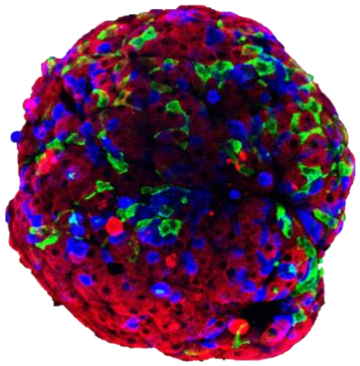

3rd Joint Meeting of the EASD Islet Study Group & Beta-cell Workshop

St Catherine's College, Oxford, UK April 1-3rd 2019

International Organising Committee:
Anna L Gloyn, UK (chair) Lena Eliasson, Sweden & Lori Sussel, USA

@ISLETSINOXFORD2019

Islet Image Credit: Elisa Vergari & Anne Clark, Oxford

Contents

Programme at a Glance

	Sunday, March 31	Monday, April 1	Tuesday, April 2	Wednesday, April 3
08:00		**Registration** Posters setup		
08:30		SESSION 1: ISLET CELL DYSFUNCTION IN AUTOIMMUNE DIABETES	SESSION 5: EPIGENETICS & GENOMICS OF ISLET CELLS	SESSION 8: BETA-CELL DEVELOPMENT
09:00				
09:30				
10: 00		Oral Poster Presentation	Oral Poster Presentation	
10: 30		Coffee break & Poster session	Coffee break & Poster session	Coffee break
11: 00		SESSION 2: ISLET CELL DYSFUNCTION IN NON-AUTOIMMUNE DIABETES	SESSION 6: MODELLING MUTATIONS IN BETA-CELL MODELS	SESSION 9: OVERVIEW & UPDATE ON COLLABORATIVE INITIATIVES IN ISLET CELL FUNCTION & DIABETES
11: 30				
12: 00				
12: 30		Oral Poster Presentation	Oral Poster Presentation	
13: 00		LUNCH BREAK	LUNCH BREAK	LUNCH BREAK
13: 30				
14: 00	*Check in from 2pm*	SESSION 3: ISLET SIGNALLING & INSULIN SECRETION	SESSION 7: ISLET -CELL MODELS & THERAPIES	SESSION 10: KEYNOTE LECTURE 2
14: 30				
15: 00				
15: 30		Oral Poster Presentation	Oral Poster Presentation	MEETING CLOSE
16: 00		Tea break & Poster session	EASD-ISG Admin meeting	Tea & Departure
16: 30	**Registration** (Bernard Sunley Building)	SESSION 4: KEYNOTE LECTURE 1 The changing beta-cell	Tea break & Poster session	
17: 00			Poster Removal	
17: 30		POSTERS & DRINK RECEPTION IN THE COMMON ROOM	OXFORD ASHMOLEAN MUSEUM VISIT and Reception	
18: 00				
18: 30				
19: 00		DINNER (in college)		
19: 30				
20: 00			Pre-dinner drinks – Common Room	
20: 30			FORMAL HALL DINNER	
21: 00				
21: 30				

Welcome from the Scientific Committee

ANNA GLOYN

On behalf of the EASD Islet Study Group & the Beta-Cell Workshop local and scientific organising committees I would like to welcome you to the 3rd Joint meeting of our societies. The ethos of the meeting is to bring together basic and clinical scientists from all over the world working on all aspects of the β-cell (and other islet cells) from their birth to their demise. It is an opportunity to hear from the Rising Stars and outstanding experts in the field and to provide plenty of opportunities for scientific discussion and networking.

Following the scientific extravaganzas in Jerusalem (2015) hosted by Professors Yuval Dor & Ben Glaser and then in Dresden (2017) by Professor Michele Solimena the bar has been set very high for delivering fantastic islet biology coupled with superb cultural and social programmes. For Islets in Oxford 2019 we have lined up an incredible list of international speakers who I am certain will delight us with a smorgasbord of islet biology accompanied by plenty of opportunities for networking washed down with an opportunity to experience life in an Oxford College, including the full Formal Hall dining experience. To ensure that we are culturally nourished there will be a visit to Oxford's celebrated Ashmolean Museum and to make sure we have not been sat still for too long there will be dancing on Tuesday night.

To mark the meeting in collaboration with Diabetologia we have assembled a special virtual edition of published papers. We hope you enjoy revisiting or discovering this selection of islet biology at its best.

To promote scientific dissemination and provide a record of the meeting I am delighted that we have our own resident artist, Alex Cagan, who will be providing "live sketching' of the meeting. Illustrations will be displayed throughput the meeting which capture the scientific activity.

I hope you have a fantastic time in Oxford and take home new scientific knowledge and great memories.

LENA ELIASSON (MALMO, SWEDEN)

Lena Eliasson is appointed Professor in Experimental Diabetes Research at Lund University and she is part of Lund University Diabetes Centre. Her research aims to understand cellular mechanisms underlying impaired islet hormone secretion contributing to the pathogenesis of diabetes. The focus is on single cell physiology involving studies of ion-channels, exocytosis and miRNA networks important in the regulation of islet cell secretion.

LORI SUSSEL (DENVER, USA)

Dr. Lori Sussel is the Director of Basic and Translational Research at the Barbara Davis Center for Diabetes at the University of Colorado. Dr. Sussel's research program focuses on pancreatic islet cell development and function, which has contributed to the understanding of the islet dysfunction that occurs during the course of diabetes. Most recently, she has made exciting discoveries in the new field of long non-coding RNAs and their regulation of islet development and function.

EASD ISLET STUDY GROUP (EASD-ISG)

The EASD Islet Study Group is a study group of the European Association for the Study of Diabetes (EASD) which focuses on research into islet biology in diabetes. It arose from discussions of participants of the islet meeting held at Coleraine 15-18 September 1991. Since this time, the Group has held meetings annually in close association with the main EASD Congress. Approximately 120 participants attend each meeting, which is of 2-days duration. The current chair is Dame Professor Francis Ashcroft FRS FMedSci (Oxford, UK).

The current other members of the committee are:

Frances Ashcroft (UK)
Timo Otontoski (Finland)
Henrik Mulder (Sweden)
Susanna Ulrich (Germany)

The first EASD-ISG meeting In Coleraine in 1991 (Photo credit Peter Flatt)

Local Organizing Committee

MARCO PONTECORVI

Marco Pontecorvi is the Scientific Project Manager for the NIHR/BRC Diabetes Theme, led by Anna Gloyn at OCDEM (Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital). His role includes supporting the grant stakeholders and their portfolio of translational research projects, managing the group website and coordinating both patient and public outreach activities for the department. He has a background in molecular and developmental biology.

SKIRMANTE TAMELYTE

Skirmante is PA to Anna Gloyn & Mark McCarthy and based at the Oxford Centre for DIabetes, Endocrinology & Metabolism.

BENOIT HASTOY

Benoit obtained his MSc and PhD from the University of Bordeaux where he worked on the mechanisms of membrane fusion in exocytosis under the supervision of Johan Lang. In 2012, he joined the University of Oxford as postdoctoral researcher in Patrik Rorsman's team where he learnt electrophysiology and worked on exocytosis. In 2015, he joined Anna Gloyn's and Mark McCarthy's teams to investigate the cellular physiology that underlies genetic predisposition for diabetes. Benoit is also involved in the characterisation of human beta cell models such as the human beta cell lines EndoC-βH1/-βH2 and the beta-like cells derived from iPSCs which are becoming essential tools to clarify the effect of T2D risk variants and beta cell dysfunction.

GRACE YU

Grace Yu is a postdoctoral scientist in Anna Gloyn and Mark McCarthy's research group at the Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM). She is also an Associate Research Fellow at Harbin Medical University, China. Her current research focuses on trying to understand the molecular mechanisms by which coding and non-coding changes in genomic sequences affect gene regulation and protein function in Type 2 Diabetes. A major component of her research includes using genetically modified animal models to study genetic variations identified in large human genome-wide associations for the risk of Type 2 Diabetes. By investigating genetic variations more closely in mice, this allows her to discover more about the basic physiology and biology, this may ultimately be translatable for therapeutic benefit.

JAKOB KNUDSEN

Jakob has a background in cellular biology and physiology from the University of Copenhagen where he also obtained his PhD, studying the role of skeletal muscle as an endocrine organ during exercise. He came to Oxford in September 2015 to start a Novo Nordisk Postdoctoral Research Fellowship with Patrik Rorsman at the Oxford Centre for Diabetes Endocrinology & Metabolism. His current research is focused on how regulation of glucagon secretion contributes to the development of diabetes. His main interest is regulation of α-cell metabolism and how perturbations of α-cell metabolism affect glucagon secretion and whole-body metabolism.

KERRY MCLAUGHLIN

Dr Kerry McLaughlin joined the Oxford Centre for Diabetes, Endocrinology & Metabolism (OCDEM) as a JDRF-funded Research Fellow in 2016. Dr McLaughlin's research interests are centred on the early development of autoimmunity in type 1 diabetes and the factors triggering progression to clinical disease. Previously, she was part of the team that identified a protein called tetraspanin-7 as a major target if the immune response in type 1 diabetes. Her current work aims to characterise the immune response to tetraspanin-7 and to determine the role of this protein in the insulin-producing beta cell.

LINFORD BRIANT

To some, Linford Briant is a mathematician posing as a biologist; to others, he is a biologist posing as a mathematician. His interests lie in understanding alpha-cell physiology in health and disease. However, he has quickly realized that he should restrict his interests to understanding alpha-cells under healthy conditions, because they are a very peculiar (and fascinating) cell type!

NICOLE KRENTZ

Nicole Krentz is a postdoctoral research fellow at the Wellcome Centre for Human Genetics at the University of Oxford and the Robert Turner Research Associate at Green Templeton College. In 2018, Nicole completed her PhD at the University of British Columbia under the supervision of Francis Lynn. Her PhD research focused on pancreas development and endocrine cell genesis using mouse embryos and human embryonic stem cell differentiation as models. In collaboration with Michael German's lab at the UCSF, Nicole discovered that the cell cycle regulates endocrine cell development by phosphorylating the transcription factor Neurog3. In 2018 Nicole moved to Oxford and joined Anna Gloyn's group where she is now investigating the role of diabetes associated genes in pancreas development using genome-editing inhuman induced pluripotent stem cell models.

VIBE NYLANDER

Vibe holds a MSc and PhD from the University of Copenhagen, where she studied the metabolic consequences of total body irradiation. She joined Anna Gloyn's laboratory at the University of Oxford as a postdoctoral researcher in 2016. Vibe's research revolves around transcriptional control, and how genetic risks variants and environmental stimuli alters the transcriptional landscape and contributes to T2D risk. Vibe works on protein-protein interaction networks and chromatin interactions of T2D GWAS loci in β-cell

Sponsors

The organizing committee would like to thank the Sponsors & Partners for their generous support of the workshop.

Platinum

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Gold

Diabetologia
Journal of the European Association for the Study of Diabetes (EASD)

EASD
European Association for the Study of Diabetes

Bronze

Smart Ephys | multichannel systems a division of Harvard Bioscience, Inc.

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SUNDAY 31ST MARCH 2019

If you have reserved accommodation at St Catherine's College you can check in from 2pm GMT at the Porter's Lodge.

There are no social events planned for Sunday evening however, there are plenty of good restaurants and pubs in the centre of Oxford to hang out in.

MONDAY 1ST APRIL 2019

From: 8:00am	**REGISTRATION**
8:30-10.30am	SESSION 1: ISLET CELL DYSFUNCTION IN AUTOIMMUNE DIABETES Chair: Sarah Richardson [UK]
8.30-9.00am	**Current status of Type 1 diabetes** John Todd [UK]
9.00-9.30am	**Development of new immunomodulatory prevention strategies** Caroline Daniel [Germany]
9.30-10.00am	**Monogenic forms of autoimmune diabetes** Sarah Flanagan [UK]

10.00-10.30am	**OP1 A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry** Nicolas Damond [Switzerland] **OP2 A Human Induced Pluripotent stem cell based in vitro model for autoimmune Type 1 Diabetes** Elad Sintov [USA]
10:30-11:00am	**COFFEE BREAK & POSTERS**
11:00 -1:00pm	SESSION 2: ISLET CELL DYSFUNCTION IN NON-AUTOIMMUNE DIABETES Chair: Maureen Gannon [USA]
11:00am	**ER Stress: Beta-Cells Rise to the Challenge** Laura Alonso [USA]
11.30-12.00pm	**Modelling PTF1A enhancer mutations which cause diabetes in mice** Irene Miguel-Escalada [Spain]
12.00-12.30pm	***SLC30A8* effects on beta-cell function** Tinnamaia Tumoi [Finland]
12.30-1.00pm	**OP3 Molecular Clockwork of Human Islets from Type 2 Diabetes** Volodymyr Petrenko [Switzerland] **OP4 GLP-1 analogs prevent and revert diabetes in Wolfram Syndrome** Maria Igoillo Esteve [Belgium]
1:00-2:00pm	**LUNCH BREAK**

2:00-4:00pm	SESSION 3: ISLET SIGNALLING & INSULIN SECRETION Chair: Anders Tengholm [Sweden]
2:00-2.30pm	**Mechanisms underlying early beta-cell dysfunction leading to loss of beta-cell identify in diabetes** Maria Remedi [USA]
2.30-3.00pm	**Insulin and glucagon granule behaviour in Type 2 diabetes** Sebastian Barg [Sweden]
3.00-3.30pm	**Using conditional approaches to understand alpha-, beta- and delta-cell functional subpopulations** David Hodson [UK]
3.30-4.00pm	**OP5 IGF Receptor-like 1 is a novel regulator of insulin signalling pathway** Heiko Lickert [Germany] **OP6 Hub cells co-ordinate 3 dimensional beta-cell Ca^{2+} dynamics in mouse islets in vivo** Victoria Salem [UK]
4:00-4:30pm	**TEA BREAK & POSTERS**
4:30-5:30pm	SESSION 4: KEYNOTE LECTURE 1 - *CHAIR: ANNA GLOYN [UK]* **The Changing Beta-Cell** Susan Bonner-Weir [USA]

5:30-7:00pm	**POSTERS & DRINK RECEPTION IN THE COMMON ROOM**
7:00pm	**DINNER IN COLLEGE**

TUESDAY 2ND APRIL 2019

8:30 - 10.30am	SESSION 5: EPIGENETICS & GENOMICS OF ISLET CELLS Chair: Lorenzo Pasquali [Spain]
8.30-9.00am	**Connecting human islet cell functional & transcriptomic heterogeneity by combined patch-clamp and scRNA-seq** Patrick MacDonald [Canada]
9.00-9.30am	**Epigenomics of diabetic islets** Tiina Ronn [Sweden]
9.30-10.00am	**Single Cell characterisation of islet cells** Andrew Pospisilik [Germany/USA]
10.00-10.30am	**OP7 A novel method enabling background-free purification of age-distinct insulin secretary granules for proteomic and lipidomic analysis** Martin Neukam [Germany] **OP8 Chromatin Interactions aid identification of effector transcripts in type 2 diabetes GWAS loci in beta-cells** Vibe Nylander [UK]
10:30-11:00am	**COFFEE BREAK & POSTERS**

11:00am-1:00pm	SESSION 6: MODELLING MUTATIONS IN BETA-CELL MODELS Chair: Adrian Teo [Singapore]
11:00-11.30am	**Modelling INS gene mutations in human IPSCs** Timo Otontoski [Finland]
11.30-12.00	**Identifying mechanisms for T2D GWAS variants in IPSCs** Nicole Krentz [UK]
12.00-12.30pm	**Understanding regulatory variation in beta-cell development** Maike Sander [USA]
12.30-1.00pm	**OP9 A 3D analysis of the structural and functional beta-cell architecture in human pancreatic islets** Louise Cottle [Australia] **OP10 4 D imaging of insulin secretory granule dynamics and secretion in primary beta cells with lattice light sheet microscopy** Andreas Mueller [Germany]
1:00-2:00pm	**LUNCH BREAK**
2:00-4:00pm	SESSION 7: ISLET -CELL MODELS & THERAPIES Chair: Jean-Christophe Jonas [Belgium]
2:00-2.30pm	**The EndoCBH Cell family** Raphael Scarfmann [France]
2.30-3.00pm	**In vitro methods for pancreatic progenitors** M. Christina Nostro [Canada]

	Designing human islet cells
3.00-3.30pm	Holger Russ [USA]
3.30-4.00pm	**OP11 Manipulating differentiation dynamics of endocrine cells from human pluripotent stem cell derived pancreatic progenitors** Yung Hae Kim [Denmark] **OP12 PRMT1 maintains mature beta-cell identify by histone arginine methylation** Joonyub Lee [Korea]
4:00-4:30pm	**EASD ISG ASSEMBLY** Fran Ashcroft [UK]
4.30-5.30pm	TEA & POSTERS *PRESENTERS PLEASE REMOVE YOUR POSTER AT THE END OF THIS SESSION*
6:30-8.00pm	OXFORD ASHMOLEAN MUSEUM VISIT (DRINKS & CANAPÉS) COACHES FROM THE PORTERS LODGE
8.00pm 8:30pm	**PRE DINNER DRINKS AVAILABLE IN THE SENIOR COMMON ROOM FOR THOSE NOT ATTENDING THE ASMOLEAN EVENT OR BACK IN TIME** **FORMAL HALL (DINNER) - DRESS CODE SMART CASUAL** *Live music & dancing in the Common Room from 9.45pm* *Carriages at midnight*

WEDNESDAY 3RD APRIL 2019

From 8:30am	SESSION 8: BETA-CELL DEVELOPMENT Chair: Lori Sussel [USA]
8.30-9.00am	**Zebrafish as a model for understanding beta-cell development** Nikolay Ninov [Germany]
9.00-9.30am	**Beta-Cell Development** Senta K Georgia [USA]
9.30-10.00am	**Neonatal Diabetes due to a failure of beta-cell development** Elisa De Franco [UK]
10.00-10.30am	**Feedback and self-assembly principles in pancreatic endocrine-cell birth and islet clustering** Chris Wright [USA]
10:30-11:00am	**COFFEE BREAK**
11:00am-1:30pm	SESSION 9: OVERVIEW & UPDATE ON COLLABORATIVE INITIATIVES IN ISLET CELL FUNCTION & DIABETES Chair: Ben Glaser [Israel]
11:00-11.30am	**STEMBANCC (IMI)** Christian Honore [Denmark]

	RHAPSODY (IMI)
11.30-12.00	Michele Solimena [Germany]
	T2DSYSTEMS (Horizon 2020)
12.00-12.30	Miriam Cnop [Belgium]
12.30-13.00	**INNODIA (IMI): Acknowledging the dialogue between the immune system and the beta-cell in T1D** Chantel Mathieu [Belgium]
1:00 - 2:00pm	**LUNCH BREAK**
2:00-3:00pm	SESSION 10. KEYNOTE LECTURE 2 - CHAIR LENA ELIASSON (SWEDEN) **Integration of human genetics and islet genomics to advance understanding and management of type 2 diabetes** Mark McCarthy (UK)
3.00-3.30pm	MEETING CLOSE Coffee available to those not heading to the airport/train station.

General Information

Meeting venue – St. Catherine's College, OX1 3UJ

St Catherine's College is the University of Oxford's newest college and also one of the largest. It developed out of the St Catherine's Society, and was founded in 1962. The College admits both undergraduates and graduates, and offers a wide range of subjects with a roughly even split between science and arts. Its modern buildings and restful, open spaces give the College a friendly and relaxed atmosphere. Situated at the end of Manor Road off St Cross Road, and surrounded by parks and water, St Catherine's College is close to University faculties and libraries. St Catherine's traces its descent from the *Scholares Non Ascripti*, or Delegacy of Non-Collegiate Students, founded by Statute on 11th June, 1868. This was established as part of an expansion of the University so that students would be able to gain an Oxford education without the costs of college membership. During the academic year 2018/2019, the College will therefore be celebrating its 150th Anniversary, in recognition of these origins. The architect of St. Catherine's, **Arne Jacobsen**, designed the college both to reflect elements of a traditional Oxford college and to be an entirely modern solution to the challenge of creating an integrated environment which would be both practical and aesthetically pleasing. At the heart of the College lies a rectangular quad, its sides formed by student rooms built in the conventional Oxford 'staircase' format, its ends consisting of the Dining Hall and Library. Read more about the College's **history**, **buildings and gardens** on our website.

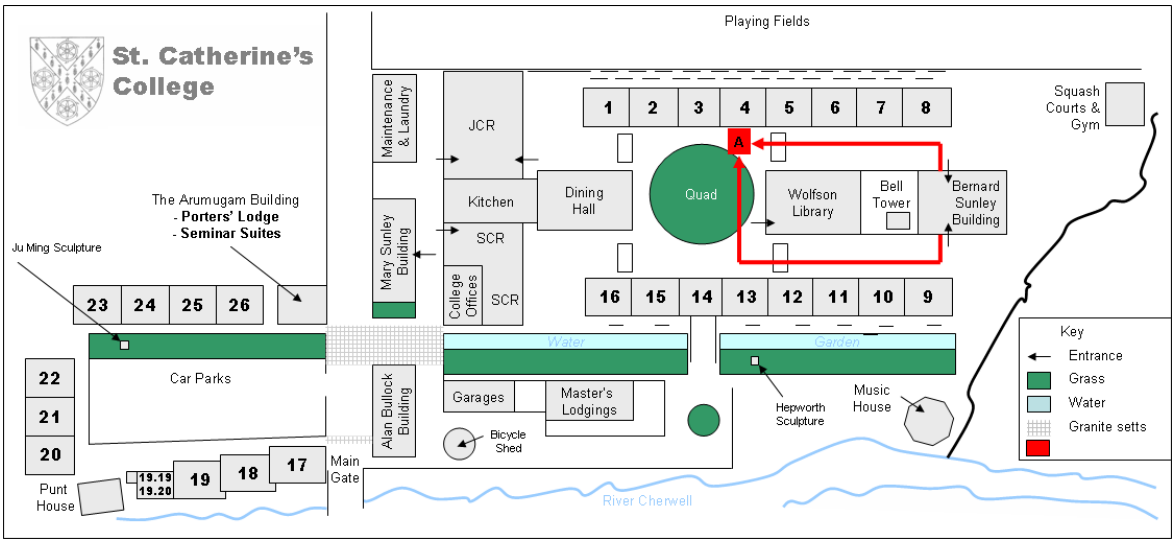
Fire Assembly Information

We all have a responsibility for the safety and security of all those resident in or visiting the College. In particular, we have roles to play in terms of protecting against theft, unauthorised visitors and damage to property. You should note that you have a duty to make yourself aware of the full fire prevention and safety instructions and procedures set out on the Safety Notice Board in this building.
You can help by reporting anything or anyone suspicious to the Lodge (01865 271700) at any time.

You are asked to ensure that all of your property is insured against theft, as College insurance does not cover personal effects for theft.

WHEN YOU HEAR THE FIRE ALARM BELL IMMEDIATELY VACATE THE BUILDING CLOSING THE DOOR(S) BEHIND YOU AND PROCEED TO THE ASSEMBLY POINT INDICATED ON THE MAP BELOW (OR TO THAT DISPLAYED ON THE BACK OF THE INDIVIDUAL ROOM YOU ARE IN). COLLEGE STAFF WILL ALSO BE ON HAND TO ASSIST.

Bernard Sunley Building, go to Staircase 4 (Point A)



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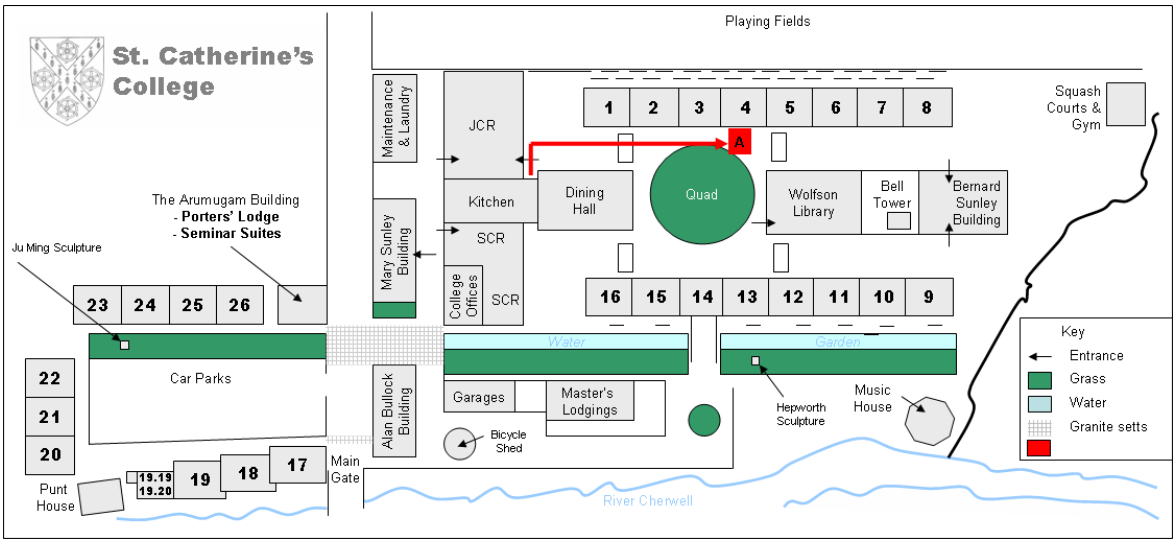
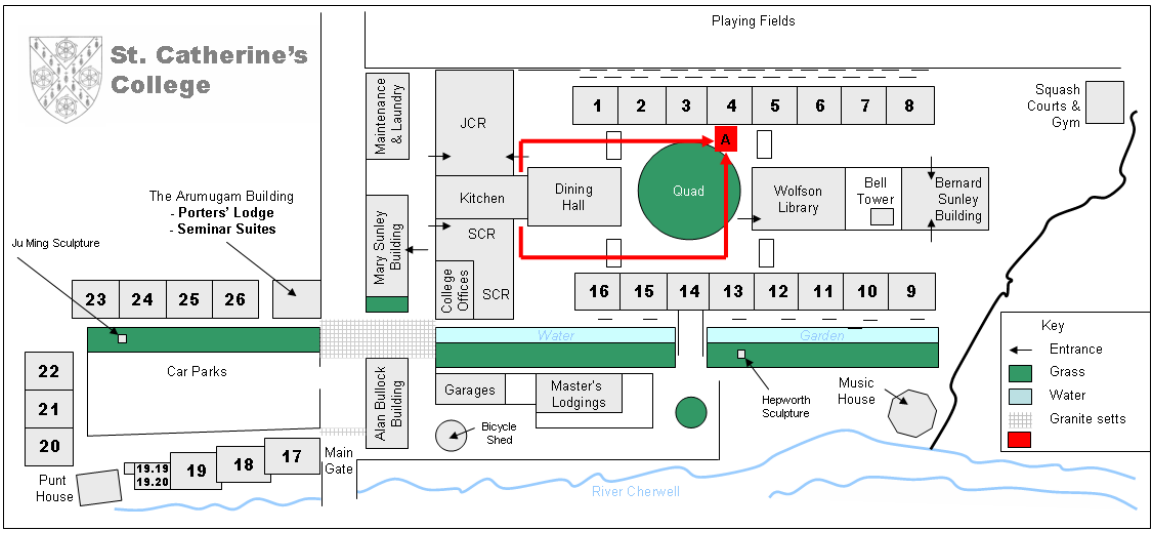
Junior Common Room, go to Staircase 4 (Point A)



Main Hall, go to Staircase 4 (Point A)



REGISTRATION

Registration material will be available at the Front Desk on Sunday from 4pm until 8pm and at the registration desk from 8am on Monday.

SOCIAL MEDIA POLICY

Our social media policy is that unless a speaker states that their presentation, or specific data within it are not for sharing then delegates are free to share the information on social media. Please be respectful and attribute data appropriately and use the meeting hastag **#isletsinox2019** . The meeting has its own Twitter ID which is @isletsinOX2019

For posters the presenters have been asked to display a NO PHOTOGRAPHS sign if they do not wish for photographs of their poster to be taken or for information to be shared on social media. Please respect their wishes.

INTERNET

Wireless internet access will be available at the meeting venue.
Networks:

1. The Cloud - https://service.thecloud.net/service-platform/
2. Eduroam
3. OWL

GUIDELINES FOR PRESENTERS

Oral Presentations

Please bring your presentation on a USB flash drive to the A/V technician in the A/V room on the second floor above the session hall in advance of your presentation. The A/V technician will load your presentation on the meeting computer. We encourage all speakers to use the provided desktop computers in the conference room, however, if you are using your own laptop, please coordinate it in advance with the technician. A spare computer will be available for presenters to check their presentations prior handing them over.

Poster Contributions

Posters should be mounted in time for the first coffee and poster break on Monday. You will be able to mount them on Sunday evening between 4-8pm if you arrive in time. We ask that you remove them on Tuesday at 5.30pm before our visit to the Ashmolean to allow the room to be reorganised for the band on Tuesday evening.

PARKING IN OXFORD

Many Oxford streets are closed to traffic and parking is severely limited. We encourage you to use the five Park and Ride bus services. Please refer to Oxfordshire County Council's up-to-date traffic information for details of all local road works and any disruptions. For information on car parks, on-street parking and costs of parking, see Oxfordshire County Council's website. **Note that there is no parking at St. Catherine's college.**

PARK&RIDE

There are five Park and Ride sites, which serve Oxford City Centre. The **Oxford Bus Company** has detailed information on Park and Ride locations, timetables and fares.

TAXIS.

A list of taxi companies can be found on **Oxfordshire's Visitor Information Centre** website.

Please note there is no UBER in Oxford!

KEYNOTE SPEAKERS

SUSAN BONNER-WEIR

The Changing Beta cell

Dr. Susan Bonner-Weir is Senior Investigator, Joslin Diabetes Center and Professor of Medicine, Harvard Medical School. She received her BA from Rice University and her PhD from Case Western Reserve University. She trained as a post-doctoral fellow at Joslin with Dr. Art Like and then later joined Joslin faculty in 1984. For over thirty years she has focused on the islets of Langerhans, from understanding their "architecture", their growth and their function. She has published over 200 peer-reviewed papers and numerous chapters and reviews. Dr. Bonner-Weir has served on numerous grant review panels (NIH, American Diabetes Association, JDRF, the Danish National Research Council, California Institute of Regenerative Medicine and the European Research Council) and editorial boards (J Biological Chemistry, Diabetes, Endocrinology, Amer J Physiology, Cell Transplantation). She has lectured around the world, including a number of named lectures such as the 19th Broidy lecture (Cedars-Sinai Hospital, CA), 21st Kroc lecture in Uppsala Sweden, W.H. Griffith lecture (St. Louis University). In 2012, she was elected a Fellow of the American Association for the Advancement of Science (AAAS) for her scientific contributions, and in 2014 she was awarded the William Silen lifetime Achievement Award in Mentoring from Harvard Medical School. Her contributions have been additionally recognized with honorary membership in the Hungarian Diabetes Association, Mary Jane Kugel Award (JDRF), the 2016 Paul Lacy Award, and the 2016 Joslin Global Achievement Award.

MARK MCCARTHY

Integration of human genetics and islet genomics to advance understanding and management of type 2 diabetes

Mark McCarthy is the Robert Turner Professor of Diabetes Medicine at the University of Oxford based at both Oxford Centre for Diabetes, Endocrinology and Metabolism and the Wellcome Centre for Human Genetics. His research group is focused on the identification and characterisation of genetic variants influencing risk of type 2 diabetes and related traits, and on using those discoveries to drive biological inference and translational opportunities.

He has played a leading role in many of the major international efforts to identify the genetic variants that influence predisposition to type 2 diabetes including DIAGRAM, T2DGENES and GoT2D. These consortia have used genome wide association and sequencing approaches to identify over 400 genetic signals for type 2 diabetes. Equivalent efforts focused on diabetes-related traits, including obesity, fat distribution and birthweight have been similarly productive.

With collaborators, his group's activities are now increasingly focused on the exploitation of these discoveries to gain insights into the biological mechanisms underlying disease development. Integration of genetic association signals with genomic annotations derived from pancreatic islets and other diabetes-relevant tissues is providing robust insights into the molecular and pathophysiological mechanisms through which many of these signals operate. This also makes it possible to use this information to open new translational opportunities through target validation, risk stratification and biomarker discovery.

INVITED SPEAKERS

Laura C. Alonso (USA)

ER Stress: Beta-Cells Rise to the Challenge

Dr. Alonso earned a BA in biochemistry from Harvard University and an MD degree from the University of Pennsylvania. She completed a residency in Internal Medicine and a fellowship in Endocrinology and Metabolism, both at the University of Chicago. Her research training began with a 5-year postdoctoral fellowship with Elaine Fuchs in stem cell biology, starting at the University of Chicago and finishing at the Rockefeller University. She began her career as an islet biologist with a junior faculty position with Andy Stewart at the University of Pittsburgh, subsequently moving to the UMass Medical School in 2013. Her research is focused on beta cell regeneration, with two principal long-term goals: to understand the molecular mechanisms behind successful beta cell mass expansion in mice, and to identify the causes of failed proliferation in human beta cells. One current molecular focus of the lab is to identify the mechanisms behind a novel concept developed by the lab: linking insulin demand with beta cell proliferation via activation of the ER stress response pathways known as the unfolded protein response. A second focus is determining how the *CDKN2A/B* T2D risk-locus impacts beta cell mass and function. She is also strongly motivated by mentoring and developing young scientists, both clinically trained and basic researchers.

Sebastian Barg (Sweden)

Insulin and glucagon granule behaviour in Type-2 diabetes

Sebastian Barg has a background in biochemistry and chemistry and a PhD in physiology (Lund U). After postdoc work at the Vollum Institute (Portland OR) and Imperial College London he started his own group at Uppsala University. The main interest is in the cell biology of hormone and

neurotransmitter secretion, with a focus on the life-cycle of insulin-containing secretory granules. The lab studies exocytosis in pancreatic ß-cells and other islet cells using advanced light microscopy (TIRF, super-resolution and single molecule imaging) in combination with patch clamp electrophysiology. The aim is to quantitatively understand the release probability of individual granules both mechanistically (as the sequential assembly of the secretory machinery from its protein and lipid components) and in the physiological context of biphasic insulin secretion in health and type-2 diabetes.

Miriam Cnop (Belgium)

Overview & Update on Collaborative Initiatives in Islet Cell Function & Diabetes: T2DSYSTEMS

Her research focuses on pancreatic cell dysfunction and apoptosis in the pathogenesis of type 2 diabetes. Her team identified endoplasmic reticulum stress as a molecular mediator of free fatty acid-induced cell apoptosis. She has contributed to the discovery and study of several new forms of monogenic diabetes, where gene mutations affect cell endoplasmic reticulum stress, mitochondrial function and tRNA biology. These patients with monogenic diabetes can be considered as human knockouts and can provide us with insight into key biological pathways important for cell development, function and survival. To gain insight into the pathways of cell failure in these monogenic forms of diabetes and to test therapies, her team is differentiating patient-derived induced pluripotent stem cells into cells. This represents a highly relevant disease-in-a-dish model, opening a range of new research avenues. She has published 90 papers that have been cited over 9000 times, with an H-index of 41. Her work has been funded by the European Foundation for the Study of Diabetes, the European Union Framework Programmes 6 and 7, the Innovative Medicines Initiative and Horizon 2020, the National Ataxia Foundation USA, the Friedreich Ataxia Research Alliance USA, and the Belgian Fund for Medical Scientific Research. The European Association for the Study of Diabetes awarded her work with a Rising Star Award in 2005 and the Oskar Minkowski Award in 2013.

