CYTOSOLIC DNA SENSING AND MITOCHONDRIAL TRANSCRIPTOMIC CHANGES AS EARLY TRIGGERS OF METABOLIC DISEASE IN DB/DB MICE

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Introduction

The animal models of diabetes, such as db/db mice, are a useful tool to decipher the genetic background of molecular changes at the early stages of disease development. Here we aimed to find the early transcriptomic changes in three tissues involved in the regulation of metabolism – adipose tissue, muscle and liver in db/db mice.

Material and Methods

Material: 9 Dock7<m>+/+Lepr<db>/J males (db/db mice) were purchased from Charles River. The animals were transferred to animal facility at the age of 6 weeks and underwent 2-week acclimatization. The animals were kept in temperature-controlled rooms (20-15°C, ±10% humidity) at the 12-hour light/12-hour dark cycle. All mice had *ad libitum* access to pelleted food and filtered water. The animals were randomly allocated into groups and examined at three stages of life – 8 weeks, 12 weeks and 16 weeks of age. Three animals were analyzed at each Time Point (Figure 1). On the day of sacrifice three metabolically important tissues (liver, muscle, adipose tissue) were immediately snap frozen or collected in RNAlater and stored at -80°C. All procedures were approved by the Local Ethics Commission for Animal Experiments in Lodz (Poland). Methods: *RNA sequencing:* Total RNA was isolated from three mouse tissues – adipose tissue, muscle and liver using Maxwell RSC Instrument (Promega) from the following groups: 8-week db/db mice (n=3; animals A, B, C), 12-week mice (n=3; animals D, E, F) and 16-week mice (n=3, animals G, H, I). Quality and quantity were checked on Quantus (Promega) and Tape Station (Agilent). 500ng of total RNA was used to prepare libraries according to SENSE mRNA-Seq Library Preparation Kit (Lexogen). The libraries were sequenced on NextSeq (Illumina). *Bioinformatic and statistical analysis:* The bioinformatic analysis consisted of the following steps: trimming the sequences using the Cutadapt tool, mapping them to the mouse reference genome GRCm38 with the STAR aligner, counting the mapped mRNA reads using HT-Seq. Following the bioinformatic analysis differential expression analysis was performed with the aid of edgeR package in R. The analysis of KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) pathways was also performed. *Immunohistochemistry:* Frozen sections were air dried for few minutes, fixed in 4% PFA (15 minutes), permeabilized in 1% PBST (10 minutes) and blocked with 1% BSA in 0.1% PBST (1,5h). Overnight incubation with primary antibody (Irf7 - #MA541165, Thermo Fischer, 1:100, +4C; cGAS - #703149, Invitrogen, 1:200, +4C) was followed by secondary antibody incubation (#96886, Abcam, conjugated with DyLight 650, 1:500, 2h, RT). Sections were co-stained with MitoView (#70054, Biotium, 1:1000, for Irf7) or dsDNA Dye (#E2670, Promega, 1: 400, for cGAS) for 30 or 20 minutes, respectively.

Results - liver

The time-dependent transcriptomic analysis has shown that most pronounced differences could be observed in liver, while muscle and adipose tissue underwent hardly any statistically significant changes in mRNA expression. We identified nine differentially expressed genes between Time Points 2 and 1 and five which differed between Time Points 3 and 2 in liver (Table 1).

The KEGG pathways analysis showed enrichment in cytosolic DNA sensing and immune response (Figure 2).

→ Table 2. The mitochondrial gene expression in liver analyzed between Time Points 2 and 1 (12 vs 8-week mice) and Time Points 3 and 2 (16- vs 12-week mice).

