Liver spheroids: a robust human in vitro system for testing the therapeutic options of cyclooxygenase 2 in NAFLD/NASH

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BACKGROUND

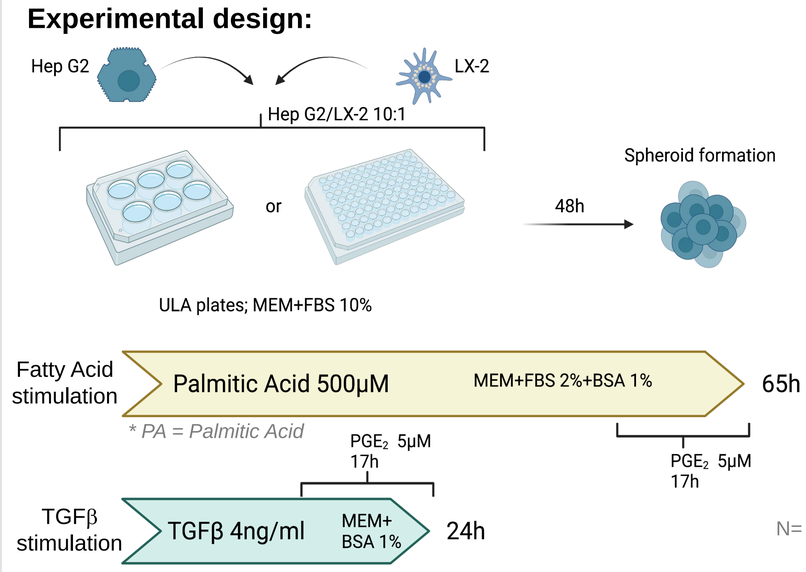
Cyclooxygenase (COX) is a key enzyme in the biosynthesis of prostanoids. Prostaglandins are involved in multiple homeostatic processes, as well as playing an important role in the onset of inflammation. COX-2 is an isoform that is expressed and induced by different stimuli in various tissues and cell types; however, in liver, COX-2 expression is restricted to those situations where proliferation and dedifferentiation occur (1). Our previous results have shown that COX-2 expression in hepatocytes protects against hyperglycemia-induced liver damage, peripheral insulin resistance and adiposity in mice fed a high-fat diet (2), and also protects against experimental non-alcoholic steatohepatitis and fibrosis (3).

AIM

Our results indicate that one therapeutic strategy for the treatment of NAFLD/NASH may be through COX-2-derived prostaglandins. Our goal is to develop a robust human in vitro system to model hepatic NAFLD/NASH. As an initial strategy, we have set up a 3D spheroid culture composed of Hep G2 and LX-2 cells to determine the effect of PGE2 on NAFLD/NASH.

MATERIALS AND METHODS

Experimental design:



Star methods used:

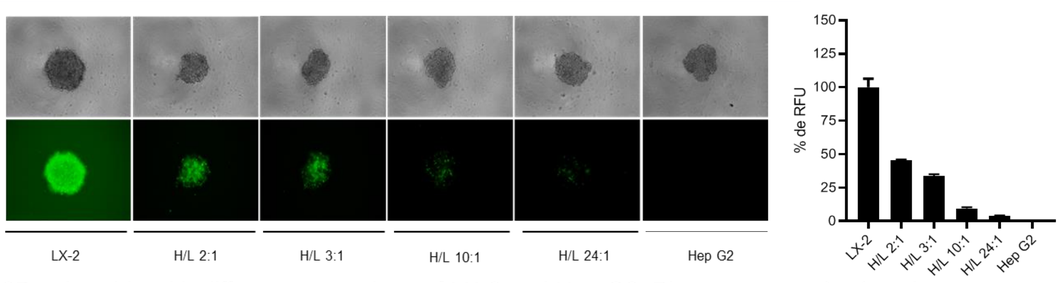
- Immunofluorescence and confocal microscopy
- Fluorescence microscopy
- SDS-PAGE and Western Blot
- Flow cytometry
- RT-qPCR

