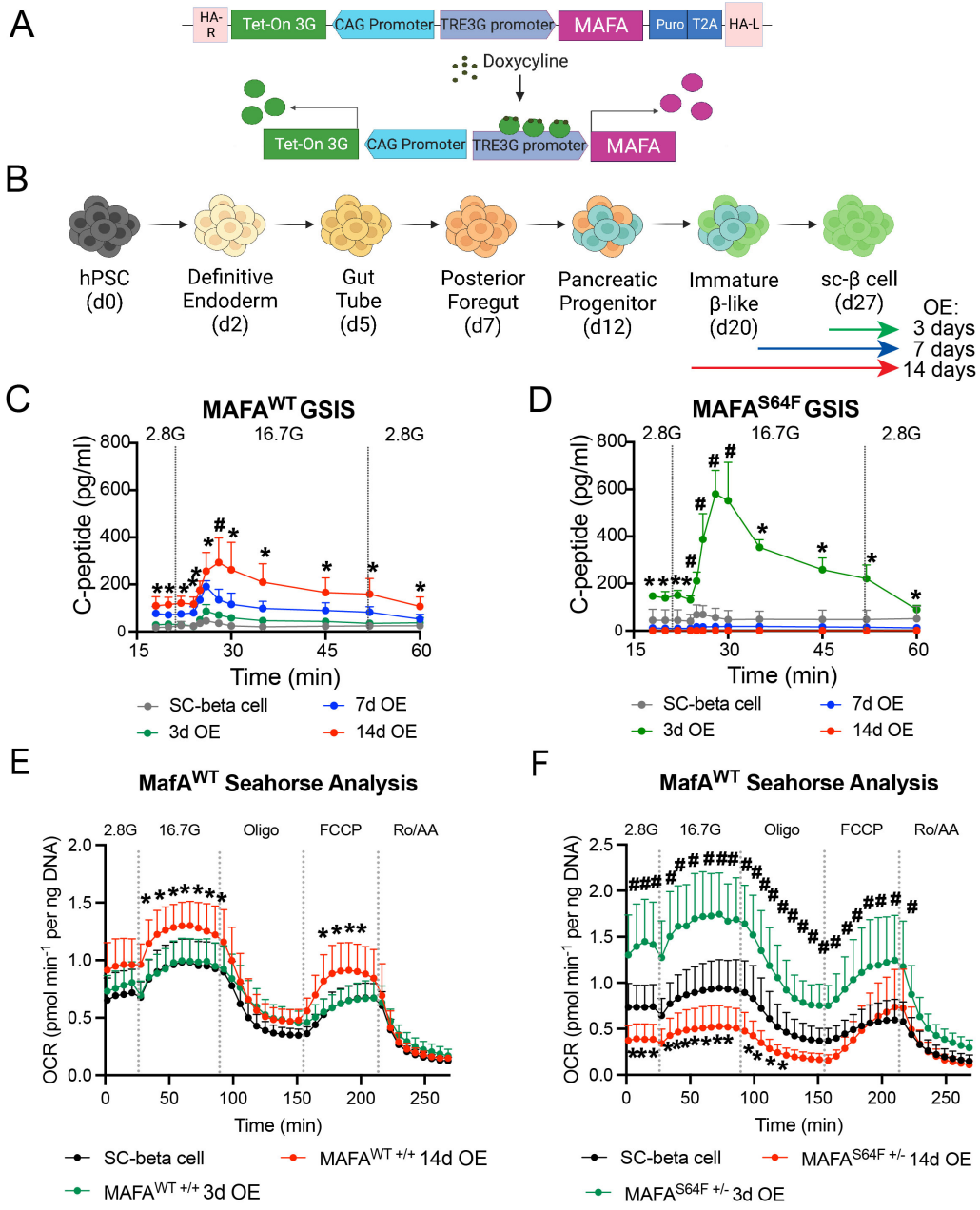


Figure 2. MafA OE SC-beta cells. A) Schematic of doxycycline-inducible MafA overexpression (OE) cassette. The hAAVS1-targeted vector contains a Tet-On bidirectional inducible expression system, in which the MAFA coding sequences are placed behind a third generation tet-responsive promote, with tet-on transactivator protein-driven by CAG promoter. B) Schematic of hPSC differentiation to SC-beta cell and OE time course. C, D) Dynamic GSIS assay of MafA^{WT} (D) and MafA^{S64F} (E), at 2.8mM and 16.7mM glucose. E, F) Seahorse analysis of MafAWT (E) and MafAS64F at 2.8mM and 16.7mM glucose, 5uM Oligomycin, 1uM FCCP, and 5uM Rotenone/Antimycin A. *p<0.001, #p<0.0001; compared to SC-beta cell.

JDRF Postdoctoral Fellowship . NIDDK R01DK090570-08.

