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Maternal diabetes in brain development: sexually dimorphic response to metformin treatment.

Miguel Ángel Serrano Lope (DIfE)

As Gestational Diabetes Mellitus (GDM) prevalence has risen during last decades, in utero exposure to anti-diabetic treatments has increased and studies about the fetal side effects are necessary. However, there is a lack of knowledge in understanding how these treatments affect the offspring's development. To address this, female mice received either CD or HFD during pregnancy and/or lactation, and anti-diabetic treatments, specifically insulin and metformin, were administered in early post-natal life. Under this paradigm, maternal HFD (mHFD) exposure did not affect glycemia in P16 offspring, while insulin and leptin levels were increased. Metformin treatment exhibited a sexually dimorphic effect: glycemia and insulin levels were reduced in males, while females showed no changes in glycemia but increased plasma insulin and leptin levels when HFD-fed. mHFD also led to AgRP fibre density reduction in anterior PVH nucleus, which could only be partially rescued by metformin treatment in males. Sexual dimorphism in AMPK activation was also observed by Western Blot. mHFD did not change AMPK activation in males, but it was increased in females. Thus, we conclude that mHFD exposure compromises several metabolic markers and hypothalamic network development in offspring mice and that these impairments do not appear to be rescued by metformin.

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The development of MC3R neurons, AgRP and POMC neuronal projections and the maintenance of intra-hypothalamic neuronal circuits.

Selma Yagoub (DIfE)

The melanocortin system has been extensively studied for its role in regulating feeding behavior and the metabolism. However, the role of this system in early development and the establishment of these neuronal circuits are less well known. We sought to study the establishment of key connections from the arcuate nucleus of the hypothalamus (ARC) neurons, namely agouti-related peptide (AgRP) and Pro-opiomelanocortin (POMC), in early development and their persistence into adulthood. In addition to this, we analyzed how the loss of the melanocortin 3 receptor (MC3R), expressed by AgRP neurons, affect these AgRP and POMC neuronal projections specifically within the hypothalamus. qPCR data collected throughout the development suggests a dynamic regulation for the MC3R expression in the hypothalamus. Therefore, using the MC3R-GFP mouse model, we were able to assess the dynamic development of MC3R neurons in the hypothalamus. These results suggest that MC3R is absolutely critical for the development of POMC and AgRP neuronal connections within the hypothalamus. Elaborating on previous literature assessing the structural development of ARC neuronal projections, we identified, throughout development, the positive labeling of the individual of AgRP and POMC neuropeptides themselves to intra but also interesting extra-hypothalamic structures. The dynamic regulation of MC3R expression and establishment of neuronal connectivity in early development underscores the probable role of the melanocortin system not only in adult feeding behavior, but also in early formation of AgRP and POMC neurocircuits.
Keywords: Hypothalamus, Melanocortin, brain development.

Abstracts of posters

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Two novel genomic loci conferring T2D protection in an additive manner

Katharina Kaiser (DDZ)

The individual susceptibility for the development of obesity and type 2 diabetes (T2D) is poorly understood. Mouse inbred strains provide the opportunity to identify novel genetic determinants of disease progression. Previous linkage analysis conducted in our research group using a T2D-susceptible New Zealand Obese (NZO) and a T2D-resistant (C3HeB/FeJ) strain revealed quantitative trait loci for blood glucose levels on chromosomes 7 (NZO blood glucose, Nbg7) and 15 (Nbg15), respectively. Both loci individually confer protection from obesity-associated hyperglycemia in an N2.NZOxC3H backcross population. Moreover, introgression of the respective C3H alleles into the NZO genome during the generation of recombinant congenic strains (RCS) demonstrated an additive protective effect of Nbg7 and Nbg15 from onset and progression of T2D. More specifically, lower blood glucose levels in Nbg7/15-C3H allele carriers were accompanied by elevated plasma insulin levels in a body weight-independent manner when compared to Nbg7/15-NZO allele carriers, indicative of a mechanism targeting the pancreatic islets of Langerhans. Furthermore, gene expression profiling and in silico analysis identified a number of candidate genes (Atp4a, Nudt19, Pop4, Kdelr3, Cbx6, Fam135b) as potentially causal for both loci. Future steps aim to analyze the interaction of genes from both loci in islet cell function.

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Nbw4 locus on mouse chromosome 4 harbors candidate genes which protect from fat accumulation and obesity

Jenny Minh-An Khuong (DDZ)

Obesity represents a major risk factor for the development of insulin resistance and T2D. The polygenic factors promoting obesity and T2D remain to be identified. Previously, we conducted linkage analyses in a backcross population generated with T2D- prone New Zealand Obese (NZO) and T2D-resistant C3HeB/FeJ mice. We detected a quantitative trait locus on chromosome 4 (Nbw4, NZO body weight on chromosome 4) that conferred protection from high-fat diet (HFD)-induced obesity. The aim of the current study is to identify the underlying genetic variants and to assess their molecular functions in energy metabolism. Repetitive backcrossing steps were performed to generate a recombinant congenic mouse line (RCS) harboring the locus on Chromosome 4 from C3H on a NZO background. RCS mice were fed a high fat-diet (45% fat/Kcal) and underwent measurements of blood glucose and body composition. At 13 weeks of age, mice were sacrificed and tissues were harvested for gene expression analysis. Blood samples were collected for plasma insulin measurements. Transcriptome analysis conducted in the parental mouse strains combined with in silico sequence analysis were used for the identification of gene variants potentially linked to the phenotype. Homozygous carriers of the Nbw4-C3H allele (C3H/C3H) demonstrated a significantly lower body weight compared to Nbw4-NZO allele carriers (NZO/NZO), starting at 6 weeks of age. Gene expression profiling in white adipose tissue (WAT) showed differential expression levels of genes from the Orosomucoid (Alpha-1-Acid Glycoprotein) family). Haplotype analysis followed by in silico predictions of gene/protein function revealed a potentially deleterious mutation R15Q in the NZO Orm1 gene variant, predicted to impair structure and function and studies in 3T3-L1 pre-adipocytes show upregulation of Orm1 during differentiation to mature adipocytes. Here we identify a novel obesity locus on Chromosome 4 with large effect size on body fat. Recombinant congenic mice carrying the C3H allele of Nbw4 on a NZO background are protected from HFD-induced obesity. Gene expression studies show strain-specific expression of the secreted Orm family genes in WAT, where expression correlated with protection from obesity.