Statistics: mean±SD (One-way ANOVA, * p<0,05, ** p<0,01, *** p<0,001 to Ctrl, # p<0,05, ## p<0,01, ### p<0,001 to AP or TGFβ)

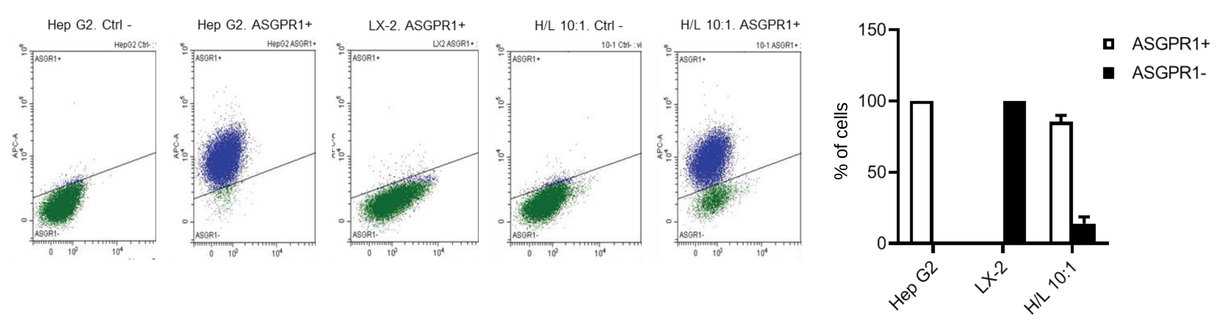
N= 3-4 for all the experiments except for immunofluorescence assays, N=4-15

RESULTS

Cellular characterization of liver spheroids

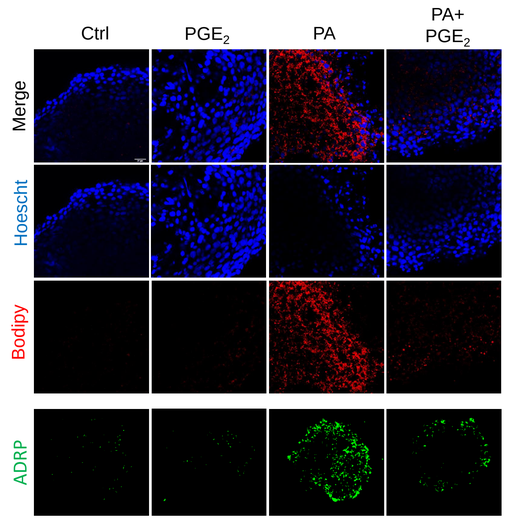


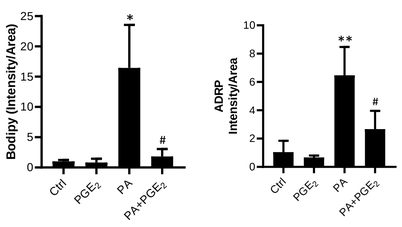
3D spheroids with different proportions of LX-2 and Hep G2. Phase contrast (top) and green fluorescence (bottom), LX-2 are labelled with the Green Cell Tracer. The HepG2/LX-2 10:1 ratio was selected for further analysis.



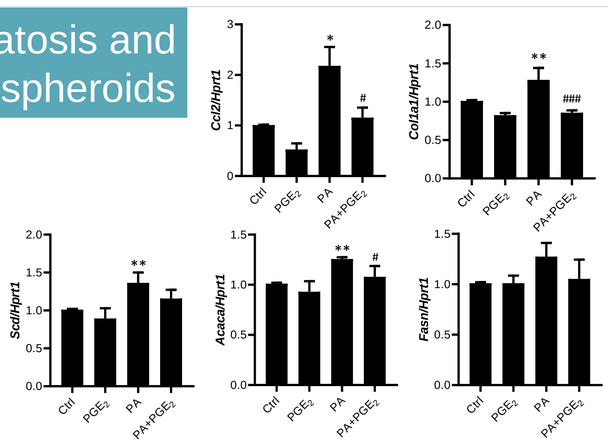
Flow cytometry of liver spheroids of Hep G2, LX-2 and H/L 10:1. ASGPR1 is used as a Hep G2 marker. ASGPR1+ populations in blue, ASGPR1- populations in green.

