Targeting TGF-β2 in chronic liver diseases

Anne Dropmann[1], Hanna Korhonen [2], Frank Jaschinski [2], Michel Janicot [2], Nadja Meindl-Beinker[1], Steven Dooley[1]

[1]Department of Medicine II, Section Molecular Hepatology, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; [2] Isarna Therapeutics GmbH, Munich, Germany

EASL The Home of Hepatology

50th THE INTERNATIONAL LIVER CONGRESS 2015

APRIL 22-26, VIENNA, AUSTRIA

BACKGROUND

Expression and function of TGF-β2 have not been investigated thoroughly in chronic liver disease (CLD) progression and HCC. After providing evidence that TGF-β2, like TGF-β1, plays a putative role in fibrogenesis, we now aim to selectively target TGF-β2 expression using antisense oligonucleotides (AONs) for attenuation or blockage of human liver disease progression.

MATERIALS & METHODS

Three CLD mouse models (CCl_4, BDL & MDR2-KO) were investigated representing different types of CLD background. Dynamics of TGF-β2 and TGF-β1 expression were compared in these models by quantitative realtime (qRT)-PCR. In vivo, we selectively inhibited TGF-β2 using specific AONs. In detail, for induction of chronic liver damage, 12 weeks old mice were injected intraperitoneally (i.p.) with 0,2 ml/kg BW CCl_4 twice per week for four weeks. After 2 weeks, subcutaneous AON application started in parallel with a dosage of 30 mg/Kg twice per week. In the MDR2-KO mouse model, the AON was administered for 4 weeks. The effect and efficacy of AON treatment was evaluated by tissue morphology and on protein and mRNA level. Typical fibrotic markers are currently investigated using qRT-PCR.

Contact Information
Anne.Dropmann@medma.uni-heidelberg.de

RESULTS

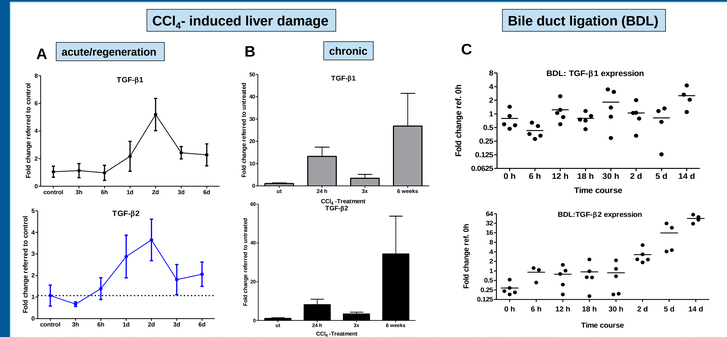


Figure 1: CCl_4- induced liver damage and fibrosis. Upon acute (A) and chronic (B) liver damage by CCl_4, TGF-β2 expression was compared to TGF-β1. (A) After injection with a single dose of CCl_4 mRNA levels of TGF-β isoforms were quantified at different time points (3h to 6d, regeneration model). (B) mRNA expression of both TGF-β isoforms was further examined 24 hours after one CCl_4 injection, after 3 CCl_4 injections and after chronic treatment with CCl_4 for 6 weeks. (n_{acute} =3 or 4; n_{ut}=12; n_{24h}=12; n_{3x}=13; n_{6wo}=12). (C) Bile duct ligation (BDL), a model for secondary biliary fibrosis, displayed an elevation of TGF-β2 expression within a time course of 14 days. Induction of TGF-β2 expression was about 8-fold stronger as compared to TGF-β1.

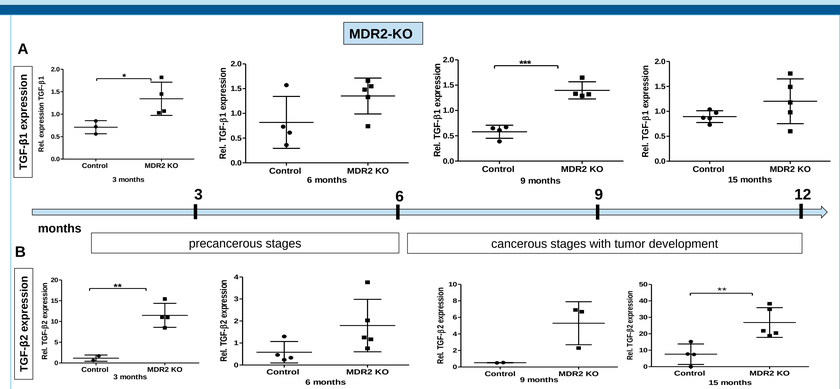


Figure 2: Fluidigm qRT-PCR analysis of (A) TGF-β1 and (B) TGF-β2 mRNA expression in MDR2-KO mice at different age as indicated. It revealed frequent TGF-β2 upregulation (B) in MDR2-KO mice within 3 to 15 month as compared to wild types. However, TGF-β1 expression (A) was clearly not as strong as TGF-β2 expression within the time course but showed significant upregulation at 3 and 9 months.

