



Samenvatting proefschrift Aleksandar Sokolović

'Pathogenesis and reversal of liver fibrosis, effects of genes and environment'

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As a normal wound-healing response to the liver injury, fibrosis is reversible - normal architecture is restored by fibrolysis, and ECM producing cells are removed by apoptosis. However, as detailed in Chapter 1, chronic liver injury increases production of fibrogenic signals by resident and infiltrating liver cells, causing an imbalance between fibrogenesis and fibrolysis, scar formation, architectural distortion, cirrhosis, and eventually liver failure. With liver transplantation as the only effective treatment, the lack of donors and surgical contraindications impel for treatments to halt the progression of disease.

HSCs, mesenchymal cells vital to hepatic function and its response to injury, play a pivotal role in the development of liver fibrosis. IGFBP5 was shown to be highly expressed in fibrotic livers of *Abcb4*^{-/-} mice. Therefore, in Chapter 2, we examined the influence of IGFBP5 on HSCs and MFs in vitro. Using gain- and loss-of-function approaches (overexpression by lentiviral transduction, or silencing by siRNA), we showed that IGFBP5 influenced the survival of human LX2 cells, a model for (partially) activated HSCs, and of hepatic MFs. Their endurance was improved without enhancing proliferation, by lowering the level of apoptosis, via an IGF1-independent mechanism. Moreover, IGFBP5 increased the expression of genes involved in ECM deposition.

The finding that IGFBP5 promotes survival of HSCs and MFs in vitro has led us to investigate its role in vivo, in *Abcb4*^{-/-} mice. These mice spontaneously progress to severe biliary fibrosis, due to absence of biliary phospholipids that leads to primary sclerosing cholangitis. *Abcb4*^{-/-} mice are also a model for human MDR3 deficiency, ranging from progressive familial intrahepatic cholestasis type 3 to adult liver cirrhosis, which makes them an attractive model for testing potential antifibrotics. In Chapter 3 we demonstrate that prolonged liver-specific overexpression of IGFBP5 alleviated the hepatocyte damage, as demonstrated by improved biomarkers of liver injury, and decreased their proliferation, possibly by arresting cell cycle, accompanied by senescence. Furthermore, overexpression of IGFBP5 reduced inflammation, indicated by decreased presence of markers for infiltrating and resident macrophages, neutrophils and monocytes. Consequential lowered release of proinflammatory cytokines may explain the decreased oxidative stress in these livers. The resulting reduced presence of activated HSC/MFs and reduced expression of collagens led to a decreased amount of ECM, ameliorating pathology in the model for chronic cholangiopathy.

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A recent study has indicated that biliary epithelium expresses IGF1R, and that IGF1 protects cholangiocytes against cholestatic injury in vitro. To establish the effect of IGF1 on the existing cholestatic injury in vivo, we subjected the *Abcb4*^{-/-} mice to a prolonged increase in hepatic IGF1 expression, by creating a transgenic animal. Chapter 4 shows that sustained overexpression of IGF1 in fibrotic livers increased cholangiocyte proliferation, enhanced inflammation and reduced matrix remodeling, bringing about an increase in deposition of scar tissue and progression of liver fibrosis. IGF1 administration therefore does not seem an option for treating fibrosis caused by chronic cholangiopathies.

The research presented in Chapters 2-4 has challenged the *Abcb4*^{-/-} mice directly, by affecting the expression of genes of interest (IGFBP5 and IGF1), to scrutinize their potential roles in the development of liver fibrosis. Along the same line, we studied how nutrient deprivation could affect liver fibrosis in the same model. In Chapter 5 we show that food deprivation causes a rapid adaptive response in *Abcb4*^{-/-} mice, already after 12h. A striking decrease in inflammation in fasted *Abcb4*^{-/-} mice seems a likely driving force for a cascade of events, including decreased hepatocyte proliferation, lowered number of activated HSCs/MFs, decreased production of ECM components, and increased expression of genes involved in tissue remodeling.

The studies described in this thesis embarked upon the problem of biliary fibrosis by delineating the roles of specific players in IGF-axis, and by introducing a fasting challenge. Though we have not eliminated the cause of fibrosis by any of the approaches (i.e. bile composition remained unchanged), we did alleviate the consequences, which leaves the door to new therapies for liver fibrosis slightly ajar. ◀