Transcriptional program of cholestatic disease elucidated from time resolved cellular and molecular responses in livers of bile duct ligated mice

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Background. Cholestasis denotes a pathological condition where the normal process of bile secretion is disrupted. Bile acids, the main component of the bile fluid, are toxic agents at higher concentrations. Thus, untreated cholestasis may result in a severe damage of the liver, which primarily is characterized by inflammation, followed by fibrosis and eventually cirrhosis, hepatocellular cancer and/or finally loss of organ function and death. One of the typical causes for cholestasis is a blockade of bile ducts (commonly referred to as biliary obstruction), e.g. due to the presence of gall stones. Obstructive cholestasis often progresses insidiously, which entails that patients generally present in the clinic with already advanced disease stages. Therefore, there is urgent need for early diagnostics and initiation of an adequate therapy. The molecular processes underlying obstructive cholestasis still remain an incompletely solved puzzle.

Methods. To better understand the cascade of histological and biochemical alterations invoked by biliary obstruction we used a classic experimental model for secondary biliary fibrosis based on bile duct ligation (BDL) in mice.

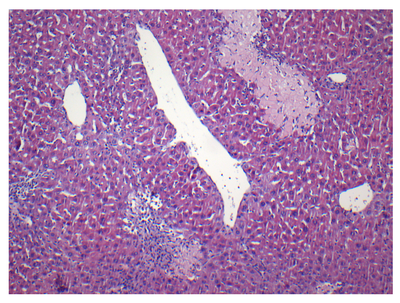
Results. A comprehensive data set of histological parameters, transcript profiles and serum markers was compiled at 8 time points of the experiment in a time range of 14 days after onset of BDL. A thorough statistical analysis of these more than 6000 data points revealed distinct temporal phases of disease progression, differing significantly in the severity of histological and the pattern of molecular changes. The histologic count of CTGF-positive cells provides the most reliable overall measure for the disease progress, and its most closely correlated transcript factor is TGFbeta (gene Tgfb2). The transcript of fibronectin (Fn1) marks the onset of the disease process best, the transcript of interleukin 2 (Il2) for the transition to the perpetuation phase, and interleukin 28 (Il28b) for the transition to the progression phase. All these genes encode excretion products and, thus, are candidates for clinical progression parameters. Prominent molecular events exhibited by strong transcript peaks are found for SHP (Nr0b2) at 6h and transin-2 (Mmp10) at 18h. By exploiting merely temporal transcript changes, we are able to construct a decision tree that allows to reliably predict these specific phases of disease progression for each single animal, suggesting the existence of a well-coordinated and individually reproducible transcriptional program.

Correlations.

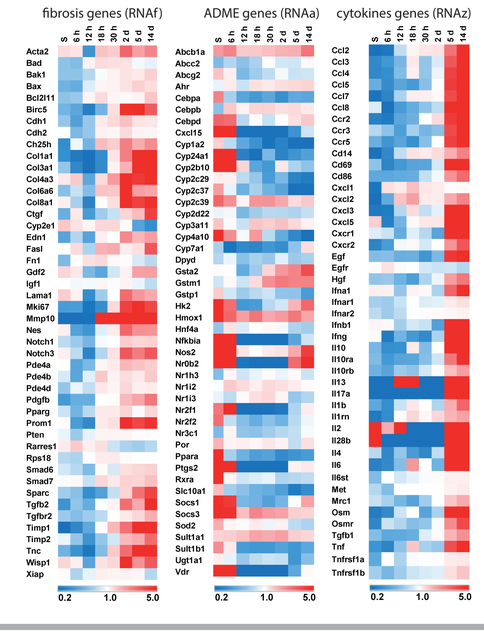
Computed for the time averages (Av), all mice (All), time points (T0h...T14d), phases (Init=6-12h. Perp=18h-2d, Progr=5d-14d) and other time frames (6-18h etc.). Consensus score: weighted average thereof, either positive or negative (anti-correlation). Clustered factors.

Bile duct ligation

35 operated and 5 control mice.
Sacrificed at 7 time points.
8 histopathological, 4 serum, 150 transcript factors.
≥6000 data points.



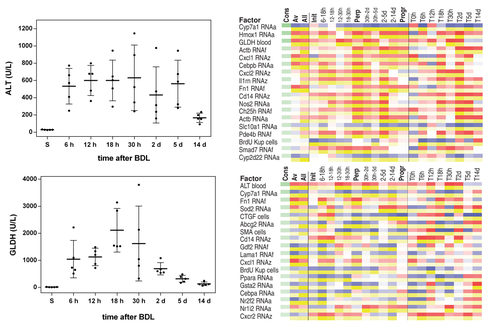
Fluidigm qPCR



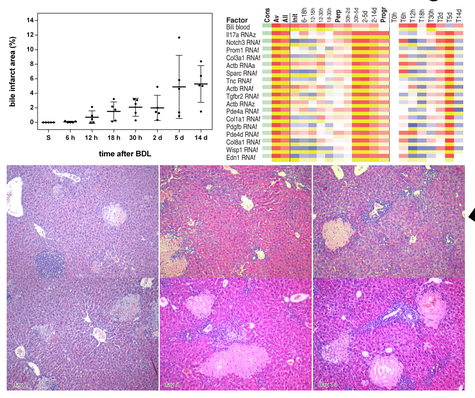
Cholestatic disease aspects,

its main diagnostic factor(s), and the most correlated other factors

Disturbance. ALT and GLDH

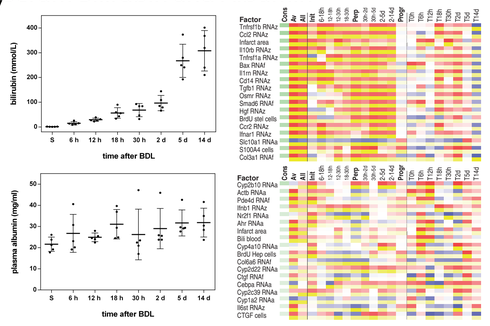


Necrosis. Infarct area in HE staining



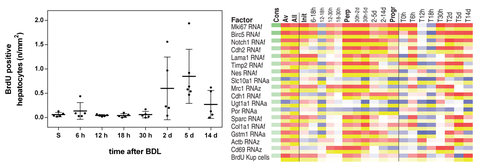
Function loss.