Carolin Daniel (Germany)

Mechanisms of regulatory T cell induction in diabetes

She obtained a Ph.D. in Immune pharmacology from the European Graduate School of the German Research Foundation in Frankfurt (Germany) and the Karolinska Institute in Stockholm (Sweden) in 2008. With a Fellowship from the Leopoldina/National Academy of Sciences Germany she did a PostDoc in Immunology at the Dana Farber Cancer Institute in the laboratory of Harald von Boehmer and a joint appointment with Harvard Medical School, Boston (MA/USA). From March 2012 – 2016 she was heading a Young Investigator Group at German Research Center for Environmental Health, Institute of Diabetes Research in Munich (Germany). Since 2017 she is a Research Group Leader (Group Immune Tolerance in Diabetes) at the Helmholtz Diabetes Center at Helmholtz Zentrum München. Her research interests focus on mechanisms of immune activation vs. tolerance in target tissues of diabetes with the goal to identify signaling intermediates that can interfere with efficient T cell tolerance induction during ongoing immune activation. To this end, her lab studies the induction and function of regulatory T cells in murine and humanized models.

Elisa De Franco (UK)

Neonatal diabetes due to a failure of beta-cell development

Dr. Elisa De Franco is a molecular geneticist working as part of the diabetes team at the University of Exeter, UK. She got a Bachelor and Master degree (with honours) in Medical Biotechnology at the University of Turin, Italy. She then went on to complete a European funded PhD on the transcriptions factors regulating human pancreatic development at the University of Exeter in 2014. She has also been a Naomi Berrie Fellow in Diabetes research (years 2015-2016) and is currently working as Postdoctoral Research Fellow at the University of Exeter, UK. To date, Dr De Franco has published 35 papers in international, peer-reviewed journals (12 of which as first or last

author) including The Lancet, Nature Genetics, Cell Metabolism and Diabetes. Her research focusses on using whole genome sequencing and other next generation genetic techniques to identify novel causes of diabetes in patients diagnosed with the disease before the age of 12 months.

Sarah Flanagan (UK)

Monogenic forms of autoimmune diabetes

Sarah is a Wellcome Trust/Royal Society-funded Sir Henry Dale Fellow at the University of Exeter Medical School. She completed her PhD in 2007 under the supervision of Professor Andrew Hattersley and Professor Sian Ellard which investigated genotype/phenotype relationships in neonatal diabetes and congenital hyperinsulinism. Sarah leads the congenital hyperinsulinism research group in Exeter and together with the monogenic diabetes team has provided genetic testing for over 4000 patients from over 100 countries around the world. Since arriving in Exeter Sarah has been involved in the discovery of 13 novel genes for monogenic disorders of insulin secretion and has led studies investigating genotype/phenotype relationships. This work has resulted in her publishing over 180 peer reviewed papers. In 2014 Sarah led the discovery of activating germline mutations in the STAT3 gene in individuals with syndromic early-onset autoimmune diabetes. This work was published in Nature Genetics and the finding sparked her interest in monogenic autoimmunity. Sarah's current work focusses on the utility of clinical features and genetic risk scores to help differentiate rare monogenic autoimmune disease from more common polygenic 'clustering'. Her team are currently undertaking gene discovery studies, immuno-phenotyping and are focussed on improving and developing new variant calling bioinformatics pipelines to allow for in-depth interrogation of next-generation sequencing data. In 2015 Sarah received the EASD-study group for the genetic of diabetes Rising Star Award and in 2018 was selected as a recipient of the G.B. Morgagni Silver Medal Young Investigator Award which recognises exceptional research in the field of metabolic disease.

Senta Georgia (USA)

Beta-Cell Development

Senta Georgia is an Assistant Professor in the Departments of Pediatrics and Stem Cells and Regenerative Medicine at the Keck School of Medicine at USC. She is a member of the Center for Endocrinology, Diabetes, and Metabolism at CHLA and the Developmental Biology and Regenerative Medicine Program at the Saban Research Institute. She attended Stanford University, earning her BS in Biological Sciences, a minor in Philosophy, and dual departmental honors in Biological Sciences and Ethics in Society. She earned her Ph.D. at UCLA in Molecular Biology. Her dissertation focused on the role of cell cycle molecules in the establishment, expansion, and maintenance of insulin cells. She was a postdoctoral fellow and Assistant Adjunct Professor at the Hillblom Islet Research Center at UCLA, where her research focused on how DNA methylation restricts progenitor cell differentiation during pancreatic organogenesis and how DNA methylation maintains insulin cell identity in adulthood. Her lab at CHLA focuses on three approaches to insulin-cell regeneration: (1) by inducing replication of existing insulin cells; (2) using epigenetic manipulation to induce cellular reprogramming into an insulin cell fate; and (3) understanding mechanisms that govern human insulin cell differentiation to make new insulin cells for patients that have diabetes. She is a wife and a mom of 4 happy kids. She enjoys being a purveyor of fine spirits, an enthusiast of thriller/horror films, and a casual gastronomist.

David Hodson (UK)

Using conditional approaches to understand alpha-, beta- and delta-cell functional subpopulations

David is Professor of Cellular Metabolism, Professorial Research Fellow and an early career researcher based at the Institute of Metabolism of Systems Research, University of Birmingham. Previously, he was a Diabetes UK RD Lawrence Fellow and Non-Clinical Lecturer at Imperial College London. David has particular interest in developing novel tools to understand beta and other cell

function both in vitro and in vivo. Current research, using mouse genetics in combination with next generation optogenetics, focuses on understanding how immature beta cell subpopulations may exert disproportionate control over insulin release. Alongside this, we continue to contribute to the field of photopharmacology, which describes the precise spatiotemporal control of receptor, enzyme and ion channel function using exogenously-applied light-activated drugs. Recent targets include KATP channels, glucagon-like peptide-1 receptors, fatty acid receptors and L-type Ca2+ channels, all with relevance for the mechanisms underlying glucose homeostasis. Finally, we are harnessing the power of enzyme self-labelling technology to target drugs to G protein-coupled receptors in body compartments and specific cell populations through reversible covalent linkage. This concept, termed 'tethered pharmacology', may contribute to our understanding of GPCR signaling in health and disease by combining the speed of pharmacology with the precision of genetics. The overarching aim of our research is to use interdisciplinary approaches to open up new avenues of exploration in metabolism research.

Christian Honore (Denmark)

Lessons from the StemBANCC consortium – Developing disease models of diabetes using induced pluripotent stem cell.

Christian Honoré was born in a suburban part of Copenhagen during the late seventies and spent most of his childhood dreaming of making it to the American basketball league NBA. Despite some early success in Danish basketball which he will be very happy to talk about, he was - for reasons still unclear to him - never drafted by an NBA team and instead turned his focus to his studies in biological sciences. He obtained his master degree in biochemistry from the University of Copenhagen and went on to study the complement system during his PhD at the University Hospital of Copenhagen. He then moved to Boston for a post doc in Professor Doug Melton's laboratory at the Harvard Stem Cell Institute. Here he was introduced to the fascinating world of pluripotent stem cells and how to differentiate these towards the pancreatic lineage. After two years in Boston he returned to Denmark for a post doc position at Novo Nordisk A/S as part of the innovative

medicine initiative academia-industry partnership StemBANCC. The main objective of StemBANCC was to establish a well-characterized bank of iPSC lines derived from patients with disorders ranging from Parkinson's, Alzheimer and various forms of diabetes. Within StemBANCC, Christian worked closely together with academic and industry partners to characterize the differentiation of iPSC towards the pancreatic lineage and to establish disease models of various forms of diabetes using patient specific iPSC lines. After the end of StemBANCC, Christian joined Novo Nordisk A/S as a scientist and now dedicates his time to translating the potential of stem cells from the culture dish to the clinic. Outside the lab, Christian spends most of his time with his family but also with friends, either on a basketball court or at a bar table.

Nicole Krentz (UK)

Identifying mechanisms for T2D GWAS variants in IPSCs

Nicole Krentz is a postdoctoral research fellow at the Wellcome Centre for Human Genetics at the University of Oxford and the Robert Turner Research Associate at Green Templeton College. In 2018, Nicole completed her PhD at the University of British Columbia under the supervision of Francis Lynn. Her PhD research focused on pancreas development and endocrine cell genesis using mouse embryos and human embryonic stem cell differentiation as models. In collaboration with Michael German's lab at the UCSF, Nicole discovered that the cell cycle regulates endocrine cell development by phosphorylating the transcription factor Neurog3. In 2018 Nicole moved to Oxford and joined Anna Gloyn's group where she is know investigating the role of diabetes associated genes in pancreas development using genome-editing inhuman induced pluripotent stem cell models.

Patrick MacDonald (Canada)

Connecting human islet cell functional and transcriptomic heterogeneity by combined patch-clamp and scRNAseq

Patrick completed his PhD at the University of Toronto in 2003, followed by postdoctoral research at Lund University and the University of Oxford. In 2006 Patrick established an independent research group in the Alberta Diabetes Institute (ADI) at the University of Alberta, where he is currently Professor of Pharmacology. Work from the group has focused mainly on understanding downstream mechanisms of excitability an exocytosis controlling insulin and glucagon secretion, and in particular the control of these processes by metabolic and receptor-mediated signalling. Current interests include understanding mechanisms of beta-cell functional adaptation, the control of spatial heterogeneity in granule fusion events across the cell surface, and signalling by neurotransmitters within the islet. The group also established a research-specific human islet isolation and distribution program, the ADI IsletCore, to process donor pancreas that is not used for clinical whole organ or islet transplant. This has improved access to human research islets, including from donors with diabetes, and now supports >80 groups in North America and Europe. The group is privileged to contribute to many collaborative efforts with investigators across the world, including efforts to link islet genomics and function; efforts to map islet cell heterogeneity in situ; and efforts to understand altered islet cell function in both type 1 and type 2 diabetes. Patrick has been fortunate to receive numerous awards for his work, most recently this year's Diabetes Canada/CIHR-INMD Young Scientist Award.

Chantal Mathieu (Belgium)

INNODIA: acknowledging the dialogue between the immune system and the beta-cell in T1D

Chantal Mathieu is Professor of Medicine at the Katholieke Universiteit Leuven, Belgium. She is Chair of Endocrinology at the University Hospital Gasthuisberg Leuven. Prof. Mathieu received her medical degree

and PhD at the University of Leuven, where she subsequently completed training in internal medicine and endocrinology. Prof. Mathieu's clinical areas of interest include the organization of diabetes care, and she is involved in several clinical trials in type 1 and type 2 diabetes. Her basic research is focused on the prevention of type 1 diabetes, effects of vitamin D on the immune system and diabetes, and functioning of the insulin-producing beta cell. Prof. Mathieu has authored or co-authored more than 350 peer-reviewed publications in international journals. In 2013, Prof. Mathieu received the prestigious InBev-Baillet Latour Prize for Clinical Research for her pioneering research on the pathogenesis of type 1 diabetes. She presently coordinates the INNODIA project on prevention and intervention in type 1 diabetes in Europe and is vice-president of EASD.and Chair of Postgraduate Education at EASD.

Irene Miguel-Escalada (Spain)

Modelling PTF1A enhancer mutations leading to pancreas agenesis in mice

Irene is a Post-Doctoral researcher specialized in Molecular Genetics with expertise in the enhancer biology and non-coding variation leading to disease. Irene completed her MRes of Reproductive Biology in Barcelona (Spain), where she studied the impact of in vitro culture on the DNA methylation patterns of preimplantation mouse embryos. Then, she moved to the UK, where she obtained a PhD in Molecular Genetics from the University of Birmingham. Her PhD work focused on transcriptional regulation, in particular, on the validation of human enhancers predicted by histone modification marks and bidirectional transcription in zebrafish embryos. During her PhD she also helped to develop a site-directed transgenesis system in zebrafish that reduces variability effects for functional testing of regulatory elements.

Irene moved to Barcelona as a Post-doctoral researcher in Jorge Ferrer's lab, where she has worked since 2014 between IDIBAPS (Spain) and Imperial College London (UK). She leads a project that assesses how the integrative analysis of 3D chromatin architecture in human islets and highly-refined epigenome maps can aid the interpretation of type 2 diabetes signals. Her long-term interest in the role of enhancers in human disease also

prompted her to start modeling regulatory mutations leading to pancreas agenesis, shedding interesting insights into pancreas development. In 2015 she was awarded with Marie Curie PostDoctoral Fellowship, funded by the European Commission to carry out her postdoctoral work. She has collaborated with international experts in the field of bioinformatics, gene regulation and genetics and was trained as a young scientist by the multidisciplinary EU-Funded Initial Training Network BOLD.

Nikolay Ninov (Germany)

Zebrafish as a model for understanding beta-cell development

Nikolay Ninov is a group leader at the Center for Regenerative Therapies Dresden at TU-Dresden and the Paul Langerhans Institute Dresden. He completed his PhD at the University of Barcelona, during which he developed methods for high-resolution live-cell cell imaging of the remodeling of the Drosophila epidermis during metamorphosis. As a postdoctoral fellow, Nikolay joined the group of Didier Stainier at the University of California at San Francisco where he studied pancreas development and regeneration in zebrafish. He applied single-cell genetic manipulations and developed new transgenic lines to visualize non-invasively the proliferation and differentiation of beta-cells, which enabled to perform the first in vivo chemical screen for inducers of beta-cell proliferation. Currently, the Ninov's group focuses on understanding beta-cell heterogeneity during development and regeneration. This research combines single-cell behavior studies and gene expression analysis to define novel factors that confer different characteristics to beta-cells. Of particular importance is the ability to monitor with a very high spatio-temporal resolution the calcium dynamics of beta-cells in their native environment in the zebrafish pancreas, as well as to follow the development of individual cells marked in different colors using the Brainbow technology.

Maria Cristina Nostro (Canada)

Modelling human beta cell development with pluripotent stem cells

Maria Cristina Nostro is a Scientist at the Toronto General Hospital Research Institute and an Assistant Professor at the University of Toronto. She holds the Harry Rosen Chair in Diabetes and Regenerative Medicine Research.

Dr. Nostro completed her undergraduate degree at the University of Florence in 2000 and received a Ph.D. from The University of Manchester in 2004. The same year, she joined Dr. Gordon Keller's laboratory at Mount Sinai School of Medicine in NY, where she used embryonic stem cells to investigate the molecular mechanisms regulating mesoderm and endoderm lineage commitment. In 2012, she started her independent career at the TGHRI. Her research is focused on identifying the molecular determinants that control endoderm specification and pancreatic lineage commitment, with the ultimate goal of generating functional β cells from human embryonic and induced pluripotent stem cells. Her long-term goal is to translate the results of these studies to the clinic and to establish in vitro culture systems for disease modeling, drug toxicity and discovery assays, through the use of patient-specific induced Pluripotent Stem Cells. Her group has defined critical pathways leading to the efficient generation of hPSC-derived pancreatic progenitors in vitro (Nostro et al., Stem cell Reports 2015) and recently, through the use of a proteomics approach, identified a specific marker that allows the purification of these β cell progenitors (Cogger et al., Nat. Communications 2017). Since 2015, Dr. Nostro has been leading a multi-investigator team aimed at developing novel transplantation approaches for Type 1 Diabetes therapy.

Timo Otonkoski (Finland)

Modelling INS gene mutations in human IPSCs

Timo Otonkoski, MD, PhD, is currently Professor of Medical Stem Cell Research and director of the Biomedicum Stem Cell Center at the University of Helsinki. He has a specialist degree in Pediatric Endocrinology and has a clinical position at the Children's Hospital of the Helsinki University Central Hospital. His main clinical specialities are childhood diabetes and hypoglycemia.

His research has focused on the growth and development of the pancreatic islets. He has also made important contributions in the field of congenital hyperinsulinism and other forms of genetic insulin secretory dysfunction. Lately, he has shifted his main area of interest into the use of human pluripotent stem cells and genome editing to study beta-cell pathophysiology. His group has made fundamental discoveries of the pluripotent reprogramming of human cells, developed novel approaches for controlling their differentiation, and used this approach successfully to elucidate several monogenic mechanisms of beta-cell dysfunction.

J. Andrew Pospisilik (Germany)

Single Cell characterisation of islet cells

Dr. J. Andrew Pospisilik is a leading expert in the study of how the epigenome regulates complex trait variation as well as disease susceptibility and heterogeneity. He earned his B.Sc. with honors and his Ph.D. in Physiology from University of British Columbia, where his work focused on type 1 and 2 diabetes. Later, as a postdoctoral fellow in the lab of Dr. Josef M. Penninger at IMBA Institute of Molecular Biotechnology, Dr. Pospisilik

performed the first genome-wide RNAi-screen for obesity in Drosophila, identifying some of the first specific developmental regulators of brown adipose tissue formation, and overturned dogma showing that compromised mitochondrial function actually buffers against diabetes. In 2010, he established his laboratory at Max Planck Institute of Immunobiology and Epigenetics in Freiburg, Germany, where his team made groundbreaking advances, including development of novel sensitized models for understanding epigenetic stability and variation in metabolic disease; the first formal proof for the existence of polyphenism in mammals (including suggestions in humans) and the first mapping of a genetic architecture for mammalian phenotypic buffering. In 2018, Dr. Pospisilik joined Van Andel Research Institute as a professor and a founding member of its Metabolic and Nutritional Programming group. He is the recipient of numerous awards and accolades, including the 2016 Novo Nordisk Helmholtz Young Investigator Award, the 2015 GSK Award for Basic Medical Research, the 2013 EASD Rising Star Award and the 2011 RISE1 Award from the Epigenesys Network of Excellence. He also is a member of the World Economic Forum faculty.

Maria Remedi (USA)

MECHANISMS UNDERLYING EARLY β-CELL DYSFUNCTION LEADING TO LOSS OF BETA-CELL IDENTIFY IN DIABETES

Maria S. Remedi was born and raised in Argentina. She obtained a Clinical Biochemistry degree and a Pharmacist degree from the University of Cordoba in Argentina, and a Ph.D in Chemical Sciences degree from the same University. Maria initially joined the Cell Biology and Physiology Department at Washington University in St Louis (USA) as a post-doc, and move up through the ranks to Instructor and Assistant Professor. In 2015 she joined the Department of Medicine at Washington University, and in 2017 she became the Associate Director of the Metabolic Tissue Function Core of the Diabetes Research Center.

Maria's research focuses on how changes in blood glucose levels regulate insulin secretion from the pancreatic beta-cell, and how this process is impaired in diabetes. The ATP-sensitive potassium (KATP) channel is a critical link between glucose

metabolism and insulin secretion. Gain-of-function and loss-of-function mutations in KATPchannel genes cause two opposite diseases: neonatal diabetes and congenital hyperinsulinism. Maria research has defined critical pathways leading to changes in pancreatic islet-identity in neonatal diabetes, and demonstrated for the first time that this process is reversible. These results challenged the current paradigm of permanent beta-cell damage in long-standing diabetes, with significant implications for therapeutic options. Her current research focus on depicting the underlying mechanisms, and temporal progression, of glucotoxicity and beta-cell failure in various forms of diabetes and devise appropriate therapies.

The Remedi lab is privileged to contribute to collaborative efforts with multiple groups nationally and internationally, hoping to elucidate the mechanisms involved in beta-cell dysfunction in human diabetes, with the expectation to improve treatments to prevent, delay or reverse these processes. Maria has been fortunate to receive numerous awards for her work

Tina Ronn (Sweden)

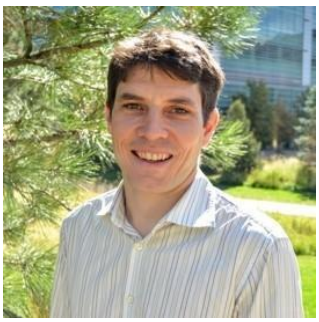
Epigenomics of diabetic islets

My background includes a Master in Medical Science (major: Biomedicine), and a PhD in Biomedicine / Diabetes & Endocrinology, defended at the Medical Faculty at Lund University in 2010 (The role of genetic variation and DNA methylation in human glucose metabolism and type 2 diabetes). The following Post Doc position was mainly focused on the interplay between exercise and epigenetic changes in human tissues.

Continuing after two rounds of maternity leave, I am currently working as an Associate Researcher at the Lund University Diabetes Centre (LUDC), Sweden, in the Epigenetics and Diabetes unit. My research focus on epigenetic alterations in human tissues important in the pathogenesis of type 2 diabetes, mainly pancreatic islets, adipose tissue and muscle. Here we investigate the epigenetic signatures in tissues from healthy vs diseased individuals and investigate the interplay with genetic as well as environmental

factors. We also explore altered DNA methylation in response to different external stimuli and the effect on different metabolic traits.

The last years we have also succeeded with whole genome bisulfite sequencing of human pancreatic islets in a case-control cohort, which can be used as a map of the human islet methylome, but also to understand gene regulation as well as a platform for exploring differences between health and disease.
Our findings propose a model where environmental factors act through epigenetic modifications, under the influence of genetic variation, on tissue specific gene transcription and consequently on metabolic phenotypes and risk of developing type 2 diabetes.



Holger A. Russ (USA)

Designing Immune Privileged Human Beta Cells

Dr. Holger A. Russ is an Assistant Professor at the Barbara Davis Center for Diabetes at the University of Colorado- Anschutz Medical Campus. Dr. Russ has been successfully working on different aspects of human beta cell biology, which has led to several original and important contributions to the fields of beta-, thymus- and stem cell- biology. He obtained his Ph.D. from Tel Aviv University in 2011 working in Prof. Shimon Efrat`s laboratory on expansion of cadaveric human beta cells. He was the first to employ the Cre/lox system, commonly only used in transgenic animals, for lineage tracing of primary human cells in culture. With this novel approach, he could demonstrate that human cadaveric beta cells dedifferentiate into a proliferative state while maintaining a cell specific epigenetic memory that could be exploited to re-differentiate expanded beta cells. In addition, he collaborated with Drs. Bar-Nur and Benvenisty, to show the maintenance of a beta cell specific epigenetic memory after reprogramming beta cells into induced pluripotent stem cells. After completing his Ph.D. studies, Dr. Russ joined the laboratory of Prof. Matthias Hebrok, Director of the Diabetes Center at UCSF, where he established a rapid, large-scale, suspension culture system for the directed differentiation of pluripotent stem cells into functional beta cells in vitro. In parallel, he was part of the research team that

demonstrate for the first time the generation of functional human thymic epithelial cells with the ability to support T-cell development from pluripotent stem cells. In fall 2016, Dr. Russ moved to Denver to establish his independent research program, focused on elucidating the underlying molecular and cellular events that result in the development of type 1 diabetes in patients, at the Barbara Davis Center for Diabetes.

Maike Sander (USA)

Understanding regulatory variation in beta-cell development

Maike Sander is the Director of the Pediatric Diabetes Research Center and Co-Director of the Center on Diabetes in the Institute of Engineering in Medicine at UC San Diego. Her laboratory has uncovered fundamental mechanisms that underlie the formation and function of insulin-producing pancreatic beta cells, which are affected in diabetes. By defining the impact of environmental cues on cell fate determination and cell plasticity, her laboratory aims to identify strategies for beta cell regeneration and replacement in order to develop novel treatments for diabetes. Dr. Sander obtained a medical degree from the University of Heidelberg in Germany and held faculty positions at the University of Hamburg, Germany and the University of California, Irvine before moving to the University of California, San Diego in 2008. Dr. Sander is an elected member of the American Society of Clinical Investigation and the German Academy of Sciences (Leopoldina), and a member of the NIH-Human Islet Research Network. She is the recipient of the Grodsky Award from the Juvenile Diabetes Research Foundation and the 2017 Humboldt Research Award.

Raphaël Scharfmann (France)

Human pancreatic endocrine cell development

Raphael Scharfmann obtained his PhD in 1989 at University Paris VII, France. He next did a post doc at the Salk institute, La

Jolla, CA (1989-1991) and obtained a permanent position at INSERM at the end of 1991. In 1999, he obtained the prestigious Minkowski Award (for distinguished research in the field of Diabetes in Europe). He is now Research Director at INSERM within the Cochin Institute, University Paris Descartes, Paris France. He is also cofounder of EndoCells/UniverCell Biosolutions, a French biotech company. The major objective of his group is to define intercellular signals regulating functional beta cell mass in rodent and human.

Michele Solimena (Germany)

An integrated approach for biomarker identification of beta cell failure in type 2 diabetes

Dr. Michele Solimena attended the School of Medicine at Univ. of Milano, Italy where he obtained his medical degree in 1986 and his PhD in Pharmacology and Toxicology in 1993. In 1988 he joined the laboratory of Prof. De Camilli in the Department of Cell Biology in the School of Medicine at Yale University working on the cell biology of glutamic acid decarboxylase and its role as autoantigen in stiff-man syndrome and type 1 diabetes. In 1994 he was appointed Assistant Professor in the Section of Endocrinology in the Department of Internal Medicine at Yale University and in 2000 he became Associate Professor in Internal Medicine and Cell Biology. In 2001 he moved as Group Leader to the Max Planck for Molecular Cell Biology and Genetics in Dresden, German. Since 2003 he is Professor of Molecular Diabetology at the Faculty of Medicine of the Technische Universität Dresden (TUD), Germany. He also directs the Paul Langerhans Institute Dresden, which he founded in 2010 and which became a satellite institute of the Helmholtz Center Munich at TUD in 2015.

Dr. Solimena's laboratory applies molecular, biochemical and imaging approaches in vitro and in vivo in order to elucidate the principles that govern the biogenesis, exocytosis and turnover of insulin secretory granules and why their cargoes are preferential autoantigens in autoimmune diabetes. He is also interested in understanding the molecular

mechanisms driving beta cell failure during the progression from normoglycemia to diabetes through the study of pancreatic islets from metabolically phenotyped pancreatectomized subjects

John Todd (UK)

Genes to clinic in autoimmune diabetes

John Todd FRS, FMedSci, FRCP Hons, PhD is Professor of Precision Medicine at the University of Oxford (until recently Professor of Medical Genetics at the University of Cambridge), Director of the JDRF/Wellcome Trust Diabetes and Inflammation Laboratory (DIL) in the University's Wellcome Centre for Human Genetics, and a Senior Investigator of the National Institute for Health Research. His PhD was in Biochemistry at the University of Cambridge. Todd researches type 1 diabetes (T1D) genetics and disease mechanisms with an aim of clinical intervention. Previously, Todd was Professor of Human Genetics and a Wellcome Trust Principal Research Fellow at the University of Oxford. Todd helped pioneer genome-wide genetic studies, first in mice and then in humans. He then went on to study the associations between mapped genomic disease-associated regions and phenotypes by founding and deploying the Cambridge BioResource. His research in genetics and diabetes has received several awards and prizes. In the latest phase of his research, to translate basic genetic and immunological knowledge to treatment and prevention, the DIL has now completed its first two mechanistic, statistically adaptive, drug dose-finding studies in T1D patients. This design and analyses have revealed several previously unknown effects of interleukin-2 (IL-2) on the human immune system, providing key information on the future possibility of using subcutaneous administration of ultra-low doses of IL-2 to preserve pancreatic islet beta-cell function to treat and prevent T1D. In Oxford the DIL is launching a programme to investigate which T1D risk regions affect beta-cell function and fragility. We are applying the latest single-cell, mass spectrometry methods, bioinformatics and statistical methods.

Todd has supervised 31 PhD students with three in progress. h-index 94, total citations over 38,000.

Elisa De Franco (UK)

Neonatal diabetes due to a failure of beta-cell development

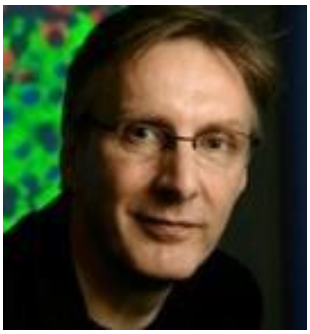
Dr. Elisa De Franco is a molecular geneticist working as part of the diabetes team at the University of Exeter, UK. She got a Bachelor and Master degree (with honours) in Medical Biotechnology at the University of Turin, Italy. She then went on to complete a European funded PhD on the transcriptions factors regulating human pancreatic development at the University of Exeter in 2014. She has also been a Naomi Berrie Fellow in Diabetes research (years 2015-2016) and is currently working as Postdoctoral Research Fellow at the University of Exeter, UK. To date, Dr De Franco has published 35 papers in international, peer-reviewed journals (12 of which as first or last author) including The Lancet, Nature Genetics, Cell Metabolism and Diabetes. Her research focusses on using whole genome sequencing and other next generation genetic techniques to identify novel causes of diabetes in patients diagnosed with the disease before the age of 12 months.

Tiinamaija Tuomi (Finland)

SLC30A8 effects on beta-cell function

Tiinamaija Tuomi, MD, PhD, is Chief Departmental Doctor at the Department of Endocrinology, Helsinki University Central Hospital and research director at Folkhalsan Research Centre and Institute for Molecular Medicine FIMM, University of Helsinki. She is also vice-director of the "Centre of

Excellence of Complex Disease Genetics – from discovery to translation" (FIMM 2018-2025). She received her PhD in 1990 on immunology of rheumatoid arthritis at the University of Helsinki. During her post-doctoral period at Monash University in Melbourne, Australia, she switched research focus to autoimmune forms of diabetes and renamed a hybrid form of type 1 and type 2 diabetes Latent Autoimmune Diabetes in Adults (LADA). She worked with the Botnia Study at Lund university during 1994-1999 as a vice-chief of the Diabetes Research Laboratory (predecessor of the Lund University Diabetes Centre), which widened the scope of her research activity to genetics, causes and clinical heterogeneity of type 2 diabetes and monogenic forms of diabetes. She is the co-PI of the Botnia Study since 1999. Her research group in Helsinki as well as Närpes, Jakobstad and Vasa in Western Finland is associated with the Folkhalsan Reserch Center, the Research Program of Clinical and Metabolic Medicine and FIMM at the University of Helsinki and the Helsinki University Central Hospital. The group works in close collaboration with the Lund University Diabetes Centre. The work of her group focuses on phenotypic and genetic characterisation of subtypes of diabetes or individuals with rare or common variants associated with diabetes.



Chris Wright (USA)

Feedback and self-assembly principles in pancreatic endocrine-cell birth and islet clustering

Christopher Wright, D. Phil., is the Director of the Vanderbilt University Program in Developmental Biology, Associate Director of the Vanderbilt Center for Stem Cell Biology, Louise B. McGavock Chair and is Professor and Vice-Chair for Faculty Affairs of the Dept. Cell & Developmental Biology at Vanderbilt University, where he began his independent research program in 1990. In 2012, he became an elected member of the American Assoc. for Advancement of Science. Wright's B.Sc. was from the University of Warwick, U.K., during which he received training with Drs. Alan Colman, Hugh Woodland, Liz Jones, Bob Old, and others. He gained his D. Phil. in 1984 in the lab of Dr.John Knowland at the University of Oxford (Linacre College) studying the biochemistry of steroid receptor control of gene expression. From 1985-1989 he carried out postdoctoral research first in Basel, Switzerland and then at UCLA, USA, under Dr. Eddy De Robertis,

discovering some of the first mammalian homeobox genes, among them Pdx1 (known first as XlHbox1). Wright's lab has studied intercellular signaling, transcriptional control of cell specification and differentiation, embryonic patterning and pancreas organogenesis. It was the first to apply lineage tracing and gene inactivation to show reallocation of stem cell properties from pancreas to duodenum (Nat. Gen. 2002). His lab has created and distributed many tools and reagents for studying pancreas development, islet cell differentiation and plasticity, pancreatic cancer, and has collaborated with many of the leading laboratories and colleagues in these fields. Under an inflammatory-signaling context of acinar injury caused by duct ligation, we showed that acini could transform into duct then endocrine cells [Pan et al., Development]. Wright was co- or lead organizer for two Keystone Diabetes meetings, and other leading developmental biology meetings. He has received research and training awards at Vanderbilt University, and is principal investigator of a 25-year duration institutional T32 award from the National Institutes of Health. He has chaired the DEV-1 Study Section for the NIH, and been standing or ad hoc member of several study sections over the last two decades. He has currently approx. 185 publications. He has trained fourteen graduate students and 12 postdoctoral fellows, many of whom have premier positions at leading globally renowned institutions.

RESIDENT ARTIST

Alex Cagan

Twitter: https://twitter.com/ATJCagan

Alex is a research scientist at the Wellcome Sanger Institute (Hinxton, UK) and also a scientific illustrator. He uses art and illustration to try and share the wonder and beauty of science.

He has been featured in Science, GENETICS, PNAS, Ecology Letters. He has worked with Wellcome, CRUK, the Genetics Society of America and many other fantastic research organisations to help tell scientific stories.

ABSTRACTS

ORAL PRESENTATIONS

OP 1 - Nicolas Damond

A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry

Nicolas Damond[1], Stefanie Engler[1], Vito R.T. Zanotelli[1], Denis Schapiro[1], Irina Kusmartseva[2], Clive H. Wasserfall[2], Fabrizio Thorel[3], Harry S. Nick[4], Pedro, L. Herrera[3], Mark, A. Atkinson[2], and Bernd Bodenmiller[1]

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Type 1 Diabetes (T1D) results from the autoimmune destruction of insulin-producing pancreatic beta cells. In humans, a comprehensive picture of the changes in islet cell composition and interactions between islet cells and immune cells is lacking due to limited post-mortem sample availability and the fact that only recently have technologies emerged that enable comprehensive tissue profiling. We analyzed pancreas sections from 12 human donors, including eight at different stages of T1D, using Imaging Mass Cytometry (IMC). This technology enabled simultaneous measurement of 35 pancreatic and immune proteins with single-cell and spatial resolution in these pancreatic tissues. We performed pseudotime analysis of islets through T1D progression in order to reconstruct the evolution of β cell loss and insulitis from the snapshot data. Our analyses revealed that β cell destruction is preceded by alteration of the β cell phenotype. In addition, the recruitment of cytotoxic and helper T cells was simultaneous and depended on both disease stage and islet profile. The approaches described here demonstrate the value of highly multiplex imaging for improving our understanding of T1D pathogenesis, and our data lay the foundation for hypothesis generation and focused follow-on experiments.

OP 2 - Elad Sintov

A Human Induced Pluripotent Stem Cell Based In Vitro Model For Autoimmune Type-1 Diabetes

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Type 1 diabetes (T1D) is an autoimmune disorder leading to the destruction of pancreatic insulin-producing β-cells. Despite recent scientific advances, questions remain regarding the initial trigger and the mechanisms of disease. The development of human induced pluripotent stem cells (hiPSCs) opened new opportunities for cell replacement therapy of T1D. Using a large-scale production strategy, therapeutic quantities of human stem cell-derived β-cells (SC-β) can be attained in vitro following a step-wise differentiation protocol. Yet, preventing immune rejection of grafted cells without the use of immunosuppressant drugs remains a major challenge. Currently, only murine systems exist for modeling T1D and no human model has been developed to sufficiently capture autoimmune responses, selectively triggered by β-cells.

In the current research, we have developed a human in vitro platform in an autologous setting that recapitulates the effector/target interactions in an autoimmune response. To create target cells for in vitro autoimmune assays, hiPSCs were reprogrammed from healthy or T1D patients, expanded in 3D suspension cultures and differentiated to human pancreatic endocrine cells by implementing SC-β and SC-α differentiation protocols.
A donor-matched response against target hiPSC-derived β-cells was achieved by co-cultures with either an autologous CD8+ T cell line specific to a known diabetogenic peptide or perihelial blood mononuclear cells (PBMCs) derived from the same donors' blood. Immune responses were quantified by means of T-cell effector activation signatures and loss of cell-type specific target cells by killing. Results show that under specific environmental conditions, both CD4+ and CD8+ T-cells exhibit a stronger activation phenotype when stimulated against iPSC-derived β-cells compared to iPSC-derived α-cells. Furthermore, HLA-I blocking experiments demonstrate that T-cell responses are mediated by T-cell receptor (TCR) to MHC-I interactions.

