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ABSTRACT BOOK

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Conclusion: The Perfusion Technology allows simple and reliable isolation of primary hepatocytes. Perfuser and appropriate reagents were optimized for parenchymal rodent liver cells but can be adapted to non-parenchymal cells. The semi-automated workflow enabling liver perfusion in a closed system is easy to apply and helps to implement the 3R principle because only one liver lobule is perfused, while others can be used for further experimentation. Nested processing allows simultaneous handling of up to 8 samples. The protocol does not require inconvenient perfusion in living animals avoiding the need of animal ethics approval.

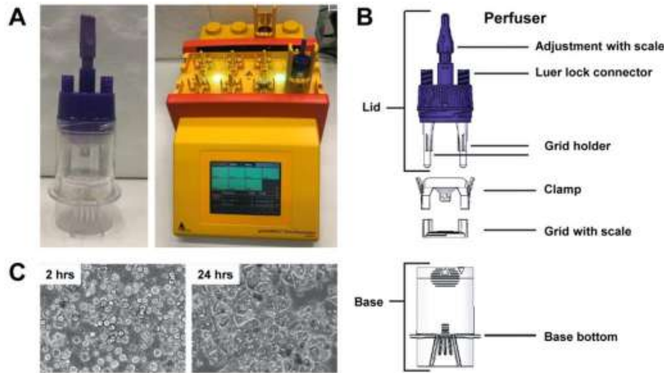

Figure: Semi-automated dissociation of liver tissue. (A) Overview of the Perfusion Technology. (B) Structure of the Perfuser used for generation of single cell cultures from rodent livers. **(C)** Hepatocytes isolated with optimized reagents after seeding for 2 hours (*left*) or 24 hours (*right*).

Conflicts of interest: Carsten Poggel, Timo Adams, Ronald Janzen, Alexander Hofmann and Olaf Hardt are employees of Miltenyi Biotec B.V. and Co. KG.

Abstract withdrawn

SAT183
Ptpn1 deletion protects oval vells against lipoapotosis by favoring lipid droplet formation and dynamics

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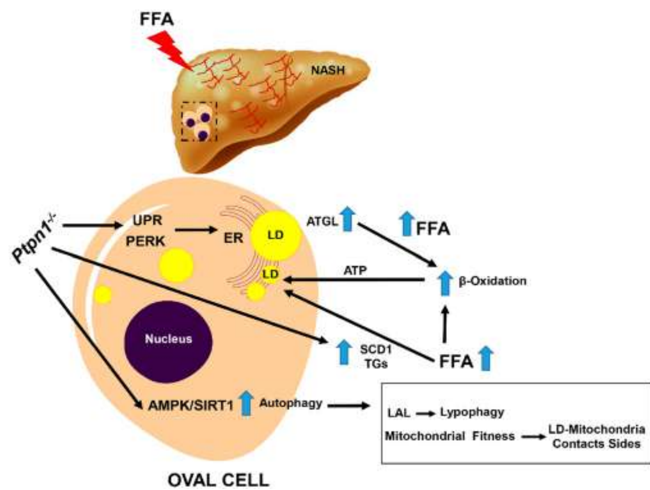
Background and aims: Activation of oval cells has been related to hepatocyte injury during chronic liver diseases including non-alcoholic fatty liver disease (NAFLD). However, oval cells plasticity can be affected by the pathological environment. We previously found a protection against hepatocyte cell death by inhibiting protein tyrosine phosphatase 1B (PTP1B). Herein, we investigated the molecular and cellular processes involved in the lipotoxic susceptibility in oval cells expressing or not PTP1B.

Method: Palmitic acid (PA) induced apoptotic cell death in wild-type ($Ptpn1^{+/+}$) oval cells in parallel to oxidative stress and impaired autophagy. This lipotoxic effect was attenuated in oval cells lacking *Ptpn1* that showed up-regulated antioxidant defences, increased unfolded protein response (UPR) signaling, higher endoplasmic reticulum (ER) content and elevated stearoyl CoA desaturase (*Scd1*) expression and activity.
Results: These effects in $Ptpn1^{-/-}$ oval cells concurred with an active autophagy, higher mitochondrial efficiency and a molecular signature of starvation, favoring lipid droplet (LD) formation and dynamics. Autophagy blockade in $Ptpn1^{-/-}$ oval cells reduced *Scd1* expression, mitochondrial fitness, LD formation and restored lipoapoptosis, an effect also recapitulated by *Scd1* silencing. Importantly, oval cells with LDs were found in livers from $Ptpn1^{-/-}$ mice with NAFLD.