Serum Bilirubin and Albumin

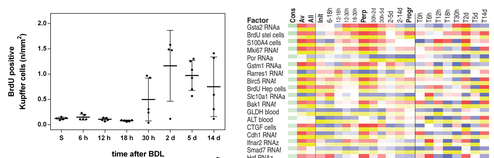


Parenchym regeneration.

BrdU⁺ hepatocytes

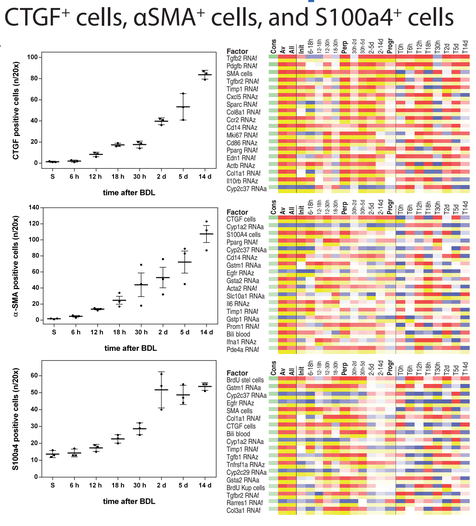


Inflammation. BrdU⁺ Kupffer cells



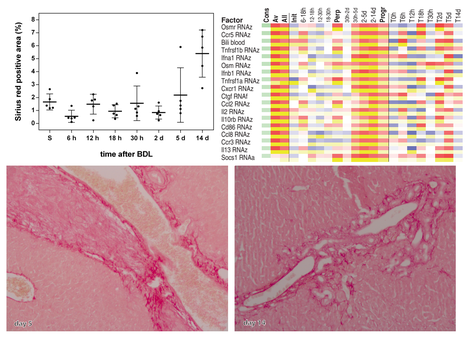
Fibrosis/microscopic.

CTGF⁺ cells, αSMA⁺ cells, and S100a4⁺ cells



Fibrosis/macroscopic.

Sirius red⁺ area



Fibrosis/activation.

BrdU⁺ hepatic stellate cells

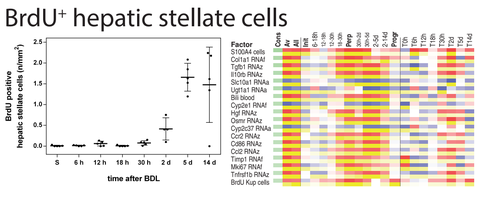
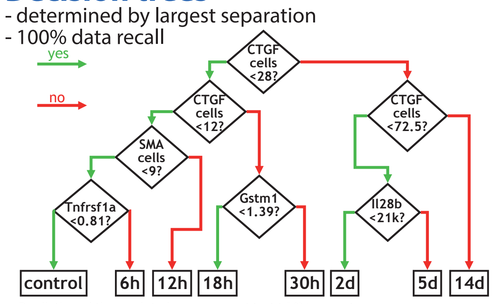


Chart of factor values by time point
strong line: average
bars: SD
dots: individual data points

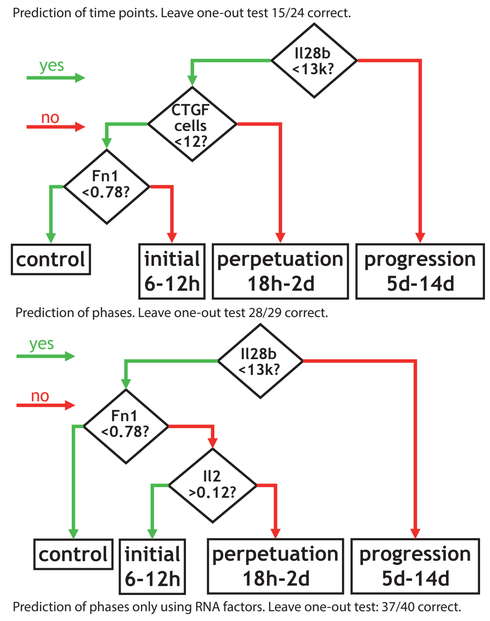
Most correlated factors sorted by consensus
shade of red: correlation
shade of blue: anti-correlation
shade of green: consensus correlation
shade of yellow: t-test significance

Decision trees

- determined by largest separation
- 100% data recall

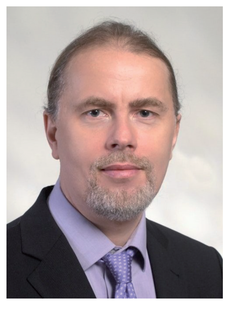


Prediction of time points. Leave one-out test 15/24 correct.



Prediction of phases. Leave one-out test 28/29 correct.

Prediction of phases only using RNA factors. Leave one-out test: 37/40 correct.



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References:

1. Eipel C, Menschikow E, Sigal M, Kuhla A, Abshagen K, Vollmar B. Hepatoprotection in bile duct ligated mice mediated by darbepoetin-α is not caused by changes in hepatobiliary transporter expression. Int J Clin Exp Pathol. 2013;6(1):80-90.

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virtual liver network

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