PGE_2 reduces PA-induced steatosis and associated damage in hepatic spheroids

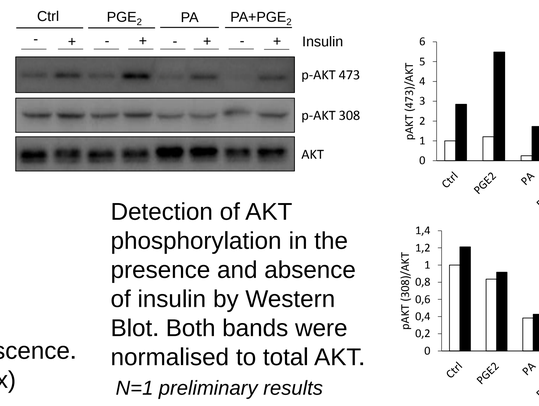




Detection of lipid accumulation with immunofluorescence. Bodipy (red, 63x) and Adipored (green, far red, 40x) signals were normalised to the spheroid area.



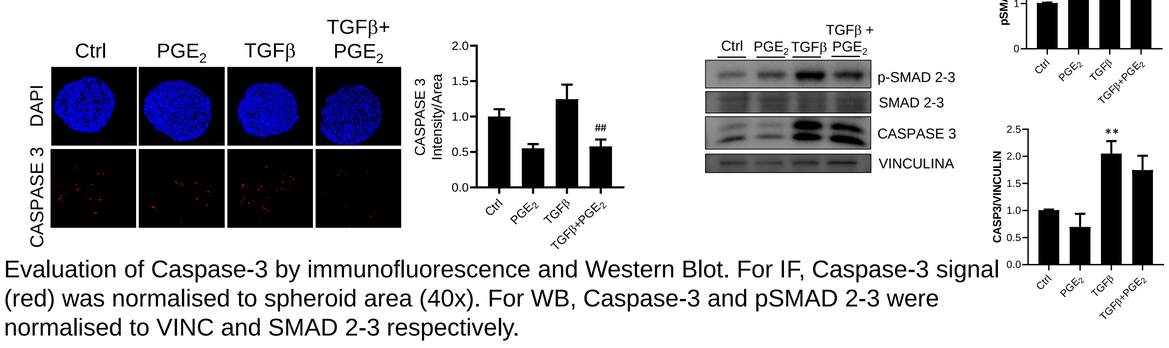
mRNA expression levels of selected genes analysed by RT-qPCR. Data are normalised to Hprt1 mRNA and its induction relative to Ctrl.



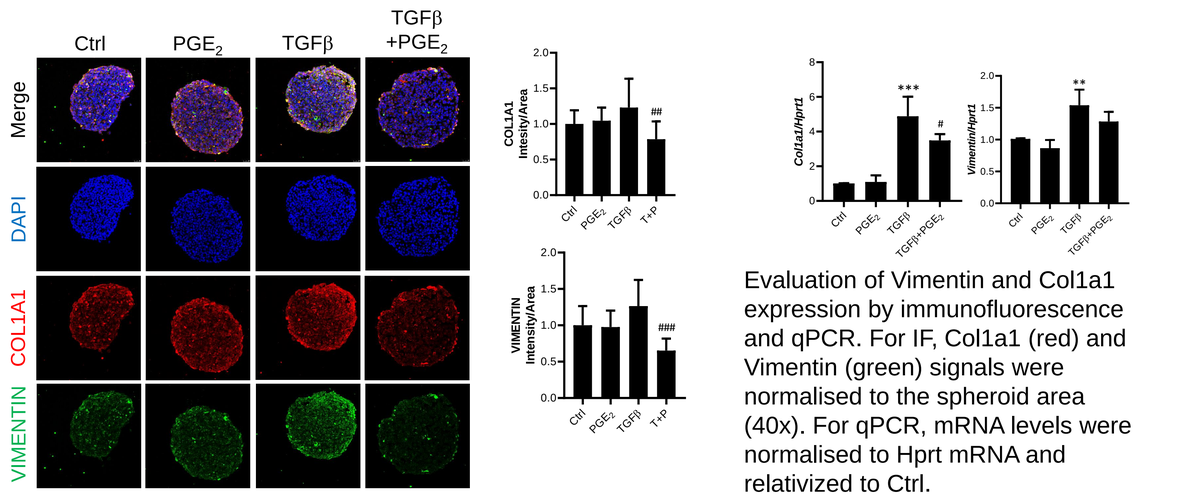
Detection of AKT phosphorylation in the presence and absence of insulin by Western Blot. Both bands were normalised to total AKT.