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Investigating the impact of FNDC4 gene variants on FNDC4 protein production, secretion and prediabetes risk

Aspasia Thodou-Krokidi (HMGU)

Background:Fibronectin type III domain containing 4 (FNDC4) is a type I transmembrane protein, which releases a soluble bioactive protein that has an anti-inflammatory effect on macrophages and adipocytes. Upon binding to GPR116 promoted insulin signaling and insulin-mediated glucose uptake in white adipocytes
Aim:Our goal is to investigate the impact of FNDC4 single nucleotide polymorphisms on FNDC4 protein production and secretion and their role in FNDC4 associated onset of prediabetes.
Methods: Analysis of GWAS and HMGU-KORA studies revealed a positive association of intronic FNDC4 SNPs with obesity and T2D in humans. In in-vitro experiments we have inserted the pre mRNA of FNDC4 in an expression vector. By point mutagenesis we have created a vector carrying the rs2303369(T:T) and rs704795(A:A) and have transfected these constructs in HeLa cells that express no endogenous FNDC4
Results: We found that rs2303369(T:T) and rs704795(A:A) occurred twice as often in severe prediabetes individuals and positively correlated with lower circulating levels of sFNDC4. Moreover, heterologous expression of those SNPs resulted in lower production and secretion of FNDC4 and a compensatory increase of newly identified shorter FNDC4 isoforms.
Conclusion: rs2303369(T:T) and rs704795(A:A) diabetes risk associated FNDC4 SNPs led to lower production and secretion of FNDC4.

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Combining next generation sequencing and metabolic phenotyping to examine molecular mechanisms of reduced mitochondrial respiration in type 2 diabetes

Martin Schön (DDZ)

Background: Single molecule real-time sequencing (SMRT-Seq) is recently established full-length transcriptomics technique, allowing precise detection of isoforms with 99% accuracy in an unbiased approach.
Aims: We aimed to apply SMRT-Seq in human skeletal muscle, and in a combination with deep metabolic phenotyping to elucidate molecular mechanisms of reduced mitochondrial respiration in type 2 diabetes (T2D).
Patients & Methods: Muscle biopsies were taken from 9 humans with T2D as well as 9 age- and body mass index-matched glucose tolerant men (CON). Whole-body insulin sensitivity (WBIS) was assessed by hyperinsulinemic-euglycemic clamps and mitochondrial respiration by high-resolution respirometry.
Results: Metabolic phenotyping revealed reduced WBIS, fatty acid-driven, complex I and complex II mitochondrial respiration in T2D than in CON. Full-length transcriptomics of skeletal muscle detected 4 transcripts of ATP synthase-related gene ATP5F1A, which encodes a subunit of ATP synthase, a key enzyme in mitochondrial respiration. Only one transcript of this gene was previously known, and only one of these 3 newly discovered transcripts was expressed in both T2D and CON, whereas two other novel transcripts were present only in CON.
Conclusions: Combination of latest sequencing methods and metabolic phenotyping can help to better understand the mechanisms underlying reduced mitochondrial respiration in T2D. In future, comparative transcriptomics may hold the potential to identify novel therapeutic targets in T2D.

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Impact of aging and adiposity on impaired skeletal muscle mitochondrial energy metabolism and regeneration

Francisco Garcia (DIfE)

Introduction: Aging is associated with a progressive decline of functional and regenerative capacity in muscle tissue. Extracellular matrix (ECM) components are extrinsic factors of the stem cell niche necessary to maintain muscle regeneration and metabolic function. Here, we hypothesize that the ECM-components secreted by muscle-resident fibro-adipogenic progenitor cells (FAPs), might play a major role in the age-associated muscle disorders that contribute to decreased muscle function.
Methods: Transcriptome analysis of aged FAPs was used to identify matrix components. Histology analysis of injured muscle in aged and loss-of-function mice models were examined. Moreover, FAPs with the inactivation of matrix components was used for transplants in aged muscle. In addition, cell culture and oxidative capacity analysis of progenitor cells were performed.
Results: Aging resulted in a severe down-regulation of ECM genes in FAPs. ECM dysregulation in aged and loss-of-function mice models resulted in a reduction of muscle regeneration and oxidative capacity. In addition, FAPs-transplants with functional ECM-secretion recovered the healthy young phenotype.
Conclusions: These results indicate that alterations in the ECM might be associated with the fibro-adipogenic switch observed in dysfunctional aged muscle. Therefore, the ECM-secreted by FAPs might serve as potential therapeutic target to treat muscle dysfunctions in elderly

Abstracts of posters

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Increased Levels of N-Lactoylphenylalanine After Exercise are Related to Adipose Tissue Loss During Endurance Training in Humans With Overweight and Obesity

Miriam Hoene (IDM)

Metabolites are gaining attention as mediators of the beneficial effects of physical activity. The pseudo-dipeptide N Lactoylphenylalanine (Lac-Phe) is produced during physical exercise and has recently been shown to decrease food intake, weight gain and adipose tissue mass in mice fed a high-fat diet.
To address whether Lac-Phe could have a similar function in humans, sedentary subjects with overweight and obesity participated in an 8-week supervised endurance intervention. Using LC-MS, Lac-Phe was quantified in blood samples of n=22 participants, both in the resting state and immediately after 30 min of bicycle exercise at 80% VO2peak. Adipose tissue volume was quantified by MRI.
The training intervention caused a decrease in BMI and adipose tissue volume. Plasma levels of Lac-Phe were not affected by the intervention but pronouncedly increased after acute exercise. Higher levels of Lac-Phe after acute exercise were associated with a greater reduction in subcutaneous abdominal adipose tissue during the intervention.
Our data provide a first hint that Lac-Phe could have a weight-lowering function in humans and support further studies to elucidate its mode of action and possible role in the prevention of type 2 diabetes and related cardiometabolic diseases.