The in vitro model designed in this research can serve as a multi-purpose platform to study mechanisms of T1D autoimmunity, functionally evaluate cell replacement therapies and screen for immunomodulatory drugs or CRISPR-edited gene perturbations.

OP 3 - Volodymyr Petrenko

Molecular Clockwork of Human Islets from Type 2 Diabetic Donors

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Circadian system drives intrinsic biological clocks with near 24-hour oscillation period regulating variety of physiological processes and behavior in most living beings, including humans. We have recently established that self-sustained cell-autonomous oscillators are operative in human pancreatic islet cells synchronized in vitro, and are required for proper insulin secretion. In view of the emerging role of the pancreas circadian clock in islet function and development of type 2 diabetes (T2D) in rodent models, we aimed at characterizing circadian clocks in human islet cells from T2D donors. We demonstrate that T2D islets synchronized in vitro exhibited longer period length and flatter amplitude of circadian bioluminescence reporters than their healthy counterparts, concomitant with altered expression levels of endogenous core-clock genes CLOCK, REV-ERBA, PER1, PER2, PER3 and CRY2. We also report that exposure of islets to high glucose lengthened the circadian reporters period length in healthy donors but not in diabetic ones. Importantly, temporal profiles of basal insulin and glucagon secretion by synchronized T2D islet cells were altered compared to the control counterparts.

Our study highlights the functional role of the islet-autonomous circadian oscillators for the islet hormone secretion, emphasizing a link between circadian clockwork and T2D. This study point out the potential role of clock components as putative therapeutic targets for modulating insulin secretion upon T2D.

OP 4 - Mariana Igoillo-Esteve

GLP-1 analogs prevent and revert diabetes in Wolfram syndrome

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Background and aims: Wolfram syndrome is a rare autosomal recessive disease due to WFS1 mutations and characterized by young-onset diabetes, optic nerve atrophy and deafness. WFS1 deficiency results in endoplasmic reticulum stress (ER), leading to neurodegeneration and β-cell demise. Glucagon-like peptide-1 (GLP-1) analogs protect β-cells from ER stress. We aimed to evaluate the effectiveness of GLP-1 analogs in Wolfram syndrome.

Materials and methods: Whole body wfs1-/- mice were treated for 4-12 weeks with the long acting GLP-1 analog dulaglutide (1mg/kg) by intraperitoneal injection every 4 days. Glucose tolerance was assessed by IPGTT. Patient's induced pluripotent stem cells (iPSCs) were differentiated into β-cells using a 7-stage protocol and transplanted into Rag2-/- mice. One Wolfram syndrome patient switched from insulin to liraglutide.

Results: Wfs1-/- and wild-type mice had comparable glucose tolerance at weaning. By age of 6 weeks, wfs1-/- mice developed glucose intolerance and by 10 weeks diabetes. Dulaglutide treatment, initiated at 4 or 7 weeks of age, respectively prevented or reverted glucose intolerance in wfs1-/- mice. iPSCs from a Wolfram patient and CRISPR/Cas9-corrected isogenic control iPSCs were differentiated into β-cells; gene expression of differentiation markers was comparable between the two iPSC lines. Exenatide protected Wolfram iPSC-β-cells from ER stress-induced apoptosis in vitro (n=4, $p<0.05$). When transplanted into Rag2-/- mice, the patient-derived pre-β-cells secreted less human C-peptide than control cells (n=4-6, $p<0.05$). In this model, the impact of GLP-1 analogs on patient β-cell function/mass is presently being assessed. A 9-year-old Wolfram patient with 5-year diabetes duration switched from insulin pump (6U/day) to liraglutide (0.3-0.6mg/day). Glycemic variability improved markedly, and his low carbohydrate diet was discontinued. Over 3 months, HbA1c decreased from 6.3 to 5.5%.

Conclusion: GLP-1 analogs are protective in iPSC-derived β-cells, prevent/revert diabetes in wfs1-/- mice and improve glucose control in Wolfram syndrome in an n-of-1 trial. GLP-1 analogs hold promise for diabetes prevention and treatment in Wolfram syndrome.

OP 5 - Heiko Lickert

IGF receptor-like 1 is a novel regulator of the insulin signaling pathway

Heiko Lickert

Systemic insulin resistance and loss or dysfunction of insulin-producing beta cells are the main causes for type 1 and 2 diabetes. Here, we have identified a previously uncharacterized regulator of insulin action, beta cell proliferation and blood glucose homeostasis. In a screen for novel regulators of endocrinogenesis we identified a differentially expressed gene that is highly expressed in the embryonic endocrine and exocrine pancreas. We named the protein IGF receptor-like 1 (IGFR-L1) as it contains an extracellular cysteine-rich domain with similarities to insulin receptor (InsR) and IGF1 receptor as well as a mannose-6-phospohate domain found in the IGF2 scavenger receptor. Strikingly, knock-out mice develop normally, but die postnatally due to hyperinsulinemia and hypoglycemia. We show that IGFR-L1 binds insulin and InsR, internalizes both via clathrin-mediated endocytosis and therefore quickly desensitizes the receptor and scavenges the ligand to effectively block insulin signal transduction. Interestingly, IGFR-R1 is upregulated in mouse and human diabetes, thus we have identified a novel drug target for insulin sensitization and diabetes therapy.

OP 6 - Victoria Salem

Hub cells co-ordinate 3-dimensional β cell Ca^{2+} dynamics in mouse islets *in vivo*

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Aim: Earlier studies of isolated murine pancreatic islets in vitro have demonstrated that insulin release is co-ordinated by a sub-population of highly connected β cells, named "hubs". We aimed to establish the existence of this connectivity *in vivo*, by studying mouse islets that express the recombinant Ca2+ sensor GCaMP6f under the insulin promoter, engrafted in the anterior eye chamber (ACE).

Methods: Pan islet calcium dynamics were imaged under isofluorane anaesthesia on a modified Nikon Ti-E spinning disc confocal microscope (20x 0.75 NA water immersion objective, 3 Hz frame rate). Calcium traces from individual β-cells (>150 regions of interest) were analysed under varying circulating glucose levels and across several planes through the islet.

Results: Co-ordinated Ca2+ waves were observed across all islets above a circulating glucose level of 4 mmol/L. Pearson statistics (R) measuring the correlation between calcium traces from all β cell pairs across a single plane of imaging rose from an average of 0.51 at low glucose (2-3 Mm) to 0.94 at medium/high glucose (7-10 Mm) [n=5; $p<0.01$]. Highly co-ordinated activity was sustained with prolonged exposure to elevated circulating glucose levels (>10 mM). High circulating glucose levels were also associated with a significant rise in 3-dimensional beta cell connectivity from 65 to 86 % (n=3; p=0.02). Notably, β-cells more than a cell layer apart retained high connectivity. Binarized signal analysis separately confirmed the presence of a very highly-connected β cell subpopulation (8.7 ± 3.6 % of cells). Granger causality analysis revealed that even during prolonged (>10 min.) stimulation, the most highly connected cells were located close to sites of Ca2+ wave initiation. Finally, analysis of published single cell transcriptomes revealed a discrete "hub cell" population.

Conclusions: These studies evidence the existence of beta cell "hubs" orchestrating the co-ordinated activity of β cells in mouse islets *in vivo*.

OP 7 - Martin Neukam

A Novel Method Enabling Background-Free Purification of Age-Distinct Insulin Secretory Granules for Proteomic and Lipidomic Analyses

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Pancreatic beta cells store the hormone insulin in secretory granules (SGs), which are released upon glucose stimulation. While the proximity of SGs to the plasma membrane influences their secretion probability, a temporal component is also critical. Glucose stimulation of beta cells favors the secretion of newly synthesized insulin SGs, or young SGs, over their older counterparts. Using novels approaches for imaging age-distinct SGs and measuring their luminal pH we established that young SGs are more motile and acidic than older SGs, but we could not yet identify molecular signatures for SG ageing (Hoboth et al, 2015; Neukam et al, 2017). To this aim, we fused the SG-specific transmembrane protein phogrin with a cytosolic CLIP-tag. Pulse-chase labeling of INS-1 cells with TMR/FITC-conjugated CLIP substrates followed by incubation of cell extracts with anti-CLIP substrate rather than anti-tag antibodies enabled the immunoabsorption on magnetic beads of labeled SGs only. Using a protease cleavage site between the CLIP-tag and phogrin we could then selectively elute intact SGs from the magnetic beads, as verified by electron microscopy. Silver staining of immunopurified and eluted SGs from cells pulsed with the TMR-conjugated CLIP substrate indicated the presence of a plethora of distinct proteins, while non-labeled control samples were virtually empty. Western blotting of the eluted SGs pointed to a very high enrichment of SG markers, while those typical for contaminating organelles, such as ER, endosomes or the Golgi, were below detectability. Preliminary mass spectrometry analysis of purified SGs identified virtually all known insulin SG proteins, supporting an excellent signal-to-noise ratio. In summary, we report here the first protocol for the immunoisolation of intact, time-resolved SGs without significant contamination by other organelles. By exploiting this know-how for proteomic and lipidomic studies we shall unravel signatures for SG ageing.

OP 8 - Vibe Nylander

Chromatin interactions aid identification of effector transcripts at Type 2 Diabetes GWAS loci in β-cells

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Identifying effector transcripts at Type 2 Diabetes (T2D) and glycaemic trait GWAS loci is a key challenge in efforts to determine molecular mechanisms influencing diabetes pathogenesis. Pancreatic islet enhancers are enriched for T2D-associated variants, indicating that T2D GWAS loci regulate gene expression. Chromatin interaction techniques can identify interactions between regulatory elements and the promoters they potentially regulate. We conducted functional GWAS analysis of GWAS credible sets from the DIAMANTE consortium, imputed to the Haplotype Reference Consortium panel using islet genomic annotations, to identify variants overlapping islet regulatory elements. Next, we used Next Generation (NG) Capture-C in the human β-cell line, EndoC-βH1 (n=3) to detect chromatin interactions of 222 regulatory elements across 42 T2D-GWAS loci, to identify chromatin interactions of regulatory elements overlapping T2D GWAS credible variants.

Chromatin interactions in β-cells, but not lymphoblasts, were enriched for islet transcription factor motifs. At the *COBLL1* locus coding variants have been identified previously, but we find that regulatory elements encompassing T2D credible variants interact with the *GRB14* promoter (FDR< 5%). At the *WARS* locus, regulatory elements encompassing glycaemic trait associated variants interacted with the *WARS* and *SLC25A29* promoters. At the *TP53INP1* locus multiple variants in the credible set, locating to different regulatory elements, were found to interact (FDR< 5%) with the *CCNE1* and *NDUFAF* promoters. At the *FBRSL1* locus only specific variants in the credible sets, locating to particular regulatory elements, interacted with the *FBRSL1* promoter (FDR< 5%).

We mapped chromatin interactions of 222 regulatory elements overlapping T2D GWAS credible sets in human β-cells. We identified interactions between regulatory elements and promoters at several loci, such as *COBLL1*, *WARS* and *TP53INP1.* Additional experiments are required to investigate if these interactions regulate gene expression. This study demonstrates that chromatin interactions can help identify functional variants in credible sets which impact on chromatin interactions and gene expression.

OP 9 - Louise Cottle

3D analysis of the structural and functional beta cell architecture in human pancreatic islets

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Previous work has shown differences in the gross structure of human islets, when compared to rodent, including the proportions of different endocrine cell types, cellular arrangement and capillary bed morphology. However, we know little about the sub-cellular architecture of beta cells within intact human islets.

Our previous work, in mouse islets, has identified a structural and functional orientation of individual beta cells. 3D analysis demonstrates that beta cells polarise with respect to the vasculature and possess distinct structural domains identified by the segregation/enrichment of proteins to distinct plasma membrane domains (Low *et al*., 2014). Functional studies also revealed preferential insulin exocytosis towards the vasculature. Whether human beta cells orientate in this way is unknown.

Using human pancreatic slices (Marciniak *et al*., 2014) and 3D confocal immunofluorescence we show beta cells orientate with respect to basement membranes (laminin) and capillaries. Synaptic scaffold proteins associated with the control of insulin exocytosis (Rim2, Liprin and Piccolo) are enriched in beta cells along the beta cell-vasculature interface. Preliminary data from islets of type 2 diabetic patients shows a similar polarised organisational structure, suggesting cell orientation is conserved in disease. Additionally, 3D functional analysis of dispersed human beta cells, cultured on Laminin-511, showed exocytic events preferentially occurred at the beta cell-basement membrane interface. This work suggests that despite inter-species differences in the cytoarchitecture of mouse and human islets, the individual endocrine cells have a consistent relationship with the vasculature. Combined with the functional exocytosis data it indicates a conserved mechanism of beta cell organization and orientation.

OP 10 - Andreas Müller

4 D imaging of insulin secretory granule dynamics and secretion in primary beta cells with lattice light sheet microscopy

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Total internal reflection microscopy (TIRFM) has been the method of choice for many years to image insulin secretory granule (SG) dynamics and secretion in primary beta cells and insulinoma cell lines. However, it only allows for imaging of SGs located <200 nm from the surface of the cell attached to the glass, thereby restricting the view only to events happening on one side of the cell. Since beta cells have a polyhedral shape with a diameter of several µm, events happening in the major part of the cell cannot be imaged by TIRFM. Furthermore, prior to TIRFM imaging pancreatic islets are usually dissociated into single cells – a procedure that affects cell-to-cell interaction, gene expression and signaling. These limitations can be overcome with novel microscopy techniques that allow for imaging insulin SGs within primary beta cells of isolated islets at sub-cellular resolution and high speed. Specifically, we have used lattice light sheet microscopy (LLSM) to resolve insulin SGs, which have a mean diameter of 250 nm. LLSM allows for fast TIRFM-like sectioning of cells in 3 dimensions with low photo-toxicity. In this way we could image age-distinct pools of insulin-SNAP^{+} SGs in isolated SOFIA (Study of Insulin Ageing) mouse islets within the whole cell volume. Use of a novel pH-sensitive SNAP-substrate further enabled us to unequivocally image insulin SGs undergoing exocytosis. Hence, this is the first report for the use of LLSM in a primary mouse tissue at sub-cellular resolution in order to address insulin SG turnover within whole beta cells. Ultimately, this approach might be exploited to study peptide hormone turnover in other model systems, thus providing novel insights into the physiology of regulated secretion in health and disease.

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OP 11 - Yung Hae Kim

Title: Manipulating differentiation dynamics of endocrine cells from human pluripotent stem cell-derived pancreatic progenitors

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Human pluripotent stem cell (hPSC)-derived beta-cell replacement would be a remarkable therapeutic approach for treating type 1 diabetes. The aim of our study is to understand the process of progenitor cell decision-making between self-renewal and differentiation and thereby control this process to boost endocrine differentiation of pancreatic progenitors derived from hPSCs. Why do not all cells differentiate in synchrony when submitted to differentiation signals introduced in the medium? Our previous study in developing mouse pancreas proposed a model with which two probabilistic parameters can determine endocrine differentiation dynamics of progenitors during organogenesis: probability of differentiation and probability of differentiation before mitosis. These parameters are calculated based on the mode of progenitor division: self-renewal, asymmetric division, and symmetric differentiative division. To test this model we use hPSCs to drive pancreatic differentiation in vitro and determine endocrine differentiation dynamics using various signaling molecules during differentiation process in both 2-D culture and 3-D organoid system. We have generated a cell line harboring both pancreatic progenitor (PDX1) and endocrine progenitor (NGN3) reporters, have established live imaging conditions, and are in the process of collecting and analyzing data. Our preliminary data indicate that hPSC-derived progenitors divide slower than their mouse counterparts and with different rates at different culture stages. In addition, we observed so far symmetric self-renewing and asymmetric divisions but no symmetric differentiative cell division, in contrast to mouse. We are investigating to what extent this is due to the culture conditions or is a species difference. We will show how different signaling molecules/media affect the modes of progenitor division and differentiation. Our study will provide insights to better control in vitro generation of beta-cells from hESCs as a therapeutic endeavor to treat type 1 diabetes.

OP 12 - Joonyub Lee

PRMT1 maintains mature β cell identity by histone arginine methylation

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Loss of functional β cell mass is an essential feature of type 2 diabetes, and recent studies indicate that β cell dedifferentiation can result in the loss of functional β cell mass. However, the mechanism underlying this β cell dedifferentiation has not yet been elucidated. Here, we demonstrate a novel function of PRMT1 in maintaining mature β cell function and identity. *Prmt1* knock-out in fetal and adult β cells induced diabetes which was aggravated by high fat diet-induced metabolic stress. Deletion of *Prmt1* in adult β cells resulted in the immediate loss of histone H4 arginine 3 asymmetric di-methylation (H4R3me2a) and the subsequent dedifferentiation of β cells. The expression of genes involved in mature β cell function and identity were robustly downregulated as soon as *Prmt1* deletion was induced in adult β cells, revealing the early feature of β cell dedifferentiation. ChIP-seq and ATAC-seq analyses revealed that PRMT1-dependent H4R3me2a increases chromatin accessibility at the binding sites for CTCF and β cell transcription factors. In addition, PRMT1-dependent open chromatin regions show a strong association with the risk of diabetes in humans. In conclusion, our results indicate that PRMT1 plays an essential role in maintaining β cell identity by regulating chromatin accessibility.

POSTER PRESENTATIONS

PP 1 Agata Wesolowska-Andersen

Transcriptome and chromatin evaluation of an HNF1-A iPSC disease model

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Maturity Onset Diabetes of the Young due to *HNF1A* mutations is the most common cause of monogenic diabetes. It results from heterozygous, loss-of-function mutations in *HNF1A* which encodes the transcription factor HNF-1A. Mice with heterozygous *HNF1A* mutations do not recapitulate the diabetic phenotype, therefore human iPSC-derived cells are more suitable for disease modelling. We have established a patient-derived *HNF1A* Pro291fsinsC iPSC model, and using CRISPR-Cas9 genome-editing have corrected the mutation to establish isogenic controls. We differentiated multiple (n=6) iPSC clones towards endocrine lineage in triplicates, and performed RNA-seq and ATAC-seq at definitive endoderm (DE), pancreatic endoderm (PE) and beta-like cells (BLC) stages.

We did not observe any expression of the HNF1A-MODY allele in the RNA-seq data, confirming *HNF1A* haploinsufficiency in the patient cell lines. We confirmed that *HNF1A* expression was restored at both the transcript and protein level in the corrected lines. Genes differentially expressed at BLC stage were significantly enriched in targets of HNF-1A identified in an independent *HNF1A* knock-down experiment in EndoC-βH1 cell line (p=4.4e-132). In line with reported effects of HNF-1A on insulin secretion, we observed up-regulation of INS (p=7.2e-03) and other insulin secretion genes in the corrected clones. At the PE stage, corrected clones showed higher expression of *SOX9* (p=7.1e-04) and *PTF1A* (p=1.5e-03), known regulators of pancreatic progenitors, suggesting that HNF-1A may be involved in establishment of this cellular population. Additionally, we identified 7811 sites at PE and 1710 sites at BLC with significant changes in open chromatin, with >90% of the sites being more accessible in the corrected clones, suggesting that HNF-1A plays a role in establishing open chromatin. In summary, the HNF1A-MODY iPSC model provides a valuable resource for studying cellular and molecular phenotypes caused by HNF-1A deficiency.

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2 Aida Martinez Sanchez

Regulation and Role of MiR-125b in β-cells

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MiRNAs are small non-coding RNAs that silence gene expression post-transcriptionally and control β-cell development and function. We have recently identified several β-cell miRNAs that are regulated by AMPK, an enzyme essential for glucose homeostasis and β-cell function. One of these miRNAs, miR-125b, controls proliferation, apoptosis and differentiation in other cell types, although its function in β-cells remains unknown. High circulating miR-125b levels associate with hyperglycaemia (HbA1c) in prediabetic, T2D and T1D subjects, suggesting miR-125b as a biomarker or contributor to the development of diabetes.

Our work aims to determine miR-125b function in β-cells and the role of glucose and AMPK in the regulation of its expression.

We found that miR-125b expression is induced by glucose in both mouse and human islets and strongly reduced in islets from mice fed a diet low in glucose (ketogenic). These effects were not observed in islets with β-cell-specific deletion of AMPK (βAMPKdKO) or its upstream kinase LKB1 (LKB1KO). Both mature miR-125b and its primary transcript were increased in βAMPKdKO islets and ATAC-seq showed increased chromatin accessibility in a Smad2/3-binding region in the promoter of *MIR125B2* in βLKB1KO islets, suggesting that AMPK negatively regulates miR-125b transcription. Whereas CRISPR-mediated miR-125b deletion in EndoCβH1 cells increased insulin secretion, miR-125b overexpression in MIN6 cells limited cytokine-induced apoptosis whilst strongly reducing insulin content. RNA-Seq on these cells suggests a role for miR-125b in metabolism, respiration and cytokine receptor interactions and RNA-IP of the miRNA-induced silencing complex revealed novel miR-125b targets such as mannose-6-phosphate receptor (MPR), important for enzyme sorting within secretory granules. We are currently investigating miR-125b role *in vivo* in animals with inducible, β-cell-specific, overexpression of miR-125b or a molecular miR-125b "sponge".

In summary, we reveal miR-125b as an important regulator of β-cell function that might contribute to the deleterious effects of hyperglycaemia on β-cells.

3 Aileen King

The KINGS *Ins2+/G32S* mouse: a novel model of diabetes and mild beta cell ER stress

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Introduction. Spontaneous hyperglycaemia in a colony of mice at King's College London (KCL) was found to be due to a G32S substitution in the *Ins2* gene. Heterozygous male mice developed overt hyperglycaemia (>16.7mM) and females were glucose intolerant. This novel model was named the KCL insulin G32S (KINGS) mouse. *Ins2* mutations can cause endoplasmic reticulum (ER) stress, an unfolded protein response (UPR) and beta cell loss. Therefore, the aim of this study was to establish the extent of beta cell ER stress, dysfunction and loss in these animals.

Methods. Transmission electron microscopy was used to investigate islet ultrastructure. qPCR for markers of ER stress: CHOP, BiP and XBP1s were measured in adult islets. Beta cells were quantified in hormone-stained pancreatic sections and insulin release measured in isolated islets.

Results. Beta cells contained swollen mitochondria and distorted ER (suggestive of ER stress), which was more severe in males than females. qPCR revealed downregulation of CHOP (a signal for apoptosis through the PERK pathway of the UPR) in KINGS males ($p<0.001$) and females (0.049, Two-way ANOVA, n=4-8). The molecular chaperone BiP was reduced in males but upregulated in females (25±3 vs 46±7, $p<0.001$). XBP1s/XBP1 (reflecting activation of the IRE1 pathway) was increased in KINGS males (0.07±0.02 vs 0.14±0.03 p=0.021) but not females. Wildtype and KINGS islets consisted of approximately 70% and 60% beta cells respectively, but KINGS islets secreted up to 91% less insulin at 20mM glucose than wildtype islets.

Conclusions. The KINGS mouse is a novel model of human diabetes. Gender differences in glycaemic control are also reflected in their islet physiology. Despite functional impairment of KINGS mouse islets, the profile of ER stress markers indicates mild ER stress, which results in limited beta cell loss. This model presents a unique opportunity to investigate how beta cells adapt to ER stress.

4 Alejandra D D Tomas Catala

Agonist binding affinity determines palmitoylation of the glucagon-like peptide-1 receptor and its functional interaction with plasma membrane nanodomains in pancreatic beta cells

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The glucagon-like peptide-1 receptor (GLP-1R), a key pharmacological target in type 2 diabetes and obesity, is known to undergo palmitoylation by covalent ligation of an acyl chain to cysteine 438 in its carboxyl-terminal tail. Work with other GPCRs indicates that palmitoylation can be dynamically regulated to allow receptors to partition into plasma membrane nanodomains that act as signaling hotspots. Here, we demonstrate that the palmitoylated state of the GLP-1R is increased by agonist binding, leading to its segregation and clustering into plasma membrane signaling nanodomains before undergoing internalization in a clathrin-dependent manner. Both GLP-1R signaling and trafficking are modulated by strategies targeting nanodomain segregation and cluster formation, including depletion of cholesterol or expression of a palmitoylation-defective GLP-1R mutant. Differences in receptor binding affinity exhibited by biased GLP-1R agonists, and modulation of binding kinetics with the positive allosteric modulator BETP, influence GLP-1R palmitoylation, clustering, nanodomain signaling, and internalization. Downstream effects on insulin secretion from pancreatic beta cells indicate that these processes are relevant to GLP-1R physiological actions and might be therapeutically targetable.

5 Alexander Hamilton

MICU2 and the critical role of mitochondrial Ca^{2+} uptake in glucose-stimulated insulin secretion

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Background: Pancreatic β-cell failure is a hallmark of type 2 diabetes with mitochondrial failure a key proponent in its manifestation. Despite this, our understanding of β-cell mitochondria and their perturbation in diabetes remains unclear. MICU2 could represent a key player in mitochondrial function. It heterodimerizes with its homologue MICU1, acting as a gatekeeper for the mitochondrial calcium uniporter. We hypothesize that MICU2 plays a significant role in glucose-stimulated insulin secretion (GSIS) via its effects on mitochondrial Ca^{2+} uptake.

Methods: To assess the role of MICU2 in β-cell function, siRNA-silencing of *MICU2* in INS1-832/13 and EndoC-βH1 cell lines was performed, followed by insulin secretion assays, mitochondrial respiration recordings and live confocal imaging.

Results: siRNA-silencing of *MICU2* resulted in a significant drop in GSIS, perturbed mitochondrial membrane hyperpolarization, attenuated mitochondrial respiration and a reduction in the ATP/ADP ratio in response to high glucose. The cause of these defects appeared to be a decline in mitochondrial Ca^{2+} uptake, which was attenuated in response to high glucose and high K^+. Despite this, unexpectedly, cytosolic Ca^{2+} was also lower in siMICU2-treated cells in response to high K^+. Mitochondria play a key role in clearing Ca^{2+} from the cytosol, including the submembrane compartment. Hence it was hypothesized that in siMICU2-treated cells, Ca^{2+} was accumulating in the submembrane compartment, resulting in desensitisation of the voltage-dependent Ca^{2+} channels, thereby lowering total cytosolic Ca^{2+}. To test this, imaging was performed using the linescan configuration to allow for Ca^{2+} to be measured in different parts of the cell. Upon high K^+ stimulation, Ca^{2+} was indeed found to be accumulating in the submembrane compartment, as compared with control.

Conclusion: MICU2 plays a critical role in β-cell mitochondrial Ca^{2+} uptake. Furthermore, it is revealed that β-cell mitochondria sequester Ca^{2+} from the submembrane compartment, allowing activation of voltage-dependent Ca^{2+} channels, thereby facilitating GSIS.

6 Alexandra-Madelaine Tichy

Chimeric GLP-1 receptor enables light-induced insulin secretion in pancreatic islets

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Maintenance of blood glucose homeostasis is orchestrated through a complex signalling network between the different cell types in pancreatic islets. Class B G-protein coupled receptors (GPCRs), such as the glucagon-like peptide-1 receptor (GLP-1R), play a fundamental role in this regulatory process. Because of the broad expression, addition of receptor agonists to pancreatic islets causes both direct as well as paracrine effects, making it difficult to study receptor function in a cell-type specific and temporally precise manner.
The aim of this project is to achieve spatio-temporally precise control of receptor function by creating light-activated GPCRs (Opto-GPCRs) with a particular focus on GLP-1R, and apply them to study class B GPCR functionality in pancreatic islets. We hypothesize that by using Opto-GPCRs we will enable i) the precise control of islet cell physiology using light and ii) the dissection of how GLP-1R activation in specific cell types affects the overall function of pancreatic islets.

We used structural and sequence-based methods to compute and engineer a light-sensitive variant of GLP-1R (Opto-GLP-1R). To determine basic functionality of this engineered receptor, we transduced primary pancreatic islets and performed glucose-stimulated insulin secretion assays.

We found that islets transduced with Opto-GLP-1R secreted more insulin when stimulated with light, comparable to stimulation with the GLP-1R agonist Exendin-4, but not when kept in the dark. This proof-of-principle experiment shows that our approach yields functional Opto-class B GPCRs able to mimic the endogenous physiology of the target receptor in primary tissues. Future experiments aim to further characterize Opto-GLP-1R in cell line and primary cell assays. Using this system, we will be able to precisely control the activity of GLP-1R and other class B GPCRs in specific cell types and for defined time spans, allowing us to elucidate the effect of precise receptor activation on islet physiology.

7 Amaya Lopez-Pascual

Discovery and functional validation of gene regulatory networks in human pancreatic islets to identify targetable Type 2 Diabetes disease mechanisms

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Deranged pancreatic islet function is a known culprit in type 2 diabetes (T2D). Islet function relies on the concerted effort of at least five different cell types whose functions are likely altered in different ways in T2D. We generated a high-quality Single-cell RNA-sequencing (scRNAseq) data set from pancreatic islet from T2D patients and controls revealing a specific loss of ghrelin-expressing cells in patients as well as further adding differentially expressed genes. It is known that the biological function of the genes may vary depending on the cellular context and, thus, it is important not only to understand which genes are up- or down-regulated, but also to see how the context of gene networks changes. In our study, the algorithm differential Gene Correlation Network Analysis (dGCNA) was used to analyze disease-related changes in gene-to-gene correlation networks. We identified 13 disease-regulated gene networks (DRGNs), representing established (e.g. mitochondria function, exocytosis and ER-stress), as well as hitherto unknown biological processes that were affected in T2D beta cells. The DRGNs included virtually all genes with previously established roles in T2D, putting them in a new disease context. We also identified node genes, i.e. genes potentially regulating many other genes, in the DGRNs. Most of the node genes have not been previously been described in T2D or beta cell biology and their significance was validated in beta cell lines. 13 out of 15 of the novel uncovered T2D-genes were found to affect insulin expression and/or glucose- and/or cAMP-stimulated insulin secretion, showing that the concerted function of a large number of genes, hitherto not implicated in T2D, is crucial for normal glucose tolerance. Our data suggests that analyzing how gene-regulatory networks change revealed emergent properties of T2D pointing to a way forward for identifying targetable disease mechanisms.

8 Amitai Mandelbaum

miR-17-92/106b regulate beta cell mitotic checkpoint and insulin secretion

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Adult beta cells in the pancreas are the sole source of insulin in our body. Beta cell loss or increased demand for insulin, impose metabolic challenges because adult beta cells are generally quiescent and infrequently re-enter the cell division cycle. miR-17-92/106b is a family of proto-oncogene microRNAs, that regulate proliferation in normal tissues and in cancer. Here, we employ mouse genetics to demonstrate a critical role for miR-17-92/106b in glucose homeostasis and in controlling insulin secretion. We demonstrate that miR-17-92/106b regulate the adult beta cell mitotic checkpoint and that miR-17-92/106b deficiency results in reduction in beta cell mass *in-vivo*. Furthermore, protein kinase A (PKA) is a new relevant molecular pathway downstream of miR-17-92/106b in control of adult beta cell division and glucose homeostasis. Therefore, contributes to the understanding of proto-oncogene miRNAs in the normal, untransformed endocrine pancreas, and illustrates new genetic means for regulation of beta cell mitosis and function by non-coding RNAs.

9 Andreas Wiederkehr

Elevated intracellular glycerol 3-phosphate in human pancreatic beta-cells following chronic high glucose culture augments basal mitochondrial respiration and blunts glucose responsiveness

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Background and aims: When exposed to chronically elevated glucose in the medium, β-cells initially improve their response to acute glucose challenge followed by a gradual loss of glucose responsiveness. Mitochondria are central for β-cell nutrient sensing and their function may be altered to different degrees depending on the glucose stress. Here we studied early metabolic and mitochondrial adaptations in human islet cell clusters cultured for 4 days in elevated 11.1mM glucose compared to 5.6mM glucose (control).

Results: Mitochondria from human islet cells cultured in 11.1mM glucose were over-activated when compared to control islet cells. Even after returning for 30 minutes to 1mM glucose basal respiratory rates remained strongly elevated but the subsequent respiratory response to glucose was weak. Glucose failed to increase the ATP/ADP ratio and glucose-induced calcium signaling was strongly impaired compared to control islet clusters. Among many metabolites measured, glycerol 3-phosphate (G3P) was the most markedly elevated by culture in 11mM glucose. When returning the cells to 1mM glucose, G3P was strongly lowered suggesting reduced synthesis or accelerated consumption of the metabolite. Whereas several metabolites of the last steps of glycolysis including pyruvate remained unusually high. After a step increase of glucose from 1 mM to 16.7 mM, G3P doubled in control but increased close to 6-fold in chronic high glucose cultured islet clusters.

Conclusion: We propose that G3P accumulated during 11mM glucose culture serves as a reservoir replenishing pyruvate levels even when returning the islet cells to 1mM glucose. This metabolic change explains accelerated respiration and the elevated ATP/ADP ratio under resting glucose conditions. The 6-fold increase of G3P during acute glucose stimulation suggests metabolic adaptations where glucose metabolism is deviated towards the synthesis of G3P rather than oxidative metabolism of pyruvate required for accelerated ATP synthesis. G3P dependent mitochondrial activation may render mitochondria subsequently unresponsive to acute glucose stimulation. Our results are relevant to understand early metabolic changes in β-cells during prediabetes.

10 Angelique Satoko Cercillieux

Role of nicotinamide riboside metabolism in pancreatic β-cells

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Nicotinamide riboside (NR), one of the NAD+ precursors, can improve oxidative metabolism and prevent high fat diet induced obesity[1]. Repletion of NAD+ intermediate, nicotinamide mononucleotide (NMN), has shown to improve insulin sensitivity and glucose-stimulated insulin secretion by pancreatic β-cells in T2D mice[2]. However, the contribution of endogenous NR mediated NAD+ biosynthesis on glucose metabolism and β-cell function is not well established. Here we describe the role of NR metabolism on β-cell function using mice that are deficient for NR's rate-limiting enzyme, nicotinamide riboside kinase 1 (NRK1)[3]. Preliminary results indicate reduced expression of insulin and glucagon with increase glucokinase mRNA expression in islets from β-cell specific NRK1 deficient mice. NRK1 whole body KO mice exhibit impaired insulin secretion after a meal or in response to a glucose overload when submitted on high fat diet. Furthermore, both insulin and glucagon content were significantly depleted in aged NRK1 deficient mice. Together, these results uncover the pancreatic islets as a key place for NR utilization and a critical role for NR metabolism in β-cell function in the setting of metabolic disease.

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11 Anna Veprik

Acetyl-CoA-Carboxylase 1 (ACC1) plays a critical role in glucagon and GLP1 secretion, and controls whole body glucose homeostasis.

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Background: One of the hallmarks of diabetes is dysregulated glucagon secretion from pancreatic alpha cells. Glucagon is cleaved from proglucagon, giving rise also to Glucagon-Like Peptide 1 (GLP1). GLP1 is secreted from gut enteroendocrine L-cells, potentiating insulin secretion, and GLP1 levels are reduced in type 2 diabetes. Despite the important role of these cell types in glycaemic control, the mechanisms regulating their secretory function are not well defined.

