Brief Communication

MOLECULAR METABOLISM

The Munich MIDY Pig Biobank — A unique resource for studying organ crosstalk in diabetes

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ABSTRACT

Objective: The prevalence of diabetes mellitus and associated complications is steadily increasing. As a resource for studying systemic consequences of chronic insulin insufficiency and hyperglycemia, we established a comprehensive biobank of long-term diabetic *INS*^{C94Y} transgenic pigs, a model of mutant *INS* gene-induced diabetes of youth (MIDY), and of wild-type (WT) littermates.

Methods: Female MIDY pigs (n = 4) were maintained with suboptimal insulin treatment for 2 years, together with female WT littermates (n = 5). Plasma insulin, C-peptide and glucagon levels were regularly determined using specific immunoassays. In addition, clinical chemical, targeted metabolomics, and lipidomics analyses were performed. At age 2 years, all pigs were euthanized, necropsied, and a broad spectrum of tissues was taken by systematic uniform random sampling procedures. Total beta cell volume was determined by stereological methods. A pilot proteome analysis of pancreas, liver, and kidney cortex was performed by label free proteomics.

Results: MIDY pigs had elevated fasting plasma glucose and fructosamine concentrations, C-peptide levels that decreased with age and were undetectable at 2 years, and an 82% reduced total beta cell volume compared to WT. Plasma glucagon and beta hydroxybutyrate levels of MIDY pigs were chronically elevated, reflecting hallmarks of poorly controlled diabetes in humans. In total, ~1900 samples of different body fluids (blood, serum, plasma, urine, cerebrospinal fluid, and synovial fluid) as well as ~17,000 samples from ~50 different tissues and organs were

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Abbreviations: CE, cholesterol ester; CPT1, carnitine O-palmitoyltransferase 1; ER, endoplasmic reticulum; FFA, free fatty acids; MIDY, mutant *INS* gene-induced diabetes of youth; PC, phosphatidylcholine; PCA, principal component analysis; SM, sphingomyelin; TAG, triacylglycerol; WT, wild-type

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preserved to facilitate a plethora of morphological and molecular analyses. Principal component analyses of plasma targeted metabolomics and lipidomics data and of proteome profiles from pancreas, liver, and kidney cortex clearly separated MIDY and WT samples.
Conclusions: The broad spectrum of well-defined biosamples in the Munich MIDY Pig Biobank that will be available to the scientific community provides a unique resource for systematic studies of organ crosstalk in diabetes in a multi-organ, multi-omics dimension.

Keywords MIDY; Hyperglycemia; Insulin insufficiency; Pig model; Biobank; Random systematic sampling; Transcriptomics; Proteomics; Metabolomics; Stereology