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Cross-talk communication between Intermuscular Adipose Tissue and Skeletal Muscle under progressing Insulin Resistance

Amare Wolide (HMGU)

Background: Intermuscular Adipose Tissue (IMAT) is associated with the insulin sensitivity (IS) of Skeletal Muscle(SM) (Sachs S, et al, 2019). Our study was initiated to understand the molecular cross-talk communications in these tissues in progressive Insulin Resistance (IR).
Methods: For this study, a total of 42 subjects from 3 different study groups were recruited. Overnight fasting blood and muscle biopsy from Vastus lateralis were taken for clinical and RNA-Seq studies. Variables such as age, gender, fat mass, fat-free mass, and body mass index were studied in One-way ANOVA. Differential combination analysis was employed to dissect the molecular crosstalk between IMAT and SM. Significant gene pairs were mapped to the Sender-Receiver Database(SRD), and matching pairs were selected for further analysis. Our SRD was curated after a rigorous database search and text mining of protein-coding genes. In this context, the sender represents a protein that could transmit information from the signalling cell to the target cell, where the receiver protein is located.
Results: We discovered increased communication from IMAT to SM in the augmented IR. Consequently, a higher number of edges and nodes were observed in T2D groups compared to the Overweight and Lean groups. T2D-specific IMAT genes enriched to functions such as lipoprotein assembly, remodelling, and clearance, and disease associated with glycosylation, lipid storage, collagen and other metabolic disorder phenotypes. The most rewiring and hub muscle genes were functionally correlated to metabolic disorders.
Conclusion: Observed genes in the communication could be a target for further study to get novel therapy for SM insulin resistance and diabetes.

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Effects of adrenergic-stimulated lipolysis and cytokine production on in vitro mouse adipose tissue–islet interactions

Estela Lorza Gil (IDM)

Inflammatory cytokines and non-esterified fatty acids (NEFAs) are obesity-linked factors that disturb insulin secretion. Here we investigate whether pancreatic adipose tissue (pWAT) is able to generate a NEFA/cytokine overload within the pancreatic environment and as consequence to impact on insulin secretion. Pancreatic fat is a minor fat depot, therefore we used high-fat diet (HFD) feeding to induce pancreatic steatosis in mice. Relative Adipoq and Lep mRNA levels were higher in pWAT of HFD compared to chow diet mice. Regardless of HFD, Adipoq and Lep mRNA levels of pWAT were at least 10-times lower than those of epididymal fat (eWAT). Lipolysis stimulating receptors Adrb3 and Npr1 were expressed in pWAT and eWAT, and HFD reduced their expression in eWAT only.
In accordance, HFD impaired lipolysis in eWAT but not in pWAT. Despite expression of Npr mRNA, lipolysis was stimulated solely by the adrenergic agonists, isoproterenol and adrenaline. Short term co-incubation of islets with CD/HFD pWAT did not alter insulin secretion. In the presence of CD/HFD eWAT, glucose stimulated insulin secretion only upon isoproterenol-induced lipolysis, i.e. in the presence of elevated NEFA. Isoproterenol augmented Il1b and Il6 mRNA levels both in pWAT and eWAT. These results suggest that an increased sympathetic activity enhances NEFA and cytokine load of the adipose microenvironment, including that of pancreatic fat, and by doing so it may alter beta-cell function.

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Exploring the role of less frequent islet cell types in type 2 diabetes pathogenesis

Pascal Gottmann (DIfE)

To understand early processes in T2D pathogenesis, we performed single cell RNA-sequencing (scRNA-Seq) of islet cells from obese mouse strains, namely diabetes-resistant B6.V-Lepob/ob (OB) and diabetes-prone New Zealand Obese (NZO) mice. In an earlier study, we observed transcriptional differences between OB and NZO β-cells particularly in response to a diabetogenic diet. Here, we aim to investigate the role of less frequent islet cells. By employing specific clustering, differences between the two mouse strains were identified, mainly for α-, δ-cells and macrophages, but not for γ-cells. The different α- and δ-cell clusters exhibited altered expression of mitochondrial genes, which might potentially affect glucagon and somatostatin secretion in NZO mice. Furthermore, NZO α-cells exhibited elevated expression of genes related to stress responses and protein folding, suggesting higher stress levels and misfolding of proteins. Concerning the clusters of macrophages, almost twice as many cells were associated with anti-inflammatory genes in cells of OB mice, indicating a putative protective effect on other islet cells. Comprehensive analysis revealed a potential ligand-receptor interaction, via the ligands AXL/IL15 from macrophages to the IL15RA receptor of OB β-cells. The receptor IL15RA is supposed to activate STAT3, which in turn, based on target prediction, might transcriptionally regulate TGFβ-signalling in β-cells of OB mice. Our data confirms that less frequent islet cell types participate in the development of T2D. For instance NZO mice exhibit low numbers of anti-inflammatory macrophages and thus deprived of their protective effect on β-cells.

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Reconstructing single cell trajectories reveals bile acid receptor signalling as an unexpected player in cell-fate decisions during endodermal organogenesis

Margirt Kamel (PLID)

It is often overlooked that healthy and diseased organs share a common origin in development. In fact, it is the molecular players that govern cell fate specification during development that can also play a role in the genetic mechanisms leading to defects in organogenesis and adult organ function. Currently, a detailed temporal map of cell fate decisions during endodermal organ development is missing. We utilized novel computational methods to generate a transcriptomic map in the form of a branching tree of the lineage decisions in the developing zebrafish endoderm. Our tree illustrated the dynamic transcriptomic segments connecting a population of multipotent progenitors to the liver, intestine and pancreas lineages. Interestingly, we observedthat the early hepatic progenitors express the bile acid receptor farnesoid X receptor (fxr). The FXR receptor, while known to have a role in bile-acid signaling, has not been implicated in liver development. Our CRISPR/Cas9-generated fxr knockout embryos showed increased hepatocyte numbers at the expense of the pancreatic domain. We also identified other metabolic perturbations reminiscent of the human condition where FXR mutations result in liver failure. Conversely, pharmacological activation of the bile-acid signaling pathway expanded the pancreatic anlage at the expense of the liver. Thus, we describe a novel role for fxr in early hepatopancreas development in zebrafish. Furthermore, this zebrafish model may allow defining the cellular mechanism underlying the human disease.