We have previously identified that Acetyl-CoA-Carboxylase 1 (ACC1), the rate-limiting enzyme of de-novo lipogenesis, plays a critical role in the growth and function of beta cells. The aim of the current project was to explore the role of ACC1 as a general modulator of secretory activity in pancreatic alpha and gut L-cell function and its effect on the organism metabolism.

Results: Pharmacological inhibition of ACC impaired glucagon secretion from transformed alpha cells, primary mouse and human islets, as well as impairing GLP1 secretion from transformed enteroendocrine cells and primary gut crypts. To investigate these mechanisms in vivo, we generated mice with ACC1 ablated in alpha and L cells using the glucagon driven Cre-Lox system (gluACC1-KO). gluACC1-KO mice showed no changes in body weight and in food administration. However, they were glucose intolerant relative to littermate control mice, with lower fasting serum glucagon levels that were unresponsive to glucose administration. Correspondingly, the secretion of glucagon, but not insulin, from islets isolated from the gluACC1-KO mice was severely impaired in low glucose condition, relative to controls, and consistent with the in vitro ACC inhibitor data. The altered secretion was unaffected by modulation of the K_{ATP} channel activity. Moreover, similar to the pharmacological inhibition, active GLP1 secretion was reduced in gut crypts from gluACC1-KO mice.

Summary: Our data reveals a critical role for ACC1 in controlling alpha and L-cell function and whole body glucose homeostasis.

12 Antje Grotz

Implementing a genome-wide CRISPR screen for pancreatic beta-cell dysfunction

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Genome-wide association studies (GWAS) have identified >400 signals at ~250 regions of the genome associated with Type 2 Diabetes risk, the majority of which are involved in pancreatic beta-cell dysfunction. A major barrier to translational insight however is uncertainty over the genes through which they exert their effect. Pooled CRISPR screens are a cost- and time-efficient approach to interrogate gene knockouts genome-wide. However, disease relevant cellular models and phenotypes suitable for pooled screening must be established. The aim of this project was to implement a pooled genome-wide CRISPR loss-of-function screen using a FACS readout for insulin content in *EndoC*-βH1, a human beta-cell line.

To determine the feasibility of CRISPR/Cas9 genome editing in *EndoC*-βH1, we generated individual knockout (KO) cell lines for genes with known roles in insulin content (*INS, PAM, IDE*). KO cells demonstrated complete protein depletion, a significant reduction on the mRNA level compared to empty vector (EV) cells (*INS*-KO: -54%; *PAM*-KO: -78%; *IDE*-KO: -66%; $p<0.001$) and the insertion/deletion frequency exceeds 87.5% for each sgRNA (TIDE). Most importantly, stable expression of Cas9 did not affect the glucose responsiveness of the cells (Fold change 2.8mM vs 15mM in WT: 2.64 vs EV: 2.48, $p=0.8$).
As a validation of the screening protocol, we performed a pooled small-scale screen using the previously individually validated sgRNAs and assessed sgRNA enrichment in the low vs high insulin content populations. *INS* and *PAM* sgRNAs were as expected enriched in the low insulin population, whereas control sgRNAs showed no enrichment in either population (Fold change low vs high, *INS*: 3.58, *PAM*: 1.32, EV: 1.07).

In summary, we have demonstrated efficient genome editing in *EndoC*-βH1 and a robust strategy for a pooled CRISPR screen to identify genes underlying pancreatic beta-cell dysfunction. We will now proceed to perform the genome-wide screen using the Toronto Knockout v3 CRISPR library.

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ABSTRACT WITHDRAWN

14 Ayat Hija

Productive cell cycle division versus non productive cell cycle division in pancreatic beta cells

Pancreatic beta cell proliferation is a key mechanism for the generation of new beta cells during postnatal life. Enhancement of beta cell proliferation, as seen in mice following diabetogenic injury is an attractive approach to increase beta cell mass in diabetic patients. However, the signals that control the decision of a beta cell to engage in the cell division cycle remain poorly understood. We have elucidated the key role of glucose metabolism in triggering cell cycle entry of quiescent beta cells. The pathway leading from glucose to beta cell replication involves glucose uptake, glycolysis, membrane depolarization, calcium signaling via calcineurin, and autocrine insulin signaling. This pathway suggests interesting points of pharmacologic intervention; for example, we have shown that treating mice with a small molecule glucokinase activator (GKA), a candidate diabetes drug currently in clinical trials, triggers cell cycle entry of quiescent beta cells in adult mice.On the other hand ,this mitogen has a harmful effect on the replicating beta cells ;this negative effect maybe a potential consequence of forcing cell cycle entry of beta cells that are not ready to engage to cell cycle. Transgenic expression of an active mutant of glucokinase causes initially cell cycle entry of beta cells, but this is followed rapidly by DNA damage, p53 activation and beta cell death. Furthermore, treatment of mice with GKA for extended periods does not lead to increased beta cell mass, despite the observation that a significant fraction of beta cells has entered the cell cycle. In addition, clinical trials with GKA have resulted in only a transient reduction of blood glucose, suggesting a toxic effect or loss of activity. I have studied the toxic effects of GKA on replicating beta cells. I found that beta cells stimulated by GKA to enter the cell cycle fail to complete the cell cycle and are eliminated as a point between S phase and mitosis. While cell death could not be observed using traditional markers of apoptosis such as TUNEL or caspase 3 activity, we could infer beta cell death from analysis of Ki67 staining, BrdU pulse-chase experiments, and the detection of circulating cell-free DNA derived from beta cells in the plasma of GKA-treated mice. GKA toxicity towards replicating beta cells depends oxidative stress, and could be partially rescued by treatment with anti oxidants. I propose that replicating beta cells are particularly vulnerable to metabolic stress, which could potentially convery a regenerative stimulus to a degenerative process.It remains unclear whether other candidates such as harmine trigger beta cell replication to effectively lead to productive beta cell divisions and increased beta cell mass. For regenerative therapy in diabetes, drugs that ensure productive beta cell division will be necessary in addition to molecules that drive cell cycle entry.

15 Melissa Adams

Roundabout receptors regulate endocrine cell type sorting and three-dimensional architecture in the islet of Langerhans, and their absence affects intra-islet synchronized insulin secretion

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The three-dimensional organization of the islets of Langerhans facilitates endocrine cells' connection with the vasculature and nervous systems, cell-cell coupling to assure synchronous hormone secretion, and directionality of intra-islet paracrine signaling. Pacemaker β cell, whose function rely on their intra-islet position, and whose activity is disrupted concomitant with the loss of islet organization, have recently been recognized. Intra-islet organization is disrupted in obesity, insulin resistance and diabetes. Moreover, recapitulating correct islet architecture during *in vitro* differentiation has emerged as a recent challenge in stem cell-based approaches to diabetes. The mechanisms governing the formation of islet organization during development, its preservation during phases of islet compensatory growth, and its disruption in diabetes are poorly understood. Here, we show that expression of the axon-guidance proteins Roundabout (Robo) receptors in β cells is essential to endocrine cell type sorting and correct islet organization. Deletion of *Robo*, either in all endocrine cells or selectively in β cells, results in disorganized islet architecture with intermingling α, β and δ cells in the islet core, and glucose intolerance. This phenotype is not due to transdifferentiation of β cells to α cells, nor is it the result of loss of β cells due to β cell death or de-differentiation. Moreover, conditional deletion of *Robo* selectively in mature β cells subsequent to islet formation results in a similar phenotype, empathizing the role of Robo in maintaining islet architecture during phases of islet expansion. Using a novel intravital imaging approach to measure Ca^{2+} oscillations in *Robo βKO* and *WT* islet *in vivo*, we further show that deletion *Robo* selectively in β cells disrupts synchronized insulin secretion within the islet, despite correct expression and localization of the gup junction protein Connexin36. Our findings suggest that Robo-mediated signaling could be harnessed to regulate islet organization and cell-cell communication.

16 Benoit Hastoy

Functional assessment of human induced Pluripotent Stem Cells (iPSC)-derived β-like cells (BLC)

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Background and aim. The limited access to human islets and the variability of preparations hamper research on human β-cell physiology. β-like cells (BLC) derived from induced Pluripotent Stem Cells (iPSC) recently raised the possibility of generating β-cells from any patient donor cell. This strategy provides a model to investigate the impact of T2D risk alleles on β-cell development and function. The BLC differentiation is a costly and challenging process and its common quality assessment focuses mainly on the expression levels of key β-cells identity markers, providing little insight on the functional properties of BLC. Our aim is to provide a deep functional characterisation of BLC using non-invasive electrophysiology on Multi-Electrode Arrays (MEAs), insulin secretion assay, and imaging (electron microscopy).

***Methods and results*.** The BLC were glucose-responsive since 20mM glucose triggered action potentials firing (perforated patch) and a 2- to 3-fold increase in insulin secretion. This effect was mimicked and reversed by K_{ATP} channels modulators Tolbutamide and Diazoxide, respectively. As in primary β-cells, biphasic kinetics of exocytosis were supported by a 10pA.pF^{-1} calcium current density. Electron microscopy revealed variable vesicle size and poly-hormonal endocrine cells. Our MEA-based bio-electronic sensor allowed automatic, multi-parameter, and real-time electrophysiological analysis at both single-cell and cell-population levels (60 simultaneous recordings). Similarly to primary human islets, repeated physiological glucose stimulations (5-10mM) in long-lasting experiments (1-2h without run-down) induced slow potentials, which demonstrated a high degree of BLC coupling. Slow potential frequencies were well-correlated to kinetics of insulin secretion measured on the same cells.

Conclusion. The complex differentiation protocol (25days) introduces some functional variabilities. However, the iPSC-derived BLC recapture key functions of native human islets. The multi-functional characterisation pipeline used here will be employed to address the impact of T2D-risk alleles on human β-cell function.

17 Berit Svendsen

GIP receptor blocking improves glucose tolerance and body composition in mice

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The incretin hormone glucose-dependent insulinotropic polypeptide (GIP) is secreted from enteroendocrine cells in response to food intake. GIP receptors (GIPR) are found in islets, adipocytes, and brain, and similar to GLP-1, GIP stimulates glucose-dependent insulin secretion from pancreatic β-cells. Unlike GLP-1, GIP has direct actions on adipocytes and α-cells, to stimulate fat accumulation and glucagon secretion, respectively. *Gipr*$^{-/-}$ mice are protected from diet-induced obesity (DIO), raising the possibility that GIPR antagonists could have a potential as anti-obesity drug. However, β cell GIPR action is strongly correlated with β cell function and decreased in people with type 2 diabetes (T2D), which could potentially limit the use of GIPR antagonists as a therapy for T2D.

The object of the current studies was to investigate the impact of a GIPR antagonist on body weight and composition in mouse models of DIO, as well as how this intervention influences β cell function and glucose homeostasis.

First, we fed 8-week-old mice a high fat diet concomitant with a long-acting GIPR antagonist to test the intervention during weight gain. Antagonizing the GIPR reduced weight gain after 12 weeks, which was exclusively due to decreases in fat mass, and continued to be effective out to 20 weeks of treatment. Here, mice treated with the GIPR antagonist demonstrated improved glucose tolerance, without apparent changes in insulin sensitivity. Furthermore, treatment with the GIPR antagonist reduced food intake compared to control group.

Together, our findings indicate that long-term antagonism of the GIPR in rodent models of DIO can improve body composition and glucose tolerance, without significant impairment of β cell function.

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18 Burcak Yesildag

A Novel *In Vitro* Human Islet Platform for Studying Type 1 Diabetes

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Type 1 Diabetes is a heterogenous group of disorders majorly characterized by autoimmune destruction of pancreatic β-cells, resulting in absolute insulin deficiency. Current research models lack many functions critical for understanding the onset and progression of this disease in humans. Although isolated primary islets are considered the standard tool for diabetes research, their experimental use is challenging due to inherent heterogeneity in their size, cellular composition and purity, as well as rapid decline in their functionality and viability *ex vivo*. Here we developed two robust *in vitro* models of varying complexity to study T1D using standardized islets: a cytokine-induced islet dysfunction assay and an islet-peripheral blood mononuclear cell (PBMC) co-culture platform. Human islet microtissues, produced by optimized reaggregation of dispersed primary islet cells, were cultured in a one-islet per well format in 96-well plates. They displayed uniform, long-term (>28 days), and robust viability and function enabling high-throughput and longitudinal studies. Treatment of the islets with various concentrations of proinflammatory cytokines resulted in decreased stimulated and increased basal insulin secretion. At higher cytokine concentrations, intracellular insulin and ATP levels were significantly lowered. Next, PBMCs in their naïve form or following T-cell specific activation, were combined in various ratios with healthy or stressed islets - preconditioned with a mild cytokine cocktail. Co-culturing of naïve PBMCs and islet microtissues had only a minor impact on β-cell function. Whereas, combination of activated PBMCs and islet microtissues resulted in a PBMC-number dependent decline in islet health shown by increased basal insulin release and decreased glucose-stimulated insulin secretion, insulin content and PDX-1 positive nuclei within each microtissue. Observed destruction correlated with the amount of CD3+ cells infiltrating the microtissues and was significantly augmented by cytokine preconditioning. The established platforms represent novel *in vitro* models for the study of T1D and high-throughput compound screening for its treatment.

19 Carina (Kaiyuan) Yang

Characterization of embryonic pancreas development in pigs

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A better understanding of human pancreatic islet development is critical for *in-vitro* production of β-cells/islets from stem cells. Pig provides an excellent model, considering its comparable pancreas/islet morphology & biology and systemic metabolism/physiology to human, and 115-day-long gestational period. We aim to comprehensively characterize pig embryonic pancreas development, identifying the differences/similarities in key lineage-specific markers' temporal-spatial expression and pancreas morphology among species (mouse, pig and human).
Currently, pig embryonic pancreases at gestational day (G) 22, 32 and 40 were analyzed. Similar to both mouse and human, pig pancreatic epithelium at G22 was marked by PDX1, FOXA2, SOX9 and GATA4, with minimal CPA1 expression. During G22-G40, NKX6.1 gradually restricted to insulin-positive cells, whereas PAX6 became restricted to both insulin- and glucagon-positive cells.

Similar to mouse pancreas, NKX2.2 was detected at all three ages in most of PDX1-positive cells (pancreatic progenitors). At G22, glucagon was the most abundant hormone detected with only a few cells insulin-positive; at G32, insulin-positive cells increased while glucagon remained more prevalent; at G40, the numbers of insulin- and glucagon-positive cells were almost equal; all suggesting α-cells appear first in the pig embryonic pancreas like mouse.
Unlike mouse that tip cells (acinar-lineage) lose SOX9 expression during tip-trunk segregation, CPA1-postive (acinar-lineage) cells in pig embryonic pancreases expressed SOX9 at all three ages; while NKX6.1 decreased at G32 and was nearly absent at G40 from SOX9+CPA1+cells.This is similar to human pancreas development.

Cells within pig pancreatic epithelial were non-polarized at G22 and acquired apical-basal polarity at G32. Apparent microlumen structures formed at G40 which were surrounded by scattered insulin-/glucagon-positive cells. Earlier and later embryonic stages will be analyzed to identify the first appearance of insulin-positive cell and islet-structure.

In summary, our results showed the unique features of pig embryonic pancreas development compared to mouse and human. These data will be complimented with single-cell RNA-sequencing analysis for in-depth understanding of endocrine formation.

20 Carol Huang

Lrrc55 is a novel pro-survival factor in pancreatic islets

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Pancreatic islets adapt to the increase in insulin demand during pregnancy by upregulating β-cells number, insulin synthesis and secretion. These changes require prolactin receptor (PrlR) signaling, as mice with PrlR deletion are glucose intolerant with a lower β-cells mass due to blunted β-cells proliferation rate. Prolactin also prevents β-cells apoptosis. Many genes participate in these adaptive changes in the islet, and Lrrc55 is one of the most upregulated genes with unknown function in islets. Since Lrrc55 expression increases in parallel to the increase in β-cells number and insulin production during pregnancy, we hypothesize that Lrrc55 might regulate β-cells proliferation/apoptosis (thus β-cells number) and insulin synthesis. Here, we found that Lrrc55 expression was upregulated by >60-fold during pregnancy in a PrlR-dependent manner, and this increase was restricted only to the islets. Unexpectedly, overexpression of Lrrc55 in β -cells had minimal effect on β-cells proliferation and glucose-stimulated insulin secretion. However, Lrrc55 protected β-cells from glucolipotoxicity-induced reduction in insulin gene expression. Moreover, Lrrc55 protects β-cells from glucolipotoxicity-induced apoptosis, with up regulation of pro-survival signals and down regulation of pro-apoptotic signals of the ER stress pathway. Furthermore, Lrrc55 appears to attenuate ER calcium depletion induced by glucolipotoxicity, which may contribute to its anti-apoptotic effect. Hence, our findings suggest that Lrrc55 is a novel pro-survival factor that is up regulated specifically in islets during pregnancy and it prevents conversion of adaptive unfolded protein response to unresolved ER stress and apoptosis in β-cells. Lrrc55 could be a potential therapeutic target in diabetes by reducing ER stress and promoting β-cells survival.

21 Carol Yang

In vivo assessment of endocrine pancreas function following perturbed autonomic innervation

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Autonomic innervation has been implicated as an important modulator of pancreas development and function. *In vivo* analyses of the role of innervation should provide insight into the nerve-endocrine interactions. The zebrafish is well suited for *in vivo* studies of pancreatic islet development and function with time-lapse imaging due to its rapid embryogenesis and transparency during embryonic/larval stages. We have recently identified key cellular events preceding the onset of pancreatic innervation. Our studies revealed the establishment of a dense network of pancreatic innervation by four days post fertilization. With the model in hand, we are focused on deciphering the functional roles of selective neurons following neuromodulation. This objective is accomplished with complementary methods that cover a wide range of spatial and temporal control, including pharmacological regulation of neurotransmitter signaling, genetically induced silencing of neurotransmitter release and targeted-ablation of neurons. With simultaneous imaging of calcium dynamics in beta, alpha and delta cells, we can interrogate the function of different endocrine cell populations upon changes in innervation architecture and activity. When zebrafish larvae were exposed to elevated glucose, we observed an increase in cellular coupling between beta cells, as has been reported from islet cultures. Likewise, expected changes in alpha and delta cell calcium signaling were observed in response to glucose. Indeed *in vivo* imaging of calcium dynamics in our zebrafish pancreas model recapitulated the effects observed in mammalian islets. Our preliminary data suggest that both chronic and acute loss of nervous control results in disordered delta cell activity and diminished beta cell activity. While the majority of functional synchronization between beta cells is mediated through gap junctions, there is a subset of cellular coupling that remains intact upon inhibition of gap junction activity. This research can have a major impact on our understanding of pancreatic innervation dynamics, signaling and function.

22 Caroline Frørup

CATHEPSIN C AND D REGULATE CYTOKINE-INDUCED B-CELL DEATH

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Aim

We recently showed that cathepsin H, a lysosomal cysteine protease, regulates β-cell function and apoptosis in response to pro-inflammatory cytokines secreted by activated immune cells. This project investigates if other cathepsin family members modulate cytokine-induced β-cell apoptosis, and which cellular apoptosis pathways they regulate.

Material and Methods

Knockdown (KD) of cathepsin expression was performed in rat INS-1E and human 1.1B4 β-cell lines using siRNA and validated by real-time qPCR. β-cell death was investigated by various apoptosis assays in response to IL-1β, IFN-γ and TNF-α. Pro-apoptotic signaling pathways were analyzed by Western Blotting, real-time qPCR and nitric oxide production. CXCL10 was measured in culture media by Luminex technology.

Results

A screening of 7 cathepsins observed to be expressed in β cells found that KD of cathepsin C and D increased cytokine-induced apoptosis, e.g. caspase 3/7 activity ($siCtsc_{INS\text{-}1E}$: FC=1.8; $siCtsd_{INS\text{-}1E}$: FC=1.9, $p<0.05$; $siCTSC_{1.1B4}$: FC=1.9; $siCTSD_{1.1B4}$: FC=1.4, $p<0.01$). Cytokine-induced activation of the MAP kinase signaling pathway was modulated upon KD in INS-1E cells: increased phospho-JNK (siCtsc: FC=1.2, $p<0.05$) and phospho-p38 (siCtsc: FC=1.3, $p<0.01$), and decreased phospho-ERK (siCtsc: FC=0.7, siCtsd: FC=0.9, $p<0.05$). There was no effect on nitric oxide. Cytokine-induced *CXCL10* mRNA expression was increased by cathepsin C KD ($siCTSC_{1.1B4}$: FC=4.3, $p<0.001$). Preliminary data suggest that cytokine-induced CXCL10 secretion was also increased after cathepsin C KD ($siCTSC_{1.1B4}$: FC=1.9).

Conclusion

Cathepsin C and D are important for the survival of β cells in response to pro-inflammatory cytokines, due to their regulation of apoptosis signaling pathways, including the MAP kinases and *CXCL10*. Dysregulation of cathepsin C and D may facilitate the autoimmune-mediated destruction of the β cells during development of type 1 diabetes.

23 Celina Pihl

Degradation of proinsulin in GRP94 deficient β-cells

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Background:
Proper insulin production and subsequent secretion is imperative to maintain pancreatic β-cell function. In a recent paper, we showed that Glucose-Regulated Protein 94 (GRP94) is essential in proinsulin handling and turnover (Mojtabi et al., 2019). Upon GRP94 deficiency, proinsulin content in β-cells is reduced significantly. However, the underlying mechanism of this reduction remains unknown.
Our aim is to investigate which pathways contribute to the decrease of proinsulin in GRP94 deficient cells, and how inhibition of these might affect the turnover of proinsulin.

Methods:
To investigate this, GRP94 KO and parental INS-1E cells were used in the following experiments:

1. Pharmacological inhibition of insulin secretion (Brefeldin-A), autophagy (Bafilomycin A1), endoplasmic reticulum associated degradation (ERAD) (Kifunensine), standard and induced proteasome degradation (MG-132 and ONX-914 respectively) in efforts to examine its effects on restoration of proinsulin content.
2. siRNA mediated knockdown of the inducible proteasome subunit LMP7 in GRP94 KO cells.

Results:
Pharmacological inhibition for 3 or 6 hours using the aforementioned inhibitors did not lead to restoration of proinsulin content in GRP94 KO nor an increase in proinsulin levels in parental INS-1E cells. Inhibition of the proteasome using siRNA targeting inducible proteasome subunit LMP7 in GRP94 KO cells led to a restoration of proinsulin content, underlining a discrepancy between the effects of pharmacological and expressional inhibition of the proteasome in proinsulin handling.

The LMP7 subunit is constitutively expressed in INS-1E cells and further upregulated upon GRP9 KO (Marzec oral communication, unpublished data). Together with this, our findings indicate that LMP7 induction upon proinsulin misfolding, may be responsible for the decrease in proinsulin content upon GRP94 deficiency.

24 Claire Berthault

Characterization of mouse fetal endocrine development by cell surface markers.

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The pancreas plays a critical role in regulating metabolism, with endocrine and exocrine cell functions. It is composed of three functionally distinct cell lineages, derived from endoderm: acinar cells excreting digestive enzymes, ductal cells and hormone-secreting endocrine cells. These three lineages differentiate from multipotent epithelial pancreatic progenitors co-expressing the transcription factors Pdx1 and Nkx6.1, that proliferate in response to factors (such as FGF10) produced by the adjacent mesenchyme.

The identification of cells at intermediate stages of development is crucial for understanding the signals driving cell-fate choice and differentiation, however this has not been fully pursued in the developing pancreas.

The aim of this study is to reconstruct mouse fetal pancreatic development by identifying cell-surface marker signatures for endocrine and exocrine progenitors, at distinct stages of differentiation. By combining six surface markers and morphology parameters, we were able to discriminate, within the fetal pancreatic epithelial fraction, endocrine and exocrine enriched fractions. Moreover, we could further separate the endocrine fraction into four subsets, with high purity: *Ngn3*-expressing early endocrine progenitors, *ChgA*-positive and *Ngn3*-negative intermediate endocrine progenitors, a *Somatostatin*- and *Ghrelin*-positive fraction, and also an *Insulin*- and *Glucagon*-positive fraction.

We are currently performing cultures and transcriptomic analyses of the two prospectively selected endocrine progenitor subsets where commitment occurs. Such analyses will improve our understanding of the signals driving lineage choice, expansion and differentiation of pancreatic progenitors into the endocrine lineage.

25 Claire Lyons

Loss of melatonin receptor 1 (MTNR1A/Mt1) results in dysregulated hormone secretion and altered whole-body metabolism

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Disruptions in the circadian rhythm and melatonin can cause dysregulated metabolism. Impaired insulin secretory capacity, increased fasting glucose and T2D risk are associated with a single nucleotide polymorphism, mapping to the melatonin receptor 1B locus. However, knowledge regarding the melatonin receptor 1A (Mt1) is still lacking. Due to its location on the alpha cells, it may be involved in regulation of glucagon secretion. Islets were isolated from wildtype (WT) and melatonin receptor 1 knockout (*Mt1*$^{-/-}$) mice. Following a starvation period, the islets were stimulated with secretagogues and islet hormone secretion measured by ELISA. Plasma insulin, glucose and glucagon levels were determined following an intravenous glucose tolerance tests (IVGTT) in WT and *Mt1*$^{-/-}$ mice. *Mt1*$^{-/-}$ islets secreted increased glucagon in response to elevated levels of glucose (16.7mM) compared to WT, which responded with an inhibition of glucagon secretion. Interestingly, upon addition of arginine (10mM) to *Mt1*$^{-/-}$ islets, glucagon secretion was normalised, suggesting involvement of the plasma membrane. Islet insulin secretion was comparable between genotypes at both low (2.8mM) and high (16.7mM) glucose. Contrary to this, *Mt1*$^{-/-}$ mice display enhanced insulin secretion and reduced glucose clearance in the IVGTT. Despite a significant increase in basal plasma glucagon levels in the *Mt1*$^{-/-}$ mice, both genotypes responded similarly to the glucose challenge. Loss of functional Mt1 signalling can influence *in vitro* glucagon secretion. Additionally, *in vivo* insulin secretion and basal glucagon secretion are deregulated. Thus, loss of Mt1 signalling alters whole-body metabolism. Further studies, utilising fluorescently labelled alpha cells in *Mt1*$^{-/-}$ mice will be conducted to determine the mechanism of action of melatonin on islet function.

26 Claudiane Guay

Identification of Scrt1, a new transcriptional regulator of pancreatic β-cell maturation using chromatin accessibility variations

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Background: Glucose-induced insulin secretion, a unique feature of fully differentiated β-cells, is only acquired after birth and is preceded by a phase of intense proliferation. These events occurring in the neonatal period are critical for the establishment of an appropriate functional β-cell mass covering the insulin needs throughout life. However, key regulators of gene expression involved in the cellular reprogramming along maturation remain to be elucidated.

Methods: This project addressed this issue by taking advantage of a new methodology called ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) permitting a fine genome-wide mapping of chromatin accessibility. ATAC-seq assay was used to compare open chromatin regions in newborn versus adult rat β-cells. These regions were then correlated with the expression profiles of mRNAs to unveil the regulatory networks governing functional β-cell maturation.

Results: We obtained a genome-wide picture of chromatin accessible sites (~100'000) among which 10 % were differentially accessible during maturation. Half of these sites are in the proximity of genes displaying differential expression in newborn and adult rat islets. An enrichment analysis of transcription factor binding sites revealed that 35 transcription factors could explain these changes. While the importance of some of them, including REST, FoxO1 and JunB, is already known, the role of others remains to be determined. We focused on Scrt1 a transcriptional repressor whose expression is upregulated in adult islets. Downregulation of Scrt1 did not affect insulin secretion in response to glucose, but restored an elevated proliferation rate in adult β-cells, suggesting an involvement of this repressor in post-natal maturation.

Conclusions: In the present study, we found several known and unforeseen key transcriptional regulators acting at cis-regulatory sites and promoters which depicted a differential accessibility and induced differential gene expression along maturation. These findings could be of interest to induce maturation of surrogate insulin-producing cells.

27 Claudia Guida

PYY plays a key role in the resolution of diabetes following bariatric surgery in humans

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Background. Bariatric surgery leads to early and long-lasting remission of type 2 diabetes (T2D). However, the mechanisms behind this phenomenon remain unclear. Among several factors, gut hormones are thought to be crucial mediators of this effect. Unlike GLP-1, the role of the gut hormone peptide tyrosine tyrosine (PYY) in bariatric surgery in humans has been limited to appetite regulation and its impact on pancreatic islet secretory function and glucose metabolism remain under-studied.

Methods and Findings. Here we have examined the changes in PYY concentrations six months after bariatric surgery in obese patients compared with healthy control subjects, and their effects on human islet functions. By means of an experimental paradigm using donor human pancreatic islets ex-vivo and sera from obese patients with T2D before and after bariatric surgery, we demonstrate that PYY is a key effector of the early recovery of impaired glucose-mediated insulin and glucagon secretion in bariatric surgery. We establish that the short chain fatty acid propionate and bile acids, which are elevated after surgery, can trigger PYY release not only from enteroendocrine cells but also from human pancreatic islets and that these metabolites can affect islet secretory function. In addition, we identify IL-22 as a new factor which is modulated by bariatric surgery in humans and which directly regulates PYY expression and release.

Interpretation. This study shows that the metabolic benefits of bariatric surgery can be emulated *ex vivo.* Our findings are expected to have a direct impact on the development of new non-surgical therapy for T2D correction

28 Daniel Oropeza

Islet Endocrine Cell Dynamics Throughout the Spectrum of β-cell Deficit

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Gradual β-cell loss is a hallmark of diabetes. However, substantial heterogeneity in β-cell loss between diabetics at all disease stages suggests β-cell regeneration might occur continuously during diabetes progression. β-cell mass homeostasis is likely achieved by a long life-span combined with low proliferation rates. Yet upon β-cell loss in rodents, islet cell type interconversion, β-cell proliferation, and possibly progenitor differentiation can regenerate β-cells to varying degrees. Unfortunately, only one β-cell decrement or regenerative process was examined at a time. Therefore, comprehensive analysis of islet cell dynamics throughout the spectrum of β-cell deficit is needed to better understand β-cell regenerative mechanisms.

We developed a simple and highly reproducible transgenic model of gradual β-cell ablation by expressing human diphtheria toxin (DT) receptor in mouse β-cells. Injecting different DT doses to adult male and female mice caused increasing and stratified levels of hyperglycemia. Females always took longer to become hyperglycemic than males. Ten days after injection (10dpDT), pancreatic insulin content (PIC) gradually decreased with increasing DT doses (~30% up to ~99% decrease). OGTTs performed 10dpDT also revealed gradual increase in glucose intolerance and decrease in insulin secretion with increasing DT dosage. Strikingly, ~30% PIC loss causes mild glucose intolerance without hyperglycemia while ~50% loss causes significant glucose intolerance with mild hyperglycemia. After a loss of up to ~85% PIC both male and female mice can recover from hyperglycemia (without insulin therapy) in similar fashion, yet their weight gain patterns are strikingly different. At 10dpDT α- and β-cell proliferation was significantly activated in both males and females after a loss higher than ~75% PIC.

The DT-dose model is a valuable tool for β-cell research. We are quantifying reprogramming, dedifferentiation, proliferation and hypertrophy of β-, α- and δ-cells after gradual β–cell loss. Results will define the thresholds linking β-cell loss, glycemic dysregulation and activation of compensatory or regenerative mechanisms in islet cells.

29 Daniela Nasteska

Prolyl-hydroxylase 3 regulates oxygen-dependent β-cell phenotype under obesogenic conditions

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Background and aims: Prolyl-hydroxylase 3 (PHD3) is an α-ketoglutarate-dependent dioxygenase involved in oxygen-dependent cell responses. Its role in metabolic pathways regulating cancer cell survival has been studied, but little is known about PHD3 involvement in β-cell metabolism. We sought to establish a connection between PHD3 and insulin secretion in β-cell-specific PHD3 knockout mice.

Methods: We generated a β-cell-specific PHD3 knockout model (βPHD3$^{-/-}$) by crossing Ins1Cre knock-in mice with those harboring floxed alleles for *Egln3*, which encodes PHD3. Body weight and glucose/insulin tolerance were measured *in vivo* under normal diet (ND) and high fat diet (HFD) conditions. *In vitro* experiments included: qPCR, Western Blot, insulin secretion, measurement of β-cell Ca^{2+}, cAMP, ATP/ADP and GC-MS-based glucose-derived metabolite tracing.

Results: βPHD3$^{-/-}$ mice were phenotypically normal under ND. Insulin secretion, Ca^{2+} fluxes, cAMP levels, ATP/ADP ratios and glucose metabolites remained unchanged *in vitro*. After 4 ($p<0.01$) and 8 weeks of HFD ($p<0.05$), glucose tolerance was impaired in βPHD3$^{-/-}$ mice. However, *in vivo* and *in vitro* insulin secretion was unexpectedly increased in 4 week HFD βPHD3$^{-/-}$ mice. Glucose tracing revealed more incorporation of $^{13}C_6$ into m+3 lactate, indicating decreased entry of glycolitically-derived Acetyl-CoA into the tricarboxylic acid cycle. Hinting at a switch to beta-oxidation, glucose-stimulated insulin release ($p=0.0512$) was further improved in βPHD3$^{-/-}$ islets incubated with the saturated fatty acid palmitate for 72 hours. By contrast, βPHD3$^{-/-}$ islets isolated at 8 weeks HFD showed both reduced Ca^{2+} fluxes ($p<0.05$) and insulin secretion ($p<0.01$) in response to glucose.

Conclusion: PHD3 loss causes metabolic remodelling in the early stages of metabolic stress (4 weeks HFD) by diverting fuel source from glycolysis to beta-oxidation, thus enabling β-cells to use stored lipids as a primary energy source to boost insulin release. However, this metabolic shift is overwhelmed as fatty acids accumulate (8 weeks HFD), leading to β-cell failure.

30 Dassine Berdous

A genetic screen in recombinant inbred mice identifies Crat as a new regulator of insulin secretion

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Pancreatic ß cells secrete insulin in response to a rise in extracellular glucose concentration to maintain normoglycemia. This secretory response is potentiated by glucagon-like peptide1(GLP1). Impaired insulin secretion is central in the onset of type 2 diabetes, a disease with a multifactorial and polygenic origin. Here, we aimed at identifying novel regulators of glucose stimulated insulin secretion (GSIS) and exendin-4 potentiation using a genetic screen in recombinant inbred mice.

We isolated pancreatic islets from a panel of 36 recombinant inbred BXD mice and characterized their insulin secretion and expressed transcripts by RNAseq. Combining QTL mapping and islet gene expression allowed us to identify six genes, on chromosome 2, whose expression was correlated to insulin secretion in response to high glucose and exendin-4. Crat was the most correlated gene (R=0.67, pvalue=6.4E-6). Moreover, a cis eQTL was found at the same location than Crat, suggesting a local gene regulation. Carnitine O-acetyltransferase (Crat) is a mitochondrial enzyme catalyzing the interconversion of acetyl-CoA and acetylcarnitine. It was extensively studied in muscle mitochondria but its role in ß-pancreatic cells is unclear. In addition, as mitochondria play a pivotal role in metabolism, many metabolic disorders are related to mitochondrial dysfunctions.

Our results showed that both transient and stable knockdown of Crat in ß-pancreatic cell lines (Min6B1 and ß-Tc-tet) reduced GSIS. Furthermore, Crat overexpression in those cell lines enhanced insulin secretion. Further, preliminary experiments suggested that cells overexpressing Crat oxidized more glucose than control cells. Currently, we are also generating mice with specific deletion of Crat in ß-cells, using Crat Ins1cre mice to characterize the role of Crat *in vivo*.

