Targeting TGF-β2 in chronic liver diseases

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Background and aims:

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

Methods

For our study three CLD mouse models (CCl_4, BDL & Mdr2-/-) were investigated representing different types of CLD background. TGF-β2 expression was compared to TGF-β1 expression by quantitative realtime (qRT)-PCR. In vivo, we selectively inhibited TGF- β2 using 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 parallely 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 on protein and mRNA level. Typical fibrotic markers are currently investigated using qRT-PCR.

CCl_4- induced liver damage

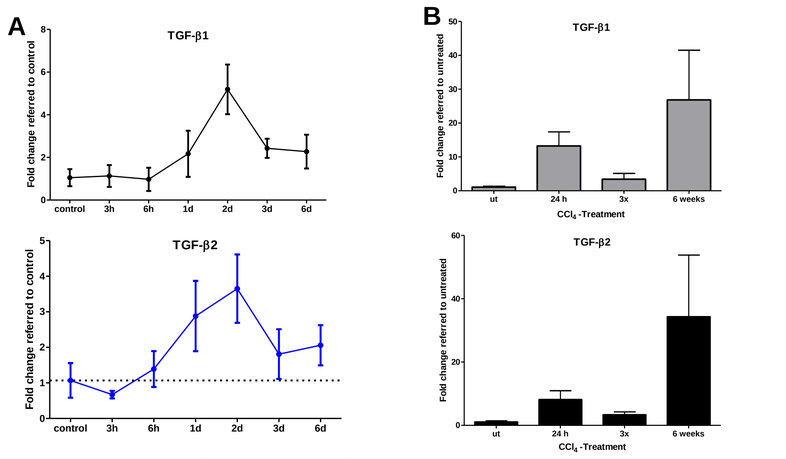


Figure 1: CCl_4- induced liver damage and fibrosis. Upon acute (Fig.1A) and chronic (Fig.1B) liver damage by CCl_4, TGF-β2 expression was compared to TGF-β1. After injection with a single dose of CCl_4 mRNA levels of TGF-β isoforms were quantified at different time points (3h to 6d, regeneration model) . mRNA expression of both TGF-β isoform 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)

Bile duct ligation (BDL)

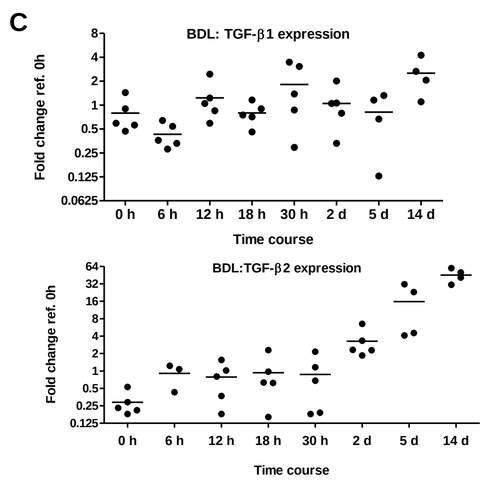


Figure 1C: Bile duct ligation (BDL), a model for secondary biliary fibrosis, displayed an elevation of TGF-β2 expression within a time course of 14 days. Induction of TGF-β2 expression was about 8-fold stronger as compared to TGF-β1.