Abstracts of posters

Maternal diabetes affects beta-cell health in offspring – lessons from a genetically diabetic pig model

Libera Valla (LMU)

Introduction: Maternal diabetes can have serious consequences on the offspring, including beta-cell dysfunction. To unravel the underlying mechanisms, we analyzed pancreas samples of wild-type (WT) offspring from genetically diabetic pigs (INSC94Y= MIDY; Renner et al., Diabetes 2013) with eGFP-labeled beta cells (Kemter et al., Diabetologia 2017) to enable beta-cell sorting by flow cytometry.
Methods: In newborn WT offspring of INS-eGFP and INS-eGFP/MIDY sows, oral glucose tolerance test (OGTT) was performed, and a comprehensive biobank was established.
Results: WT offspring from diabetic mothers showed higher insulin and glucose blood concentrations prior and during OGTT, with males being more severely affected than females and having a distinct delayed insulin response curve. scRNAseq analysis of beta cells revealed a higher proportion of INShigh vs. INSlow cells in offspring from diabetic vs. non-diabetic mothers. Holistic proteome analysis of pancreas revealed an increase of insulin and somatostatin and a decrease of glucagon and pancreatic polypeptide content. Newborn WT offspring from diabetic mothers had a distinct increase of urea, uric acid, triglycerides, lactate and lipase, and decreased aspartate transaminase blood concentration.
Conclusion: Maternal diabetes induced multiple molecular alterations of beta cells in the offspring, associated with changes in insulin secretion and altered glucose and lipid metabolism.

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Methylglyoxal Induces Endothelial Dysfunction via a Stunning-like Phenotype

Thomas Fleming (UK Heidelberg)

"Introduction & Open Questions
Elevated levels of methylglyoxal (MG) have been reported to be associated with progression and development of numerous pathological conditions, of which diabetes is the most predominant. However, it still remains unclear, what the cellular effects of MG are and how such effects are induced.

Material & Methods
MG and the associated post-translational modification, MG-H1, were studied in murine cardiac endothelial using immunoassay and mass spectrometry. Cells were stimulated with increasing concentrations of exogenous MG and the effects on cell viability markers, proliferation, metabolism and endothelial phenotype assessed.

Results:
Stimulation with MG induced a loss in proliferation, as well as endothelial dysfunction. These effects were only evident once the intracellular MG was 12-fold higher than the basal levels, leading to an equivalent increase in MG-H1. This represents an in vitro threshold which needs to be reached in order for MG to induce a cellular effect. Increasing the levels above this threshold was shown to be associated with the induction of DNA damage and cytotoxicity.

Conclusions:
MG-induced cellular stunning describes a new hallmark for cellular dysfunction which could lead to alterations in tissue homeostasis as well as cell-to-cell interactions, thereby contributing to the pathogenesis of late diabetic complications, such as cardiovascular disease.

Abstracts of posters

MATERNAL HYPERGLYCEMIA CAUSES METABOLIC ALTERATIONS IN THE LIVER OF NEONATAL OFFSPRING

Bachuki Shashikadze (LMU)

So far, diabetes-related fetal programming has been mainly observed by epidemiological studies and neonatal or postnatal adverse outcomes at the proteomics level remain almost unexplored. To address this, we used a clinically diabetic pig model (INSC94Y = MIDY; Renner et al., Diabetes 2013) and compared molecular profiles of wild-type (WT) piglets (n=9) born to hyperglycemic mothers with those of piglets born to WT mothers (n=10). To address molecular consequences in the liver, we compared mass spectrometry-based hepatic proteome profiles of piglets developed in hyperglycemic vs. WT mothers. In total, we identified 61284 unique peptides that could be mapped to 6313 protein groups with high confidence (FDR < 0.01). Quantitative analysis revealed several proteins to be significantly altered in abundance between conditions among them key proteins related to amino acid (e.g. branched chain amino acid transaminase 1 and tyrosine aminotransferase) and fatty acid metabolism (e.g. acetyl-CoA acyltransferase 2, which catalyses the last step of mitochondrial beta-oxidation). In line, targeted metabolomics revealed alterations of several classes of metabolites among them amino acids, acylcarnitines, glycerophospholipids and sphingolipids. In conclusion, multi-omics analysis suggests that crucial metabolic pathways are affected in the liver of the neonates piglets developed in hyperglycemic mothers.

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PROTEOMIC SIGNATURES OF THE HEART IN INSULIN-DEFICIENT DIABETES MELLITUS

Bachuki Shashikadze (LMU)

To explore the molecular effects of chronic insulin deficiency and hyperglycemia on the heart, we performed a label-free liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS) of samples of two compartments of the heart (left atrial appendage (LAA) and left atrial free wall (LAFW)) from a clinically diabetic pig model (MIDY, n = 5) and WT (n = 5) controls. We identified 30140 and 31873 unique peptides from 3769 and 3902 protein groups at a false-discovery rate < 0.01 in LAA and LAFW, respectively. Quantitative analysis of LAA vs. LAFW data revealed specific proteome profiles with prominent differences in the abundance of a plethora of proteins. Statistical analysis of MIDY and WT data further demonstrated compartment-specific proteome alterations due to insulin deficiency and chronic hyperglycemia. However, also proteins commonly regulated in both compartments were detected, among them key proteins involved in, cardiac contraction, and development of insulin resistance and atherosclerosis. Interestingly, the two most significantly altered proteins in both compartments were myosin light chain 1 (MYL1) and troponin I1, slow skeletal type (TNNI1). Alteration of these proteins may point to ongoing diabetic cardiomyopathy.

