A ^{1}H-MAS-NMR BASED METABOLOMICS STUDY OF PTP1B DEFICIENCY INFLUENCE ON HEPATIC REGENERATION IN MICE

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1. INTRODUCTION

- Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of tyrosine kinase growth factor signaling.
- Levels of PTB1B may exert a pivotal role in maintaining the balance between survival and death in hepatocytes.[1]
- Recently, it has been proposed that PTP1B deficiency accelerates hepatic regeneration in mice.[2]

2. GOALS

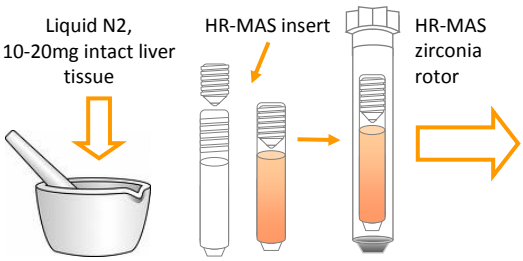
This work is primarily aimed to analyze the differences between PTP1B$^{-/-}$ and WT mice in the early metabolic events produced upon partial hepatectomy (PH) and liver regeneration.

3. EXPERIMENTAL DESIGN

A ^{1}H-MAS-NMR metabolic profiling pilot study in a strain comparative strategy within the first 24 hours after PH is performed

Time	KO	Replicates
0 hours after PH	WT	n=4
	PTPB1$^{-/-}$	n=4
24 hours after PH	WT	n=4
	PTPB1$^{-/-}$	n=4

4. SAMPLE HANDLING

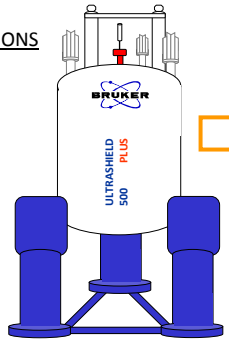


Liver biopsies were crushed in liquid nitrogen using a frozen mortar and pestle and subsequently homogenized in buffer D_2O saline (0.9%) and packed into a 4-mm-diameter zirconia MAS rotor with a top insert to give a final volume of 15-25 μL.

5. 1H-MAS-NMR EXPERIMENTS

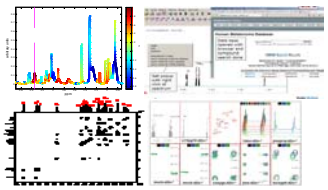
HR-MAS-1H-NMR CONDITIONS
Bruker Avance DRX-500
operating at 500.13 MHz
T=280K
Spinning rate=4 kHz.
Eretic signal calibration

PULSE SEQUENCES
NOESY
CPMG



6. DATA ANALYSIS

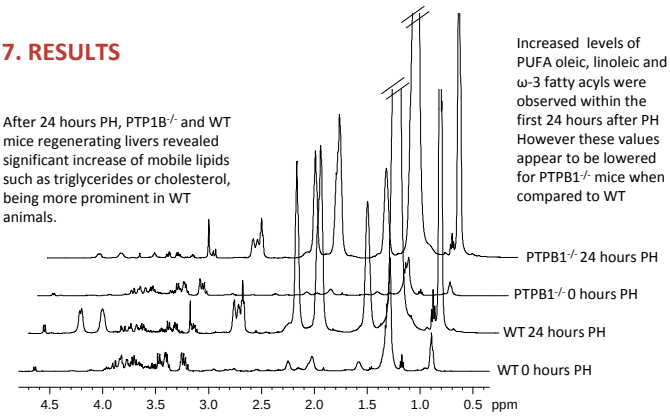
Metabolites ID were based on previous reported literature, BRUKER database, 2D-spectra and STOCSY



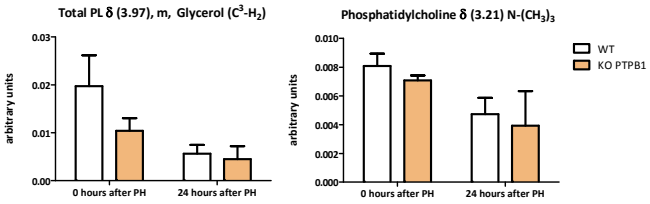
After metabolite identification, metabolite regions were integrated using in-house Matlab scripts. Selected regions were scaled to ERETIC signal and further on to mg of liver tissue.

7. RESULTS

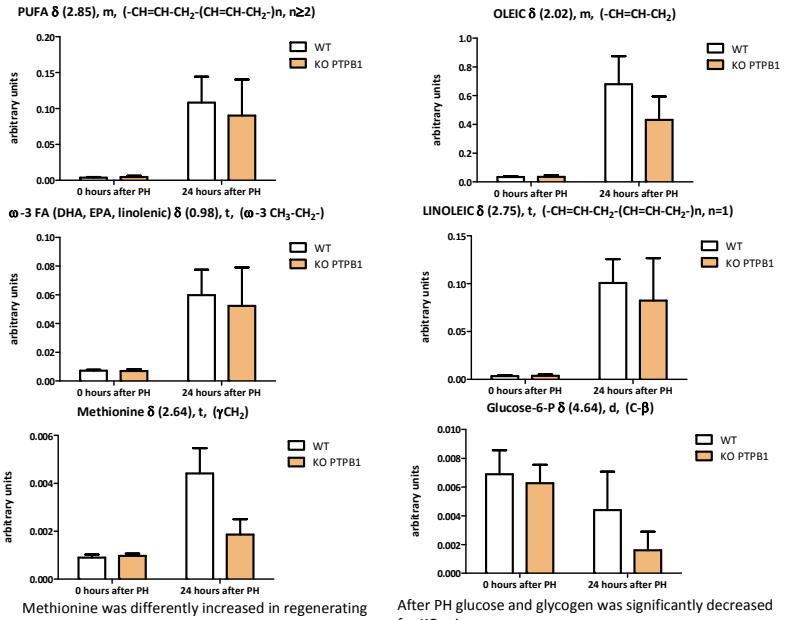
After 24 hours PH, PTP1B$^{-/-}$ and WT mice regenerating livers revealed significant increase of mobile lipids such as triglycerides or cholesterol, being more prominent in WT animals.



Increased levels of PUFA oleic, linoleic and ω-3 fatty acyls were observed within the first 24 hours after PH However these values appear to be lowered for PTPB1$^{-/-}$ mice when compared to WT



Depleted levels of PL in general and phosphatidylcholine in particular were detected within 24 hours after PH



Methionine was differently increased in regenerating livers according to genotype (2-factor ANOVA p<0.05).

After PH glucose and glycogen was significantly decreased for KO mice.

8. CONCLUSIONS

- The decreased content of lipid unsaturation for PTP1B$^{-/-}$ after 24h would appear as signal promoting the liver regeneration within this strain.
- Lowered levels of methionine in PTP1B$^{-/-}$ mice might be indicative of lowered levels of SAM (S-adenosine methionine). A drop in SAM levels is required for the sensitization of liver cells to hepatocyte growth factor (HGF), a key mitogenic signaling molecule in the regeneration process.[3,4] Such evidence supports the thesis that PTPB1 deficiency accelerates hepatic regeneration.
- Taken together these findings demonstrated that a coordinated pattern of biochemical changes occur with and after hepatic regeneration.
- ^{1}H-HR-MAS NMR is able to provide a biochemical snapshot of such changes allowing for a rapid assessment of the regeneration status in the liver with minimal sample preparation requirements and in less than 15 minutes.
- Further work is in progress with this dataset in order to confirm such evidences in ^{1}H-NMR liver extracts.

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