Abstract 6: Islet versus enterochromaffin lineage allocation of hPSC-derived pancreatic progenitors can be modulated at both pre-progenitor and post-progenitor stages

Paraish Misra[1,2,3], Emily McGaugh[2,3], Haiyang Huang[2,3], Alex Cho[2,3], Amanda Oakie[2], Valéria Fabríciová[4], Romana Bohuslavová[4], Gabriela Pavlínková[4], M. Cristina Nostro[2,3].
[1]Department of Medicine, University of Toronto, Toronto, ON, Canada; [2]McEwen Stem Cell Institute, University Health Network, Toronto, ON, Canada; [3]Department of Physiology, University of Toronto, Toronto, ON, Canada; [4]Laboratory of Molecular Pathogenetics, Institute of Biotechnology CAS, Vestec, Czech Republic

Introduction: Little is known about the molecular mechanisms that underlie the allocation of human pluripotent stem cell-derived pancreatic progenitors (hPSC-PPs) into specific endocrine lineages, particularly enterochromaffin (EC)-like cells, which are often believed to be an aberrant islet cell type. Herein, we describe our efforts to understand mechanisms regulating islet-like vs EC-like lineage allocation.

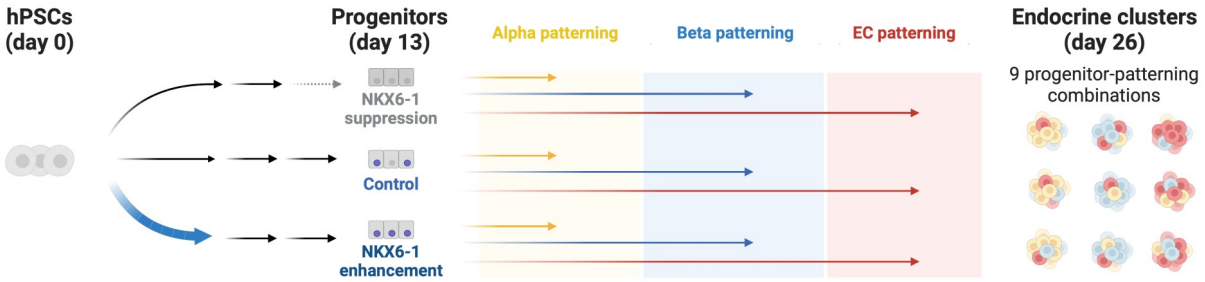
Methods: We systematically interrogated the components of pancreatic differentiation media to identify factors that can modulate primitive streak, pancreatic progenitor, and endocrine differentiation. To determine whether EC-like vs islet-like lineage allocation can occur due to molecular changes after pancreatic progenitor specification, we characterized the endocrine cells arising within the islets of mice with a conditional knockout of Isl1 following onset of Neurod1 expression.

Results: We identify methods that robustly increase Brachyury expression in hPSC-derived cells at the primitive streak stage, and further elaborate protocols to increase NKX6-1 expression at the hPSC-PP stage, but find that these alone have minimal effects on modulating the endocrine composition of hPSC-derived islets. We then test the effects of altering the signaling cues provided after progenitor specification and find that several pathways can be manipulated to shift hPSC-PPs between islet and non-islet endocrine lineages. We further find that the method of hPSC-PP specification integrates with these later cues in a way that can be leveraged to selectively increase the frequencies of specific islet lineages or EC-like cells. Using a model of disrupted murine islet development that gives rise to pancreatic EC-like cells, we identify prolonged NEUROG3 expression as a conserved feature associated with both human and murine pancreatic EC-like cell differentiation.

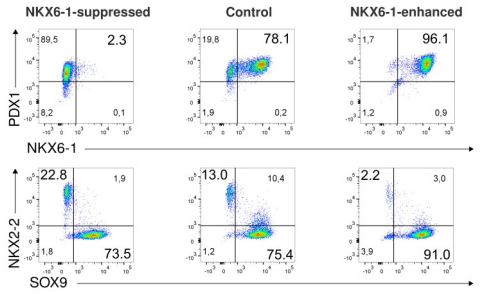
Conclusion: These findings expand our understanding of how pancreatic progenitors commit to different endocrine lineages. Our work highlights the significant phenotypic plasticity of endocrine cells arising from pancreatic differentiation of hPSCs, provides some of the first tools to fine-tune the endocrine composition of hPSC- islets for research and therapeutic applications.