1. INTRODUCTION

Diabetes mellitus is a complex metabolic disease with markedly increasing prevalence worldwide (http://www.diabetes.org/diabetes-basics/statistics/). Acute hyperglycemia may lead to life-threatening diabetic ketoacidosis, chronic hyperglycemia is associated with macrovascular complications, increasing the risk for myocardial infarction and stroke, and microvascular complications leading to diabetic nephropathy, retinopathy, and neuropathy (reviewed in Ref. [1]). The molecular disease mechanisms behind these multi-organ changes are only partially understood.
Molecular profiling techniques on the transcriptome, proteome, and metabolome levels facilitate the investigation of intermediate molecular phenotypes in disease-related cells, tissues, and organs (reviewed in Ref. [2]). Systems biology approaches such as integrative analyses of multi-omics data sets aim to provide novel mechanistic insights and to identify therapeutic targets and biomarkers.
Central gene expression data repositories such as NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and EMBL-EBI ArrayExpress Archive (http://www.ebi.ac.uk/arrayexpress/) are important sources for capturing transcriptome alterations in diabetic patients (e.g. Ref. [3]), but are mostly limited to one or few tissues per study (e.g. blood cells and adipose tissue in Ref. [4]). Recently, the Human Diabetes Proteome Project (HDPP) was launched with an initial focus on islets of Langerhans, insulin-producing cell lines, and blood samples from diabetes-related patient cohorts [5]. Moreover, targeted and non-targeted metabolomics approaches are available for diabetes research and have been used for analyzing human samples and samples from model organisms (reviewed in Ref. [6]).
Although cross-tissue networks with a limited spectrum of tissues have been constructed in several studies, integration of multi-omics data with expanded tissue coverage would markedly benefit disease-related network analyses on an organism-wide scale [2]. This is particularly true for metabolic diseases such as diabetes and obesity, for which multiple tissues/organs may be causally involved in and/or affected by disease-relevant tissue crosstalk (reviewed in Ref. [7]).
For ethical reasons, the spectrum of tissues available from diabetic patients is limited. In addition, confounding factors such as age, comorbidities, and variance introduced by tissue sampling and storage procedures may complicate the analysis and interpretation of omics data from human samples. Samples from diabetic rodent models are less variable, but the amount of tissue available for multi-omics analyses is limited.
Pigs are interesting models for diabetes and obesity research and can be genetically engineered to mimic human disease mechanisms (reviewed in Ref. [8]). Transgenic pigs expressing the mutant insulin C94Y are a model for permanent neonatal diabetes [9], now termed mutant *INS* gene-induced diabetes of youth (MIDY) (reviewed in Ref. [10]). Corresponding *INS*/*Ins2* mutations that disrupt the C(B7)-C(A7) interchain disulfide bond of the insulin molecule exist also in humans and in the widely used Akita mouse model (reviewed in Ref. [10]). Expression of the mutant *INS*/*Ins2* leads to impaired trafficking of normal proinsulin by formation of high-molecular weight complexes with misfolded (pro)insulin, accumulation of misfolded insulin in the endoplasmic reticulum (ER), and ER stress, which finally triggers beta-cell apoptosis (reviewed in Ref. [10]). Accordingly, MIDY pigs are characterized by impaired insulin secretion, increased fasting glucose levels, and progressively decreasing beta cell mass [9].
To generate a unique resource for studying consequences of chronic insulin insufficiency and hyperglycemia in a multi-tissue, multi-omics approach, we generated a complex biobank of more than 50 different tissues and body fluids from two-year-old MIDY pigs and WT littermate controls (highlighted in Ref. [11]). A comprehensive standardized protocol, taking the principles of systematic uniform random sampling into account, was established [12] to ensure uniform high quality of representative samples for a broad spectrum of analyses, including molecular profiling as well as qualitative and quantitative morphological investigations.

2. MATERIAL AND METHODS

2.1. MIDY pig model

A cohort of 4 female MIDY pigs and 5 female WT littermates was maintained for two years. Animals were housed under controlled conditions and had a once-daily feeding regimen (Supplementary Figure 1a) and free access to water. Treatment of MIDY pigs with a combination of long-acting insulin (Lantus®; Sanofi) and short-acting insulin (NovoRapid®; NovoNordisk) was started at age 2 months aiming for moderate hyperglycemic levels to mimic suboptimal insulin treatment (Supplementary Figure 1b). Blood glucose levels were determined once or twice daily using a Precision Xceed® glucometer and Precision XtraPlus® test stripes (Abbott) to control treatment [9] (Supplementary Figure 1c). WT and MIDY sows were estrus synchronized [13] and inseminated 12 days prior to necropsy to exclude estrous cycle related effects on molecular profiles of tissues and body fluids and to facilitate collection of conceptuses. All experiments were performed according to the German Animal Welfare Act with permission from the responsible authority (Government of Upper Bavaria), following the ARRIVE guidelines and Directive 2010/63/EU for animal experiments.

2.2. Metabolic characterization, clinical chemistry, targeted metabolomics, and lipidomics

Blood samples were taken regularly using EDTA coated tubes (Monovette® blood collection system, Sarstedt). Plasma was separated by centrifugation and stored at −80 °C. Plasma insulin, C-peptide and glucagon levels were determined using specific RIAs (Merck Millipore) or ELISAs (Mercodia). Clinical chemical parameters in plasma were determined using an AU400 (Olympus) or AU480 autoanalyzer (Beckman−Coulter) and adapted reagent kits from Olympus, Beckman−Coulter, or Sentinel (fructosamine).
Targeted metabolomics analysis of plasma samples was done by liquid chromatography-electrospray ionization-tandem mass spectrometry and flow injection analysis-electrospray ionization tandem mass