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PROTEOMIC AND LIPIDOMIC SIGNATURES OF THE LUNG IN INSULIN-DEFICIENT DIABETES MELLITUS

Bachuki Shashikadze (LMU)

Growing evidence shows the lung as an organ prone to diabetes-induced alterations. However, the molecular consequences of diabetes in lung tissue are not fully understood. To study chronic effects of insulin deficiency and hyperglycemia, we performed proteome and metabolome analyses of lung tissue samples from long-term (2 years) diabetic MIDY pigs and wild-type (WT) littermate controls. In MIDY lung, we found an elevated protein level of pulmonary surfactant-associated protein A (SP-A) – a known biomarker of lung injury. Furthermore, the abundance of proteins involved in humoral immune response was altered. Interestingly, polyunsaturated fatty acid lipoxygenase (ALOX15) was significantly reduced in MIDY lung, which was further confirmed by immunohistochemical staining. In line with this, quantitative eicosanoid analysis demonstrated reduced levels of lipid products that are produced by ALOX15 and are known to be involved in inflammation resolution. Such an aberrant production of lipoxygenase products in the MIDY lung further points to a deleterious effect of hyperglycemia on the lung immune system. In summary, we generated the first comprehensive multi-omics analysis of lung samples from a pig model for insulin-deficient diabetes, providing a valuable resource for future comparative and translational studies.

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Fat tissue inflammation in a diet-induced obese pig model - insights from multi-omics analyses

Simone Renner (LMU)

The metabolic syndrome is a cluster of metabolic dysregulations including obesity and diabetes mellitus. Obesity is commonly associated with chronic low-grade inflammation that is considered a crucial risk factor for the development of insulin resistance and type 2 diabetes. However, the triggers for adipose tissue inflammation are still poorly understood.
Young adult female ovariectomized Göttingen minipigs were fed a high-fat/high-energy diet for a period of 70 weeks. Body weight, body composition (evaluated by dual-energy X-ray absorptiometry and magnetic resonance tomography), blood parameters, intravenous glucose tolerance were determined at regular intervals. Transcriptome and proteome analyses were performed in different adipose tissue compartments (subcutaneous and visceral) of obese and lean pigs.
Diet-induced obese (DIO) Göttingen minipigs developed severe subcutaneous and visceral adiposity (body fat 53.8 ± 0.6 vs. 22.2% ± 2.3 of body mass, $p<0.001$), increased plasma cholesterol, triglyceride and free fatty acid levels, insulin resistance (HOMA-IR 5.2 ± 0.4 vs. 2.2 ± 0.3, $p<0.001$), impaired glucose tolerance. Interestingly, extensive adipose tissue inflammation and adipocyte necrosis were observed in visceral, but not subcutaneous adipose tissue compartments. Transcriptome and proteome analyses can give more insight into the pathophysiology and potential triggers of fat tissue inflammation.

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Investigation of Diabetes-induced Atrial Remodeling in a Preclinical Large Animal Model

Sebastian Clauß (LMU)

Introduction: Diabetes mellitus is associated with an increased risk for arrhythmias such as atrial fibrillation (AF). The underlying mechanisms are incompletely understood, but may include remodeling processes establishing a proarrhythmic atrial substrate.
Objective: To evaluate diabetes-induced proarrhythmic remodeling in a preclinical large animal model.
Methods: At the age of six months, transgenic pigs expressing a mutant insulin gene (INSC94Y) and their wildtype littermates were studied in-vivo including left/right heart catheterization and electrophysiology (EP) studies. AF-Inducibility was tested by burst pacing. In left atrium (LA) fibrosis was assessed histologically and expression of extracellular matrix genes/proteins was studied by qPCR.
Results: INSC94Y pigs showed significantly elevated blood glucose levels indicating a diabetic phenotype. In both groups, hemodynamics and left ventricular function were normal. EP studies revealed significantly shorter refractory periods and Wenckebach cycle length in diabetic pigs. Furthermore, diabetic pigs were more susceptible to develop AF. Histologic assessment revealed significant LA fibrosis accompanied by fibronectin upregulation in INSC94Y pigs. Further analysis showed an upregulation/activation of the profibrotic TGF-beta pathway in diabetic pigs.
Conclusion: Diabetic pigs show enhanced susceptibility for AF. Further studies suggest an activation of profibrotic TGF-beta signaling leading to a structural substrate as a potential underlying mechanism of diabetes-associated arrhythmogenesis.

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Plasma lipidomic n-6 Polyunsaturated Fatty Acids and Type 2 Diabetes Risk in the EPIC-Potsdam Prospective Cohort Study

Marcela Prada (DIfE)

Background: The evidence on plasma n-6 polyunsaturated fatty acids (PUFAs) and type 2 diabetes (T2D) risk is inconsistent. We examined the associations of lipid class-specific PUFA concentrations and estimated delta-5 desaturase (D5D) activity with T2D risk.
Methods: In a case-cohort study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort (subcohort=1,137; T2D cases=775; median follow-up 6.5 years), we measured plasma fatty acids 18:2, 20:3, and 20:4 concentration in 12 lipid classes. Associations were estimated with Cox proportional-hazards models per SD.
Results: Higher 18:2 concentrations were inversely associated with T2D risk, particularly in diacylglycerols (HR 0.68), triacylglycerols (HR 0.52), and lysophosphatidylcholines (HR 0.69), however, monoacylglycerol(18:2) was positively associated (HR 1.40). Higher concentrations of 20:3 in phospholipids (HRs 1.14–1.63), free fatty acids (HR 1.28), cholesterol esters (HR 1.45), and monoacylglycerols (HR 1.22) were linked to higher T2D incidence. Higher 20:4 concentrations were positively associated in free fatty acids (HR 1.30) and phosphatidylethanolamines (HR 1.36). The D5D activity was associated with lower T2D risk when estimated as 20:4/20:3-ratio in phospholipids and cholesterol esters.
Conclusion: n-6 PUFAs were associated differently with T2D incidence depending on FA and the lipid class.