The identification of Crat as a new positive regulator of insulin secretion will allow a better understanding of ß cell function, which in turn may provide new therapeutic targets for type 2 diabetes.

31 Decio L. Eizirik

The RNA-binding landscape of SRp55 and its impact in human β-cells

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Purpose: Master splicing factors modulate functionally related transcripts into regulatory networks. Our group has previously shown that *GLIS3*, a candidate gene for both types 1 and 2 diabetes, modulates β-cell apoptosis via regulation of the splicing factor SRp55. In the present study we aimed to identify the global SRp55-regulated splicing networks and decode the SRp55 functional RNA binding elements in human β-cells.

Methodology: Human insulin-producing EndoC-βH1 cells were subjected to RNA sequencing (RNAseq) (control conditions or following SRp55 knock down (KD) for 48h) to identify transcriptomewide alternative splicing events regulated by SRp55, and individual-nucleotide resolution UV crosslinking and immunoprecipitation followed by high-throughput sequencing (iCLIP) to identify SRp55-RNA binding sites. Bioinformatics/integration tools were used to identify direct RNA targets and infer a position-dependent regulatory model. Targeted biological experiments using specific siRNAs validated the RNA-seq findings in both EndoC-βH1 cells and human islet cells.

Results: RNA-seq evidenced that SRp55 KD modifies the splicing of 4055 different genes in EndoC-βH1 cells. Initial comparison against the iCLIP data indicated that 1279 of these genes are directly bound by SRp55. SRp55 crosslinking sites occurs preferentially on exonic regions. Functional enrichment analysis showed that these genes are involved in regulation of cell survival, insulin secretion and JNK signalling. Additional experiments, based on KD of the SRp55-modulated splice variants, indicated that SRp55 KD augments expression of the pro-apoptotic variants BIMs and BAXβ and exacerbates JNK signalling. The parallel KD of SRp55 and BIMs, BAXβ or JNK prevented, at least in part, the β-cell apoptosis secondary to SRp55 KD, confirming that BIMs, BAXβ and JNK are mediators of β-cell apoptosis following SRp55 KD. The extended comparison between the SRp55 binding profile and confirmed splicing events is presently under validation.

Conclusions: SRp55, a splicing regulator downstream of the diabetes candidate gene GLIS3, is a master splicing factor in human pancreatic β-cells. The integration between RNA-seq and CLIP-seq data should unveil the global SRp55 regulatory map in human ®-cells.

32 Denise Berti

Enrichment of Insulin producing cells derived from hESCs large scale cultures using novel cell surface markers

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Despite the gradual improvement in islet transplantation procedures achieved in the last decades, the limiting factor for the wide acceptance of this procedure remains the low numbers of available islets for transplantation. Therefore, alternative abundant sources for islet transplantation are needed. This has shed light on the usage of human Pluripotent Stem Cells (hPSCs) as a potential renewable source for insulin producing cells. Based on their almost unlimited proliferation capabilities and their potential to differentiate into all cells of the three primary germ layers, hPSCs are attractive candidates for cell replacement therapy for diabetes. Utilizing embryonic developmental pathways, hPSCs were differentiated into pancreatic and specifically into insulin producing cells. The newly formed Islet-Like-Clusters (ILCs), grown in dynamic suspension conditions, contain a heterogeneous population of poly hormonal, pancreatic progenitors and insulin producing cells. In the present work, we identified a cell surface markers combination that can potentially enrich for the relevant insulin producing cells population. Using MACS technology, the enriched population was tested both in vitro and in vivo. In vitro, sorted ILCs exhibited an improved mature pancreatic gene expression profile, demonstrating reduced levels of alpha-fetoprotein (AFP) and cytokeratin 19 (CK19), in addition to increased levels of insulin and transcription factors MafA, and NKX6.1. In vivo, sorted ILCs were macro encapsulated and transplanted under the skin of SCID-Beige mice. Seven days after transplantation, human insulin secretion was detected at physiological levels. Interestingly, the sorted ILCs transplanted group presented superior activity when compared to the non-sorted ILCs control group. Therefore, this strategy can be used to improve ILCs efficiency at the same time that it reduces the therapeutic mass, required for the encapsulation devices loading capacities.

33 Elaine Cowan

Nudix Hydrolase 2 (Nudt2) Is Critical For Physiological Glucose Stimulated Insulin Secretion From Ins-1 832/13 β–Cells

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Defective insulin secretion from pancreatic β-cells has been suggested as the ultimate trigger for type 2 diabetes (T2D). Using a bioinformatical approach we have identified NUDT2, a nudix hydrolase that hydrolyses Ap4a (an energy rich nucleotide) to yield ATP, as a potential target to improve β-cell function. Previous research shows *Nudt2* interacts with transcription factors, important for physiological β-cell function, and, Ap4a has been implicated in regulating KATP channel activity in β-cells. These findings strongly suggest that NUDT2 and Ap4a, are involved in β-cell function. Here, we investigate effects of *Nudt2* knockdown in clonal β-cells. Rodent INS-1 832/13 β-cells were transfected with *Nudt2* small interfering (si)RNA's (150nM). Protein and mRNA knockdown were subsequently determined by western blot and RT-qPCR, respectively, while functional evaluation of *Nudt2* silencing was performed (siRNA2 only) by assessing insulin secretion responses to low glucose (2.8mM), high glucose (16.7mM), pyruvate (10mM) and K^+ (35mM). Insulin content was also quantified in NUDT2-deficient cells. NUDT2 protein and mRNA were potently reduced upon silencing ((91%(siRNA1) & 87%(siRNA2); P<0.0001; n=2 (72h)) and ((72%(siRNA1) & (82%(siRNA2); P<0.01 & P<0.001; n=5(48h)) respectively. Insulin secretion (ng insulin/mg total protein/h) was significantly inhibited at 16.7mM glucose (30±7 vs 63±9; n=5; P<0.01), 10mM pyruvate (35±3 vs 65±7; n=3; P<0.01) and 35mM K^+ (25±5 vs 52±8; n=3; P<0.05) thereby highlighting the importance of NUDT2 for β□cell function and demonstrating NUDT2 mechanisms heavily involve mitochondrial metabolism and influence of insulin exocytosis. Insulin content was also significantly reduced (45%; n=5; P<0.001) suggesting possible effects on insulin gene expression and biosynthesis. In summary, our findings show that *Nudt2* knockdown results in loss of function in INS-1 832/13 cells. This is indicated by a severely diminished ability of silenced cells to produce and secrete insulin. Clearly, targeting NUDT2 (and its mechanisms) is a potentially promising strategy for improving β-cell function in T2D.

34 Elizabeth Haythorne

Hyperglycaemia adversely affects mitochondrial function in pancreatic islets

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Aims: Metabolically generated ATP is essential for glucose-stimulated insulin secretion from pancreatic beta cells. There is some evidence to suggest that beta cell mitochondrial metabolism is impaired in type 2 diabetes mellitus (T2DM), and contributes to reduced insulin secretion. As hyperglycaemia is common to all forms of diabetes, we aimed to determine if hyperglycaemia adversely affects mitochondrial function in pancreatic islets.

Methods: We utilised the βV59M mouse model in which hyperglycaemia (>20mmol/l) can be initiated via a tamoxifen-inducible K_{ATP} channel activating mutation in pancreatic beta cells. Oxygen Consumption Rate (OCR) was monitored using the extracellular flux analyser (Seahorse Bioscience, Inc.). A fluorescent sensor for Mg^{2+} (Mg-Green, ThermoFisher) was used as a surrogate for ATP.

Results: Islets from diabetic mice displayed an attenuated OCR response when ambient glucose was raised from 2 to 20mmol/l (diabetic = 48.44 ± 8.98 vs control = 91.29 ± 7.26% increase in OCR, $p<0.005$; n=9-12, 6 animals/genotype). Oligomycin produced significantly less inhibition of OCR in islets from diabetic mice compared to control, indicating hyperglycaemia reduces the activity of ATP-synthase (diabetic = 88.72 ± 6.43 vs control = 123.05 ± 5.59 % decrease in OCR, $p<0.05$; n=5-7, 3 animals/genotype). ATP increase in response to 20mmol/l glucose was reduced in diabetic islets by 56 ± 6% (control = 1147 islets, diabetic = 423 islets).

Conclusions: Our results demonstrate that hyperglycaemia leads to impaired mitochondrial respiration and ATP production in pancreatic islets. Mitochondrial metabolism is essential for the stimulation of insulin secretion by glucose and therefore hyperglycaemia-induced mitochondrial dysfunction is likely to contribute to beta cell failure in T2DM.

35 Emily McGaugh

Using hESC to model pancreatic patterning and specification

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Human embryonic stem cells (HESC) can be differentiated to pancreatic progenitors (PPs) and to β-like cells, offering a potential therapy for patients with Type 1 Diabetes (T1D). Despite progress in directed differentiation, development of functional β-cells from hESCs is inefficient due to poor yields and poor glucose responsiveness. I speculate that primitive gut tube patterning and pancreatic specification may impact the successful differentiation of HESC-derived β-cells. To test this hypothesis and identify signalling pathways important for pancreas development, I mined our mass spectrometry database to identify growth factor receptors expressed by PPs. Fibroblast Growth Factor Receptor 3 (FGFR3) was identified as a marker exclusively expressed by PPs and its expression pattern was validated by QPCR and flow cytometry. FGFR3 was detected from stages 2-4 of differentiation, during PDX1 endoderm and NKX6.1+ induction. Interestingly, mouse studies indicate that Fgfr3 signalling may inhibit Pdx1+ expansion, but its role in human pancreas development remains unknown. Based on these data, I hypothesize that FGFR3 inhibits human pancreatic development and by using FGFR3 inhibitors at specific developmental stages I can improve β-cell differentiation.

As expected, activation of FGFR3 at stage 3 of differentiation using FGF2, FGF4 and FGF9 resulted in a significant decrease in the percentage of stage 4 PDX1+NKX6.1+ PPs and stage7 NKX6.1+CPEP+ β-like cells. Importantly, the percentage of PPs can be restored using small molecule inhibitors and shRNA for FGFR3, suggesting that FGFR3 may indeed inhibit human pancreas development. Furthermore, FGF2 treated cells express higher levels of the foregut marker, SOX2, suggesting that FGFR3 stimulation may direct hESC differentiation towards foregut lineages at the expense of a pancreatic phenotype. In summary, these data suggests that FGFR3 signaling may play a role during patterning of the primitive gut tube and that inhibition of endogenous FGFR3 signalling could lead to improved pancreatic differentiation in vitro.

36 Emmanuel Ampofo

Erythropoietin pretreatment accelerates revascularization of transplanted pancreatic islets

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Background/Purpose: Pancreatic islet transplantation is a promising approach for the treatment of diabetes type 1. A major prerequisite for the survival of grafted islets is their rapid revascularization after transplantation. Erythropoietin (EPO), the primary regulator of erythropoiesis, promotes angiogenesis. Therefore, we investigated in this study whether EPO pretreatment improves the revascularization of transplanted islets.

Methods: Islets were isolated from FVB/N-TgN (Tie2/GFP) donor mice and syngeneically transplanted into dorsal skinfold chambers of wild type recipient animals. The mice were pretreated for 6 days with EPO (500 IU) or vehicle and the revascularization of transplanted islets was analyzed by repetitive intravital fluorescence microscopy over 14 days. Finally, the dorsal skinfold chamber tissue was excised and the formation of new blood vessels within the grafts was analyzed by additional immunohistochemical stainings.

Results: Pretreatment of mice with EPO significantly accelerated the revascularization and engraftment of the transplanted islets, as indicated by a significantly higher take rate as well as an enhanced functional capillary density and endocrine revascularization when compared to controls. The immunohistochemical analyses revealed an increased density of microvessels in islets of EPO-pretreated animals on day 6. In addition, EPO pretreatment caused vasodilation during the first 6 days. Of note, pretreatment of mice with EPO did not elevate the hematocrit levels of the animals.

Conclusions: EPO pretreatment effectively accelerates the revascularization of transplanted islets without increasing systemic hematocrit levels. These novel findings indicate that EPO pretreatment may represent a promising therapeutic approach to improve the clinical outcome of islet transplantation.

37 Esteban Gurzov

Stat3 Contributes To Pancreatic Beta Cell Adaptation In Obesity

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Background: While reduction of β-cell mass and function can be observed in obese diabetic patients, most obese and insulin resistant individuals do not develop diabetes. This is the result of the capacity of β-cells to adapt and produce enough insulin to cover the needs of the organism. The underlying mechanism of β-cell adaptation in obesity, however, remains unclear. Previous studies have suggested a role for STAT3 in mediating β-cell development and function, but little is known about its role in β-cell adaptation in obesity.

Methods: To address the functional role of STAT3 in adult β-cells, we generated a tamoxifen-inducible STAT3 β-cell specific knockout model in MIP-CreERT transgenic mice (βSTAT3KO mice) and fed them a high fat diet before analysis.

Results: We treated βSTAT3KO homozygous, heterozygous and control mice with oral gavages of tamoxifen (6 mg/mouse for 3 times every other day) at 10 weeks of age to induce complete or 50% STAT3 gene deletion respectively. The mice did not show any metabolic phenotype (glucose homeostasis) after 14 weeks when fed a chow diet (n=5-9). We challenged βSTAT3KO and control mice with a high fat diet as model of obesity. Body weight, oxygen consumption, respiratory exchange rate, energy expenditure, food intake and ambulatory activity were similar in 12 weeks high fat fed βSTAT3KO and control mice (n=8-14). Interestingly, homozygous and heterozygous βSTAT3KO mice showed glucose intolerance in oral and intraperitoneal glucose tolerance tests when compared to controls (n=10-14, $p<0.05$-0.001). The serum insulin concentration in βSTAT3KO mice was 2.1-fold lower than in control mice after glucose administration (n=9, $p<0.05$). No difference was observed in percentage insulin area between βSTAT3KO and control mice, which suggests no changes in β-cell mass. Genetic analysis showed reduced (30-35%) expression of mitochondrial genes *Nd4*, *Nd5* and *Cytb* in FACS-purified β-cells from βSTAT3KO mice (n=4-5, $p<0.05$). We then knocked down STAT3 with siRNAs in human EndoC-βH1 cells and found impaired mitochondria activity (n=4, $p<0.05$-0.01), suggesting a mechanism for STAT3-regulated β-cell function.
Conclusion: Our results implicate STAT3 in mediating β-cell adaptation in obesity. We propose a novel role of STAT3 in the regulation of mitochondrial activity during glucose induced insulin secretion in β-cells.

38 Estela Lorza Gil

Differential expression and activation of FFA2 and FFA3 determines the effect on insulin secretion

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Glucose is the main metabolic parameter regulated by insulin but it is not the sole regulator of insulin secretion. To understand molecular mechanisms, rodent models are used allowing the maintenance of genetic background, age, sex and environmental factors. Nevertheless, the translation to the human situation remains often elusive. Contradictory effects of short chain fatty acids (SCFA) on insulin secretion have been observed using genetically modified mice and specific FFA2 and FFA3 receptor agonists. In mice, SCFAs inhibit glucose-stimulated insulin secretion (GSIS) through FFA3/GPR41, but stimulate or inhibit GSIS through FFA2/GPR43. This study aims to clarify the regulation of GSIS by SCFAs.
In isolated mouse islet cells both receptors are expressed.

Using FACS sorted GFP-positive beta-cells and mT-marked non-beta-cells we detected FFA2 mRNA in beta and non-beta cells. FFA3 was expressed predominantly in beta cells although at lower levels. In isolated mouse islets SCFAs had no significant effect on insulin secretion. The FFA2 agonist 4-CMTB stimulated GSIS at 10 µM, but inhibited GSIS at 100 µM. The FFA3 agonists TUG-816 and 1-MCPC had no effect on secretion. In INS-1E cells, a rat cell line expressing mainly FFA3, SCFAs and FFA3 agonists inhibited insulin secretion. This effect was abrogated by siRNA against FFA3. In human pseudoislets, mRNA levels of FFA2 were higher than of FFA3. However, the SCFAs effect on GSIS was less uniform. Thus, in three human islet preparations SCFAs stimulated secretion, in other three SCFAs had no effect, and in one preparation they inhibited GSIS. The FFA2 agonist 4-CMTB inhibited secretion at 100 µM, but at lower concentrations (1-10 µM) was effectless. The inhibitory effect of 4-CMTB correlated with FFA2 expression level. The FFA3 agonists did not affect secretion.

These results suggest that the relative expression of FFA2 and FFA3 in beta-cells determines stimulation or inhibition of GSIS by SCFAs.

39 Evgeniy Panzhinskiy

Overexpression of eukaryotic translation initiation factor 2A (eIF2A) in pancreatic beta cells attenuates diabetes in Akita mice

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Endoplasmic reticulum (ER) stress is a key mechanism mediating beta-cell apoptosis. Previously, we demonstrated that overexpression of an alternative translation initiation factor eIF2A is up-regulated as part of the unfolded protein response and protects beta cells *in vitro* from ER stress-induced apoptosis. Therefore, we investigated the protective mechanism of eIF2A in beta cells *in vivo* using mice that carry the 'Akita' *Ins2* allele that leads to spontaneous diabetes due to ER stress-induced apoptosis. For beta-cell specific overexpression, we designed adeno-associated virus 6 (AAV6), encoding either eIF2A-GFP or control GFP and driven by an insulin promoter. Virus was injected into the pancreatic duct of 6-week old *Ins2*$^{Akita/WT}$ female mice randomized into two groups. *Ins2*$^{Akita/WT}$ mice given GFP-control AAV6 showed increased fasting blood glucose levels with age, but this increase was attenuated by eIF2A overexpression in beta cells. *Ins2*$^{Akita/WT}$ mice with eIF2A overexpression had lower fasting blood glucose levels compared to GFP control mice at 3 weeks (11.5±1.0 vs 17.0±0.8 mmol/l) after AAV6 injections. *Ins2*$^{Akita/WT}$ mice overexpressing eIF2A also showed significantly improved glucose tolerance when compared with controls (AUC 1631±140 vs 2222±85). Overexpression of eIF2A in *Ins2*$^{Akita/WT}$ led to increased insulin secretion post glucose challenge (0.73±0.09 vs 0.47±0.07 ng/ml) in comparison to control mice. qPCR analysis revealed a decrease in mRNA levels of pro-apoptotic ER stress marker CHOP in pancreatic islets isolated from *Ins2*$^{Akita/WT}$ mice with eIF2A overexpression compared to controls. To uncover the molecular mechanisms behind protective effects of eIF2A overexpression in unbiased way, we performed the total RNA sequencing of MIN6 cells overexpressing eIF2A and exposed to ER stressor thapsigargin. We identified that eIF2A overexpression lowered amount of aldehyde dehydrogenase 1a3 mRNA, that was recently linked to β cells failure in diabetic mice. We conclude that overexpression of eIF2A in pancreatic beta cells preserves beta cell function during ER stress.

40 Federica Fantuzzi

Use of 3D culture systems to generate human induced pluripotent stem cell-derived β-cells *in vitro*

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Background and aims. Pancreatic β-cell failure is central in the pathogenesis of diabetes. *In vitro* differentiation of human induced pluripotent stem cells (hiPSCs) into β-cells is a novel cell source in diabetes research, allowing for instance mechanistic studies of β-cells in monogenic forms of diabetes. This differentiation requires suspension culture, which is technically challenging and results in heterogeneously sized aggregates with central necrosis in large ones. Here we developed and implemented 3D culture in size-controlled microwells to uniformize maturity and function of hiPSC-β-cells.

Methods. hiPSCs were differentiated into β-cells as previously described[1]. Up to the pancreatic progenitor stage cells were cultured on Matrigel-coated plates, after which they were transferred either to rotating suspension culture or to microwells. Key markers of β-cell development were assessed by qPCR and immunocytochemistry. hiPSC-β-cell function was assessed by glucose- and forskolin-stimulated insulin secretion.

Results. The success rate of 3D culture was higher in microwells (100%) than in suspension (40%), due to formation of large cell clumps in the latter. The suspension aggregates were larger and more heterogeneously sized (230-600 μm) compared to microwell aggregates (75-120 μm). Successfully transferred hiPSCs differentiated with similar efficiency in microwell and suspension aggregates, as determined by mRNA expression. The yield of insulin-positive β-cells was higher in microwell aggregates (43% versus 33%, $p<0.05$) and there were fewer glucagon-positive α-cells (5% versus 13%, $p<0.005$). Aggregates obtained by both culture systems did not augment insulin release in response to glucose (16.7 mM versus 1.7 mM), but high glucose plus forskolin induced insulin secretion by 3-4-fold ($p<0.001$).

Conclusions. Microwell culture allowed improved and more homogeneous hiPSC-β-cellsdifferentiation and aggregate formation, as compared to suspension culture. The higher experimental success rate of microwells reduces costs and, pending additional research, will provide an invaluable supply of human β-cells for mechanistic studies of β-cell failure in diabetes.

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41 Fernando Abaitua

Evaluation of platforms for karyotype assessment of human Induced Pluripotent Stem Cell diabetes models post CRISPR-Cas9 genome-editing

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Advances in *in vitro* differentiation protocols for Induced human pluripotent stem cells (iPSC) coupled with genome-editing offers new opportunities for exploring the impact of diabetes associated genetic variants on islet cell development and function. Clonal selection post genome-editing is known to lead to intrinsic genomic instability in iPSCs, which means that quality control steps are essential before differentiating the edited clones. We selected 37 clones from three independent iPSC lines (SB, KOLF-C2 and SFC854) including several CRISPR-edited clones and their parental cell lines and used a variety of methods (chromosome counting, KaryoLite BoBs [PerkinElmer], CytoSNP [Illumina], hPSC Genetic Analysis Kit [StemCell Technologies] and our "homemade" CNV qPCR-based system) to assess karyotype.

We evaluated both cost and complexity of the analysis. CytoSNP provided the most comprehensive analysis but was expensive (£185/sample; 23 samples) and analysis was technically challenging. Although the KaryoLite BoBs kit was user friendly (39 samples were analyzed with a cost of £24/sample), it required a specialized analyser and was vulnerable to issues with technical reproducibility. For both the commercial and in-house qPCR-based methods (£18 and £7 per sample respectively), there were reproducibility and control-bias issues due to DNA quality, which required alternative DNA extraction methods to resolve. The low-resolution and throughput chromosome counting approach (£15/sample) was the most reliable method, but required optimization of cell density plating. In terms of concordance, the best was between CytoSNP and chromosome counting, where all the clones with aberrant regions detected by CytoSNP also exhibited abnormal chromosome counting. KaryoLite failed in most cases to identified clones with abnormal chromosome counting, though in two cases detected abnormalities in clones that had normal chromosome counting.

Our data support the use of chromosome counting for routinely assessing karyotypes post genome-editing and the use of CytoSNP if an in-depth analysis is required for selected samples.

42 Frankie Poon

Deciphering the signaling pathways driving β-cells from human pluripotent stem Cells

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Type 1 diabetic patients suffer from hyperglycemia due to the lack of insulin-producing β-cells. Human pluripotent stem cells (hPSCs) represent a potential infinite source of transplant-suitable β-like cells. However, the differentiation process has proven to be challenging, as current *in vitro* protocols are characterized by low yields and poor efficiency. To ensure the safety of these hPSC-derivatives for future therapeutic uses, it is critical to be able to produce β-like cells in a reproducible and efficient manner. Using a 6-stage protocol, our lab has previously demonstrated that nicotinamide (NA), in combination of EGF and Noggin, can efficiently induce NKX6-1 expression in PDX1+endoderm to generate pancreatic progenitors (PPs) at stage 4. These PPs can be further differentiated into insulin-producing β-like cells at stage 6. In this study, we demonstrated that NA can be replaced by tankryase inhibitors (TI) to generate PPs at similar efficiency. Interestingly, these TI-derived PPs gave rise to higher percentages of β-like cell population with higher INS expression. Further characterization confirmed that *in vitro* generated TI-derived aggregates at stage 6 consisted of similar endocrine cell type distribution and maturity compared to NA. Subcutaneous transplantation of TIderived PPs into immunocompromised mice was used to assess TI-derived PP developmental potential. Strikingly, H&E and immunofluorescence stainings revealed the presence of multiple islet-like structures, with minimal exocrine contribution, suggesting TI-derived PPs maybe prone to enter endocrine lineage. Interestingly, transcriptional profiling of TI-derived PPs indicated higher expression of integrin genes compared to NA-derived PPs. All together, these results showed the importance of tankyrases during the NKX6-1 induction step and implicated integrin signaling as a possible driving force for endocrine lineage commitment.

43 Gaurav Verma

Role of DIMT1, a potential methyltransferase, role in insulin secretion in pancreatic β-cells

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Background and Aims: Bioinformatics and comparative biology led us to identify *DIMT1* (Dimethyladenosine Transferase 1 Homolog), exhibiting 30% homology with *TFB1M* (Transcription Factor B1, Mitochondrial), expressed in human islets and regulated by glucose. The role of *DIMT1* is unknown in β-cells but it could act as a methyltransferase of 18S ribosomal RNA.

Aim: To resolve the unknown function of *DIMT1* in pancreatic β-cells.

Materials and Methods: Cultured INS 832/13 and EndoC-βH1 cells were transfected with scramble or *DIMT1* siRNA (50–200 nM) and the knockdowns were confirmed with qRT-PCR and western blotting. Insulin secretion was measured using ELISA kit, mitochondrial parameters were visualized using fluorescent microscopy and protein synthesis was measured using Puromycin-based protein synthesis kit.

Results: At 50 to 200nM siRNA, we observed 40-70% depletion of *DIMT1* mRNA levels and 60-80% of proteins levels. All knockdown experiments (n=5), were found to be highly significant (***p<0.0001). We further observed reduction in insulin secretion from 1800 ng/mg protein/h in control cells to 800 ng/mg protein/h in siRNA treated cells (n=3,***p<0.001). We also found altered mitochondrial function in siRNA treated cells (n=3, ***p<0.001). In addition, we also found 40% reduced protein synthesis in *DIMT1* siRNA treated cells as compared to control one suggesting its role in translational machinery.

Conclusion: We identified DIMT1, expressed in human, rodent islets and clonal β-cells. Preliminary data suggest DIMT1-deficiency causes mitochondrial dysfunction and perturbs β-cell function. As of yet, we have not identified a mechanism for this β-cell dysfunction, but we are currently identifying targets for DIMT1 activity in β-cells.

44 Gerard Gradwohl

Role of the winged helix transcription factor Rfx6 in islet and enteroendocrine cell differentiation and function

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Homozygous mutations in the winged helix transcription factor RFX6 have been identified as the cause of Mitchell-Riley syndrome (MRS) which is characterized by neonatal diabetes, intestinal atresia, and intractable diarrhea. Heterozygous RFX6 protein truncating variants have also been associated with MODY. Our goal is to decipher RFX6 function by disease modeling in mice. Like in MRS patients, beta cells do not develop properly in Rfx6-/- embryos leading to diabetes in neonates, supporting conserved function of Rfx6 in mouse and human. By conditional inactivation of Rfx6 in beta cells, we revealed that Rfx6 is important for insulin secretion and beta cell identity in the adult. We have now identified Rfx6 bound regions at the genome level in mouse islets and determined whether they map to active or poised enhancers. Rfx6 acts as transcriptional repressor or activator and only 20% of Rfx6 regulated genes are direct targets of Rfx6 in beta cells. Rfx6 directly and positively regulates *Mlxipl/Chrebp* transcription by binding to an enhancer in intron 1. MRS RFX6 mutant proteins fail to transactivate *Mlxipl* suggesting that *Mlxipl* is downregulated in patient's beta cells. Rfx6 is also expressed in the gut endoderm and subsequently restricted to intestinal endocrine progenitors and their hormone+ descendants. We found gastric heterotopia in the small intestine of Rfx6-deficient embryos but no intestinal atresia. Both in the embryo and adult, the differentiation of peptidic enteroendocrine (PEPC) cells, and hormone production is impaired, explaining intestinal malabsorption, while the number of Serotonin producing enterochromaffin cells (EC) is increased. Combining single cell and bulk RNA-Seq, we identified Rfx6 regulated genes in PEPC and EC lineages. Our study provides unprecedent information on Rfx6 function and Rfx6-dependent gene regulatory networks in the pancreas and intestine and thus on possible mechanisms of diabetes and intestinal failures in human Rfx6-defiencies.

45 Grace Yu

Causal genetic variant identified in the *PROX1* regulatory region using large-scale GWAS is associated with increased risk of type 2 diabetes and alteration in gene expression

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Genome wide association studies of type 2 diabetes (T2D) have identified multiple independent association signals near *PROX1*. The primary association signal at this locus is represented by a non-coding variant, rs340874-C (risk allele frequency 56%). Fine-mapping analysis of this signal in over 900k Europeans ascribed causality to a single variant, rs340874 (>99% posterior probability of association). This was further confirmed using a larger trans-ethnic meta-analysis of >1million individuals (~50% non-Europeans). The underlying functional mechanism at this locus, however, still remains unknown.

To understand the plausible mechanism of how rs340874 influences T2D-risk, we examined quantitative trait associations using publicly available summary statistics. The risk-allele C was associated with higher levels of fasting glucose (p=4x10-14) and HbA1c (p=3x10-11), and lower HOMA-B (p=3x10-5) indicating that variant likely affects beta cell function. Integration with epigenome maps (based on histone post-translational modification) of tissues relevant to T2D we mapped the variant to a promoter (characterized by H3K27ac and H3K4me3 histone marks) of PROX1 in both human pancreatic islets and liver. In islets, this variant also overlapped open chromatin (ATAC-seq) and DNA hypomethylated sites. We further determined the relative expression level of PROX1 across multiple tissues and found that PROX1 is highly expressed in the liver and islets.

Finally, we evaluated the functional impact of rs340874 on luciferase reporter gene expression. We sub-cloned a 304bp genetic fragment encompassing rs340874 into the pGL3-promoter vector and accessed its impact *in vitro* using transient transfection assays in two cell models; hepatocytes (HepG2) and pancreatic β–cells (EndoC-βH1). Luciferase assays showed increased rs340874-C allele transcriptional activity in both HepG2 (40% increase, p=0.002) and EndoC-βH1 cells (26% increase, p=0.03) compared to the non-risk rs340874-T allele.

In conclusion, our results implicate *PROX1* in mediating the T2D association in this region, with higher PROX1 expression in the liver and islets contributing directly to T2D susceptibility.

46 Guy A Rutter

Deletion of the tumour suppressor Liver kinase B1 (LKB1/STK11) leads to altered β-cell identity and impaired intercellular connectivity: a single cell transcriptomic analysis

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Introduction: The role of the tumour suppressor and Ser/Thr protein kinase LKB1, and the downstream kinase AMP activated protein kinase (AMPK), in the control of insulin secretion, are incompletely understood. We have previously reported (Kone et al, FASEB, J, 2014) that deletion of LKB1 highly selectively from the β cell leads to changes in β cell identity, and an up-regulation of non-canonical pathways of glucose-stimulated insulin secretion. Here, we perform RNASeq and connectivity analyses at single cell resolution to understand these changes.

Methods: Islets were isolated from 12 week old male C57BL/6 mice carrying either floxed *Lkb1* alleles alone (WT), or the latter plus the *Cre* recombinase gene (βLKB1KO) introduced at the *Ins1* locus (Thorens *et al,* Diabetologia, 2015). Isolated islets were dispersed and single cells subjected to RNA sequencing (RNASeq) on the 10x Genomics platform. Analyses were run using the R package Seurat and RNA trajectories with Monocle. Ca^{2+} imaging and connectivity analyses (Pearson R) were as in (Hodson *et al*, JCI, 2013).

Results: Graph-based clustering of endocrine cells from both wild-type and LKB1-deleted islet cells revealed six clusters (numbered 0-5). LKB1-deleted islets contributed fewer cells to the β-cell cluster (cluster 3) than wild-type islets. LKB1 deletion also moved cells within both the α cell (from 0 to 1) and δ cell (from 2 to 4,5) clusters. Pseudotime ordering revealed a trajectory with a central node and three branches, with βLKB1KO cells enriched in the tips. Ca^{2+} imaging revealed significantly lowered cell-cell coordination during stimulation of βLKB1KO versus WT islets with either 17 mM glucose ($p<0.01$) or 20 mM KCl ($p<0.05$).

Conclusions: LKB1 deletion changes the identity of β and, unexpectedly, other islet cells and is associated with impaired intra-islet coordination. Altered LKB1 or AMPK signalling may contribute to defective intercellular coordination and insulin secretion in type 2 diabetes.

47 Haopeng Lin

SENP1 in Compensation and Failure of Insulin Secretion with High-Fat Diet

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Background: Pancreatic β -cells adjust their ability to secrete insulin according to metabolic status. Functional up-regulation of β -cell insulin secretion may precede increases in islet mass in mice on high fat diet (HFD). We previously identified the enzyme SENP1 as a key factor that amplifies insulin secretory responses by deSUMOylating protein targets at the exocytotic site. Interestingly, increasing SENP1 activity rescues insulin exocytosis in β -cells from human donors with type 2 diabetes (T2D). However, the involvement of SENP1 in the regulation of β -cell functional compensation, and decompensation in T2D, remains unclear.

Methods: We studied β -cells and islets of human donors, and wild-type (WT) or islet-specific SENP1 knockout mice (iSENP1-KO) on chow and high fat diet (HFD) for between 2 days and 4 weeks. We measured glucose tolerance, insulin secretion, single-cell exocytosis, and intracellular Ca^{2+} responses. We also performed structure-function studies of recombinant SENP1 activity and redox-sensing.

Results: In humans without diabetes, the exocytotic capacity of β-cells is increased at elevated BMI (>25), but this is only apparent in cells from young donors (<45 years). In WT mice fed HFD for 2-days (before any change in islet mass or glucose tolerance), and up to 4 weeks, plasma insulin responses and in vitro insulin secretion is up-regulated. This is not due to increased β-cell Ca^{2+} responses, but rather to a cell-autonomous increase in β-cell exocytotic capacity. This response is dynamic and dependent on cellular redox state. We find that SENP1 expression is increased in islets of HFD mice, where it acts as a redox-sensor to control β-cell exocytotic capacity. Structure-function studies demonstrate that oxidation/reduction of a key cysteine residue (C535) near the SENP1 catalytic site modulates enzyme activity and exocytotic function. Finally, iSENP1-KO mice lack compensatory increases in β-cell exocytotic capacity and show rapidly worsened glucose tolerance following short term HFD.

Conclusion: β-cells up-regulate their capacity to mount an exocytotic response upon metabolic challenge. This requires SENP1 as a redox-dependent mediator of compensatory insulin secretion during HFD in mice due its ability to amplify insulin exocytosis.

48 Helena Sork

Purification of extracellular vesicles from cell culture medium and plasma to study virus-induced ß-cell damage in type 1 diabetes etiology

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The mechanisms by which beta-cell injury and an autoreactive immune response arise in type 1 diabetes susceptible individuals is still not fully understood, yet increasing evidence suggests the involvement of enteroviruses. Considering the inaccessibility of the target organ and scarcity of research-oriented tissue samples, an extended knowledge on the viral component in diabetes progression and onset could be gained from non-invasive sampling of diseased individuals, virus-infected animal models and cell culture material. During the past decade interest has increased in cell-derived extracellular vesicles (EVs), playing a central role in cell-to-cell communication both in physiological as well as pathophysiological conditions. In addition to the molecular information of the cell-of-origin, EVs are known to carry and spread pathogen-derived molecules, including viral macromolecules. Here we present a selection of methods which can be used for the enrichment of EVs from cell culture medium (including human pancreatic ß-cell culture), the cultures of pancreatic islets and plasma from type 1 diabetes mouse models, at pre- and postinfection with Coxsackieviruses. In-depth analysis of this bioactive extracellular material could advance our understanding on the role of enteroviruses in type 1 diabetes development and can hopefully provide therapeutic targets to reduce virus induced ß-cell loss.

