

Liver transcriptomics analysis unravels the sequentiality of liver steatosis mechanisms in metabolic dysfunction-associated steatotohepatitis

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Introduction: Hepatic steatosis is secondary to a complex-metabolic dysregulation of lipid homeostasis. Pathophysiological mechanisms are numerous and include dysregulations in lipid uptake, de novo synthesis, oxidation and secretion. Specific drugs targeting lipid metabolism are currently evaluated in clinical trials with inconsistent results. We suggest that the pathophysiology of steatosis may evolve with MAFLD progression and the mixed effects may reveal a mismatch between the deregulated mechanisms and the drug used.

Aim: To analyse the evolution of gene expression of key lipid metabolism genes during MASLD-MASH disease progression in mice.

Methods: *Foz/foz* mice (FOZ) were fed normal diet (ND)(3.39 kcal/g, 16% of lipids, A03 Research Diets) or high-fat diet (HFD)(5.24 kcal/g, 60% of lipids, D12492 Research Diets). Liver tissue was harvested at baseline (control group, N=5) and after 4 (FOZ ND=3, HFD=4), 12 (FOZ ND=6, HFD=8) or 32 (FOZ ND=6, HFD=6) weeks. Formalin-fixed paraffin-embedded liver samples were used for histological scoring (SAF score) using hematoxylin & eosin staining. Total RNA from liver samples was extracted and purified using Qiagen® mini kits prior to bulk RNA sequencing (Macrogen Europe). Sequencing data were normalized according to timepoint and diet and analyzed RStudio. Differential gene expression and enrichment analysis (Gene Ontology database, biological process) were performed as follows: FOZ ND week 0 (control group) *versus* FOZ HFD week 4, FOZ ND week 0 *versus* FOZ HFD week 12, FOZ ND week 0 *versus* FOZ HFD week 32. Differentially expressed genes (DEG) were defined by a p adjusted value (padj) < 0.05 and |log2FoldChange| > 1. Enrichment results are presented by Normalized Enrichment Score (NES) and p adjusted value.

Results: FOZ mice fed HFD reached maximal liver weight to body weight ratio at 32 weeks ($p < 0.001$). Insulin resistant is present at 4 weeks of HFD as assessed by the HOMA-IR index ($p < 0.01$). FOZ mice fed HFD exhibited the whole MASH spectrum with fluctuating transcriptomics changes over time. At week 4, liver histology highlighted isolated steatosis with 284 DEG involved in 685 enriched pathways including fatty acid

biosynthetic process (NES = 13.9, padj = 1.2e-4)(Ppar γ , Hacd4, Obp2a), lipid transport (NES = 9.7, padj = 8.0e-8)(Ppar γ , Vldlr, Pltp), lipid storage (NES = 21.5, padj = 3.0e-6)(Cav1, Clstn3, Mup1) and fatty acid oxidation (NES = 9.4, padj = 0.02)(Ppar γ , Obp2a, Sox9). At week 12, liver histology reported steatohepatitis with 469 DEG involved in 504 enriched pathways including lipid transport (NES = 14.7, padj = 3.6e-15)(Cd36, Ppar γ , Ppar δ , Pltp), lipid storage (NES = 24.2, padj = 1.66e-7)(Fitm1, Bltp1, Cidec) and fatty acid oxidation (NES = 22.2, padj = 2.7e-7)(Acaa1b, Ehhadh, Ppar δ). At week 32, fibrosing steatohepatitis was observed with 750 DEG involved in 1167 enriched pathways including lipid transport (NES = 11.3, padj = 6.3e-21)(Cd36, Apom, Ppar δ), lipid storage (NES = 24, padj = 1.4e-15)(Fitm1, Bltp1, Cidec; Ppar γ) and fatty acid oxidation (NES = 11, padj = 7.4e-7)(Hao2, Ppar γ , Ppar δ). At the gene level, the lipid metabolism key regulator Ppar γ was downregulated in a time-dependent manner (Log2FC = -1.6, padj = 9e-5 at week 4; Log2FC = -1.8, padj = 9e-6 at week 12 and Log2FC = -2.35, padj = 6.6e-9 at week 32). Fatty acid oxidation related genes are all downregulated at week 12, such as Obp2a (Log2FC = -3.17, padj = 0.003) and Acaa1b (Log2FC = -1.64, padj = 9.9e-9). Lipid storage genes expression also greatly change over time. Cidec and Fitm1 are involved in lipid droplet homeostasis preventing lipotoxicity of free fatty acids. Both Cidec and Fitm1 are significantly downregulated at week 12 (Log2FC = -2.13, padj = 3e-6; Log2FC = -1.37, padj = 1.68e-7 respectively) and at week 32 (Log2FC = -2.7, padj = 2e-5; Log2FC = -1.02, padj = 2e-4 respectively).

Conclusions: we report the dynamic changes of pathophysiological mechanisms potentially involved in hepatic steatosis occurring in a MASH mouse model over time using bulk RNAseq. These time-dependent shifts in gene expression of potential therapeutic targets may significantly influence the response to MASLD treatments.