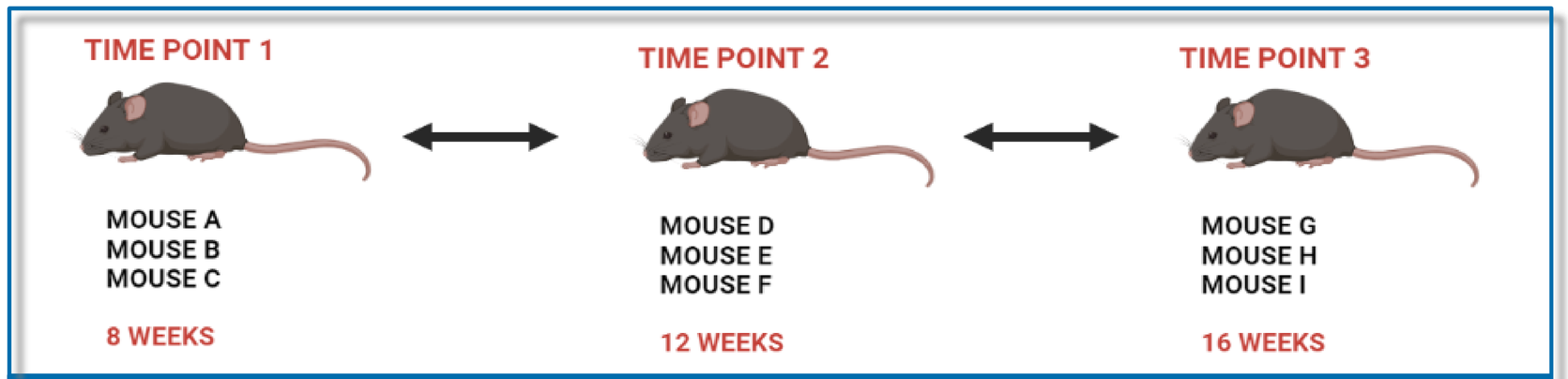

Figure 1. The scheme of the experiment.

Gene	logFC	Average expression	p-value	adj. p-value
LIVER 2 vs 1				
Irf7	3.28	4.77	1.27E-07	0.001
Ly6a	2.31	3.93	3.72E-06	0.022
Ly6g6d	2.53	1.24	1.67E-05	0.044
H2-Dma	1.73	2.96	1.97E-05	0.044
Pld4	2.42	1.33	2.09E-05	0.044
Ly86	1.39	4.47	2.52E-05	0.044
Fcer1g	1.63	1.87	2.61E-05	0.044
Ly6e	2.18	8.17	3.03E-05	0.045
Idi1	-2.02	2.99	3.58E-05	0.047
LIVER 3 vs 2				
Irf7	-3.33	4.77	1.00E-07	0.001
Plac8	-2.06	2.39	4.44E-06	0.026
Ifi44	-3.77	1.05	1.14E-05	0.033
Xaf1	-2.54	3.67	1.38E-05	0.033
Ly6a	-2.02	3.93	1.31E-05	0.033

Table 1. Differentially expressed genes in liver between analyzed Time Points.

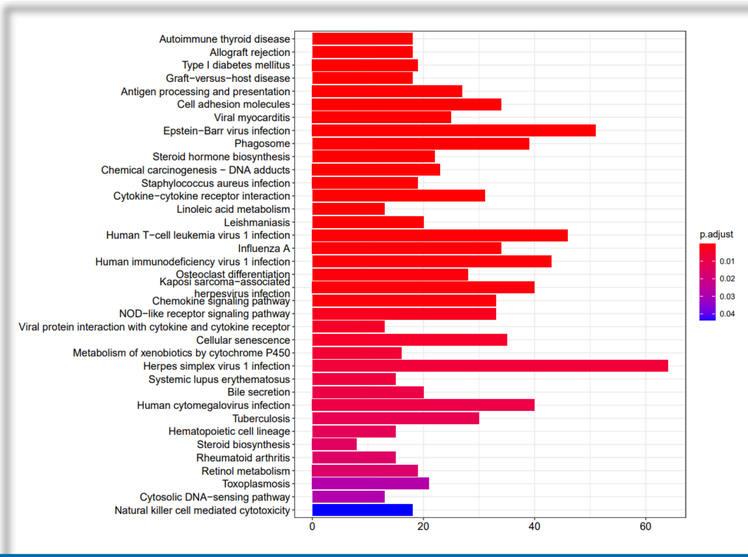

Figure 2. Significantly enriched KEGG pathways between Time Points 2 and 1 (12 vs 8-week mice) in liver.

Results – liver - mitochondria

When we looked specifically into mitochondrial gene expression, we have found two genes, which expression was significantly downregulated between Time Points 2 and 1, and five differentially regulated genes between Time Points 3 and 2 (Table 2).

Gene	logFC	Average expression	p-value	adj.p-value
LIVER 2 vs 1				
Ckmt2	-5.36	2.36	4.43E-05	0.0221
Cox6a2	-5.42	1.77	2.77E-05	0.0221
LIVER 3 vs 2				
Mavs	1.33	5.73	0.0001	0.0318
Tomm40L	1.80	2.71	0.0001	0.0318
Mtfp1	1.85	2.92	0.0001	0.0318
Ckmt2	-4.94	2.35	0.0001	0.0318
Cox6a2	-5.69	1.77	1.33E-05	0.0132

Immunofluorescence

We have also checked Irf7 and cGAS protein expression levels. The preliminary results comfirm the highest Irf7 expression at Time Point 2 comparing to Times Points 1 and 3 (Figure 3), and a steady increase of cGas expression with time (Figure 4).

