TGF-β2 in Chronic Liver Disease and Hepatocellular Carcinoma

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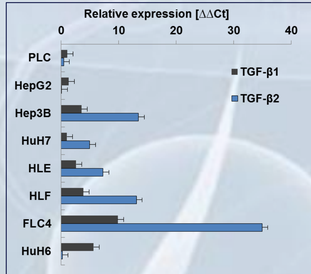
BACKGROUND and AIMS

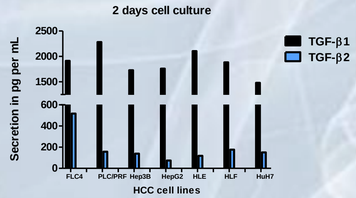
TGF-β is a prominent cytokine in diseased liver, orchestrating a complex cell-cell communication in wound healing and regeneration, inflammation, fibrogenesis as well as carcinogenesis. Although multiple preclinical approaches directed towards the prominent family member TGF-β1 provided promising results, none were successfully translated into clinics. We here demonstrate that TGF-β2 may represent an alternative therapeutic target in CLD and HCC.

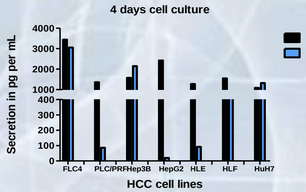
METHODS

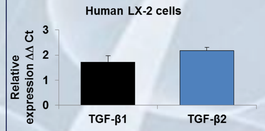
TGF-β1/2 expression, autocrine stimulation, Smad and non Smad signaling were performed in hepatic stellate cells (HSC), HCC cell lines and mouse models for chronic liver diseases, using real time PCR, immunoblotting, ELISAs and reporter assays .

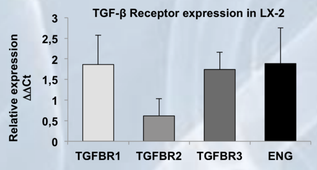
RESULTS



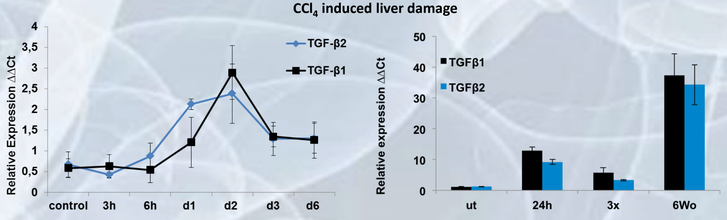


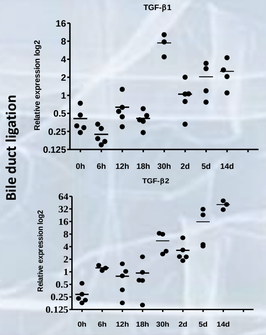




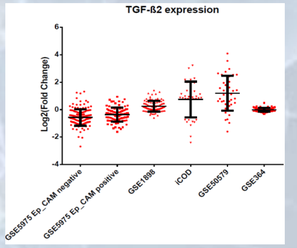


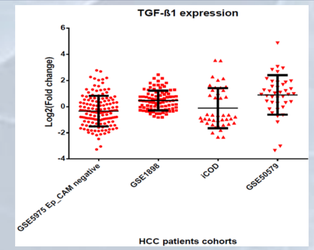
TGF-β2 vs TGF-β1 mRNA expression levels were investigated in 8 hepatocellular carcinoma cell lines. Higher levels of TGF-β2 were found in 5, whereas it was equal to TGF-β1 in the other three. Cell lines with higher TGF-β2 mRNA levels also secreted higher amounts of TGF-β2 protein (sandwich ELISAs). In the human HSC cell line LX-2, TGF-β2 vs TGF-β1 mRNA expression was found to be slightly elevated. TGF-β receptor expression indicated that all components for TGF-β2 and TGF-β1 mediated signaling are present.





We analyzed three mouse models of CLD (CCl4, BDL & Mdr2-/-) and one model of liver regeneration (1xCCl4) for TGF-β2 vs TGF-β1 expression. In liver regeneration and fibrosis upon CCl_4, TGF-β2 expression is transiently upregulated at least equally strong as TGF-β1, along with HSC activation and collagen expression. Upon bile duct ligation (BDL), damage/fibrogenesis dependent TGF-β2 expression is induced even 10-fold higher as compared to TGF-β1.





Gene	N° Cohorts significant	N° Cohorts up-regulated	N° Cohorts down-regulated
TGF-β1	4	2	2
TGF-β2	5	3	2

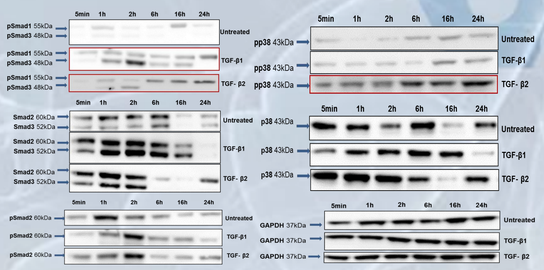
Data from average expression within the cohorts

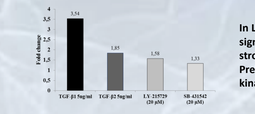
N_{total}= 596	% HCC patients with significant changes	% HCC patients with up-regulation	% HCC patients with down-regulation
TGF-β1	36	53	47
TGF-β2	38	41	59

Data from single patients expression within the cohorts

In order to study TGF-β1 and TGF-β2 isoform expression in different patients collectives, data from 5 HCC patient cohorts (iCOD database, GSE5975, GSE1898, GSE50579 and GSE364) with a total number of 596 HCC patients with different etiologies, referred to independent normal liver samples and/or surrounding tissue samples were analyzed. The distribution of TGF-β1 and TGF-β2 expression in patients from different cohorts are displayed in scatter plots, representing each single patient. The GSE5975 cohort is divided in 2 groups (Ep_CAM positive and Ep_CAM negative). Mean expression, as well as intra and inter-variation in the cohorts were determined and represented are those patients, where the isoforms are significantly expressed (p-value < 0.001). Presented data show the expression tendency in patients and allows further separation into clusters as well as correlation with clinical outcome. The table displays the information collected from the cohorts, that is mean fold change within the cohorts, expression tendency and number of patients showing this tendency.

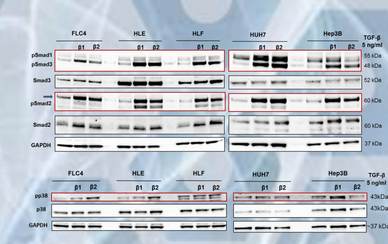
Human LX-2 cell line





In LX-2 cells, TGF-β1/2 can activate canonical Smad signaling, whereby SBE reporter assay revealed stronger induction by TGF-β1 than TGF-β2. Preliminary data suggest non-canonical MAPK-p38 kinase signaling is induced by TGF-β2 in LX2.

Human HCC cell lines



Smad and non-Smad TGF-β signaling in human HCC cell lines. FLC4, HLE, HLF, HUH7 and Hep3B cells were TGF-β1 and –β2 stimulated for 1h. Activation of Smad and MAPK-p38 signaling were investigated. Our first studies reveal that there is no distinct signaling pattern of the different HCC cell lines upon TGF-β1 and –β2 stimulation.

CONCLUSIONS

Significant upregulation of TGF-β2 in line with TGF-β1 in CLD and HCC suggest TGF-β2 as promising therapeutic target to tackle fibrosis and HCC. Molecular details and specific outcome remain to be estimated further.