49 Honey Modi

Imaging *Ins2* gene activity and single-cell RNA sequencing reveal heterogeneous β-cell states

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Background: Functional β-cell heterogeneity is well established, including cellular specialization for islet synchronization, insulin secretion, insulin production, and marker gene expression. Previous work from our group identified dynamic states marked by fluorescent proteins driven by the promoters of insulin and Pdx1. In order to study β-cell heterogeneity of insulin production with more accuracy, we turned to an *Ins2*^{GFP} knock-in/knockout mouse line, and other knock-in alleles. Here, we used live-cell imaging and single cell RNA sequencing to characterize heterogeneous β-cell states.

Materials and methods: Based on our preliminary data showing heterogeneity of GFP expression in the *Ins2*^{GFP} knock-in islets, we crossed this line with Ins1-mCherry transgenic mice that are known to show relatively stable mCherry expression in >90% of β-cells. Dispersed islet cells from the resulting double-mutant *Ins2*$^{GFP/wt}$:Ins1-mCherry were studied over ~3 days using ImageXpress^{MICRO} live-cell, high-throughput imaging systems. Single cell RNA sequencing, using the 10X Genomics platform, was performed on FACS purified GFP-positive and GFP-negative β-cells from younger as well as older *Ins2*^{GFP} knock-in/knockout mice.

Results: Analysis of pancreatic tissue sections from *Ins2*^{GFP} knock-in mice showed that, at any given time, only about half of all β-cells were robustly GFP-positive, suggesting that not all β-cells have active transcription at the *Ins2* locus *in vivo*. FACS analysis confirmed *Ins2* mRNA and pre-mRNA were increased in GFP-positive cells. *In vitro* perifusion of islets isolated from *Ins2*$^{GFP/GFP}$ knock-in/knockout mice showed reduced insulin secretion at 20 mM glucose, compared to heterozygous *Ins2*$^{GFP/Wt}$ knock-in/knockout mice and control *Ins2*$^{Wt/Wt}$ mice. Live-cell imaging of dispersed cells from *Ins1*^{mCherry}:*Ins2*$^{GFP/wt}$ mice revealed that GFP fluorescence flashed on and off in a sub-set of cells, suggesting bursts of transcription at the *Ins2* gene locus rather than stable heterogeneity. Using Cell Profiler software and custom R scripts, we tracked individual cell GFP activity and found that 153 out of 547 cells show flickering GFP activity. Principal component analysis identified 3 distinct clusters of *Ins2* gene activity cell behaviours. Single-cell RNA sequencing on FACS purified GFP-positive and GFP-negative population from islets isolated from young as well as old homozygous *Ins2*$^{GFP/GFP}$ and heterozygous *Ins2*$^{GFP/Wt}$ knock-in/knockout mice identified significantly up-regulated, and down-regulated genes between low-GFP and high-GFP β-cells, which further gives insight about molecular features of this β-cell state.

Conclusion: Our results demonstrate the *Ins2*^{GFP} knock-in mice are a useful tool for studying β-cell heterogeneity, state transitions and plasticity. Understanding the dynamics of insulin production has relevance for understanding the pathobiology of diabetes and for regenerative therapy research.

50 Hyeongseok Kim

Lactation improves glucose homeostasis by enhancing β cell mass and function through serotonin production

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Parous women who did not breastfeed has increased risk of diabetes compared to nulliparous women, but this risk is normalized by lactating for more than 3 months. Human epidemiologic studies also support the beneficial effects of lactation. However, the mechanism of the beneficial effects of lactation has not been thoroughly evaluated. In the present study, we established lactating mouse model to analyze metabolic changes by lactation. Female C57BL6/J mice that gave birth were randomized to either lactating or non-lactating whose pups were immediately removed at birth. Glucose tolerance and β cell features were evaluated at postpartum 3 weeks. We observed that lactating mice had improved glucose tolerance and increased β cell mass compared to non-lactating mice. Lactating mice maintained high serum prolactin levels for milk production, which induced serotonin production in β cell via prolactin receptor - STAT5 - tryptophan hydroxylase 1 (TPH1) signaling cascade. Similar to pregnancy increased β cell serotonin facilitated β cell proliferation and enhanced β cell function. In addition, serotonin protected β cells from oxidative stress. Metabolic improvements and β cell features by lactation were diminished by β cell specific *Tph1* knockout. In conclusion, serotonin contributes to the beneficial metabolic effects of lactation by enhancing β cell mass and function.

51 Ines Cebola Cebola

Coupling of 3D enhancer-promoter interaction maps with genome editing uncovers novel diabetes regulatory links

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Genetic variants associated with type 2 diabetes (T2D) risk are often located within pancreatic islet active transcriptional enhancers. Due to the complex nature of transcriptional enhancers, which can regulate very distal genes and be bidirectional, assigning T2D-associated variants to true disease susceptibility genes has remained a challenge. Using promoter-capture Hi-C (pcHi-C), we have created a map of 3D interactions between genes and distal sequences, which allowed us to link >80% of diabetes-associated enhancer variants with putative target genes, including genes located hundreds of kilobases away. Further analysis of the 3D interactions also revealed that islet enhancers that carry T2D-associated variants often reside within 3D *enhancer hubs*, regulating multiple genes in the same cell population. In this study, we validated 10 T2D-related enhancer-promoter assignments using CRISPR/Cas9-mediated deletions, as well as CRISPR inhibition and CRISPR activation of distal enhancers. This study reveals human islet 3D chromatin architecture coupled with genome editing as a powerful framework for interrogation and interpretation of T2D GWAS signals.

52 Izortze Santin Gomez

The T1D-associated lncRNA *Lnc13* modulates pancreatic β cell inflammation by allele-specific stabilization of *STAT1* and *STAT2* mRNA.

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The vast majority of type 1 diabetes (T1D) association signals lie in non-coding regions of the human genome and many have been predicted to affect the expression and secondary structure of lncRNAs. However, the contribution of these lncRNAs to the development of the disease and the molecular mechanisms by which these molecules may contribute to the pathogenesis of T1D remain to be clarified.

Preliminary results of our Group have demonstrated that the expression of several T1D-associated lncRNAs in pancreatic β cells is modified by diabetogenic stimuli, such as viral infections and pro-inflammatory cytokines. Of special interest are the results obtained in the functional characterization of *Lnc13*, a lncRNA expressed and upregulated by viral dsRNA in pancreatic β cells that harbors a SNP associated with T1D (rs917997).
Overexpression of *Lnc13* in the human Endoc-βH1 cell line led to an increase in STAT1/2 pathway activation that correlated with increased production of pro-inflammatory chemokines. Interestingly, when the *Lnc13* harboring the risk allele for T1D (rs917997*C) was transfected, the increase in STAT1/2 signaling was more pronounced than in cells transfected with the plasmid encoding the *Lnc13* with the non-risk allele for T1D (rs917997*T). In addition, the effect of *Lnc13* upregulation on pro-inflammatory chemokine production was also allele-specific.

Our studies have shown that intracellular PIC induces *Lnc13* translocation from the nucleus to the cytoplasm, promoting the interaction between *STAT1* and *STAT2* with a protein named PCBP2 that regulates the stability of *STAT1* and *STAT2* mRNA molecules.

In conclusion, our results show that *Lnc13* participates in pancreatic β-cell inflammation via regulation of the STAT1/2 signaling pathway in an allele-specific manner. The STAT1/2 signaling pathway plays a crucial role in T1D-related β cell dysfunction, so the fact that *Lnc13* affects the activation of this pathway supports its potential functional implication in the pathogenesis of the disease

53 Jessica Chaffey

The protein phosphatase inhibitor, PPP1R1A, is differentially expressed in the islets of individuals with T1D, dependent on duration of disease and age at diagnosis

Aims: Increasing evidence implicates a persistent enteroviral infection as a potential trigger for the development of Type 1 diabetes (T1D). Protein Phosphatase 1, Regulatory, Inhibitory Subunit 1A (PPP1R1A) controls the activity of MDA5, a viral dsRNA sensor. Our aim was to study PPP1R1A expression in islets of non-diabetic controls, and individuals with T1D, to establish whether alterations in PPP1R1A correlates with islet autoimmunity.

Methods: Formalin-fixed, paraffin-embedded pancreas samples from 6 newly diagnosed individuals with T1D (3 diagnosed <7 years, 3 diagnosed >13 years) and 2 individuals (diagnosed >13) with longer duration of T1D were immunostained for PPP1R1A, insulin and glucagon, alongside 8 similarly aged controls (range 3-25 years). Expression of PPP1R1A was analysed in accordance to insulin and glucagon staining in each case.

Results: In controls, PPP1R1A expression was abundant, and restricted to β-cells. By contrast, in newly diagnosed individuals' <7y at onset, PPP1R1A was markedly depleted from a subset of β-cells but retained in most. Among individuals >13y at diagnosis, PPP1R1A was lost from almost all β-cells and the α-cells remained PPP1R1A negative. In individuals who were diagnosed with T1D >13y but who had a longer duration of T1D (mean 4.25years), PPP1R1A remained absent from β-cells but, surprisingly, was clearly expressed in many α-cells.

Conclusions: PPP1R1A is selectively expressed at high levels in the β-cells of non-diabetic individuals. In people with T1D, PPP1R1A expression is altered in an age and disease duration-dependent manner, first depleted from β-cells and then expressed in α-cells. These changes may contribute to the selective establishment of a persistent enteroviral infection in β-cells by reducing the effectiveness of anti-viral signalling in β-cells while enhancing the resistance of α-cells to infection.

54 Jessica Hill

Immunomodulatory protein expression in Type 1 Diabetes – are the islets fighting back?

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The pathophysiology of type 1 diabetes (T1D) remains uncertain although it is known that beta cell loss is associated with islet inflammation (insulitis). This involves the infiltration of lymphocytes and the development of a dialogue between the resident beta cells and these infiltrating immune cells. To gain an improved understanding of this process, we have examined the expression of key immunomodulatory proteins on islet endocrine cells and lymphocytes.

Pancreas sections from subjects with recent-onset T1D and control subjects of similar ages were studied. Sections were probed with antisera directed against relevant immunomodulatory molecules (PDL-1, PD1, HLA-E and IRF1) and islet hormones. Immunopositivity was evaluated by immunofluorescence microscopy. The expression of PDL-1 on cultured EndoC-βH1 cells was also studied using total cell extracts and extracellular vesicles harvested from the cell culture medium.

PDL-1 was absent from control islets but expressed abundantly in a subset of islets from patients with T1D. Expression of PDL-1 correlated with the intensity of insulitis, suggesting that the upregulation of PDL-1 represents a response of the beta cells to the development of an inflammatory milieu. PDL-1 was also seen on some alpha cells in inflamed islets although the majority did not express this protein. The presence of PDL-1 correlated with marked increases in IRF-1 within the nuclei of a subset of beta cells. PDL-1 was also detected by Western blotting in lysates of EndoC-βH1 cells treated with interferon-alpha and it was shed from these cells in extracellular vesicles. HLA-E was upregulated in the insulin-containing islets (ICIs) of T1D vs controls, with highest expression in alpha cells, although some beta cells were also stained.

The increased expression of immunomodulatory molecules by islet endocrine cells in T1D could represent a protective response which tempers the activity of activated immune cells during islet infiltration.

55 Joan Mir

A Novel Standardized *In Vitro* Model For Highly-Efficient and Homogeneous Viral Transduction of Pancreatic Islets

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Modification of gene expression in human pancreatic islets can be a powerful strategy for dissecting the pathophysiological mechanisms involved in the onset and progression of type 1/type 2 diabetes and represents a valuable tool for the research of novel therapeutic strategies. However, access to pancreatic endocrine cells to modify gene expression results challenging due to the highly compact structure of pancreatic islets. Here we describe a newly developed method for adenoviral transduction of reaggregated 3D InSightTM human islet microtissues. This platform represents a highly efficient tool for homogeneous *in vitro* genetic modifications of pancreatic endocrine cells. An adenoviral vector encoding for three different fluorescent reporters from a single back-bone was used to label human endocrine cells during three different experimental windows: during cell dispersion, reaggregation and post reaggregation at 3 increasing MOI. Transduced 3D InSightTM human islet microtissues were cultured in one-islet per well format and their insulin secretory function, insulin content and cell viability were evaluated. 3D confocal microscopy was used for quantification of transduction efficiency and viral penetration. High transduction efficiency (>75 %) was achieved during cell dispersion and cell reaggregation, which displayed high uniformity between the core and the periphery of the islets. Approximately 80-95 % of transduced cells were endocrine cells, from which 50-63 % corresponded to β-cells. Islet microtissues exhibiting high transduction efficiency displayed reductions in chronic (35-50 %), basal (55-62 %) and stimulated (65-75 %) insulin secretion, while maintaining a significant fold induction in response to glucose, as well as unaltered insulin and ATP content. In summary, here we present a method for efficient genetic manipulation of functional reaggregated islets by viral transduction as a novel tool for diabetes research.

56 Jocelyn Manning Fox

Open Access Sharing of Human Islet Donor Characteristics and Functional Parameters.

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Human islets are a valuable resource for the study of islet cell biology in heath and diabetes, and are in increasingly high demand[1]. The Alberta Diabetes Institute (ADI) IsletCore serves the research community by isolating, distributing, and biobanking human islets from deceased organ donors for research purposes. In addition, the ADI IsletCore and its collaborative network perform quality assurance and phenotyping on each islet preparation, including isolation, transcriptomic, and functional parameters.

The importance of accessible and transparent collecting and reporting of human islet donor characteristics and phenotyping has recently been highlighted[2] and is now a requirement for publication in major journals in the field. To this end, the ADI IsletCore has created a publicly accessible and searchable website to display data from its 300+ human islet preparations. Open access information from individual preparations, including basic donor information and quality measures, are easily searchable. The latter, including cold ischemia times, purity, insulin secretion and end user feedback, is shown in relationship to the entire data set for rapid evaluation of preparation quality.

Registration with an academic email address allows deeper database access. This includes further donor information, isolation technical parameters, quality assessment, and detailed insulin content/secretion values assayed at 4 glucose concentrations. Registration also provides access to a biobank inventory search function, allowing users to view the availability of paraffin-embedded tissue/islet blocks, cryopreserved islets, and snap frozen islets with user-defined donor characteristics.

Finally, as a pilot study we are examining the utility of this platform for the decentralized deep phenotyping of individual human islet preparations by allowing remote integration of data that includes transcriptomic datasets, dynamic secretion assays, and gene panels, into this pipeline. With the public release of this website, we will improve accessibility to human islet donor information and actively support the reporting of these for publication. In addition, we provide a medium for the sharing of functional phenotyping data within the islet biology community and a user-friendly tool for searching the ADI IsletCore's biobank inventory.

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57 Jonathan Baldan

Human pancreatic acinar cell plasticity – potential for endocrine neogenesis?

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Rodent pancreatic acinar cells retain differentiation plasticity *in vitro* and *in vivo* and showed reprogramming efficiency towards insulin-producing beta cells. In contrast, human pancreatic acinar cell plasticity was less well investigated. We found that acinar-to-ductal metaplasia and epithelial-to-mesenchymal transition occur when human acinar cells are cultured in 2D monolayer culture. In contrast, reprogramming towards an embryonic $CD142^+GP2^+PDX1^+SOX9^+$ cellular state can be appreciated in 3D suspension culture. Exocrine cells present at start of culture rearrange themselves as pancreatospheres, i.e. smooth spheroids composed of $CA19.9^+$ duct-like and $CD142^+GP2^+$ embryonic-like cells. The acinar origin of the observed embryonic-like $CD142^+GP2^+PDX1^+SOX9^+$ cells was robustly confirmed by acinar-specific lineage tracing using FITC-conjugated UEA1 lectin, FACS sort, qRT-PCR and immunofluorescent analysis. Co-expression of CD142-GP2 surface markers and PDX1-SOX9 transcription factors were only observed during human foetal development *(Ramond, C. et al. 2018).* Furthermore, addition of Alk5iII, a TGF-beta signalling inhibitor, induced transition from a quiescent to an active proliferative state in the embryonic-like $CD142^+GP2^+PDX1^+SOX9^+$ cell population, i.e. 28-fold increased KI67 labelling. TGF-beta, Notch and BMP signalling pathways are involved in the differentiation of pancreatic progenitors towards endocrine cells as inhibition induced differentiation towards a beta-like cell phenotype (*Rezania, A. et al. 2014*). We observed, at day 4 of 3D suspension culture, increased phosphorylated Smad2 signalling in the pancreatospheres, which was abrogated by the addition of Alk5iII. Inhibition of the Notch signalling, by addition of the gamma secretase inhibitor compound E, inhibited the proliferative effect of Alk5iII indicating activated Notch signalling in the TGF-beta inhibitor condition. Furthermore, Notch inhibition induced increased BMP2 transcription. This indicates an interplay between TGF-beta, Notch and BMP in the 3D-cultured exocrine fraction. It remains to be demonstrated whether manipulation of these signalling pathways can induce “chemical” reprogramming of acinar cells to endocrine cells.

58 Jonathan Esguerra

Glucocorticoid induces human beta cell dysfunction and involves the riborepressor GAS5 lincRNA

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Glucocorticoids (GCs) are highly-potent class of steroid hormones in the frontline of various clinical therapy procedures due to their anti-inflammatory and immunomodulatory properties. However, a widely recognized metabolic side-effect of GC therapy is steroid-induced diabetes mellitus (SIDM), in which rapid onset of hyperglycemia is observed in up to 80% of patients receiving high-dose GC treatment. Furthermore, the incidence of new onset diabetes mellitus (DM) in these patients is estimated to be ≈50%. The diabetogenic effects of GC have been mainly ascribed to its adverse action in liver, muscle, adipose and bone tissues collectively manifested as dyslipidemia, insulin resistance and glucose intolerance. However, studies on the molecular basis of GC-induced pancreatic beta cell dysfunction in human beta cells is lacking. Here, we report on the deleterious effect on insulin secretion in risk patients undergoing chronic high dose GC therapy, as opposed to augmented insulin secretion observed in GC-treated healthy individuals. More importantly, we used human islets and the human beta cell line, EndoC-βH1, to demonstrate the involvement of long intergenic non-coding RNA (lincRNA) GAS5 in GC-mediated beta cell dysfunction. Modulation of GAS5 in the human beta cell alleviated the GC-induced insulin secretion defect demonstrating the potential of this non-coding RNA as a novel therapeutic target in countering GC-mediated beta cell dysfunction.

59 Jonna M Saarimäki

MANF knockout results in impaired function of stem-cell derived human beta cells in humanized mice

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MANF is an endoplasmic reticulum (ER) stress inducible protein highly expressed in secretory cells, such as beta cells. MANF-KO mice develop severe diabetes due to impaired expansion and increased apoptosis of beta-cells, associated with elevated ER stress. Exposure of human islets to cytokines induces MANF expression, while exogenous recombinant MANF protects beta-cells from apoptosis. MANF expression, along with unfolded protein response markers (BIP), is upregulated in patient iPS cells-derived beta-cells carrying a mutation causing INS misfolding.

To further study the role of MANF in developing beta-cells, we engineered MANF-KO human pluripotent stem cells by deleting *MANF* exon 1 using CRISPR-Cas9. MANF-KO cells differentiated to the pancreatic lineage similarly as WT control cells in vitro, consistent with the postnatal diabetic phenotype of the KO-mice. To study the MANF-KO impact in functional mature human beta-cells, in vitro-differentiated (stage 6- 7) islet-like clusters were implanted under the kidney capsule of immunodeficient mice. The grafts excised at 3-4 months after implantation presented increased BIP immunoreactivity in INS positive cells. Blood glucose levels of mice implanted with both WT and KO cells decreased to human levels (appr. 4 mM). Interestingly, MANF-KO implanted mice presented reduced glucose tolerance compared to controls, a difference that was exacerbated after eradication of mouse beta cells using streptozotocin. This was associated with a significantly lower increase in human C-peptide secretion in response to glucose challenge (AUC p=0.009, n=7 in both groups) and significantly decreased glucose clearance (AUC p=0.0008). Graft removal resulted in immediate hyperglycemia in all transplanted mice, validating graft functionality in our humanized mouse model. Studies on beta-cell ER-stress, proliferation and apoptosis in these grafts are ongoing.

These preliminary results further highlight the important role of MANF in modulating ER-stress levels for proper beta-cell expansion and functionality, especially as their workload increases.

60 Joseph P Harris

The impact of activating AMP-activated kinase on insulin secretion from the pancreatic beta cell

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Aims: AMP-activated protein kinase (AMPK) is a hetero-trimeric (α, β and γ subunit) Ser/Thr protein kinase that is activated by a decrease in energy availability. Dysregulation of AMPK contributes to insulin resistance and type 2 diabetes. Whilst activation of the enzyme in extrapancreatic tissues improves glucose homeostasis (Cokorinos et al, Science, 2017), the effect of activating AMPK in the pancreatic beta cell is still debated. Here, we investigated the effects of the pharmacological AMPK activators PF-06409577 (β1 isoform-selective) and RA089 (β1 and β2 binding) on glucose-stimulated insulin secretion (GSIS) *ex vivo*.

Method: Islets were isolated from wild type C57/BL6 mice by collagenase digestion and GSIS assessed during static incubations (30 min.) in modified Kreb's-Ringer medium containing 3 or 17 mM glucose, or 30 mM KCl. Ca^{2+} dynamics were assessed using the intracellular dye fluo-8, and confocal imaging.

Results: PF-06409577 significantly augmented GSIS at 5μM and 25μM (2 fold, $p<0.005$ and 1.7 fold, $p<0.05$) whereas no differences were observed in response to KCl at any concentration tested. Activation of AMPK with RA089 resulted in significantly higher GSIS at 25μM (1.7 fold, $p<0.005$), and an increased response to KCl at 25μM RA089 (1.7 fold, $p<0.005$). Neither activator significantly affected glucose-induced Ca^{2+} dynamics at concentrations of <5μM, though 25μM RA-089 significantly slowed the time to peak.

Conclusion: PF-06409577 and RA089 significantly increase GSIS, indicating that the activation of either β1 or β2-containing AMPK complexes enhances insulin release, without increasing Ca^{2+} influx. The possible mechanisms involved, and the physiological ramifications, will be discussed.

61 Jovana Vasiljevic

Post-transcriptional co-regulation of insulin and secretory granule protein expression in β-cells

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Pancreatic β-cells produce insulin and store it within insulin secretory granules (SGs). Hyperglycemia stimulates SG exocytosis and insulin release to restore normoglycemia, and also initiates SG biogenesis by increasing the transcription, stability and translation of *Insulin* mRNA. However, in the first two hours following glucose stimulation, upregulation of insulin production occurs without changes in its mRNA levels, pointing to the importance of post-transcriptional mechanisms for insulin expression

Post-transcriptional regulation relies on proteins binding to regulatory elements in mRNAs. Several such elements are conserved in human, mice and rat *Insulin* transcripts. Moreover, functionally related mRNAs can be co-regulated through sequences recognized by the same RNA binding proteins (RBPs). Previously, we proposed that SG protein expression is post-transcriptionally coordinated. However, an overview of RBPs for mRNAs coding for SG cargoes was missing.

Therefore we combined *in vitro* RNA pull-downs and mass spectrometry to identify RBPs binding to mouse *Insulin1, Insulin2,* spliced *Insulin2, PC2* and *ICA512* mRNA 5'-UTRs, as well *γ-tubulin* mRNA 5'-UTR, in resting and stimulated MIN6 cells.

We discovered several RBPs that are shared among the tested transcripts for SG cargoes and enriched compared to the control *γ-tubulin* mRNA. Notably, the sets of RBPs which control mRNAs for SG proteins in resting and glucose stimulated cells were distinct.

One of the novel RBPs for *Insulin* mRNA was hnRNP A2/B1. We identified sequences homologous to hnRNP A2/B1 response elements (A2REs) within the mouse *Insulin1* mRNA 5'-UTR that are partially conserved in human and rat *Insulin* transcripts. Mutation of A2REs in the 5'-UTR of *Insulin1* mRNA reduced its binding to hnRNP A2/B1. *Hnrnpa2b1* knockout MIN6 cells displayed reduced *Insulin1* mRNA, proinsulin and insulin levels, and insulin secretion. These results point to hnRNP A2/B1 as a likely regulator of *Insulin1* mRNA stability. Further studies will expand on the mechanisms of post-transcriptional regulation in β-cells.

62 Juha Torkko

ICA512 RESP18 homology domain is protein condensing factor and insulin fibrillation inhibitor

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Type 1 diabetes islet cell autoantigen ICA512)/IA-2/PTPRN is a catalytically inactive receptor tyrosine phosphatase involved in the biogenesis and turnover of insulin secretory granules (SGs) in the β-cells of the pancreatic islets. Whereas the ICA512 proximal luminal ectodomain has been functionally and structurally characterized, the role of its preceding N-terminal luminal segment, termed regulated endocrine-specific protein 18-homology domain (RESP18HD) encompassing amino acids 35-131 remains largely unknown. Here we show that ICA512 RESP18HD C-terminal motif including residues 91-131 contains an intrinsically disordered region (IDR), which is critically required for RESP18HD function as a pH and Zn^{2+} dependent condensation and reversible aggregation factor for insulin and other β-cell proteins. At variance with other SG cargoes with aggregating properties, the condensing activity of ICA512 RESP18HD is displayed at pH values close to neutral, as found in the early secretory pathway, while being resolved at acidic pH and in the presence of high Zn^{2+} concentrations, as found in mature insulin SGs. Moreover, we show that the ICA512 RESP18HD portion including amino acids 35-90, i.e. the region preceding the IDR, inhibits insulin fibrillation in vitro. Finally, we show that glucose-stimulated release of ICA512 RESP18HD from the SGs is associated with proteolysis of its IDR, conceivably to prevent its aggregation upon exposure to neutral pH in the extracellular milieu. Taken together, these findings point to ICA512 RESP18HD being a condensing factor for SG protein sorting and granulogenesis early in the secretory pathway and for prevention of insulin fibrillation.

63 Kailun Lee

Deletion of Xbp1 in β-cells causes decompensation and diabetes during high fat feeding in mice.

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Pancreatic β-cells hypersecrete insulin to maintain normoglycaemia under metabolic stress conditions in a process called β-cell compensation. The role of endoplasmic reticulum (ER) stress and its associated unfolded protein response (UPR) in β-cell compensation is not clear. The transcription factor XBP1 regulates the adaptive UPR, which acts to alleviate ER stress by increasing protein folding capacity. The aim of this study was to examine the role of XBP1 in β-cell compensation. We generated β-cell-specific XBP1 knockout (β-Xbp1$^{-/-}$) mice by crossing Xbp1^{flox} and Pdx1-Cre^{ER} mice. Control (β-Xbp1$^{+/+}$) and β-Xbp1$^{-/-}$ mice were fed chow or high-fat diet (HFD) for 4 weeks. Compared to β-Xbp1$^{+/+}$ mice, β-Xbp1$^{-/-}$ mice fed HFD displayed increased fasting blood glucose levels and severe glucose intolerance. Isolated islets from β-Xbp1$^{-/-}$ mice fed HFD displayed reduced glucose-stimulated insulin secretion and insulin content. Furthermore, the expression of genes important for β-cell function and the adaptive UPR were reduced, whereas pro-apoptotic UPR (*Trib3*), proinflammatory cytokine and macrophage gene expression was increased. This was associated with increased β-cell apoptosis in β-Xbp1$^{-/-}$ mice fed HFD. Moreover, defective proinsulin processing in β-Xbp1$^{-/-}$ mice fed HFD was indicated by elevated serum proinsulin and decreased prohormone covertase-2 (*PC-2)* expression. To investigate the mechanisms, β-Xbp1$^{+/+}$ and β-Xbp1$^{-/-}$ islets were cultured *ex vivo* under metabolic stress conditions induced by chronic (72h) palmitate (0.4mM) and high glucose (25mM) treatment. Apoptosis was potentiated in β-Xbp1$^{-/-}$ islets in association with reduced antioxidant gene expression, suggesting a diminished response to oxidative stress. The potentiated cell death in β-Xbp1$^{-/-}$ islets was prevented by co-treatment with an antioxidant, N-acetyl-cysteine. These studies suggest that XBP1 is required for β-cell compensation by regulating the UPR, β-cell function and survival and the antioxidant response. XBP1 may protect against type 2 diabetes by promoting insulin secretion and β-cell survival during metabolic stress.

64 Katia Mattis

Using genome-edited human beta-cell models to unravel the role of the transcription factor RREB1 in islet cell development and function

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Human genetics can aid us in understanding Type 2 diabetes (T2D) pathogenesis. Genome-wide association studies (GWAS) have identified several independent signals for T2D risk at the *RREB1* locus. *RREB1* encodes the Ras-responsive element binding protein-1, a zinc finger transcription factor (TF) which binds to RREs of gene promoters. The biology driving the association signals is currently unknown, as the role of RREB1 in islet-cell development and glucose homeostasis is largely unexplored.

To investigate whether RREB1 contributes to beta-cell development and/or function, we used both human induced pluripotent stem cells (hiPSC) and the EndoC-βH1 cell line in combination with CRISPR/Cas9 to generate various *RREB1* KO models. Four KO and three *RREB1* wild-type (WT) hiPSC lines were differentiated along the pancreatic endocrine lineage. Transcriptome profiling was performed at seven stages during *in vitro* islet differentiation and compared to RNA-Seq data received from the EndoC-βH1 *RREB1* KO model.

RREB1 was expressed at all stages of development. 11759 differentially expressed genes (DEGs, $q<0.01$) were identified across all stages between *RREB1* KO and WT lines. Fifty percent of the DEGs at each stage matched predicted RREB1 target genes. Additionally, DEGs of the final differentiation stage correlated ($r=0.7274$, $p<0.0001$) with those observed from the EndoC-βH1 *RREB1* KO model. Motif activity response analysis (ISMARA) highlighted RFX2 and RFX3 as key transcription factors driving the gene expression changes. Consistent with this finding, the RFX family has previously been implicated in regulation of islet-cell differentiation and insulin secretion. Whether there is a direct link between RFX TFs and RREB1 remains to be elucidated.

In conclusion, combining different *RREB1* KO models and transcriptomic datasets can help prioritise follow-up studies to ultimately decipher the biology underlying the T2D GWAS hits at the *RREB1* locus.

65 Laura Marroqui Esclapez

BCL-XL, but not complement C3, protects alpha-cells against metabolic stress- or proinflammatory-induced apoptosis

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Aims/hypothesis: Diabetes is a chronic disease that affects glucose metabolism, either by autoimmune-driven beta-cell loss (type 1 diabetes, T1D) or by progressive loss of beta-cell function due to prolonged metabolic stresses (type 2 diabetes, T2D). Alpha- and beta-cells are exposed to the same stressors, such as proinflammatory cytokines, viruses and palmitate, but only alpha-cells survive. The mechanisms involved in this alpha cell resistance remain to be clarified. Here we investigated whether BCL-XL and complement C3 are part of these alpha cell defence mechanisms.

Methods: Human insulin-producing EndoC-βH1 cells, and primary rat alpha- and beta-cells, were exposed to cytokines (IL-1β + IFN-γ) and/or palmitate in order to model stresses present in respectively T1D and T2D. Viability was assessed by Hoechst/Propidium Iodide staining. Small interfering RNAs (inhibition of >50%) were used to silence gene expression, while adenoviral vectors were used to overexpress BCL-XL and C3. Protein and mRNA expression were evaluated by Western blot and RT-PCR, respectively.

Results: Primary rat alpha-cells presented higher mRNA expression of BCL-XL and C3 compared to beta-cells (6-fold and 70-fold change, respectively). Cytokines induced C3 expression (70-fold increase) in beta- but not in alpha-cells. Conversely, palmitate did not change BCL-XL expression in both cell types. BCL-XL silencing increased palmitate-induced alpha-cell death, whereas its overexpression protected beta-cells against palmitate-induced apoptosis. C3 inhibition did not change cytokine-induced alpha-cell apoptosis while C3 overexpression did not protect EndoC-βH1 cells against cytokine-induced cell death.

Conclusions/interpretation: These data suggest that BCL-XL, but not C3, is part of the defence mechanism used by alpha-cells to evade metabolic stress- or proinflammatory-induced apoptosis in pre-clinical models of T1D and T2D.

66 Lina Sakhneny

Pericytic BMP4 is Required for Glucose-Stimulated Insulin Secretion

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Beta-Cells were shown to depend on the activity of the BMP4 receptor, BMPR1A, for their proper function. In addition, elevating BMP4 levels *in vivo* improved mice glucose response and promoted the mature beta-cell phenotype. However, the source(s) of BMP4 in the adult pancreas is yet to be determined. Beta-Cell function and maturity are regulated by multiple interactions with cells in their microenvironment including pericytes. Published data from our lab revealed that pericytes express BMP4 in a Tcf7l2-dependent manner to support glucose response. Here, we show that pericytic BMP4 is required for glucose homeostasis and beta-cell function. Transgenic mice in which the *Bmp4* gene was selectively deleted in their pancreatic pericytes exhibited impaired glucose tolerance without affecting their insulin sensitivity. In addition, deletion of pericytic BMP4 resulted in impaired glucose-stimulated insulin secretion *in vivo* and *ex vivo*, whereas beta-cell mass and islet vascularization remain unaffected. Our data suggest that pericytes are a significant source of BMP4 in the pancreas, which is required for proper beta-cell function and glucose regulation. Thus, we propose the BMP4/BMPR1A pathway mediates the direct interaction of beta-cells and pericytes.

67 Lisa Nalbach

Improvement of islet transplantation by the incorporation of adipose tissue-derived microvascular fragments into pseudo-islets

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Introduction
A major issue for the poor outcome of the transplantation of pancreatic islets for type 1 diabetic patients is the insufficient engraftment of the transplanted islets. To overcome this problem, we co-cultured pancreatic islet cells with adipose tissue-derived microvascular fragments (adMVF) to generate prevascularized pseudo-islets (PI).

Materials & Methods
Pancreatic islet cells and adMVF from C57BL/6 donor mice were co-cultured in liquid overlay technique to form prevascularized PI. Native islets and non-prevascularized PI served as controls. The morphology, cellular composition and viability of the grafts were analyzed using scanning electron microscopy, immunohistochemistry and flow cytometry. Insulin secretion was assessed by an insulin-ELISA. In vivo, the revascularisation of the grafts was determined by the mouse dorsal skinfold chamber model in combination with intravital fluorescence microscopy.

Results
Stable and compact PIs were generated within 5 days of cultivation. The number of endocrine cells did not differ between the groups. However, the fraction of endothelial cells was significantly higher in prevascularized PI when compared to controls. The incorporation of adMVF did not affect cellular viability and insulin secretion within the grafts. In vivo, prevascularized PI exhibited a significantly accelerated vascularization, as indicated by a higher functional capillary density throughout the 14-day observation period.

Conclusion
These findings demonstrate that the incorporation of adMVF improves the angiogenic potential and the engraftment of prevascularized PIs. Hence, the herein introduced prevascularization approach may represent a promising future strategy to increase the success rates of clinical islet transplantation.