MDR2-KO: model of inflammation-associated HCC

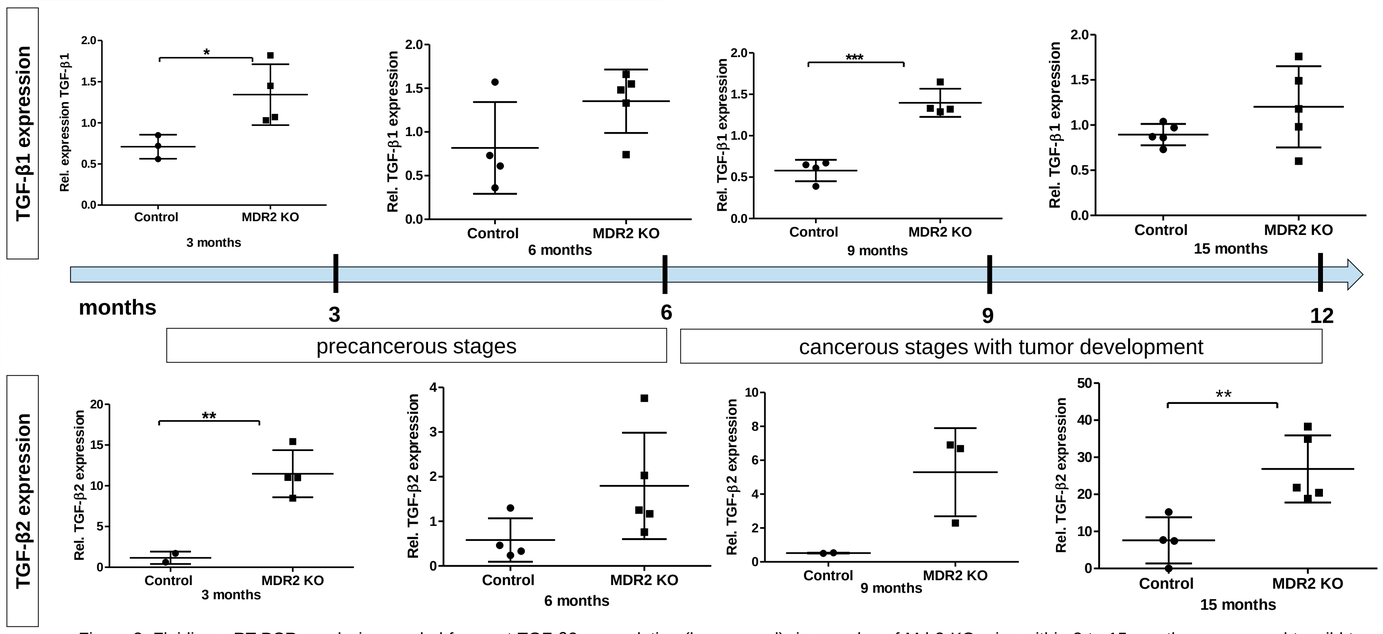


Figure 2: Fluidigm qRT-PCR analysis revealed frequent TGF-β2 upregulation (lower panel) in samples of Mdr2-KO mice within 3 to 15 month as compared to wild types. However, TGF-β1 expression (upper panel) was clearly not as strong as TGF-β2 expression within the time course but showed significant upregulation at 3 and 9 months.

MDR2-KO: systemic treatment with a selective TGF-β2 -AON

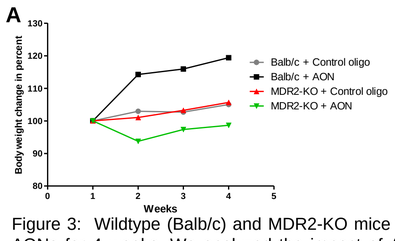
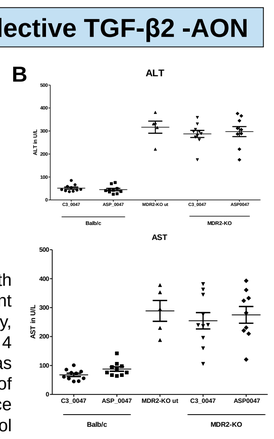


Figure 3: Wildtype (Balb/c) and MDR2-KO mice were treated with AONs for 4 weeks. We analyzed the impact of AONs on different physiological parameters in comparison to control oligos. Generally, MDR2-KO mice gain less weight than wild-type animals within 4 weeks. Further, AON-treatment leads reduced weight gain as compared to animals treated with control oligos (Fig3A). Analysis of liver function parameters in the serum revealed no difference between animals treated with AONs vs. those treated with control oligos (Fig3B). Of course, ALT and AST values are elevated in MDR2-KO mice as compared to wild-type.



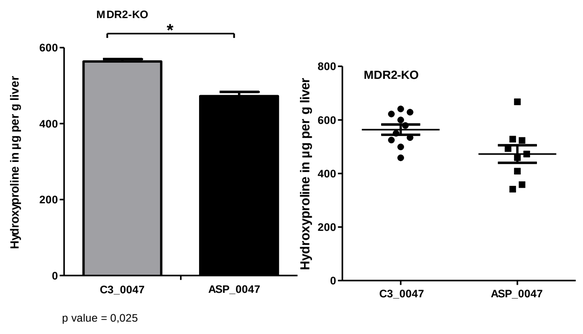


Figure 4: Measurements of Hydroxyproline content to determine the collagen amount displayed significant collagen reduction in AON-treated animals when compared to controls.