N=1 preliminary results

PGE_2 administration slows the progression of TGFβ-induced liver fibrosis in hepatic spheroids

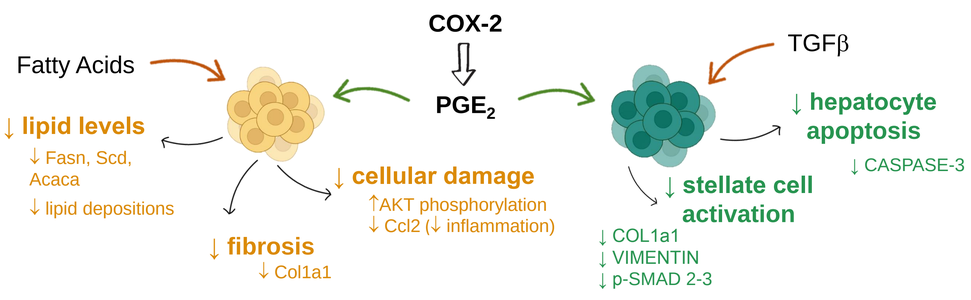


Evaluation of Caspase-3 by immunofluorescence and Western Blot. For IF, Caspase-3 signal (red) was normalised to spheroid area (40x). For WB, Caspase-3 and pSMAD 2-3 were normalised to VINC and SMAD 2-3 respectively.

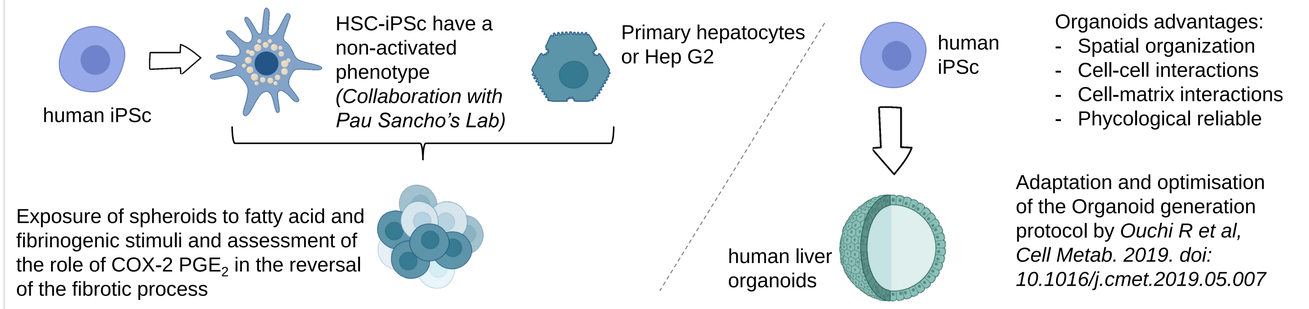


Evaluation of Vimentin and Col1a1 expression by immunofluorescence and qPCR. For IF, Col1a1 (red) and Vimentin (green) signals were normalised to the spheroid area (40x). For qPCR, mRNA levels were normalised to Hprt mRNA and relativized to Ctrl.

TAKE HOME MESSAGE



IN PROGRESS / FUTURE WORK



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