68 Lorenzo Pasquali

The impact of pro-inflammatory cytokines on the β-cell regulatory landscape provides new insights into the genetics of type 1 diabetes

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In type 1 diabetes (T1D) early inflammation of the pancreatic islets (insulitis) by T and B cells contributes to both the primary induction and secondary amplification of the immune assault with pro-inflammatory cytokines contributing to the functional suppression and apoptosis of β cells. Nevertheless, the precise mechanisms by which autoimmunity is triggered and aggravated remain to be clarified. GWAS have made a substantial contribution to the knowledge of T1D genetic architecture uncovering >60 loci containing thousands of associated genetic variants. However, translating variants to function remains a main challenge to achieve for T1D as well as for other complex diseases. Consistent with a major role of the immune system recent studies showed that T and B cells enhancers, unlike islet regulatory elements are enriched for T1D association signals.

We now show that exposure to pro-inflammatory cytokines unmasks a marked plasticity of the human β-cell regulatory landscape. By mapping stimulus-responsive enhancers we expand the repertoire of human islet regulatory elements. We link the newly mapped regulatory elements to the β-cell transcriptome, proteome and 3D chromatin structure.

Our data indicates that the islet response to cytokines is mediated by the induction of novel regulatory regions as well as the activation of primed regulatory elements pre-bound by islet-specific transcription factors. Such changes are coupled with the formation of novel enhancer-promoter loops promoting the activation of genes target of stimulus-responsive enhancers.
We found that T1D-associated loci are enriched of the newly mapped cytokine–responsive islet regulatory elements. We identify trait-associated variants disrupting cytokine-responsive enhancer activity in β cells and implicate two candidate causal genes, *TNFSF18*, with known immunomodulatory function, and *DEXI*, of unknown function.

Our study illustrates how β cells respond to a pro-inflammatory environment and implicate a role for stimulus-response islet enhancers in the susceptibility to the development of T1D.

69 Magdalena Mazur

Type III interferons (IFNLs) are expressed in the human type 1 diabetes pancreas at disease onset, upregulate HLA class I expression and protect beta cells from Coxsackievirus infection

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Type I and II interferons (IFNs) have been proposed to contribute to beta cell destruction in human T1D. Type III IFNs (IFN-lambda 1-4) have only recently been described and they are produced by both immune and parenchymal cells during inflammation and infection. We have previously shown that type III IFNs are expressed by human pancreatic islets infected with Coxsackievirus *in vitro*, that type III IFNs induce a type I IFN-like gene transcriptional signature and protect human islets from Coxsackievirus infection.

In this study, we examined the impact of type III IFN stimulation on human beta cell HLA class I expression and analyzed the expression of genes encoding proteins involved in antiviral defense and/or frequently detected in the islets of enterovirus positive individuals with T1D. We also investigated the expression of type III IFNs in laser captured islets from recent onset T1D patients (DiViD study) and matched controls (nPOD network). RNA was isolated, amplified and transcriptome analysis performed with Affymetrix Human 2.0ST arrays.

Type III IFNs were expressed in the human T1D pancreas at disease onset. Exposure of human beta cells (EndoC-bH1) to type III IFNs led to HLA class I hyperexpression, a characteristic feature of human T1D. Type III IFNs also induced the expression of PKR, a protein commonly expressed in beta cells from enterovirus positive type 1 diabetes cases, as well as MDA5 and CXCL10, and strongly reduced beta cell permissiveness to CVB infection. The expression levels of IFN-L1 ($p<0.001$) and IFN-L2 ($p<0.01$) were increased in DiViD islets compared to controls. The DiViD islets were previously reported to have increased expression of HLA class I.

Collectively, these studies highlight that type III IFNs may be important players in mediating both protective and potentially dangerous signals during the development of human T1D.

70 Maria Marques de Lima

***In vitro* generation of functionally mature beta-cells from adult human iPSCs**

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Islet transplantation has demonstrated that replacement of the beta-cell mass in diabetic patients is able to restore endogenous glycaemic control, but it is currently limited by the shortage of available donor tissue. Stem cell therapies hold great promise for generating a replenishable supply of insulin producing beta-cells for transplantation. Despite the progress achieved over the last decade, existing *in vitro* beta-cell differentiation methods require refinement regarding efficiency and cell maturation.

In the present studies, we have used human iPSCs to generate functionally mature beta-cells *in vitro*. This population can be enriched by FACS sorting up to 50% of beta-cells in a scalable 3D culture system. The *in vitro* generated beta-cells display mature features including insulin content close to that of *bona fide* beta-cells, 95% proinsulin processing, Pdx1, Nkx6.1 and MafA expression, calcium-dependent insulin release and mature insulin granules. Furthermore, the i*n vitro* differentiated beta-cells exhibit glucose regulated insulin secretion, displaying the first and second insulin release phases characteristic of adult islets. Following transplantation into immunocompromised mice, human C-peptide was detected 2 weeks post-implantation and graft functionality was sustained for 20 weeks. These findings pave the way for the generation of in vitro beta-cell models for personalised medicine strategies to improve metabolic health.

71 Marie-Sophie C Nguyen Tu

The type 2 diabetes GWAS gene *Tcf7l2* is required in murine adipocytes for normal pancreatic insulin secretion

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Background. Genome-wide association studies (GWAS) have identified over 200 loci that are associated with type 2 diabetes risk. A single nucleotide polymorphism, rs7903146, in the Tcell factor 7 like-2 (TCF7L2) gene, is amongst the most strongly associated of the identified common variants. We have previously shown that deletion of *Tcf7l2* from the mouse pancreatic β-cell leads to glucose intolerance, and TCF7L2 is required for normal β-cell expansion and insulin secretion under metabolic stress. TCF7L2 is a downstream regulator of the Wnt/β-catenin signalling pathway, involved in a variety of cellular processes, including adipocyte development. Our aim here was to investigate whether there is a role for TCF7L2 in the adipocyte in regulating glucose homeostasis.

Methods. We achieved adipocyte-selective deletion of *Tcf7l2* in mice by breeding C57BL/6 animals bearing *floxed Tcf7l2* alleles to transgenic mice bearing *Cre* recombinase inserted at the *adiponectin* locus (Adipoq-Cre).

Results. Deletion of *Tcf7l2* in adipose tissue (aTCF7L2KO) caused impaired whole-body glucose tolerance compared to controls (Adipoq-Cre-) after oral administration of glucose ($p<0.05$). No differences were seen between genotypes after intraperitoneal glucose injection. Insulin sensitivity, body weight, fat mass and fasting glycemia were also unchanged. In contrast, insulin release, measured *in vivo* by monitoring circulating insulin levels after glucose administration, was lower in aTCF7L2KO versus control mice ($p<0.05$). Correspondingly, glucose-stimulated insulin secretion from isolated pancreatic islets was reduced in aTCF7L2KO islets, and cytoplasmic Ca2+ dynamics were impaired. Circulating levels of GLP-1 ($p<0.05$) and GIP ($p<0.001$) were lowered in aTCF7L2KO mice, whilst FABP4 levels were elevated ($p<0.05$).

Conclusions. We demonstrate a complex role for adipose tissue TCF7L2 in regulating pancreatic β-cell function, involving altered incretin production from enterendocrine cells, and adipokine production from fat. Altered levels of TCF7L2 in fat cells in carriers of disease associated variants may thus influence the activity of multiple metabolically-relevant tissues to determine diabetes risk.

72 Mark Russell

Signal Regulatory Protein Alpha (SIRPα) is a novel regulator of pancreatic beta-cell viability.

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Introduction

Treatment of pancreatic beta-cells with anti-inflammatory cytokines such as interleukin (IL)-13 can protect them from a range of cytotoxic stimuli. This response is dependent on the transcription factor, STAT6, and we have identified a number of genes, including signal regulatory protein α (SIRPα), whose expression is increased upon activation of STAT6. In the current work we have studied the induction and functional effects of SIRPα in pancreatic beta-cells.

Results

qRT-PCR analysis revealed that *SIRPA* mRNA was upregulated by ~3-fold upon treatment of INS-1E cells with IL-13. Studies of protein expression confirmed a robust increase in SIRPα in INS-1E cells, EndoC-βH1 cells or human islets exposed to IL-13. siRNA-mediated knockdown of STAT6 abrogated the elevation in SIRPα protein and mRNA.

The function of SIRPα in pancreatic beta-cells was assessed either by depletion of *SIRPA* expression using siRNA or by over-expression in cells transfected with a plasmid encoding *SIRPA*. Knockdown of SIRPα reduced INS-1E cell viability under both serum containing (22.50±1.82%, siSIRPα: 34.89±2.1%; p<0.0001) and serum deprived (SW) conditions (SW: 48.87±0.97%, SW+siSIRPα: 70.24±1.48%; p<0.0001). Conversely, SIRP over expression significantly protected INS-1E cells from serum withdrawal (SW: 51.87±2.0%, cell death; SW+SIRPα: 38±1.1%; p<0.0001).

In immune cells, SIRPα becomes functionally active upon interaction with CD47 expressed at the cell surface. We, therefore, studied the expression of CD47 in beta cells and found, by Western blotting, that CD47 is expressed in INS-1E cells and human islets. Moreover, in common with SIRPα, CD47 expression was elevated after treatment of INS-1E cells with IL-13. Co-immunofluorescence staining of human pancreatic tissue confirmed that CD47 is predominantly localised to beta-cells.

Conclusion

These data reveal that STAT6 controls the expression of SIRPα in pancreatic beta-cells and suggest that SIRPα exerts a previously unrecognised function in the regulation of beta-cell viability. This may be mediated, at least in part, by its interaction with CD47.

73 Marta Pérez Alcántara

Chromatin accessibility patterns of a hiPSC model of islet development and type 2 diabetes risk.

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Most variants associated with type 2 diabetes (T2D) predisposition in genome-wide association studies (GWAS) act through defects in insulin secretion, which could result from deficiencies in islet development and/or mature islet function. While functional studies have focused on the latter, we have explored the contribution of disturbed islet development to T2D pathogenesis. We used ATAC-Seq to characterise patterns of open chromatin in three human fibroblast-derived iPSC lines (from different donors) differentiated across seven developmental stages towards beta-like cells. We obtained ATAC-Seq profiles of 133,210 peaks, which were clustered by weighted gene co-expression network analysis (WGCNA). Peaks within the resulting 18 modules (median=1351, range=7753) that were highly correlated (Pearson's r *p*-value < mode [r *p*-value]) with their respective module profile were tested for enrichment in T2D-associated SNPs from the European ancestry DIAMANTE analysis (~ 900,000 participants) using fGWAS. Seven clusters of ATAC-Seq profiles were enriched in T2D-associated signals, reaching maximum peak counts at varying degrees of development, including middle (posterior foregut) and late stages (endocrine and beta-like cells), with $\log_2$ fold enrichment values from 2.18 (95% CI 0.17–3.25) to 4.01 (95% CI 2.99–4.71). Peaks within these modules were enriched (p-value ≤ 10^{-10}) in several transcription factor (TF) binding motifs (as found by HOMER), including those bound by TFs implicated in islet development (e.g. *PDX1*, *MAFA*, *FOXA2*; with RNA-Seq confirming their expression). 1007 ATAC-Seq peaks contained at least one variant from the 99% credible set, 47 with an individual posterior probability of association (PPA) above 10%. Several were near known important islet development genes (*HNF1A* [98%], *HNF4A* [52%]) and candidates such as *WDR72* (99%) or *PROX1* (99%).

These results highlight how integration of ATAC-Seq, RNA-Seq and other epigenetic data from this differentiation model allows exploration of the contribution made by genome regulation to disturbed human islet development and T2D pathogenesis.

74 Mawieh Hamad

Estrogen-induced suppression of hepcidin synthesis enhances intracellular iron efflux and minimizes oxidative stress and apoptosis in pancreatic β-cells

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It is well accepted that elevated levels of estrogen (17-β estradiol, E2) associate with significant protection against diabetes mellitus (DM). This has been attributed to the ability of E2 to enhance β-cell proliferation, increase insulin secretion and reduce gluconeogenesis among other mechanisms. However, the exact molecular mechanism underlying this effect of E2 remains unclear. In this context, several studies have demonstrated that elevated levels of E2 suppress hepcidin synthesis and enhance intracellular iron efflux in breast, ovarian and liver cell lines. Hence, E2-driven intracellular iron efflux may limit the production of reactive oxygen species (ROS) and minimize oxidative stress in β-cells. This is particularly relevant given that increased oxidative stress in β-cells induces apoptosis and leads to DM. To test this idea, rat clonal β-cells (INS-1/832/13) were treated with E2 and/or apoptosis-inducing cytokines for 24-48 hrs. and assessed for intracellular iron status, mitochondrial function and apoptosis. Treatment of INS-1 cells with 20 nM E2 for 24-48 hrs. resulted in a significant reduction in hepcidin synthesis and a transient depletion of intracellular labile iron. It also associated with a significant depolarization of mitochondrial inner membrane, which suggests reduced ROS production. Levels of ferritin protein were significantly lower ($p<0.05$) and those of Hif-1α were significantly higher ($p<0.05$) in cells on E2 + cytokines as compared with cell on cytokines alone. Apoptosis in E2 + cytokines-treated cells was significantly lower ($p<0.05$) than that in cytokines alone-treated cells. Lastly, expression of p^{53} and p^{21} proteins was significantly increased ($p<0.01$) and that of BIRC5 (survivin) was significantly reduced ($p<0.05$) in cells on E2 + cytokines as compared with cells on cytokines alone. Work is underway to replicate these studies in cadaveric human islets and to further characterize the anti-apoptotic effects of E2. These findings suggest that E2-driven intracellular iron efflux reduces oxidative stress and enhances β-cell survival.

75 Milan Patra

Chromatin modifications involved in senescent beta cell function.

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Pancreatic beta cell failure is a hallmark of Type 2 Diabetes (T2D)[1-3] Replenishment of beta cells through compensatory replication is restricted by the limited proliferative capacity of these cells, and this capacity dramatically declines with age, potentially contributing to pathogenesis[1-3] Importantly, inherited polymorphisms in the *CDKN2A* locus, encoding p16 (a senescence marker), are amongst the most highly associated with T2D[4] . Our lab recently discovered a previously unknown mechanism in which the senescence program regulates beta-cell function [5]. Senescence, an irreversible cell cycle arrest, was shown, in addition to blocking proliferation, to boost glucose-stimulated-insulin-secretion. Senescence of beta-cells could also contribute to recruitment of immune cells, and thereby contribute to the development of diabetes, representing a negative flip-side of the same program. The roles of senescence in diabetes are, however, unknown. I propose that genome-wide changes in specific gene-regulatory networks during beta-cell senescence can reprogram beta cell function during T2D development and pathogenesis. I recently found that senescence induction in human beta like cells (EndoC-βH2 and EndoC-βH3) alters organization of H3K27Me3/H3K9Me3 heterochromatin marks in the nucleus and gene expression of key signaling molecules. Furthermore, multiplexed ChIP-seq analysis with key chromatin marks indicates differential binding profiles of senescent and non-senescent beta cells. Interestingly, knockdown of p16 reverses some of the senescence profiles. Further in-depth analysis of gene expression, chromatin modifications and nuclear organization associated with beta cell senescence using in vitro and in vivo model system, will lead us to understand the molecular dynamics of beta cell senescence and how they affect insulin secretion.

76 Mireia Ramos Rodriguez

The *Islet regulome browser*

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The pancreatic islet is a highly specialized tissue embedded in the exocrine pancreas whose primary function is that of controlling glucose homeostasis. Thus, understanding the transcriptional control of islet cell types may help to puzzle out the pathogenesis of glucose metabolism disorders. Integrative computational analyses of transcriptomic and epigenomic data allow predicting genomic coordinates of putative regulatory elements across the genome and decipher tissue-specific functions of the non-coding genome.

The Islet Regulome Browser (IRB) (www.isletregulome.com) is a tool that serves the scientific community fast access and exploration of pancreatic islet epigenomic and transcriptomic data produced by different labs worldwide. This open access browser includes links to the raw data as well as post hoc analyzed datasets to provide an integrated view of pancreatic islet genomic data. More specifically the IRB allows interactive exploration of a wealth of information, allowing the visualization of different classes of regulatory elements, together with enhancer clusters, transcription factor binding sites, and open chromatin maps, which are integrated with publicly available GWAS datasets. We now launch a new version of the IRB with a new intuitive interactive layout. Importantly the new version includes new pancreatic islets genomic and epigenomic data including virtual 4C data.

The IRB facilitates the access to pancreatic islet genomic and epigenomic datasets promoting data integration and analysis and boosting functional genomics studies in glucose metabolism related traits.

77 Natasha Hui Jin NG

HNF4A haploinsufficiency in MODY1 abrogates liver and pancreas differentiation from patient-derived iPSCs

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Maturity onset diabetes of the young 1 (MODY1) is a monogenic diabetes condition caused by autosomal dominant mutations in the *HNF4A* gene. Although *Hnf4α* is expressed during embryonic foregut development, the consequences of MODY1 mutations on foregut endoderm development have not been reported. Additionally, rodent models are unable to recapitulate the MODY1 phenotype as heterozygous or conditional knockout mice do not present with diabetes. To determine if *HNF4A* haploinsufficiency affects human foregut development which can subsequently impact liver and pancreas development and function, we generated hiPSCs from members of a MODY1 family harbouring a heterozygous HNF4A p.Ile271fs mutation and differentiated them down the foregut endoderm lineage. In MODY1-derived hepato-pancreatic progenitors, which expressed reduced *HNF4A* levels and mis-localized HNF4A protein, numerous foregut pancreas- and liver-related genes were downregulated whereas hindgut-specifying *HOX* genes were upregulated. Changes in hepatic and β cell gene signatures were also observed in MODY1-derived hepatocyte-like and β-like cells respectively, using independent differentiation protocols. Molecular studies revealed that the MODY1-HNF4A mutation neither resulted in complete nonsense-mediated decay based on allele-specific transcript expression assays, nor exerted a dominant negative effect. However, mutant HNF4A protein demonstrated impaired transcriptional activation of downstream gene targets such as the MODY3-associated gene *HNF1A*. Collectively, our patient-derived iPSC disease model combined with mechanistic investigations suggested that in MODY1, liver and pancreas development may be perturbed early on due to impaired transcriptional regulation by HNF4A. This potentially contributes to altered hepatic proteins and β cell defects in MODY1 patients.

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78 Neda Mousavy Gharavy

Title: Roles for the Type 2 Diabetes-Associated Genes *C2CD4A* and *C2CD4B* in the Control of Glucose Homeostasis and Insulin Secretion

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Background: Single nucleotide polymorphisms (SNPs) on human chromosome 15, near *C2CD4A* and *C2CD4B,* are associated with increased Type 2 Diabetes (T2D) risk at genome-wide significance. In human subjects, risk allele rs7163757 is associated with altered *C2CD4A* and *C2CD4B* mRNA levels. Although the expression levels of these genes was shown to be elevated by cytokines in endothelial cells, and localised to the nucleus, their role in pancreatic β-cells is unclear. Here, we explore this question using CRISPR/Cas9 mediated gene deletion in human β-cells and in mice.

Methods: *C2cd4b*^{null} mice were generated by the International Mouse Phenotyping Consortium (IMPC). A deletion was made at the first 100bp of *hC2CD4A* in EndoC-βH1 cells, using a lentivirus-based CRISPR/Cas9 system. Sub-cellular analysis of FLAG-tagged constructs was performed by immunohistochemistry using a spinning disk microscope (Nikon-ECLIPSE-Ti). Insulin secretion measured using HTRF kit (Cisbio).

Results: *C2cd4b*^{null} female mice showed a significant increase (area-under-the curve, AUC, p=0.031, 20 weeks) during intraperitoneal glucose tolerance tests. This was associated with a significant reduction in plasma insulin, measured at 5min. (p=0.0203) and 15min. (p=0.0102) after injection of glucose. Maintained on a high fat, high sugar diet, both male and female *C2cd4b* null mice displayed marked glucose intolerance (AUC, p=0.033). Deletion of *C2CD4A* from human-derived EndoC-βH1 cells caused a significant (p=0.016) decrease in glucose- and 3-isobutyl-1-methylxanthine (IBMX)-stimulated insulin secretion. Examined in rodent and human derived β-cell lines, C2CD4A and –B were localised in the cytoplasm and plasma membrane as well as the nucleus.

Conclusions: Our data suggest that changes in *C2CD4A* and/or *–B* expression in the β-cell drive enhanced T2D risk in variant carriers in a sexually dimorphic manner. These actions result from altered glucose-regulated insulin secretion. The sub-cellular localisation of these proteins suggests that they may regulate extra-nuclear signalling events in β-cells, in contrast to earlier findings in other cell types.

79 Neta Harari

Pancreatic pericytes originate from the embryonic pancreatic mesenchyme

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Despite their central role in pancreas physiology, the embryonic origin of pancreatic pericytes is still unknown. Here, we show that adult pancreatic pericytes originate from the embryonic pancreatic mesenchyme. Our analysis indicates that pericytes of the adult mouse pancreas originate from cells expressing the transcription factor Nkx3.2. In the embryonic pancreas, Nkx3.2-expressing cells constitute the multilayered mesenchyme, which surrounds the pancreatic epithelium and supports multiple events in its development. Thus, we traced the fate of the pancreatic mesenchyme. Our analysis reveals that pancreatic mesenchymal cells acquire various pericyte characteristics, including gene expression, typical morphology, and peri-endothelial location, during embryogenesis. Importantly, we show that the vast majority of pancreatic mesenchymal cells differentiate into pericytes already at embryonic day 13.5 and progressively acquires a more mature pericyte phenotype during later stages of pancreas organogenesis. Thus, our study indicates the embryonic pancreatic mesenchyme as the primary origin to adult pancreatic pericytes. Our findings further points to a coordinated development driven by epithelial-mesenchymal interactions that play different roles during various stages of pancreas development.

80 Ofer Elhanani

REST is a negative regulator of exocrine to endocrine cell reprogramming that restricts PDX1-mediated activation of endocrine genes in exocrine cells

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In recent years it has become clear that terminally differentiated cells in the pancreas retain a surprising degree of plasticity. Thus, under a variety of experimental conditions, de-differentiation and trans-differentiation / reprogramming have been described. The molecular mechanisms underlying these processes remain poorly understood.

The goal of our studies is to employ an *in vitro* system that allows dissection of the molecular events involved in the process of reprogramming adult pancreatic acinar cells into insulin-producing β cells. We show that adenoviral delivery of PDX1, NGN3 and MafA (PNM) into primary mouse exocrine cells *in vitro*, results in appearance of insulin-expressing cells. Employing fluorescent labeling techniques, allowed the specific sorting of enriched populations of insulin-positive reprogrammed cells.

RNA sequencing of these populations revealed many similarities to native β cells. Further analysis of the molecular events occurring specifically in reprogrammed cells, allowed the identification of the RE-1 silencing transcription factor (REST) as a negative regulator of reprogramming. Accordingly, during reprogramming of exocrine cells, REST is downregulated, whereas overexpression of REST in reprogrammed cells, results in repression of endocrine genes. Furthermore, REST loss of function in acinar cells, results in induction of endocrine genes in a manner that synergizes with PDX1 expression. This was associated with increased histone acetylation and PDX1 binding to its endocrine targets which were dependent on REST loss of function. These findings suggest that REST functions as an important repressor of endocrine gene expression in acinar cells, through generation of a repressive local chromatin environment that prevents activation by endocrine transcription factors such as PDX1.

Collectively, these studies have identified REST as an important inhibitor of exocrine cell reprogramming to β-cells. Improved understanding of this process is important for future application in regenerative and cell therapy-based treatment of diabetes.

81 Paraish Misra

Determining The Ability Of Hla-Deficient Human Pluripotent Stem Cells To Generate Insulin-Producing Cells

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Background: Stem cell-derived insulin-producing “beta-like” cells can now be generated in high quantities *in vitro* from human pluripotent stem cells (hPSC), and may present a scalable alternative to pancreatic transplantation for the treatment of diabetes mellitus. However, allogeneic cells derived in this way would require systemic immunosuppression to prevent allorejection, limiting the therapeutic benefit of this therapy. HLA-deficient stem cell-derived beta-like cells may effectively engraft in allogeneic hosts without immunosuppression, but the ability of HLA-deficient pluripotent stem cells to differentiate into beta-like cells remains to be determined.

Objective: We sought to determine whether hPSCs lacking both HLA class I and II retain the potential to differentiate into beta-like cells.

Results: We first optimized a 6-stage differentiation protocol for the generation of pancreatic beta-like cells. With this protocol, HLA-deficient hPSCs successively differentiated with high efficiency into endoderm (median CXCR4 and CD184 double-positive 94.5%), pancreatic progenitors (median PDX1 and NKX6.1 double-positive 93.3%), and insulin-producing cells (median C-peptide and NKX6.1 double positive 33.1%). We next profiled HLA expression in wild-type and HLA-deficient stem cells. The H1 line expressed robust interferon gamma-inducible HLA class I expression throughout all stages of differentiation, but never demonstrated significant surface expression of HLA class II. As expected, HLA-deficient stem cells expressed no surface class I or class II HLA at any stage of differentiation.

Conclusion: HLA-deficiency does not affect the ability of hPSCs to differentiate into insulin-producing beta-like cells. Although this portends well for their clinical potential, the immunogenicity and functionality of these cells still remains to be determined.

82 Richard Norris

Gene regulatory networks in pancreatic islets and insulinomas.

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Insulinomas are extremely rare tumors that develop from the β cells of the pancreas and feature dis-regulation of insulin secretion, and aberrant proliferation. Tumour development has been associated with genetic and epigenetic alterations leading to loss of cell fate. Combining molecular biology techniques with bioinformatics analyses we herein aim to unmask β-cell physiological and aberrant regulatory networks, across different layers of gene regulation. We collected a set of 13 insulinomas and 10 unaffected human islet samples. ATAC-seq, to map open chromatin and ChIP-seq of H3K27ac, a mark of active enhancers and promoters, were performed to infer their regulatory landscape. Transcriptome maps were charted by RNA-seq. We profiled the genomic regions enriched for H3K27ac and calculated the number of individual samples in which a regulatory region overlaps with the master list. We demonstrate that this data is sufficient to approximate saturation in detecting all gene regulatory elements active in insulinomas.

By comparing unaffected pancreatic islets and insulinomas we identified widespread changes in enhancer activity. We found more than 9,000 regulatory elements that are differentially active in insulinoma compared to normal islets and linked them to gene expression activity. 95% of these 'insulinoma-specific' regions are distal to the nearest transcription start site. Further characterisation of these enhancers and the gene regulatory networks that contribute to insulinoma development may lead to new insights into β cell identity and β cell proliferation.

83 Roland Stein

Lipid droplet accumulation in human pancreatic islets is dependent upon both donor age and health

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Human but not mouse islets transplanted into immunodeficient NSG mice accumulate lipid droplets (LDs). Because chronic lipid exposure is associated with islet β cell dysfunction, we investigated LD accumulation in mouse and human islets over a range of ages and states of diabetes. Very few LDs were found in normal human pancreatic acinar and islet cells until around 8 years after birth, with LD numbers then increasing throughout adulthood. While accumulation appeared to be distributed randomly between acinar and islet cells in normal donors, LDs were enriched in islet α and β cells from donors with Type 2 diabetes mellitus (T2D). These patterns were detected by electron microscopy and using lipophilic dyes, with the islet LD-enriched perilipin 2 and 3 proteins co-staining with these dyes. In contrast, LD accumulation was nearly undetectable in the rodent pancreas, even in hyperglycemic and hyperlipidemic T2D models. Taken together, this study suggests that LD buildup in humans is due to the functional and molecular differences reported between the adolescent and adult pancreas cell populations. The observed age and disease dependent accumulation of LDs in human pancreatic islet cells is consistent with LD enrichment influencing islet cell function. Ongoing efforts to investigate the functional consequence of LDs to these cells will be presented.

84 Sarah Richardson

Comprehensive histopathology of insulitis in young-onset Type 1 diabetes

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Purpose: Worldwide, fewer than 80 pancreata are available from individuals who were <10y at onset of type 1 diabetes and had short duration (<1y) of disease. Most are autopsy cases collected >30 years ago and, since deaths close to diagnosis are now very rare in young children, these samples are invaluable for study of disease aetiopathology. In the present study, we have examined 30 pancreas sections from two different historical collections, the Exeter Archival Diabetes Biobank and Seattle Childrens' Hospital (n=17; <10y at onset). Triple chromogen immunohistochemical staining was optimised to assess islet inflammation and insulin immunopositivity in a blinded manner, using a state-of-the-art image analysis platform.

Results: A total of 5714 islets were assessed, of which 1270 (22.2%) contained insulin (ICI) and 368 (6.4%) were inflamed. The sections were sub-classified according to the average number of infiltrating B-cells per ICI (<3 versus >3) and two groups defined. The median age at diagnosis in Group 1 (n=10) was 19y and among the residual ICIs, 16.9% were inflamed (mean of 9.8±2.6 CD45+ cells & 0.21±0.01 CD20+ cells per islet, respectively). By contrast, subjects in Group 2 (n=20) had a median age of 5y at diagnosis; all were under 11y, except 1 case (17y) with pancreatitis. Fewer residual ICIs were present in Group 2 (14.0% vs 35.8%) and most (72.8%) were highly inflamed (mean of 61.1±7.8 & 28.6±6.7 CD45+ cells and CD20+ B cells per ICI, respectively).

Conclusions: Pancreata collected >30 years ago from two independent sources can be utilised to investigate the immunopathology of T1D using state-of-the-art image analysis platforms. The results strengthen and expand previous observations revealing that individuals diagnosed with Type 1 diabetes before age 13y have a more aggressive insulitis (with high numbers of both CD8+ & CD20+ lymphocytes) and retain a smaller proportion of ICIs than those diagnosed >13y.

85 Sergiy Korol

Exploring the $GABA_A$ receptor-mediated currents and expression of hormone mRNAs in multiple hormone transcript-expressing cells in intact human pancreatic islets

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The primary cell-types in pancreatic islets are α-, β- and δ-cells, producing glucagon (GCG), insulin (INS) and somatostatin (SST), respectively. However, emerging evidence indicates there are islet cells expressing more than one hormone transcript being the cells with mixed identity. Such mixed-identity cells may express hormone transcripts in various combinations: *GCG/INS* (in α/β cells), *INS/SST* (β/δ cells), *GCG/SST* (α/δ cells) or *GCG/INS/SST* (α/β/δ cells), and have different expression levels of the hormone transcripts in the cells. They potentially represent different developmental stages of the primary cell-types or may appear under various conditions e.g. obesity, diabetes, surgery, reprogramming[1].

Elements of the different neurotransmitter signaling machineries are found within human pancreatic islets and one of them is the γ-aminobutyric acid (GABA) signaling system[2,3]. Here, we extended our previous islet GABA signaling studies[3,4] and examined what multiple hormone transcript-expressing cells exist in intact pancreatic islets from non-diabetic (ND) and type 2 diabetic (T2D) donors and what the relative hormone transcript expression levels are in such mixed-identity cells. We further studied the activity patterns of single-channel $GABA_A$ receptor ($GABA_AR$) currents in the cells using the whole-cell patch-clamp technique. Single-cell RT-PCR analysis of the expression levels of insulin (*INS*), glucagon (*GCG*) and somatostatin (*SST*) transcripts was done to determine the type of cell we recorded from. Mixed-identity cells varied in the $GABA_ARs$ activity pattern. α/β cells with lower *GCG*-to-*INS* expression ratio had higher frequency of $GABA_AR$ openings and similar to what was recorded from β-cells[4]; in contrast, α/β cells with higher *GCG*-to-*INS* expression ratio had low $GABA_AR$ opening frequency, similar to α-cells[4].

In summary, the cells with multiple hormone transcripts exist in pancreatic islets from ND and T2D donors. The single-channel frequency of $GABA_AR$ openings correlated with the ratio of the expression levels of the hormone transcripts in mixed-identity cells.

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86 Shahana Sengupta

Characterisation of a novel, *de novo* candidate causal variant in *PAM* as a cause of neonatal diabetes mellitus through pancreatic β cell dysfunction

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Peptidylglycine α-amidating monooxygenase (PAM) catalyses the α-amidation of glycine-extended peptides, thereby maximising their biological potency. We recently showed that coding alleles in *PAM* mediate Type 2 Diabetes (T2D) risk through alterations to PAM enzyme activity and expression in the β cell. Identifying alleles along a continuum of functional severity informs on the relationship between protein perturbation and clinical effect. We hypothesised that rare, loss of function *PAM* alleles could cause severe β cell dysfunction presenting as neonatal diabetes.

Sequencing of 15 probands presenting with diabetes at ≤ 6 months of age and their unaffected family, identified a novel *de novo* nonsynonymous coding variant (p.R36S) in *PAM* in a single proband. We investigated the impact of R36S-PAM on amidating activity, protein stability and localisation as a measure of PAM function and the consequences on β cell viability.
Recombinant R36S-PAM exhibited normal amidating activity *in vitro*, consistent with PAM activity measured in patient serum (WT: 391pmol/μl/hr vs. R36S: 305pmol/μl/hr). In human β cells (EndoC-βh1) the secreted luminal R36S-PAM isoform displayed reduced protein expression (-78%, p=0.004) compared to WT-PAM, which was rescued by inhibiting the proteosomal pathway; whilst the non-secreted membrane-integral isoform was retained in the endoplasmic reticulum (ER). We observed that overexpression of R36S-PAM elevated levels of ER stress (p-IRE1: +196%, p=0.02; CHOP: +114%, p=0.051) and pro-apoptotic markers (ASK1: +144% p=0.03; Phospho-JNK: +202%; caspase-3: +143%) compared with WT-PAM. This is a greater response than with S539W-PAM (Phospho-JNK: +97.3%; caspase-3: -72.7%, p=0.07), a known T2D-risk allele with ER mislocalisation.

We have identified a novel, *de novo* coding variant, in a gene with a proven role in T2D risk, in a single patient with neonatal diabetes. Functional studies provide evidence for protein mislocalisation, instability and support a role for this allele in ER stress and β cell dysfunction consistent with neonatal diabetes pathogenesis.

87 Sophie Emilie Bresson

Identification and functional analysis of interacting amino acids in the GRP94-proinsulin complex

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2)

Background: Proinsulin-folding is a critical process for the synthesis and secretion of mature insulin. Our recently published data suggest that the chaperone Glucose-Regulated Protein 94 (GRP94) is of vital importance in this process (Ghiasi et al., 2019). We found that decreased GRP94 activity or expression lead to diminished glucose-stimulated insulin-secretion, decreased half-life of proinsulin and lower proinsulin- and insulin-levels.
Direct interactions between endogenous GRP94 and proinsulin and the effects of GRP94 rescue remains to be demonstrated. We hypothesize that GRP94-proinsulin interaction depends on the C-terminal protein interacting-domain of GRP94.

Aims:

1. Predict interacting residues in the GRP94-proinsulin complex using in silico modelling
2. Investigate the interaction of endogenous proinsulin and GRP94

Methods

1. *In silico* modelling based on established crystal structures of GRP94 (5ULS, Huck et al., 2017) and proinsulin (2KQP, Yang et al., 2010), using ZDOCK docking software.
2. Investigation of endogenous proinsulin and GRP94 interactions in insulin-secreting INS-1E cells by cross-coIP.
3. Rescue of phenotype of CRISPR-Cas9 GRP94 KO INS-1E cells by transfection with wild-type proinsulin by SDS-PAGE and Western blotting.

Results: *In silico* modelling predicted that the C-terminal domain of GRP94 is the main interacting domain in the GRP94-proinsulin complex (most frequent predicted interactions between GRP94 amino acids 575-678 and proinsulin's 75-83). Of special interest are residues M658 and M662 known to be critical in for the binding of other GRP94 clients.
Endogenous GRP94 cross-coimmunoprecipitated with endogenous proinsulin.Reintroduction of wild-type GRP94 into GRP94 KO cell lines reconstituted proinsulin levels.

