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Implication of the Patatin-like phospholipase domain-containing-3 I148M mutation on Hepatic Stellate Cells mitochondrial dysfunction and profibrogenic potential

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1 Introduction

The I148M variant of the Patatin-like phospholipase domain-containing 3 (PNPLA3) protein is a well validated risk locus for hHSC-driven fibrogenic progression in chronic liver diseases, particularly in NASH. Mitochondrial dysfunction has also been shown to play a key role in NASH development.

2 Aim

In this study we investigated the impact of PNPLA3 I148M mutation on mitochondrial dysfunction in hHSCs in 2D and 3D culture models.

3 Method

Primary hHSC were isolated (n=23 donors) and cultured in 2D, then genotyped for PNPLA3(I148M) variants CG/GG. RNAseq data were analysed on 3 donors/genotype with Ingenuity pathway analysis (IPA). Cell behaviour of PNPLA3(WT) hHSC and PNPLA3(I148M) hHSC was evaluated in 3D decellularized scaffolds from human healthy and cirrhotic liver. Cells were cultured for 13 days and stimulated 3x48h with TGFbeta1. QRT-PCR, western blot and cytochrome-c-oxidase activity assay was performed.

4 Results

IPA associated the PNPLA3(I148M) hHSC variant to the "NRF2 mediated oxidative stress response" and "Oxidative phosphorylation" signalling pathways. A possible derangement of intracellular anti-oxidant response was also suggested by qPCR on 3D cultured hHSC, with a significant decreased expression in VARS2, a mitochondrial enzyme, and GSTT1, a Glutathione-S-Transferase in PNPLA3(I148M) hHSC compared to PNPLA3(WT) hHSC with/without TGFB1 treatment ($p<0.05$). This was further confirmed by protein expression of VARS2 and cytochrome-c-oxidase subunit MTCO1, which was significantly downregulated in PNPLA3(I148M) hHSC compared to PNPLA3(WT) hHSC ($p<0.05$) and in PNPLA3(WT/I148M) hHSC when cultured in healthy scaffolds compared to cirrhotic scaffolds ($p<0.05$). The lower expression of MTCO1 protein also determined a significant reduction in enzymatic activity of the cytochrome-c-oxidase in PNPLA3(I148M) hHSC compared to PNPLA3(WT) hHSC when measured in 2D and healthy scaffolds ($p<0.005$ and $p<0.05$).

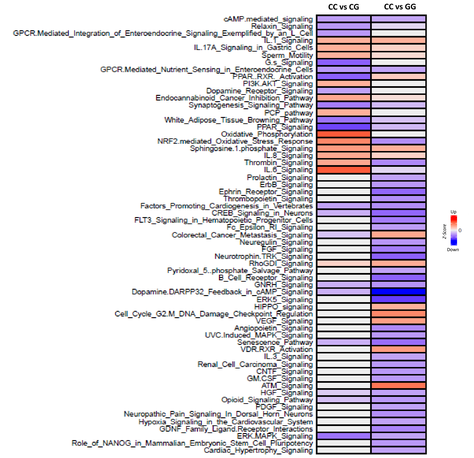

Figure 1. Prediction of canonical pathways significantly enriched and predicted as activated (red) or inhibited (blue) according to IPA on gene expression profiled by NGS of primary HSCs genotyped for PNPLA3 and identified as wild type (WT/CC), heterozygous mutant (HET/CG) or homozygous mutant (HOM/GG). Data are shown as a heat map matrix format representing the activation (Z) score prediction by IPA (-1 ≤ Z-score ≥ 1 for at least one group.

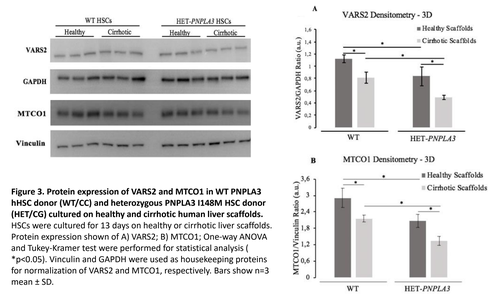

Figure 3. Protein expression of VARS2 and MTCO1 in WT PNPLA3 hHSC donor (WT/CC) and heterozygous PNPLA3 I148M HSC donor (HET/CG) cultured on healthy and cirrhotic human liver scaffolds. HSCs were cultured for 13 days on healthy or cirrhotic liver scaffolds. Protein expression shown of A) VARS2; B) MTCO1; One-way ANOVA and Tukey-Kramer test were performed for statistical analysis (*p<0.05). Vinculin and GAPDH were used as housekeeping proteins for normalization of VARS2 and MTCO1, respectively. Bars show n=3 mean ± SD.

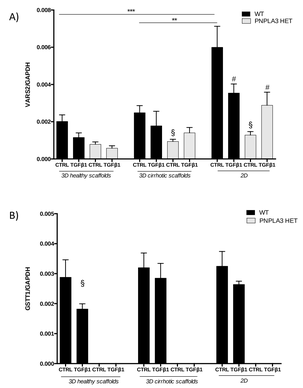

Figure 2. Gene expression of VARS2 and GSTT1 in WT PNPLA3 hHSC donor (WT/CC) and heterozygous PNPLA3 I148M HSC donor (HET/CG) cultured on healthy and cirrhotic human liver scaffolds or 2D plastic culture. HSCs were cultured for 7 days on healthy or cirrhotic liver scaffolds and treated with TGFβ1 (5 ng/mL) for 3x48 hours. HSCs were cultured for 2 days on plastic dishes and treated with TGFβ1 (5 ng/mL) for 3x48 hours. Gene expression shown of A) VARS2; B) *GSTT1*; (§§/§*0.05/0.01 WT vs HET; ***/**P<0.01/0.005 healthy 3D scaffold vs 3D cirrhotic scaffold vs 2D; #P<0.05 control vs TGFβ1 treated) Bars show n=4 mean ± SD.

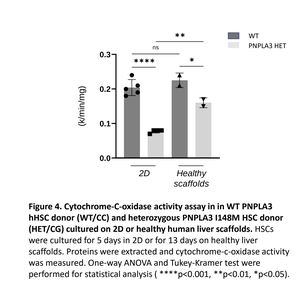

Figure 4. Cytochrome-C-oxidase activity assay in in WT PNPLA3 hHSC donor (WT/CC) and heterozygous PNPLA3 I148M HSC donor (HET/CG) cultured on 2D or healthy human liver scaffolds. HSCs were cultured for 5 days in 2D or for 13 days on healthy liver scaffolds. Proteins were extracted and cytochrome-c-oxidase activity was measured. One-way ANOVA and Tukey-Kramer test were performed for statistical analysis (****p<0.001, **p<0.01, *p<0.05).

5 Conclusions

In this study, following IPA analysis on the genetic background of hHSC carrying different variants of the PNPLA3 I148M mutation, mitochondrial function of hHSC was investigated in a 3D model recapitulating the ECM microenvironment of normal and cirrhotic human liver. Results indicate that the PNPLA3(I148M) variant is linked to a disrupted expression and activity of different mitochondrial proteins, including a key enzyme of the respiratory chain. This leads to a dysfunctional hHSC mitochondrial phenotype which is worsened by the fibrotic ECM.

6 Acknowledgements

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7 References

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