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The German Diabetes Risk Score: Estimating individual 10-year Type 2 Diabetes Risk

Catarina Schiborn (DIfE)

Background: The German Diabetes Risk Score (GDRS) currently enables prediction of the individual type 2 diabetes (T2D) risk of the following five years. The aim of this study is to extend the prediction period of the GDRS to 10 years and to perform external validation.
Methods: In data from the EPIC-Potsdam study (n = 25 393), Cox proportional hazards regression was used to reweight the points of the five-year GDRS. Two population-based prospective cohorts (EPIC-Heidelberg n = 23 624, BGS98 cohort n = 3717) were used for external validation. Discrimination was represented by C-indices, and calibration by calibration plots and the expected-to-observed (E/O) ratio.
Results: Predictive performance in EPIC-Potsdam was very good (C-index for the non-clinical model: 0.834) and was confirmed in EPIC-Heidelberg (0.843) and in the BGS98 cohort (0.851). The models were very well calibrated in EPIC-Potsdam (E/O ratio for the non-clinical model: 1.08), slightly overestimated the risk in EPIC-Heidelberg (1.34), and predicted T2D very well in the BGS98 cohort after recalibration (1.06).
Conclusion: Extending the prediction period of the GDRS' non-clinical version and the HbA1c extension to 10 years enables the even longer-range, evidence-based identification of high-risk individuals with many different applications, including medical screening.

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Novel drug candidates to foster regulatory T cells in Type 1 Diabetes

Maike Becker (HMGU)

Islet autoimmunity, the presymptomatic phase of Type 1 Diabetes (T1D), is characterized by aberrations in T cell activation vs. tolerance. Regulatory T cells (Tregs) are the main mediators of immune tolerance. In the last years, we have identified a multitude of impairments in immune tolerance and various manifestations of aberrant immune activation during the development of murine and human T1D. This dysregulated immune tolerance triggers islet autoimmunity and the loss of insulin-producing β-cells in T1D.
Immune targeting approaches aimed at fostering Treg cells with the goal to interfere with ongoing autoimmune activation and progression provide a promising target for restoring immune tolerance in T1D, but so far, the identification of relevant compounds is a big challenge.
To this end, in collaboration with the Assay Development and Screening Platform at Helmholtz Munich, we performed a high-throughput screening of 25.000 compounds using a novel antigen-specific in vitro Treg induction system with primary murine T cells. We identified 48 candidates that significantly improved the Treg-induction capacity.
The candidates were challenged in several assays, mimicking conditions of aberrant T cell activation as observed during ongoing islet autoimmunity in T1D. Interestingly, the top three candidates significantly increased Treg frequencies and numbers, even in the presence of pro-inflammatory cytokines and strong T cell activation, a scenario where well-established Treg inducers such as the mTOR inhibitor Everolimus fail.
In summary, the here presented data suggest that one of the top candidates might open a new path for Treg targeting to regain immune homeostasis during ongoing islet autoimmunity.

Abstracts of posters

miRNA 150 targets Akt3 signaling to shape th9 differentiation in models of type 1 diabetes

Giulia Boschi (HMGU)

miRNA 150 targets Akt3 signaling to shape th9 differentiation in models of type 1 diabetes
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Type 1 diabetes (T1D) is characterized by an impairment in T cell tolerance, normally maintained in the periphery by Foxp3+ regulatory T cells (Tregs). Several miRNAs are involved in guiding aberrant immune activation and impaired Treg induction, function and stability during islet autoimmunity. Among them, miRNA 150-5p is found to be differentially regulated in children with and without ongoing islet autoimmunity. Bioinformatic prediction tools and HITS-Clip data indicate that miR 150-5p targets components of the PI3K pathway, thereby controlling Th9 cell differentiation and function. In line with the finding that NOD mice present with overshooting PI3K activation, we observe an impairment in Th9 induction in insulin autoantibody positive NOD mice. Given the pleiotropic characteristics of Th9 cells with pro- vs. anti-inflammatory functions, here we aim at testing the hypothesis that a miR150-5p/Pi3K axis is involved in guiding Th9 induction.
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Targeting aberrant immune activation in autoimmune Type 1 Diabetes

Hannah Hipp (HMGU)

In autoimmune Type 1 diabetes, a loss of immune tolerance and aberrant immune activation promotes the progressive destruction of insulin-producing beta cells in the pancreas. Peripheral immune tolerance is mediated by regulatory T cells suppressing self-reactive T cells. Critical Treg impairments observed during ongoing islet autoimmunity highlight the need for innovative strategies to efficiently target Tregs and their induction during aberrant immune activation such as islet autoimmunity. Here, we study the drug candidates IMU-838 and IMU-935 (Immunic AG, Germany). IMU-838 is a dihydroorotate dehydrogenase (DHODH) inhibitor, an enzyme which catalyzes the de novo pyrimidine synthesis and is essential for activated lymphocytes. IMU-935 is a potent RORγt inverse agonist, while it can also inhibit, although to a lower extent, DHODH. We show that IMU-935 increases in vitro Treg induction from murine naïve T cells. Importantly, both compounds enhance Treg induction under challenging conditions, mimicking autoimmune activation. In addition, preliminary in vivo studies using IMU-838 indicate reduced incidences of T1D and reduced frequencies of activated T cells in a model of accelerated T1D induced by adoptive transfer. Together with their positive effect on Tregs under pro-inflammatory conditions, these compounds provide a potential means to foster Tregs and reduce immune activation during overt islet autoimmunity.