Biodistribution of TGF-β2 targeting AON in vivo

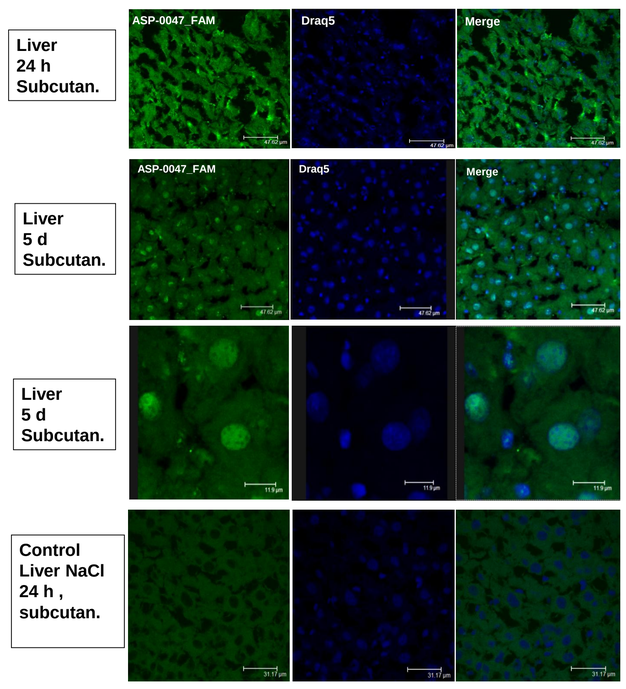


Figure 5: Biodistribution of the TGF-β2 targeting AON was analyzed after different application methods. Signal intensities were high enough to detect the AON ex vivo after 24 hours as well as after 5 days. The strongest signal was observed in liver and kidneys and seemed not to affect other organs.

CCL_4 – induced liver damage with parallel AON treatment in vivo

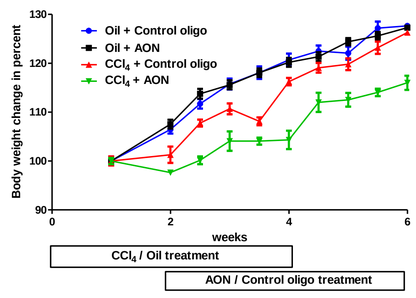


Figure 6: Physiological parameters. After selective inhibition of TGF-β2 using AONs in CCl_4- damaged mice, we observed that those mice slower gain body weight during AON treatment.

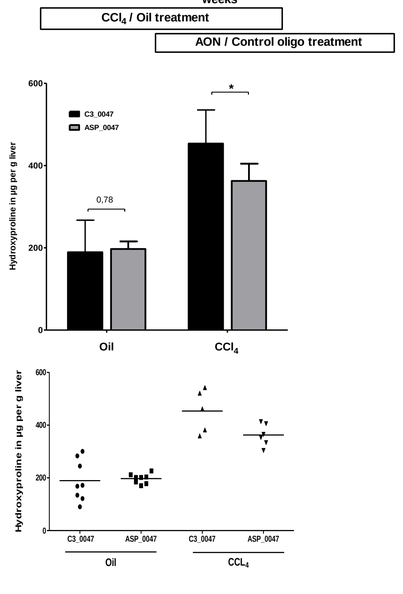


Figure 7. Hydroxyproline. Hydroxyproline content as a surrogate marker for collagen was determined in tissue homogenates of AON-treated, CCl4-challenged mice. Hydroxyproline content was significantly reduced in injured AON-treated animals as compared to controls.

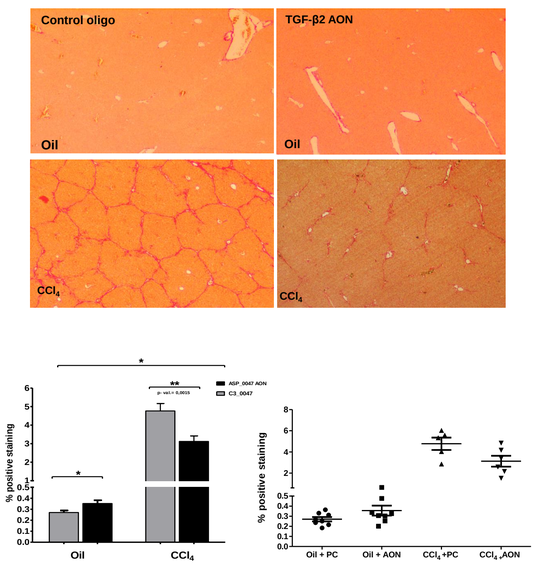


Figure 8. Sirius Red staining. Immunohistological assessment of mouse liver tissue by Sirius Red staining revealed a significant reduction (~34 %) of collagen deposition in AON-treated mice.

Conclusion

Taken together, our results indeed suggest a role of TGF-β2 in the process of CLD. We further conclude that in vivo application of TGF-β2 directed AON to CLD mouse models attenuate fibrogenesis. 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.