Figure 1

A


hPSCs
(day 0)
Progenitors
(day 13)
Alpha patterning
Beta patterning
EC patterning
Endocrine clusters
(day 26)
9 progenitor-patterning
combinations
NKX6-1
suppression
Control
NKX6-1
enhancement

B


NKX6-1-suppressed
Control
NKX6-1-enhanced
PDX1
NKX6-1
NKX2-2
SOX9
89.5
2.3
8.2
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19.8
78.1
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0.2
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22.8
1.9
1.8
73.5
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1.2
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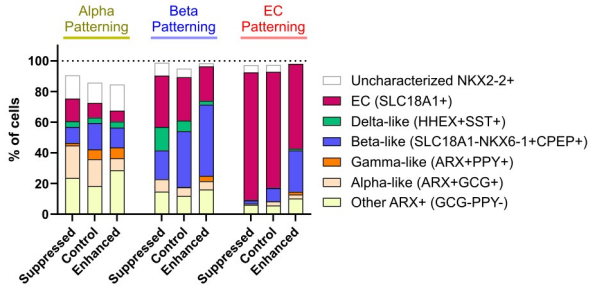
2.2
3.0
3.9
91.0

C


Alpha
Patterning
Beta
Patterning
EC
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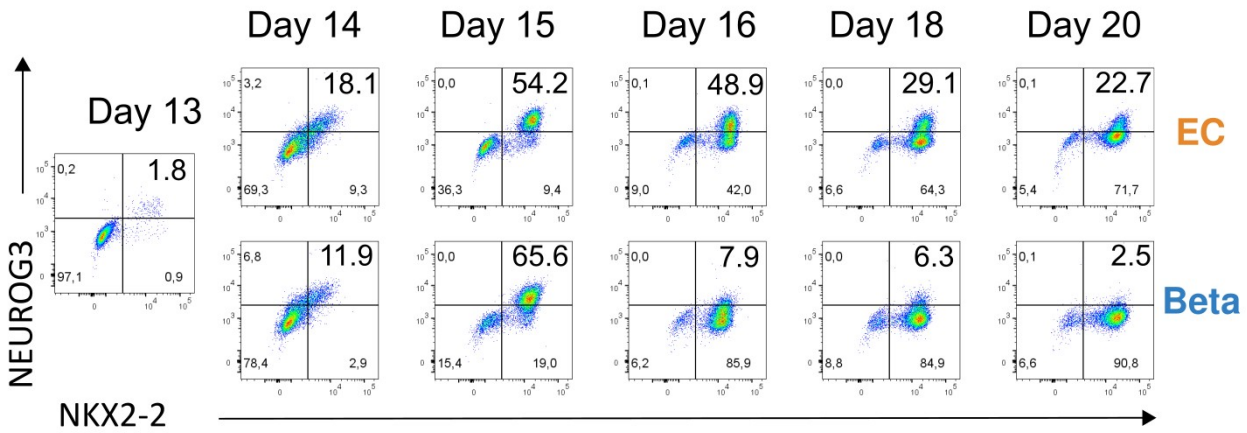
% of cells
100
80
60
40
20
0
Suppressed
Control
Enhanced
Uncharacterized NKX2-2+
EC (SLC18A1+)
Delta-like (HHEX+SST+)
Beta-like (SLC18A1-NKX6-1+CPEP+)
Gamma-like (ARX+PPY+)
Alpha-like (ARX+GCG+)
Other ARX+ (GCG-PPY-)

Figure 2

A


Day 14
Day 15
Day 16
Day 18
Day 20
Day 13
EC
Beta
NEUROG3
NKX2-2
0,2
1.8
97,1
0,9
3,2
18.1
69,3
9,3
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54.2
36,3
9,4
0,1
48.9
9,0
42,0
0,0
29.1
6,6
64,3
0,1
22.7
5,4
71,7
6,8
11.9
78,4
2,9
0,0
65.6
15,4
19,0
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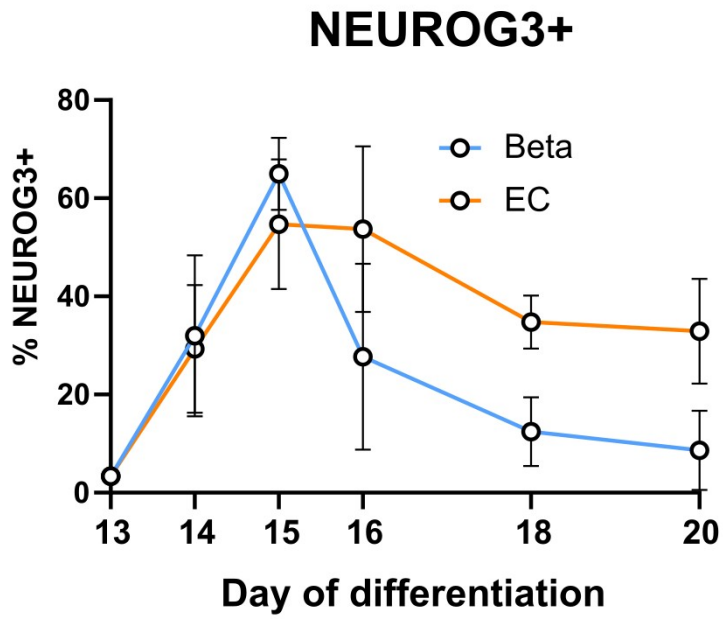
B


NEUROG3+
% NEUROG3+
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60
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0
13
14
15
16
18
20
Day of differentiation
Beta
EC

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