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Expression of a major histocompatibility complex class II protein by pancreatic β-cells as a type 1 diabetes model in zebrafish

Alisa Hnatiuk (PLID)

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by destruction of beta-cells in the pancreatic islets leading to insulin deficiency and hyperglycemia. Pancreatic beta-cell destruction is influenced by genetic factors, environmental factors and autoimmune factors. MHC class II molecules under most circumstances are expressed on professional antigen-presenting cells (APCs) and present foreign antigens to CD4+ T cells. Recently, an increased expression of MHC class II and MHC class II-related genes, including the Class II-invariant chain CD74, was shown in pancreatic beta-cells from T1D donors. This has led to the hypothesis that aberrant expression of MHC class II on beta-cells can lead to their immunological "unmasking" and selective targeting by T-cells. To test this hypothesis, we expressed cd74 under insulin promoter in zebrafish model. We observed that this expression led to the death of cd74 cells tagged by fluorescent mCerulean at early juvenile zebrafish stage. In the same time islet immune-cell infiltration was observed, including macrophages and regulatory T cells. However, due to its high regenerative capacity, with time zebrafish can recover from insulin loss and restore its blood glucose level using hybrid cells as a source of insulin. Additionally, with high degree of genetic, anatomical and functional similarities with humans, this model presents a promising tool for studying not only the mechanisms involved in T1D, but also the ones involved in recovery from insulin loss with further possibilities to translate the findings to human.

Abstracts of posters

Fr1da in Saxony - Screening for presymptomatic type 1 diabetes

Anja Loff (PLID)

The identification of type 1 diabetes in an asymptomatic stage is possible via detection of islet autoantibodies, which precede the onset of clinical type 1 diabetes. In September 2021, islet autoantibody screening of children commenced in Saxony via the Fr1da study. The aims of the study are to improve early diagnosis and treatment of type 1 diabetes, the reduction of severe complications during manifestation (e.g. ketoacidosis), education of the parents about the disease and its treatment and participation in intervention trials. Since study start a network of cooperating pediatrician practices was established with 45 registered practices. Screening for islet autoantibodies has been completed in 700 children, and of these, the majority also consented to test for SARS-CoV-2 antibodies. Five (0.7%) children were identified with presymptomatic type 1 diabetes. The frequency of SARS-CoV-2 antibody positivity increased from around 20% in 2021 to over 80% in July 2022. Education, staging and follow-up was offered to families of the children with presymptomatic type 1 diabetes; all have agreed and 3 have had an educational session and the first follow up visit for staging. We have successfully established a presymptomatic type 1 diabetes screening program in the Saxonian general childhood population.

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Does sex matter? Differences in glucose homeostasis and insulin secretion between male and female C57BL/6N mice

Celina Funke (PLID)

As the incidence of diabetes increases worldwide dramatically every year, the German Diabetes Association described the disease as a worldwide epidemic in 2021.
Similar to other diseases there is rising evidence of sex-related differences in glucose homeostasis as well as in age-dependent incidence and progression of diabetes. Those differences need to be further investigated, especially in light of optimizing therapies and medication in a sex-specific way.
Different factors are already known to influence glucose homeostasis. One of the most obvious are the sex hormones beta-Estradiol and Dihydrotestosterone.
Taking this into account we hypothesize that the biological sex itself affects islet cell function, independent of systemic physiological circumstances.
To investigate this question, we performed glucose and insulin tolerance tests on three different aged groups (15, 20, and 30 weeks) of C57BL/6N mice, together with the measurement of plasma C-peptide levels in vivo. Additionally, we assessed insulin secretion kinetics in situ using the acute pancreatic tissue slice approach, which enables the study of islets under close to physiological conditions.
The analysis showed that female mice of three different aged groups have a better glucose tolerance than male mice when given a weight-adjusted glucose load. In parallel plasma C-peptide levels in vivo, and insulin secretion of the pancreatic slices in situ showed a strongly increased insulin secretion of male islets to different glucose concentrations as well as to GLP-1 stimulation compared to their female counterparts.
To investigate these differences in more detail we plan to evaluate calcium dynamics in beta-cells during different glucose stimulations using pancreatic slices as well as analyze the islet architecture of the two sexes.
Our data illustrate the need of considering sexual dimorphism in glucose homeostasis when it comes to the treatment of the so-called "Diabetes Epidemic".

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Role of insulin degrading enzyme (IDE) in insulin turnover and beta-cell stress

Lisa-Marie Kretschmar (PLID)

Introduction: Insulin degrading enzyme (IDE) is a ubiquitously expressed Zn2+ metalloprotease. It degrades several small peptides associated with glucose homeostasis, including insulin. Studies suggest that IDE has a multifunctional role in cell homeostasis. Nevertheless, the intracellular localisation of IDE in beta cells and how it targets insulin for degradation remains unclear. Furthermore, genetic analysis identified that polymorphisms in the IDE locus are associated with susceptibility to type 2 diabetes.
Materials and Methods: Experiments were performed in insulinoma INS1 wildtype and ide knockout ide -/- cell lines. Western Blots and immunostainings were used for protein analysis of secretory granule- and ER cell stress related proteins. Insulin content and secretion were measured with HTRF ELISA and SNAP-ELISA. Luciferase assays were performed to investigate proteasome and caspase 3/7 activity. Age-defined labelling of of wildtype and knockout cells transfected with hIns-SNAP was performed to investigate insulin secretory granule turnover Immunofluorescence and electron microscopy were used to elucidate intracellular localisation of IDE as well as insulin secretory granule turn-over.
Results: Ide -/- INS1 cells show increased level of insulin compared to wildtype cells although glucose stimulated Insulin secretion was not altered. Immunostainings indicate an increased number of secretory granules in ide -/- cells. Measurement of old (24-36h) and young (2-4h) secretory granule content revealed an accumulation of old secretory granules under IDE deficiency as well as increased insulin biogenesis. Ide -/- cells show low level ER stress and decreased proteasome activity.
Conclusion: Ide -/- cells display elevated levels of secretory granule components, including insulin. Increased biogenesis and accumulation of secretory granule precursor cargoes could potentially lead to ER stress. Insulin secretory granule degradation is altered. Ide -/- cells cells exhibit decreased proteasome activity. Overall, our studies could help to clarify the role of levels of IDE can confer a risk for type 2 diabetes.