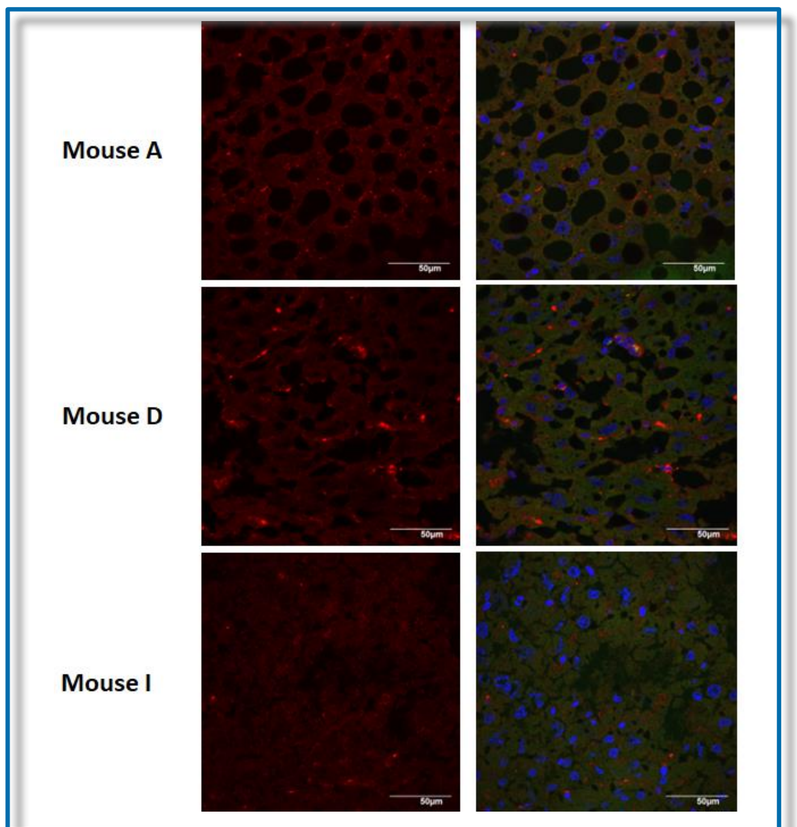

Figure 3. Immunofluorescence analysis of Irf7 expression.

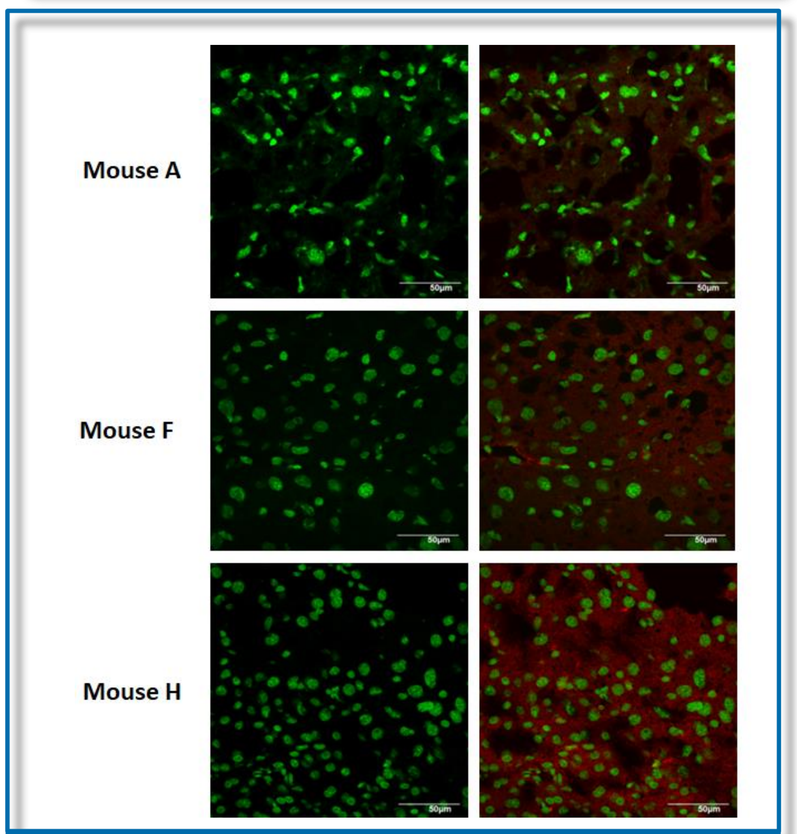

Figure 4. Immunofluorescence analysis of cGAS expression.

Conclusions

Our results suggest an important contribution of immunological response, mainly cytosolic DNA sensing, and mitochondria at the early stages of diabetes and obesity development.

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