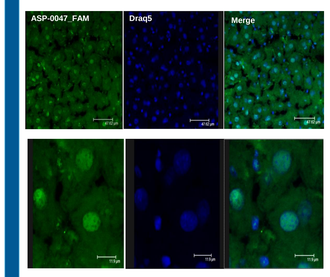
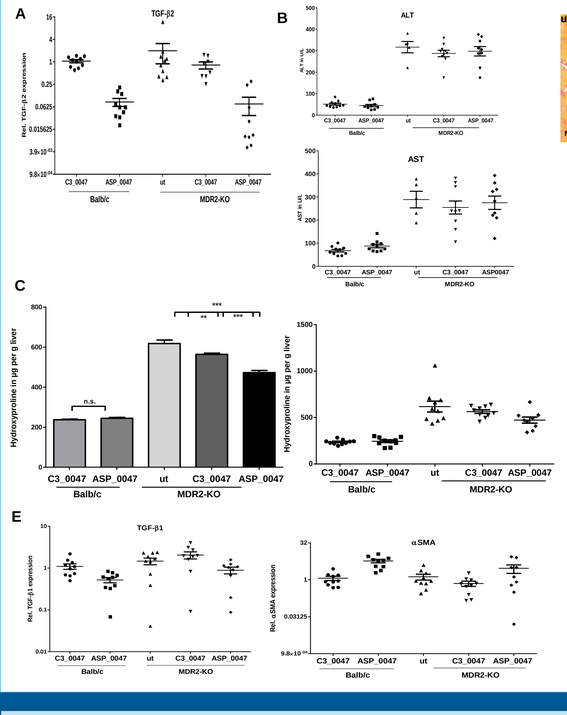


Figure 3: Biodistribution of the TGF-β2 targeting AON in liver tissue was analyzed after different application methods. Signal intensities were high enough to detect the AON ex vivo after 24 hours (not shown) as well as after 5 days. The strongest signal was observed in liver and kidneys (not shown) and seemed not to affect other organs.

TGF-β2 selective AON treatment of MDR2-KO mice reduces liver damage



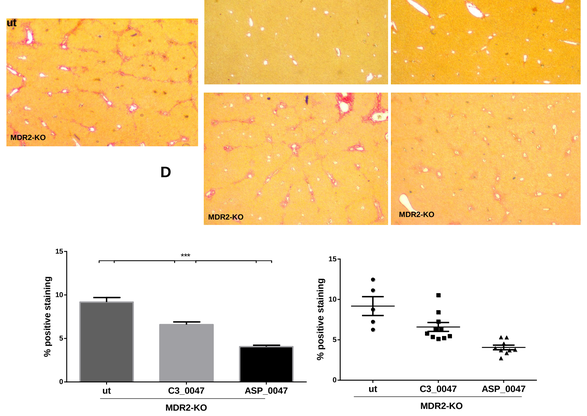


Figure 3: Wildtype (Balb/c) and MDR2-KO mice were treated with AONs for 4 weeks. (A) Efficient TGF-β2 downregulation due to AON treatment could be shown on mRNA level . We analyzed the impact of TGF-β2 selective AONs (ASP_0047) on different physiological parameters in comparison to control oligos (C3-0047). (B) Analysis of liver function parameters in the serum revealed no difference between animals treated with AONs vs. those treated with control oligos. Of course, ALT and AST values are elevated in MDR2-KO mice as compared to wildtype. (C) Measurement of the Hydroxyproline content indicated significant collagen reduction in AON-treated animals when compared to controls. (D) Immunohistological assessment of mouse liver tissue by Sirius Red staining revealed a significant reduction (~56 %) of collagen deposition in AON-treated mice. (E) Fibrotic markers, e.g. TGF-β1 and α-SMA were as well affected by AON treatment in MDR2-KO mice suggesting an important role of TGF-β2 in the fibrotic process.

CCl_4 – induced liver damage is attenuated after selective TGF-β2 AON treatment in mice

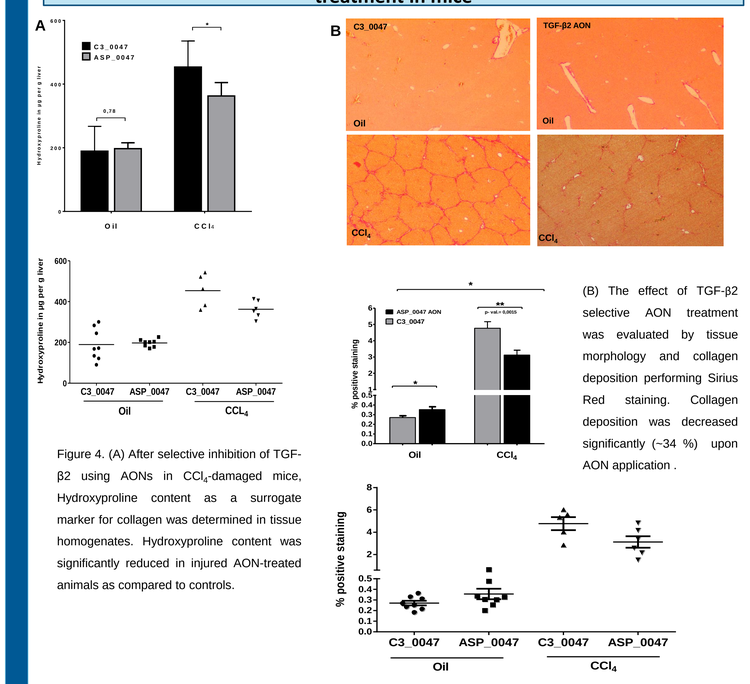


Figure 4. (A) After selective inhibition of TGF-β2 using AONs in CCl_4-damaged mice, Hydroxyproline content as a surrogate marker for collagen was determined in tissue homogenates. Hydroxyproline content was significantly reduced in injured AON-treated animals as compared to controls.

(B) The effect of TGF-β2 selective AON treatment was evaluated by tissue morphology and collagen deposition performing Sirius Red staining. Collagen deposition was decreased significantly (~34 %) upon AON application .

CONCLUSIONS

Taken together, our results suggest a role of TGF-β2 in the process of CLD. We further conclude that in vivo application of a TGF-β2 directed AON to CLD mouse models could contribute to fibrogenesis attenuation. Further studies are currently performed to determine mechanistic details of AON effects and define specifications of a potential AON based treatment of CLD, e.g. dosage and stage of disease, when application is feasible.