Conclusion: *Ptpn1* deficiency restrained lipoapoptosis in oval cells through a metabolic rewiring towards a "starvation-like" fate, favoring autophagy, mitochondrial fitness and LD formation. Dynamic LD-lysosomal interations likely ensured lipid recycling and, overall, these adaptations protect against lipotoxicity. The identification of LDs in oval cells from $Ptpn1^{-/-}$ mice with NAFLD opens new therapeutic perspectives to ensure oval cells viability and plasticity under lipotoxic liver damage.

SAT184
Accurate detection of HBV splice variant DNA by using long-read sequencing
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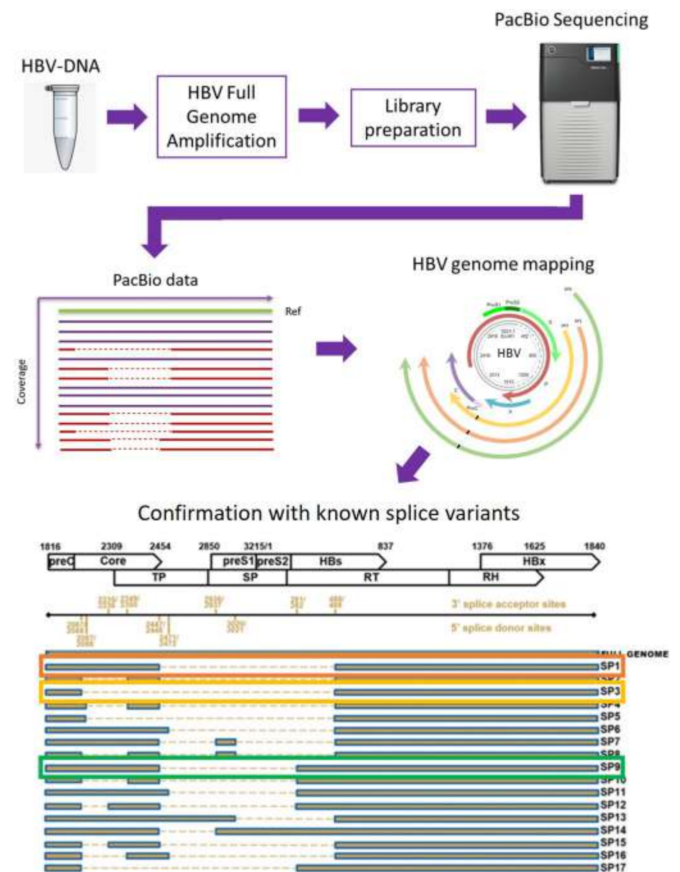
Background and aims: Hepatitis B virus (HBV) RNA splice variants might play an important role in the HBV life cycle and pathogenesis. Although the biological and clinical significance of splice variants remains to be defined, some studies have linked HBV spliced variants to impaired response to interferon-α therapy. In depth HBV genome analysis may help to understand the role of splice variants in disease pathogenesis. With long-read sequencing, a spliced HBV genome can be covered by a single read, in contrast to short reads where the spliced genome is sequenced by multiple fragmented shorter reads. In a mixed population sample with both splice variant and full genomes, these shorter reads may originate either from the spliced template or the original full length genome template. In this study, we report our findings on the accurate detection of HBV splice variant DNAs in plasma (derived from RNA splice variants generated in the liver) with PacBio long-read sequencing.

Method: Starting material for the analysis were plasma sample from HBV-infected patients. Nucleic acid extraction was performed by using the QIAamp MinElute Virus Spin Kit (QIAGEN). The isolated DNA was amplified for HBV full genome by PCR and nested PCR, using the Expand high-fidelity PCR kit (Roche Molecular Systems). Sequencing libraries were prepared using the SMRTbell Express Template Prep Kit 2.0 (Pacific Biosciences). All sequencing reactions were performed on the PacBio Sequel System with the Sequel Sequencing Kit 3.0 chemistry (Pacific Biosciences). Sequencing was performed by using the circular consensus sequencing (CCS) mode, creating HiFi consensus reads. Splice variant analysis was done by using our in-house bioinformatics pipeline Athena.
Results: A first indication of the presence of splice variants is obtained by visualizing the amplification products on gel and looking at the read length distribution after sequencing. In our samples we observed multiple amplicons with varying lengths. After sequencing the read length distribution showed samples with full length HBV genome CCS reads (~3200 bp) and shorter CCS reads of varying size (~1600, ~2000, ~2400 bp). CCS reads were bioinformatically mapped against an HBV reference sequence using minimap2. Consequently, the read mapping was parsed with separate scripting on individual read level, resulting in read counts for each unique start and end splice junction combination. The read counts for potential splice variants were summarized in a table and the most prevalent ones were compared with literature. All identified dominant splice variants were virtually identical to the splice variants described in literature. In the example shown, we specifically identified DNAs, derived from SP1, 3 and 9 (Figure 1).



Conclusion: HBV splice variant DNA can be accurately detected with PacBio long-read sequencing.