Liver fibrosis in 2015

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LIVER FIBROSIS IN 2015

Crucial steps towards an effective treatment

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In 2015, new tools were developed to modulate fibroblast and macrophage activity to halt liver fibrogenesis and stimulate resolution. Essential factors for resolution were identified and clinical trials yielded potential new antifibrotic drugs. Although innovations were made this year, clinical trials are still hampered by the lack of methods to monitor disease progression.

The best way to treat liver fibrosis is to remove the inciting stimulus. The spectacular advancements made with the use of direct-acting antiviral agents against hepatitis C, and agents against hepatitis B and C continued in 2015, and since fibrogenesis seems to be quite reversible after removing the cause, resolution of liver fibrosis in patients with viral hepatitis might now be achieved. However, NAFLD is gradually emerging as the major cause of liver fibrosis in Western society and as yet limited treatment strategies are available, it is unlikely that the incidence of liver fibrosis will drop in the coming years. Nevertheless, the successes in treating liver diseases have taught us that liver fibrosis is reversible even at a late stage of disease and in 2015 we learned much about factors involved in the regression of fibrosis (FIG. 1).

Within the fibrotic liver, hepatic stellate cells and portal fibroblasts are activated and transformed into myofibroblasts. These cells were identified many years ago as the main producers of collagens and other extracellular matrix components, marking them as the main target for antifibrotic therapies. Transforming growth factor (TGF)β, produced by many cell types including activated myofibroblasts, is one of the key activators of a positive feedback loop that accelerates tissue repair. Termination of this TGFβ-induced feedback loop is essential to stop fibrogenesis, but the mechanism behind this signalling pathway was unclear. Palumbo-Zerr *et al.*[1] discovered in 2015 that nuclear receptor 4 A1 (NR4A1), expressed in (myo)fibroblasts, terminates TGFβ signalling. NR4A1, which is induced by TGFβ itself, recruits a protein complex to the promoters of several TGFβ target genes, such as the collagen type 1 genes, and inhibits their expression. However, persistent TGFβ exposure leads to a histone-deacetylase-mediated reduction in the expression of *NR4A1* and an AKT-mediated inactivation of NR4A1 through phosphorylation in (myo)fibroblasts. High levels of phosphorylated NR4A1 were found in human and mouse fibrotic liver tissue samples, which provides a mechanism for the persistent high expression of profibrotic genes observed in these tissues. In addition, the NR4A1 agonist cytosporone B attenuated fibrogenesis in human dermal fibroblasts and in mouse models of skin, pulmonary, renal and liver fibrosis. This interesting study provides novel insights into the derailment of tissue repair processes and identifies new opportunities for therapies. Although complete inhibition of myofibroblast activity might stop progression of the disease, most patients present themselves only after excessive deposition of scar tissue. Removal of the already deposited matrix is then required to restore organ function. In 2015, two studies (neither centring on myofibroblasts) provided fascinating data to explore that option.

A study by Kantari-Mimoun *et al.*[2] showed the importance of angiogenesis in the resolution of fibrosis. This study is intriguing because many studies have shown the close association between angiogenesis and the progression of liver fibrosis, suggesting the use of anti-angiogenic compounds for the treatment of liver fibrosis. However, by cell-selective deletion of the gene encoding vascular endothelial growth factor A (*Vegfa*) in myeloid cells the authors showed that Vegf-A production, particularly by myeloid cells, is essential for the resolution of liver fibrosis in mice. Deletion of *Vegfa* in myeloid cells did not affect liver fibrogenesis itself, but prevented the resolution of liver fibrosis in the recovery phase

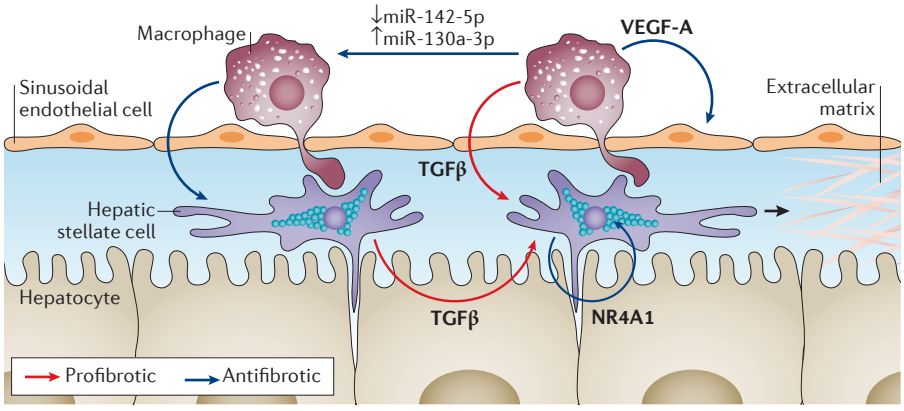

Figure 1 | **Schematic representation of the architecture of the liver depicting intrahepatic cells and the newly discovered pathways involved in the regulation of fibrogenesis.** Hepatic stellate cells, Kupffer cells, macrophages and endothelial cells all act in concert to generate profibrotic and antifibrotic mediators. Effects of NR4A1, miR-130a-3p and VEGF in their designated target cells counterbalance the profibrotic activities of other mediators. miR, microRNA; NR4A1, nuclear receptor 4 A1; TGFβ, transforming growth factor β; VEGF, vascular endothelial growth factor.