In conclusion, endogenous GRP94 binds endogenous proinsulin and this interaction is predicted to center around the C-terminal domain of GRP94. Furthermore rescue of proinsulin production is possible by GRP94 introduction in KO cells.

88 Susanne Ullrich

Modulation of TGFβ-1 signalling affects differentiation of pancreatic beta-cells

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Activation of TGFβ receptors changes cellular fates by inducing differentiation and inhibiting proliferation. TGFβ has been described to stimulate insulin secretion and inhibit β-cell proliferation. A natural antagonist of TGFβ signalling is the hepatokine fetuin-A. Fetuin-A is a foetal glycoprotein and its abundance declines after birth. In adults increased plasma fetuin-A levels link to fatty liver. Previously we described that fetuin-A inhibits glucose-induced insulin secretion in human islets. This study aims to examine the role of fetuin-A as TGFBR antagonist in β-cell differentiation and function.

Using pig neonatal islet cell clusters differentiation was induced *in vitro* under defined culture conditions in the absence of serum and fetuin-A. The addition of fetuin-A inhibited the time-dependent increase of *INS* and *PDX1* mRNA levels. In parallel, fetuin-A inhibited SMAD2/3 phosphorylation. The addition of TGFβ reduced *ALDOB* mRNA levels, while the inhibitor of TGFBR1, SB 431542, increased ALDOB expression. Fetuin-A did not affect *ALDOB* mRNA but increased mRNA levels of AKR1B1, a downstream target of the polyol pathway. In adult human islets fetuin-A inhibited SMAD2/3 phosphorylation, an effect not reversed by TGFβ. Fetuin-A augmented basal insulin secretion masking the stimulatory effect of glucose. In humans, plasma fetuin-A levels associated with mRNA levels of *AKR1B1* of LCM-cut islets. These results suggest that fetuin-A interferes with islet differentiation and glucose-induced insulin secretion (GIIS), effects associated to the inhibition of TGFβ signalling although distinct to the effects of TGFβ and SB431542. Both, fetuin-A and SB431542 stimulate the expression of enzymes of the polyol pathway. These effects may affect basal secretion due to bypassing glucokinase, the rate limiting enzyme of glycolysis. In conclusion, these observation suggest that increased fetuin-A levels favour dedifferentiation and dysfunction of β-cells during the development of fatty liver-linked T2DM.

89 Tatiana Danilova

MANF is a promising therapeutic candidate for diabetes

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Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an evolutionarily conserved secreted protein (18kDa) with protective and restorative properties in different animal models of neurodegenerative diseases and heart ischemia. MANF is mostly located in the endoplasmic reticulum (ER), and its expression and secretion are regulated by ER stress. To reveal the physiological roles of MANF in mammals, we created conventional MANF knockout (*Manf*$^{-/-}$) mice which develop diabetes due to postnatal loss of β-cells mass caused by reduced β -cell proliferation and increased β -cell death accompanied by sustained ER stress. Similarly to *Manf*$^{-/-}$ mice, the absence of MANF in the pancreases of conditional *Pdx-1Cre*$^{+/-}$::*Manf*$^{fl/fl}$ mice resulted in diabetes. Consequently, tamoxifen-induced ablation of MANF from β-cells in adult *MIP-1Cre*^{ERT}*::Manf*$^{fl/fl}$ mice resulted in reduced β-cell mass and diabetes caused largely by β-cell ER stress and apoptosis, possibly accompanied by the β-cell de-differentiation and reduced rates of the β-cell proliferation. In vitro, MANF-deficiency in cultured islets leads to the activation of pro-inflammatory NF- κB, p38, JNK signaling cascades whereas addition of recombinant MANF protein to islets alleviated ER stress and increased AKT phosphorylation in β -cells. In addition, MANF protein induced β-cell proliferation in islets from young and aged mice and protected them from hyperglycemia-induced ER stress. Importantly, our recent studies revealed that MANF also protected human primary β-cells from cytokine-induced cell death and induced their proliferation together with small molecule TGF- β inhibitor *in vitro*. In order to test the therapeutic potential of MANF in diabetes, we developed a doxycycline-inducible transgenic mouse line that specifically overexpresses MANF in the β -cells. We identified that MANF overexpression in mouse pancreatic β -cells protects from streptozotocin-induced β -cell death and diabetes *in vivo*, thus making it a promising therapeutic candidate for the treatment of diabetes.

90 Tenna Holgersen Bryde

Is Proinsulin Dimerization Dependent On Grp94 Chaperone?

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Background: Insulin availability is an essential determinant of glucose homeostasis, and insufficient insulin production to meet insulin demand is the underlying cause of diabetes. Insulin, like any other secreted protein, needs folding in the endoplasmic reticulum to attain its proper tertiary structure, but the details of this process remain understudied. Recently, we described the first proinsulin chaperone, termed GRP94 (Ghiasi et al Diabetes 2019). GRP94 knockout leads to proinsulin misprocessing evident by aberrant proinsulin conversion and impaired formation of mature secretory granules containing electron-dense insulin crystals, suggesting that GRP94 deficiency inhibits proinsulin processing and dimerization.

Hypothesis: GRP94 presence in the endoplasmic reticulum is required for proinsulin dimerization.

Aim: By size exclusion chromatography to analyze proinsulin dimer-formation within parental and GRP94 knockout cells.

Material and Methods: INS1-E parental as well as GPR94 knockout cell lysis was optimized for size exclusion chromatography (SEC). Whole cell lysates (300 μg) were fractionized and the obtained fractions were heat-denatured and reduced, and proteins separated via SDS-PAGE. Proinsulin and insulin levels were detected by Western blotting.

Results: We were able to detect proinsulin and insulin as monomeric proteins. However, both of the proteins were predominantly found as dimers and, to a lesser extent as hexamers, confirming that SEC can be used to asses the formation of proinsulin and insulin multimers. Finally, proinsulin dimer levels were reduced in GRP94 knockout cells.

Conclusions: SEC based separation of cellular lysates is a useful method to analyze proinsulin and insulin homo- and heteromeric complexes. Our initial data indicates that GRP94 presence is required for assembly of proinsulin multimers.

91 Thomas Aga Legøy

To bead or not to bead?: Alignate encapsulation improves hiPSC to pancreatic β-cell differentiation

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Introduction

Today we observe alarming increase of diabetes incidence worldwide, which exerts a strong pressure for urgently finding new treatments. One suggested treatment is to generate insulin producing cells from the patients and give them as implants. Unfortunately, there are multiple challenges to generate a homogenous fully mature β-cell population *in vitro*. One suggestion to improve differentiation is to mimic the clustering observed in endocrine cells development. In this study we aim to assess if the encapsulation of differentiated iPSCs (3D environment) could generate higher number of, and more mature, β-cells.

Material and methods

Skin biopsies were collected from one healthy patient and reprogrammed to iPSCs by Tekara Bio Inc. The iPSC line was differentiated according to a previously published seven step protocol, either on matrigel-coated plates (representing the 2D environment), or in alginate beads. For the encapsulation we used ultra-pure high-G (67 percentage G) sodium alginate and an electrostatic bead generator. We performed whole mount immunofluorescence on S7 cells encapsulated at S0, S5 or S7. In addition, we performed proteomic analysis of S5 and S7 cells differentiated either on matrigel or in alginate beads and compared to isolated human islets.

Results

By immunofluorescence we observed that the proportion of cells expressing insulin, glucagon and somatostatin were increased in S7 cells that were encapsulated at S5 compared to the ones grown only on matrigel-coated plates. This condition had also a higher number of NKX6.1 and PDX1 positive cells, in addition to more insulin cells co-expressing these β-cell factors. Cells encapsulated through the entire differentiation protocol also had more glucagon and somatostatin positive cells, but not insulin. By proteomic analysis we observed that encapsulated cells expressed proteins patters closer to human islets compared to cells differentiated on matrigel only.

Conclusion

Encapsulation of the cells at later stages of differentiation promotes the pancreatic-islet differentiated profile in S7-cells.

92 Thomas Hill

Effects of kisspeptin on insulin secretion and glucose homeostasis

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Aims: The placental peptide, Kisspeptin (KP), has been suggested to increase glucose-stimulated insulin secretion (GSIS) in rodents and humans. However, whether this is mediated through its cognate G-protein coupled receptor-54 (Gpr54), or some other receptor on the β-cells is unclear. We used an inducible β-cell-specific Gpr54 knockout mouse (βGpr54KO) to investigate the effects of KP on insulin secretion *in vitro* and glucose homeostasis *in vivo.*

Methods: Following induction of Gpr54 knockdown with tamoxifen (TMX), insulin secretion from isolated islets in response to 20 mM glucose and KP-10 was quantified by radioimmunoassay. Glucose homeostasis of adult βGpr54KO and control mice following exogenous KP-10 administration was assessed by i.p. glucose tolerance tests. Controls included both mice without TMX (Cre+ve/TMX-ve) and Cre-ve animals.

Results: GSIS from islets of Langerhans isolated from both βGpr54KO and control animals was equivalent. However, exogenous KP (1 µM) potentiated GSIS (AUC; 20 mM Glucose + KP vs 20 mM Glucose; 56.5 ± 13.6 vs 28.5 ± 1.58; $p < 0.01$) from control islets, but not in islets from βGpr54KO animals ($p > 0.05$). *In vivo* studies using control and βGpr54KO mice demonstrated that i.p. KP (100 nM) improved glucose clearance in control mice (Blood Glucose AUC; Control vs Control + KP; 625.5 ± 115.2 vs 236.6 ± 103.0; $p < 0.05$) but not in equivalent βGpr54KO animals (AUC, βGpr54KO vs βGpr54KO + KP; 644.3 ± 115.2 vs 466.5 ± 115.1; $p > 0.05$).

Conclusions: These data suggest that the potentiation of GSIS and improvement in glucose tolerance in mice in response to kisspeptin are due to a direct effect on β-cell Gpr54.

93 Tina Fløyel

Lysosomal cathepsin proteases are regulated by proinflammatory cytokines in human β-cell models

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Background and aims:
We previously showed that the type 1 diabetes (T1D) candidate gene *CTSH* (lysosomal protease cathepsin H) regulates β-cell function, apoptosis, and disease progression in children with newly-diagnosed T1D. As proinflammatory cytokines downregulate *CTSH* in human islets, we speculated if dysregulation of other members of the cathepsin protease family is implicated in β-cell dysfunction in T1D. The aim of the study was to investigate the expression of the cathepsin proteases in human model systems of β-cell dysfunction.

Materials and methods:
Expression of the cathepsins was examined in untreated and cytokine-treated human islets (*n*=5), EndoC-βH1 cells (*n*=4), and 1.1B4 cells (*n*=4) by RNA sequencing or real-time qPCR. siRNA-mediated knockdown of cathepsin expression was performed in 1.1B4 cells (*n*=4-6) and apoptosis was investigated using Caspase-Glo 3/7 assay.

Results:
In human islets, 13 of the 15 human cathepsins were expressed and five cathepsins were differentially expressed after treatment with IL-1β+IFNγ for 48h ($log2FC=-1.77_{CTSF}$, $log2FC=-2.50_{CTSH}$, $log2FC=-0.94_{CTSK}$, $log2FC=1.40_{CTSO}$, $log2FC=3.17_{CTSS}$, $p<0.05_{Bonferroni\text{-}adjusted}$). Twelve cathepsins were expressed in EndoC-βH1 and 1.1B4 cells. In EndoC-βH1, five cathepsins were differentially expressed after IL-1β+IFNγ for 48h ($log2FC=0.35_{CTSB}$, $log2FC=-0.51_{CTSF}$, $log2FC=4.04_{CTSO}$, $log2FC=10.51_{CTSS}$, $log2FC=-1.14_{CTSV}$, $p<0.05_{FDR\text{-}adjusted}$). In 1.1B4, seven cathepsins were differentially expressed after IL-1β+IFNγ+TNFα for 24h ($log2FC=0.54_{CTSB}$, $log2FC=1.44_{CTSC}$, $log2FC=-0.60_{CTSD}$, $log2FC=-0.91_{CTSF}$, $log2FC=-1.37_{CTSH}$, $log2FC=1.42_{CTSO}$, $log2FC=4.98_{CTSS}$, $p<0.05_{Bonferroni\text{-}adjusted}$). *CTSH* and *CTSS*, being the most downregulated and upregulated cathepsin respectively, were knocked down in 1.1B4 cells to assess their effect on cytokine-induced apoptosis. *CTSS* knockdown had no effect but *CTSH* knockdown aggravated cytokine-induced caspase-3/7 activity (2-fold, p<0.01), which is in line with previous data showing that overexpression of *CTSH* protects INS-1 cells.

Conclusion:
Most of the cathepsin proteases are expressed in human β-cell models, and several cathepsins were differentially expressed after cytokine exposure. Dysregulation of the cathepsin family may therefore play a role in the immune-mediated destruction of the β-cells that occurs during development of T1D.

94 Tom Barsby

Probing the Functional Immaturity of Pluripotent Stem Cell Derived Beta Cells

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Pancreatic islet beta cells can be derived from human pluripotent stem cells (hPSCs) using multistage protocols that mimic *in utero* tissue development. The hPSC-derived beta cells are transcriptionally close to primary beta cells and are equipped with functional exocytotic machinery that can release insulin following pharmacological stimuli. However, the coupling of glucose metabolism to insulin release is inefficient. Our aim is to systematically identify the key metabolic components underlying this functional immaturity and develop strategies to overcome it.

hPSC-derived islet-like clusters were differentiated *in vitro* using a 7-stage protocol before implantation into immunocompromised mice. 10X single cell RNA-sequencing (scRNAseq) analysis was used to compare the transcriptomic profiles of pre- and post-implanted islet-like cells. Respiration rates and mitochondrial kinetics of islet clusters were probed using a Seahorse XFe96 Analyzer. The metabolic flux of radiolabeled ^{13}C-glucose in hPSC-derived islet cells was tracked using liquid chromatography-mass spectrometry (LC-MS).

The scRNAseq data provides strong evidence for endocrine cell maturation of islet-like clusters following implantation, and has many overlaps with published primary beta cell transcriptomic data. Genes such as G6PC2, a proposed mature beta cell marker and enzyme associated with glucose metabolism, are highly differentially expressed between pre- and post- implanted beta cell clusters. Mitochondrial respiration experiments show low levels of glucose coupled mitochondrial activation from pre-implanted islet clusters. Mitochondrial kinetics are also unchanged after treatment with activators of the cAMP/PKA and PLC/PKC amplification pathways, as well as the mTORC1 and AMPK signaling pathways. Metabolic flux data also indicates the low level of mitochondrial involvement in glucose metabolism in immature pre-implanted islet-like clusters.

These data form a positive preliminary report, but with the combination of ongoing transcriptomic and metabolomic experiments we expect our rational approach to reveal the underlying metabolic pathways of beta cell maturation.

95 Väinö A Lithovius

ABCC8 -mutant stem cell derived islet cells recapitulate the features of congenital hyperinsulinism

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Mutations in the genes encoding the beta cell K_{ATP}-channel subunits of are the most common cause of congenital hyperinsulinism (CHI) and cause a drug resistant, severe form of the disease, characterized by continuous insulin secretion regardless of blood glucose concentration. Our objective was to recapitulate this CHI phenotype with patient induced pluripotent stem cell (iPSC) derived beta cells *in vitro* and in a humanized mouse model and to use the model to investigate the developmental consequences of K_{ATP}-channel inactivation.

iPSCs were derived from a patient with severe, diazoxide unresponsive diffuse CHI caused by homozygous *ABCC8*-V187D -mutation. iPSCs from a healthy donor and the patient iPSCs corrected with CRISPR-Cas9 technology were used as controls. The cells were differentiated into islet-like clusters (ILCs) that were studied *in vitro* with sequential exposures to glucose and insulin secretion modifying pharmaceuticals. The ILCs were also implanted into immunocompromised mice that were subjected to an insulin tolerance test 4 months after implantation. The endocrine cell populations and proliferation rates were determined in the final stage ILCs and graft explants by a custom automated segmentation pipeline on the CellProfiler software.

Mutant ILCs had higher basal insulin secretion and failed to respond to K_{ATP}-channel acting pharmaceuticals.
The CHI-mice had lower fasting blood glucose and higher circulating human c-peptide. Most importantly, the human c-peptide secretion was not inhibited by insulin-induced hypoglycemia in the CHI-mice.
The mutant ILCs had a larger proportion of insulin monohormonal (50,8% SD 2,4% *vs.* 34,3% SD 5,2% p=0,019) cells among the endocrine cells. This difference was not explained by increased proliferation of insulin positive cells.

In conclusion we have recapitulated the CHI phenotype in beta cells derived from patient iPSC in vitro and in a humanized mouse model. Our data also suggests that K_{ATP}-channel inactivation biases the developmental trajectory of islet cells towards beta cells.

96 Vikash Chandra

TYK2 Knockout iPSC Lines For T1D Modelling

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Type 1 Diabetes (T1D) is caused by the autoimmune destruction of pancreatic beta cells. Genome wide association and fine mapping studies to identify causal variants predicted several loci in the human genome that are associated with T1D risk. One such candidate gene is a member of the JAK (Janus Kinase) family of tyrosine kinases, TYK2, which plays a critical role in intracellular signaling stimulated by cytokines through STATs. Loss-of-function variants of TYK2 are associated with protection against T1D. In line with this, silencing of TYK2 in beta cells attenuated inflammatory signaling pathways and apoptosis. To study the role of TYK2 in human beta-cell development, we now generated TYK2- knockout (KO) human iPSC lines using CRISPR-Cas9 genome editing and induced them to differentiate to pancreatic endocrine lineage. Our results suggest that absence of TYK2 does not compromise the pluripotency or the early pancreatic endoderm differentiation potential of iPSC. However, in the later stages of differentiation, the mRNA levels of key pancreatic TFs (*PDX1* (55±17%, *p=0.03*), *NGN3* (65±13%, *p=0.008*)), *RFX6* (58±14%, *p=0.014*), *NKX6.1* (39±19%, p=0.114) and *NKX2.2* (68±7%, *p=0.0008*)) were significantly reduced in the TYK2-KO cells. In the final stages of controlled induction to endocrine lineage TYK2-KO lines expressed lower levels of *INS* (53±9%, *p=0.004*), *GCG* (70±10.5%, *p=0.002*) and *SST* (62±8.6%, *p=0.002*) compared to control lines (mean ± SEM of 3 independent experiments). Furthermore immunostaining for INS, GCG and SST confirmed a lower number of endocrine hormone positive cells in TYK2-KO lines. In contrast to the wild-type controls, the TYK2-KO iPSC-derived pancreatic cells displayed no activation of STAT1 and STAT2 and showed impaired STAT3 activation in response to IFNα or stimulation by polyinosinic-polycitidilic acid treatment, a viral infection mimetic. The present study suggests that TYK2 plays an important early role in pancreatic endocrine differentiation.

97 Wan Jun Gan

Insulin granule fusion is tightly restricted along beta cell-vasculature interface in rodent pancreatic slices

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Loss of insulin secretion in islet beta cells is a recognised characteristic of diabetes. It is therefore important to understand how insulin secretion is controlled in the beta cells. Our previous work shows that insulin secretion is targeted towards the vasculature (Low et al., 2014). This targeting is regulated by vascular basement membrane proteins-activated integrin beta 1 and focal adhesion (Gan et al., 2018). All of these works are however done in isolated islets and dispersed beta cells. An agarose-perfused pancreatic slice preparation that preserves the native islet microenvironment has been recently developed (Marciniak et al., 2014). This method should better reflect the islet physiology *in vivo*. Here, we therefore assess the targeting of insulin secretion and underlying regulatory mechanisms in the slices.

In the islets prepared using the slicing method, approximately 92% of beta cells are in contact with the vasculature. Along the beta cells-vasculature interface, immunostaining and 3D confocal imaging show an enrichment of integrin beta 1 and activated focal adhesion proteins such as p-paxillin and p-FAK. Using two photon live cell imaging, insulin granule fusion upon 15mM glucose stimulation is more tightly concentrated along the vasculature in the islets of the pancreatic slices, as compared to the isolated islets that already exhibit a bias towards the vasculature. Use of FAK inhibitor Y15 and FAK-silencing shRNA in the slice suppress the vascular-targeting of insulin granule fusion. This result suggests that in the slice, the targeting of insulin granule fusion is also regulated through the focal adhesion-mediated mechanism as previously reported in the dispersed beta cells.

As a summary, our report of vascular-targeted insulin secretion is more tightly focus in an intact islet microenvironment similar to that *in vivo*.

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98 Zeina Drawshy

Accurate quantification of beta-cell fraction in islet preparations

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Measuring Glucose Stimulated Insulin Secretion (GSIS) *ex-vivo* in mouse and cadaveric human islets is the gold standard for assaying beta-cell function. Insulin secreted is usually normalized to protein or insulin content. Such normalization might be inadequate specifically in cases where protein or insulin content is changed. An alternative is to measure insulin secretion on a per-islet basis or normalize to DNA. The caveat with this method is that islets contain other cell types beside beta-cells and that both islet cell composition and function can be affected by conditions such as prolonged hyperglycemia. We reasoned that a more appropriate method would be to normalize insulin content or secreted insulin to beta-cell DNA. To this aim we developed a sensitive and specific Taqman-ddPCR assay using bisulfite converted islet DNA as a template to quantify beta-cell DNA in islets. The detection of beta-cell DNA is based on the presence of differentially methylated CpG sites in the mouse Ins2 and human INS genes. These sites are specifically unmethylated in beta cells while almost fully methylated in non-beta-cells.

We show that this method is quantitative, specific and sensitive enough to measure beta-cell DNA in mouse and human islets. We are currently calibrating this procedure to assess the fraction of alpha-cells in human islets.

Our ultimate goal is to use this strategy to quantify the main cell-type fractions (beta, alpha and delta) in islets. Such a resolution will be useful to monitor the effects of islet cell composition on beta-cell function.

99 Zhe Jin

GABA modulates immune cell function in type 1 diabetes

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The γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain. However, accumulating evidences suggest GABA as an emerging immune and inflammatory modulator. GABA is synthesized from glutamate via the enzyme glutamic acid decarboxylase (GAD 65 and 67). In type 1 diabetes (T1D), GAD65 is one of the major humoral autoantigens. We aimed to study how GABA affects the release of inflammation-related proteins (IRPs) and immune cell proliferation in T1D. Plasma and peripheral blood mononuclear cells (PBMCs) were isolated from fresh blood samples and $CD4^+$ T cells from buffy coats of nondiabetic (ND) individuals and T1D patients. The GABA concentration and IRPs were measured in plasma by GABA ELISA and multiplex Proximity Extension Assay (PEA) assays, respectively. The plasma GABA concentration and 26 IRPs were significantly increased in plasma from T1D patients as compared to ND individuals. Isolated PBMCs and $CD4^+$ T cells were stimulated with anti-CD3 antibody to examine the effect of GABA on cell proliferation and IRPs release in the culture media. GABA (100 nM) significantly inhibited proliferation of PBMCs from T1D patients but not ND individuals. In addition, GABA (100 nM) decreased the release of 47 and 16 IRPs in the medium from stimulated PBMCs of T1D patients and ND individuals, respectively. Interestingly, stimulated $CD4^+$ T cells from ND individuals can be divided into GABA responder and non-responder groups based on the response to GABA (100 and 500 nM) in the proliferation assay. In the non-responder T cells, GABA only regulated release of 8 IRPs. In contrast, GABA decreased cell proliferation and release of 37 IRPs in the responder T cells. Our results suggest the immunomodulatory function of GABA varies among immune cell subpopulations, is concentration-dependent and altered in T1D (Bhandage AK, Jin Z et al., EBioMedicine. 2018, 30:283-294).

100 Zuzana Marinicova

A novel highly specific and sensitive mAb against capsid protein VP1 to study the involvement of Enteroviruses in type 1 diabetes.

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Background: Enteroviruses (EVs) have been linked to pathogenesis of type 1 diabetes (T1D), but their causality in beta cell autoimmunity remains debated. EV presence in beta cells from subjects with recent-onset T1D has been shown by immunostaining with the monoclonal antibody (mAb) 5D8/1 against the VP1 capsid protein of a broad spectrum of EVs. However, the availability of additional antibodies against distinct EV epitopes may be very valuable to corroborate such findings, and thus the viral hypothesis of T1D pathogenesis.

Methods: VP1 from Coxsackievirus B5 (CVB5) Faulkner and MCA strains were expressed fused with GST in E. Coli and used for mouse immunization. mAbs from several hybridomas were screened for recombinant VP1 detection. Reactivity against various EV-infected cells was assessed by immunoblotting, ELISA, immuno-histo/cytochemistry and -fluorescence.

Results: One of the anti-VP1 mAb (termed Veronica) showed strong reactivity against recombinant VP1 and several EV serotypes, including CVA9, CVB1-6, Echo-9-Barty, -Hill and -DM, -11 and -30, and Polioviruses 1-3 by ELISA. Independent tests on formalin-fixed paraffin-embedded (FFPE) EV cell microarrays demonstrated that Veronica mAb recognized Echoviruses -3, -4, -6, -9, -11 and CVB6 more strongly than 5D8/1 mAb. Unlike 5D8/1 mAb, however, it did not recognize CVA2, 5, 6, and 10, and only weakly CVA4 and AdenoC. No background was observed in uninfected A549, HPeV1, Vero and Hela and RD cells. Analysis of FFPE uninfected and CVB4-infected neonatal heart tissue confirmed the specific reactivity of Veronica mAb with CVB4. No background was detected in uninfected tissue.

Conclusion: Differences in their ability to detect various EVs suggest that Veronica and 5D8/1 mAbs recognize distinct VP1 epitopes. Hence, their combined use for staining of tissue specimens from T1D could be valuable to validate the correlation between EV infection of beta cells and their autoimmune destruction.

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101 Linford Briant

Counter-regulatory glucagon secretion evoked by hypoglycaemia is mediated by arginine-vasopressin

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Abstract:
Glucagon is released by islet alpha-cells to counter a major threat to survival: hypoglycaemia. The mechanisms by which this stressor evokes glucagon secretion are generally thought to be due to intrinsic sensing of hypoglycaemia by the islet, although this view may be considered 'islet-centric'. Here, the regulation of glucagon by arginine-vasopressin (AVP), secreted from the posterior pituitary, is investigated. Activation of AVP neurons *in vivo* using DREADD approaches (hM3Dq) increased circulating AVP and evoked hyperglycaemia that could be blocked by pharmacological antagonism of either the glucagon receptor or vasopressin 1b receptor (V1bR). In *ex vivo* mouse and human islets, V1bR was detected at the mRNA level. Incubating islets in AVP increased intracellular Ca^{2+} oscillations, DAG, action potential firing and glucagon secretion. Monitoring brain Ca^{2+} activity (GCaMP6s) using *in vivo* fibre photometry demonstrated that AVP neuron activity is increased in response to hypoglycaemia. Furthermore, pharmacological approaches revealed that AVP signalling is central to glucagon secretion in response to counter-regulatory challenges, including fasting, exogenous insulin, and neuroglucopaenia evoked using the non-metabolisable glucose analogue 2-DG. By combining *in vivo* fibre photometry and DREADD approaches (hM4Di), we were able to show that the A1/C1 neurons of the medulla oblongata provide the excitatory input to AVP neurons in response to hypoglycaemia. Central mechanisms therefore contribute significantly to glucagon secretion evoked by hypoglycaemia, with AVP having an important role in mediating this counter-regulatory response.

102 Miri Stolovich-Rain

Differential regulation of cell size by nutrients and fasting in the endocrine and exocrine pancreas

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The dynamic control of cell size is an important yet understudied aspect of tissue homeostasis. PI3K-mTOR signaling pathway, integrating intracellular information about availability of nutrients with extracellular input transduced via cell surface receptors, is thought to be a universal regulator of cell size. We report that within the pancreas, cell size in the endocrine and exocrine compartments is regulated very differently. Various manipulations of PI3K-mTOR signaling (including gain and loss of function mutations, as well as treatment with the mTOR inhibitor rapamycin) have a considerable impact on beta cell size, but do not affect acinar cell size. By contrast, fasting leads to a rapid and dramatic reduction in acinar cell size, while beta cell size remains unchanged. We propose that these effects reflect the key role of intracellular nutrient sensing for beta cell size control, and the reliance of acinar cells on PI3K-independent hormonal input for cell size control. Mechanisms determining the beta cell size set point might be novel targets for enhancement of beta cell functional mass in diabetes.

103 Mawieh Hamad

Estrogen-induced suppression of hepcidin synthesis enhances intracellular iron efflux and minimizes oxidative stress and apoptosis in pancreatic β-cells

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It is well accepted that elevated levels of estrogen (17-β estradiol, E2) associate with significant protection against diabetes mellitus (DM). This has been attributed to the ability of E2 to enhance β-cell proliferation, increase insulin secretion and reduce gluconeogenesis among other mechanisms. However, the exact molecular mechanism underlying this effect of E2 remains unclear. In this context, several studies have demonstrated that elevated levels of E2 suppress hepcidin synthesis and enhance intracellular iron efflux in breast, ovarian and liver cell lines. Hence, E2-driven intracellular iron efflux may limit the production of reactive oxygen species (ROS) and minimize oxidative stress in β-cells. This is particularly relevant given that increased oxidative stress in β-cells induces apoptosis and leads to DM. To test this idea, rat clonal β-cells (INS-1/832/13) were treated with E2 and/or apoptosis-inducing cytokines for 24-48 hrs. and assessed for intracellular iron status, mitochondrial function and apoptosis. Treatment of INS-1 cells with 20 nM E2 for 24-48 hrs. resulted in a significant reduction in hepcidin synthesis and a transient depletion of intracellular labile iron. It also associated with a significant depolarization of mitochondrial inner membrane, which suggests reduced ROS production. Levels of ferritin protein were significantly lower ($p<0.05$) and those of Hif-1α were significantly higher ($p<0.05$) in cells on E2 + cytokines as compared with cell on cytokines alone. Apoptosis in E2 + cytokines-treated cells was significantly lower ($p<0.05$) than that in cytokines alone-treated cells. Lastly, expression of p^{53} and p^{21} proteins was significantly increased ($p<0.01$) and that of BIRC5 (survivin) was significantly reduced ($p<0.05$) in cells on E2 + cytokines as compared with cells on cytokines alone. Work is underway to replicate these studies in cadaveric human islets and to further characterize the anti-apoptotic effects of E2. These findings suggest that E2-driven intracellular iron efflux reduces oxidative stress and enhances β-cell survival.

104 Muhammad Saad Khilji

Constitutive expression of non-standard proteasomes and its further upregulation in insulin producing cells with low dose of Interleukin 1-β

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Non-standard proteasomes (intermediate- and immune-proteasomes referred here as i-proteasomes) differ from standard proteasomes by being expressed in response to oxidative stress or cytokines in cells other than that of hematopoietic origin. I-proteasomes degrade non-ubiquitinated small proteins and generate a peptide repertoire different from standard proteasomes to be presented on MHC-I. We investigated the presence of these inducible subunits (β1i, β1i and β5i) in INS-1E cells and human and mouse islets under normal and stressed conditions, i.e. exposure to the cytokines interleukin 1-beta (IL-1β) and interferon gamma (IFN-γ). I-proteasome subunits are constitutively expressed in INS-1E cells and induced by IFN-γ. Further, IL-1β at low dose (15pg/ml) alone is capable of upregulating inducible subunits in INS-1E cells and human islets. All three proteasomal activities (trypsin-, chymotrypsin- and caspase-like) were demonstrated in INS-1E cells. Selective inhibition of β5i subunit by ONX-0914, β5i specific inhibitor, reduced β5i-dependent chymotrypsin activity in non-induced INS1-E cells further demonstrating constitutive expression and proteolytic activity of i-proteasomes in these cells. Exposure to cytokines such as IL-1β and IFN-γ further increased i-proteasomal enzymatic activities.

We conclude that i-proteasomes are not only present under basal conditions but also active in β-cells and upregulated in response to cytokine exposure including interleukin 1-β.

105 Reinaldo Sousa Dos Santos

Differential effects of chronic exposure to bisphenol A on ion channel activity and expression in mouse pancreatic β-cells

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Aims/hypothesis: Bisphenol A (BPA) is a widespread endocrine-disrupting chemical used to make food and beverage cans, polycarbonate plastic, and cash register receipts. It has been shown that low doses of BPA modify pancreatic β-cellsfunction and induce insulin resistance, among other metabolic effects. Here we investigated whether low doses of BPA regulate ion channel expression and function in mice pancreatic islets.

Methods: Microarray gene profiling of isolated islets from vehicle- and BPA-treated (100 µg/kg/day for four days) mice was performed using Affymetrix GeneChip Mouse Genome 430.2 Array. Expression level analysis was performed using the normalization method based on the processing algorithm named robust multi-array average. Whole islets or dispersed islets from C57BL/6 or oestrogen receptor beta (ERβ) knockout (βERKO) mice were treated with vehicle or BPA (1 nM) for 48 h. Whole-cell patch-clamp recordings were used to measure sodium and potassium currents. mRNA expression was evaluated by quantitative RT-PCR.

Results: Microarray analysis showed that BPA modulated the expression of genes, encoding important sodium and potassium channel subunits, such as *Scn9a*, *Kcnb2*, *Kcnma1*, and *Kcnip1*. These findings were confirmed by quantitative RT-PCR. Electrophysiological measurements showed a decrease in both sodium and total potassium currents in BPA-treated islets. The pharmacological profile indicated that BPA reduced $K_v2.1/2.2$- and $K_{Ca}1.1$-mediated currents in agreement with BPA effects on gene expression. β-cells from βERKO mice did not present BPA-induced changes, suggesting that BPA effects involved ERβ.

Conclusions/interpretation: Our data suggest that BPA modulates the expression and function of sodium and potassium channels via ERβ in mice pancreatic islets. Altogether, these BPA-induced changes in beta cells might play a role in the diabetogenic action of BPA described in animal models.

106 Aimée Bastidas-Ponce

Deciphering mechanisms of β-cell failure and regeneration by single cell RNA sequencing

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Insulin-dependent diabetes is characterized by progressive β-cell loss or dysfunction. Recently, β-cell dedifferentiation has been proposed as a major mechanism of β-cell failure in both forms of diabetes. If dedifferentiated or lost β-cell mass can be regenerated by pharmacological intervention is ill defined. Here, we used the multiple low dose streptozotocin (mSTZ) β-cell ablation model to reveal mechanisms of islet cell failure and regeneration. Massive parallel single-cell RNA sequencing (scRNAseq) revealed that immature and mature β-cells co-exist in healthy mouse islets. Upon mSTZ treatment and chronic hyperglycemia, most β-cells die, but surprisingly some remain dedifferentiate in a dysfunctional and embryonic-like state. Dedifferentiated β-cells are characterized by the upregulation of a large number of ER stress and dedifferentiation markers. Furthermore, STZ-mediated β-cell killing induced islet cell plasticity and changes in core transcription factor (TF) programs of non-β- and β-cells to become polyhormonal contributing to islet cell dysfunction. Daily treatment with a long acting insulin analog or a glucagon-like peptide-1 / estrogen conjugate (GLP-1/estrogen) for 100 days ameliorated mSTZ-induced hyperglycemia. Strikingly, both GLP-1/estrogen-mediated ER-associated protein degradation (ERAD) and insulin-mediated signaling activation in β-cells restores TF programs and triggers β-cell regeneration to restore glycemic control. Altogether, our study deciphers paths and mechanisms of islet cell failure and reveals markers and drugable pathways for regenerative therapy.

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