Abstracts of posters

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3D ultrastructure of the beta cell primary cilium

Andreas Müller (PLID)

Primary cilia are protrusions of the plasma membrane with an underlying array of microtubules called axoneme. These specialized cell compartments are usually non-motile and their microtubule structure follows a 9+0 scheme. The majority of cell types in our body have one primary cilium which plays important roles in signaling acting as a "cellular antenna". In pancreatic beta cells they contribute to signal transduction within the islet. Furthermore, perturbation of ciliary genes has been linked to the development of diabetes mellitus. Here, we provide the first high resolution 3D reconstructions of mouse and human beta cell primary cilia. By 3D electron microscopy we resolve and reconstruct the axoneme, basal body and ciliary membrane. We show that the regular 9+0 structure is not maintained throughout the whole length of the cilium. Furthermore, we observe close interactions of cilia with the ones of neighboring cells as well as with their plasma membranes with some cilia even "pinching" neighboring beta or alpha cells. Finally, we find that in mice and humans beta cells can contain up to three primary cilia. Overall, this structural information can be valuable to understand the role of cilia in beta cells in health and diabetes.

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Re-internalized Phogrin/IA-2beta is routed to young insulin granules destined for degradation

Inna Kalaidzidis (PLID)

Abstracts of posters

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Beta cell turnover orchestrates the inflammatory milieu of the islet by modulating the balance between resident macrophages and Tregs

Mohammad Nadeem Akhtar (PLID)

Programmed cell death is a regulated form of cellular demise that is critical to maintain tissue homeostasis. Upon receiving signals from dying cells, surveilling immune cells are rapidly homed to the target tissue in order to phagocytose the dead cells. How developmentally-regulated beta-cell death influences the immune-cell repertoire and the inflammatory status of islet cells remains unknown. Here, using zebrafish as a model organism, we show that beta-cells undergo extensive turnover during the juvenile stage of development, which is necessary for the recruitment and the establishment of islet-resident macrophages. Genetic inhibition of apoptotic beta-cell death by overexpressing p35, a baculovirus caspase inhibitor, leads to a dramatic reduction in the population of islet-resident macrophages. While the population of macrophages is reduced, we observe an increase in regulatory T cells (Tregs) and lower levels of islet inflammation upon caspase inhibition. Preliminary data using foxp3-deficient zebrafish suggest that Tregs are essential for the promotion of an anti-inflammatory islet-milieu. Overall, our study shows that the increase in beta-cell death in the juvenile period modulates the levels of islet inflammation by regulating the balance between macrophages and Tregs. We are currently exploiting these findings to identify the unknown chemoattractant that recruits islet-resident Tregs, opening up new therapeutic avenues for the treatment of type 1 diabetes.

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Novel monoclonal antibody for β cell analysis and targeting

Priyadharishini Ayyappan (HMGU)

Diabetes mellitus is a multifactorial disease characterized by progressive loss or dysfunction of the insulin-producing β cells in the pancreas. Targeted regeneration of resident β cells holds great promise to prevent or reverse disease progression. However, currently, there is a lack of knowledge of the processes involved in in vivo β cell regeneration and a lack of technology that can facilitate β cell specific targeting. In our lab, we generated a large set of novel monoclonal antibodies (mAbs) targeted against human pancreatic cell types to study and potentially target these cell types in vivo. From the screen, we identified the 5E1 mAb, which marks the insulin-producing β cell population in both healthy and T2D human pancreas sections. We also observed that the 5E1 mAb is internalized via clathrin-mediated endocytosis, which is crucial for the targeted therapy approach. Here we demonstrate that our novel monoclonal 5E1 antibody, specific for human β cells, can be used therapeutically to isolate and characterize β cell subpopulations in diabetic patients. This 5E1 mAb is a promising tool for generating β cells regeneration therapy.

Abstracts of posters

A novel sandwich ELISA to quantify SNAP tagged proteins

Oleksandra Topcheva (PLID)

Pancreatic beta cells secrete insulin in response to various stimuli. In this regulated, exocytotic process, only a small fraction of secretory granules is secreted, while the remaining granules are stored or degraded. Selection for their fate is not random but influenced by several factors, including subcellular localization and their age. Early studies using radioisotope labeling to differentiate time-resolved insulin could show preferential secretion of young secretory granules compared to older ones. Since radiolabeling is not suitable for microscopy, our laboratory has developed a reporter fusing the self-labeling protein tag (SNAP) to insulin for subsequent labeling with fluorescent substrates (TMR). Using this and similar reporters, we could show that motility, degradation, acidity, and composition of secretory granules correlate strongly with their age. Yet, microscopy is not suitable for the quantitative assessment of secretion of insulin of distinct ages making alternative approaches necessary. To close this gap, we developed a sandwich ELISA recognizing total and dye-labeled SNAP tag. Using this assay, we could measure the content and secretion of age-distinct pools of insulin-SNAP in mouse insulinoma MIN6 K8 cells. The preliminary data confirms the preferential secretion of young secretory granules, which is in agreement with previous studies and validates our assay.

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Generation and Characterization of Pseudo-Islets for Beta-cell Replacement

Hany Abdelgawad (PLID)

Pancreatic islet transplantation is a safe and minimally invasive curative treatment option for patients with insulin-dependent diabetes. However, the availability of this treatment remains limited due to the need for immunosuppression and the shortage of donors. To overcome these limitations, our group has developed immune-shielding strategies for islet macro-encapsulation and exploited the utilization of alternative cell sources such as xenogeneic adult and neonatal pig islets [1]. In the macro-encapsulation setting, it is essential to use intact islet clusters with high purity and ideal size in order to reduce oxygen demand, maximize applicable density, and obtain optimal diffusion characteristics. However the most important question which needs to be addressed is "what are the similarities of the metabolomic machinery between the pig islets and human islets ? " Answering this question is a pivotal step in xenotransplantation to understand glucose/insulin algorithm to avoid putting the patients in risks of hyper or hypoglycemia. Here, we managed to overcome the major obstacles that render the reproducible generation of pseudo-islets "PIs" from adult pig or neonatal pig islets-cells like clusters (pNICCs). Then we proceeded to decipher the metabolomic machinery of this system