Key advances

- The nuclear receptor nucelar receptor 4 A1 has a key role in terminating the profibrotic feedback loop induced by transforming growth factor β in fibroblasts, offering new targets for antifibrotic therapies[1]
- Scar-infiltrating myeloid cells produce vascular endothelial growth factor A, which is essential for the resolution of fibrosis[2]
- The clinical testing of experimental antifibrotic compounds requires definition of clear end-points in clinical trials, patient stratification and, most of all, noninvasive tests to monitor liver fibrosis at an early stage[6]
- microRNAs 142-5p and 130a-3p are found to regulate macrophage profibrogenic activities which opens up options for the resolution of liver fibrosis through modification of macrophage polarization[4]
- Microbiome analysis in stool or saliva might provide innovative tools in the prognostication of the cirrhotic process[7]

(after cessation of carbon tetrachloride injections). Re-introduction of wild-type myeloid cells, capable of producing Vegf-A, restored the resolution process whereas infusion of *Vefga*$^{-/-}$ myeloid cells could not. This study shows that scar-infiltrating myeloid cells stimulate angiogenesis and matrix degrading activity in sinusoidal endothelial cells. This process parallels fibrogenesis and should not be inhibited in antifibrotic therapies, but rather stimulated to enable resolution of fibrosis.

An additional option for the resolution of fibrosis was offered by modification of macrophage profiles. In the past few years, several studies have already highlighted the dual role of macrophages in the process of tissue remodelling[3]. Macrophages can polarize into a pro-inflammatory cell type with matrix-degrading activities (M1 phenotype) upon stimulation by IFNγ or bacterial components such as lipopolysaccharide. Alternatively, macrophages can polarize into an M2 phenotype, associated with tissue repair and fibrosis, after stimulation by IL-4 and/or IL-13. Moreover IL-10 can induce an anti-inflammatory profile in macrophages that produces profibrotic cytokines such as TGFβ[3]. Although the factors that modulate macrophage polarity are known, modulation of macrophage plasticity *in vivo* to induce regression of fibrosis has been difficult to achieve. In 2015, a study by Su *et al.*[4] characterized microRNA (miR)-142-5p and miR-130a-3p levels in lung and liver fibrotic tissue in mice and humans. IL-4 and IL-13 caused an upregulation of miR-142-5p and a downregulation of miR-130a-3p levels in macrophages, which was associated with the induction of profibrotic activities in these cells. Subsequent administration of miR-142-5p antisense oligonucleotides and agents that mimic miR-130a-3p led to a strong reduction in matrix deposition within lungs of bleomycin-treated mice and livers of carbon-tetrachloride-treated mice. This study opens up a valuable option for the resolution of liver fibrosis by modification of macrophage polarization. We have not fully gained the fruits of the discovery of small interfering RNAs (siRNAs) and miRNAs yet, mainly due to delivery problems. However, Calvente *et al.*[5] delivered siRNA against procollagen using nanoparticles and they achieved resolution of liver fibrosis in mice and this approach could be an important step towards the therapeutic application of such RNA molecules.

Are we getting near an effective pharmacotherapy for liver fibrosis? Hundreds of experimental drugs directed against fibrogenic cells have been tested in experimental models and in clinical trials, but none of them has reached the market yet. Out of the many drugs that are directed against myofibroblasts, only very few have reached phase III clinical trials in patients with liver fibrosis. The fact that only drugs already on the market for other diseases are the ones that reach phase III in patients with cirrhosis is no coincidence; the safety profile of these drugs have already been established, which is a decisive factor in clinical trials that are inevitably long in duration for these patients. It might be that, similar to anticancer and antiviral therapies, a combination of drugs is required for this multifactorial disease — making trials even more complex. In 2015, a summary of the American Association for the Study of Liver Diseases emerging trends conference "strategies and endpoints of antifibrotic drug trials," organized to address issues surrounding clinical trial design was published in *Hepatology*[6]. The main conclusions of the conference were the need for better patient stratification to enroll homogenous groups of patients with a high risk of cirrhosis, standardization of drug discovery and validation of biomarkers, standardization of clinical trials including patient subgroup definitions and use of end points. Finally, and related to all of the above, the development of reliable non-invasive tests for fibrosis. This latter development would shorten the very long clinical trials that are needed to show effects of antifibrotic compounds, which represents a major hurdle for all clinical trials in this field. Approximately one-third of ongoing clinical trials in patients with liver fibrosis are now dealing with biomarkers, either serum markers or imaging techniques, illustrating the urgent need for such tools. Bajaj and colleagues[7] reported on a surprising and innovative method of analysing the saliva microbiome, which seemed to disclose prognostic information about the liver. The topic of microbiome analysis in stool and saliva samples elicited much debate[8] and holds promising perspectives. The data also fits with the growing evidence that the gut microflora has an important role in the progression of liver fibrosis to end-stage liver failure[9].

New tools that reliably monitor intrahepatic fibrogenesis would help to give this disease the attention it deserves as it imposes a heavy burden on society. Promising antifibrotic drugs have first been tested in patients with idiopathic pulmonary fibrosis, renal or skin fibrosis or cancer, yielding pirfenidone and nintedanib as the only antifibrotic compounds on the market today[10], but not for the treatment of liver fibrosis. As outlined here, the complexity of this chronic disease, the long clinical trials that require huge investments and the lack of non-invasive methods for surveillance of disease activity and stratification of patients, underlies the lack of drugs against liver fibrosis to date. It is about time that clinical trials on the treatment of liver fibrosis also progress towards their end goal.

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Competing interests statement
K.P. is co-founder and minority shareholder in BiOrion Technologies. The antifibrotic compounds developed by BiOrion Technologies are not the subject of this Year-in-Review and are not mentioned in any other way in the article.