The Role of Scar-Associated Macrophages in Liver Fibrosis

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Abstract: Liver fibrosis is a critical condition closely linked to liver cirrhosis and hepatocellular carcinoma, primarily due to the accumulation of extracellular matrix proteins produced by activated myofibroblasts during chronic liver injuries. Scar-associated Macrophages (SAMs), a recently identified subgroup of macrophages, play a pivotal role in facilitating the transition of hepatic stellate cells (HSCs) into activated myofibroblasts. Understanding the functions of SAMs could potentially uncover new therapeutic targets for treating liver fibrosis. This review synthesizes current knowledge on the origins of activated myofibroblasts and SAMs, the interactions between HSCs and SAMs,and the therapeutic targets associated SAMs in the progression of liver fibrosis.
***Keywords*:** liver fibrosis, scar-associated macrophages, hepatic stellate cells, myofibroblasts

1. Introduction

Liver cirrhosis is a major cause of mortality worldwide,characterized by fibrosis. Liver fibrosis emerges as a consequence of enduring liver damage, which can be categorized into hepatocyte damage-induced and cholestasis-induced liver damage. hepatocyte damage-induced damage are typically caused by viral infections such as hepatitis B (HBV) and hepatitis C (HCV), excessive alcohol intake, or metabolic disorders that result in non-alcoholic steatohepatitis (NASH). Cholestasis-induced liver damage, caused by obstructions in bile flow, are also observed in primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and biliary atresia (BA)[1]. Extracellular matrix proteins, secreted by activated myofibroblasts, are responsible for the development of fibrous scars in the progression of the liver fibrosis. HSCs and portal fibroblasts (PFs) are recognized as the primary sources of these activated myofibroblasts. A significant body of research has focused on the transformation of HSCs and PFs into activated myofibroblasts, which play a critical role in the fibrotic process. Recently, a distinct macrophage population of SAMs, characterized by TREM2+CD9+ markers, has been identified through single-cell mRNA sequencing of liver cirrhosis tissues and is considered a principal driver of myofibroblasts activation[2]. The populations of SAMs are significantly increased in the liver of patients with fibrosis and cirrhosis compared to healthy individuals. The specific mRNA transcripts expressed by SAMs, such as SPP1, FABP5, and GBNMP, are linked to the increased expression of genes responsible for extracellular matrix components, including COL1A1 and COL2A1[3]. Furthermore, several mechanisms of fibrosis driven by SAMs have been identified, providing potential therapeutic targets for managing liver fibrosis.

2. The Origins of Activated Myofibroblasts

Myofibroblasts are crucial in the normal wound healing process, where they temporarily exist to facilitate wound contraction and the repair of connective tissues. However, in tissues affected by fibrosis, myofibroblasts persist, leading to an over-abundance of extracellular matrix and acting as a key cellular driver in fibrotic conditions. Myofibroblasts originate from various sources, including resident HSCs, portal fibroblasts (PFs), fibrocytes, and mesenchymal stem cells from the bone marrow. HSCs and PFs are the predominant sources of myofibroblasts, constituting over 90% of the myofibroblasts in both hepatocyte damage-induced and cholestasis-induced liver fibrosis scenarios. In the context of liver fibrosis triggered by hepatocyte injury, HSCs consistently serve as the primary source of myofibroblasts[4]. During the onset of cholestasis-induced liver fibrosis, the initial inflammatory cells activates PFs around the portal tracts. PFs are the main contributors to the myofibroblasts population. However, as the inflammation progresses towards the central regions of the liver lobules, HSCs become activated and eventually take over as the primary source of myofibroblasts. Studies from animal models of cholestasis-induced liver fibrosis indicate that within the first five days of fibrosis initiation, Myofibroblasts derived from PFs are in the majority. Subsequently, HSCs become activated and eventually become the main source of myofibroblasts[5].

3. The Origins of SAMs

Single-cell transcriptomic pseudotime analysis has shown that peripheral blood monocytes differentiate into SAMs, other

macrophages, and dendritic cells (DCs). Single-cell data from liver fibrosis also indicate that monocytes are chemotactically drawn to the fibrotic tissue, where they may further differentiate into SAMs[2]. In experimental studies, an increase in SAMs was observed in a mouse model of DDC (diethylnitrosamine-induced) cholangiocyte injury. The study used experimental methods to label myeloid-derived monocytes with tdTomato and found that approximately 90% of SAMs originated from tdTomato+ cells[6]. However, the transcriptional regulatory mechanisms involved in this differentiation process are still not well understood and require further research to confirm.

4. The interaction of SAMs and Myofibroblasts

Studies utilizing spatial transcriptomics and immunofluorescence have discovered that SAMs are localized to fibrotic regions and co-localize with HSCs, indicating a spatial proximity that facilitates interaction between HSCs and SAMs. In another mouse model involving DDC, it was observed that the number of SAMs increased with the severity of fibrosis. Conversely, as fibrosis resolved, the number of SAMs decreased, eventually returning to the normal levels. Moreover, the expression of key marker genes specific to SAMs cell subpopulations, such as CD9, TREM2, SPP1, GPNMB, FABP5, and CD63, was found to be elevated in livers affected by fibrosis[3]. These genes also showed a positive correlation with the fibrosis-associated gene COL1A1, which sufficiently demonstrates a close relationship between SAMs and the process of fibrosis.

In patients with liver fibrosis, the expression of CCL2 is increased, primarily produced by damaged bile duct cells, liver progenitor cells, and mesenchymal liver cells in the fibrotic areas. CCL2 recruits monocytes to the fibrotic region, while also promoting the differentiation of CCR2+ monocytes into myeloid cells, some of which differentiate into SAMs[7]. Additionally, PFs secrete CSF1, which can promote the differentiation of monocytes into macrophages and maintain the survival of macrophages[8].

TGF-β is a crucial signal for activating HSCs and PFs, and it is highly expressed on M2-activated macrophages. Similarly, SAMs also highly express TGF-β signals to activate HSCs and PFs. The TGF-β signaling pathway includes both Smad-dependent and Smad-independent routes. TGF-β superfamily members start signaling through different combinations of type I (TβRI) and type II (TβRII) transmembrane receptors. The Smad family, key transcriptional activators in the TGF-β signaling pathway, is split into receptor-activated Smads (R-Smads), the common mediator Smad (Co-Smad), and inhibitory Smads (I-Smads). Beyond the Smad pathways, TGF-β can also signal through non-canonical ways by activating the mitogen-activated protein kinase (MAPK) family, which might further control Smad proteins or carry out Smad-independent TGF-β responses. This leads to the activation and proliferation of myofibroblasts[9].

During the process of HSCs transforming into myofibroblasts, fibrosis-associated genes like COL1A1, COL1A2, COL3A1, and TIMP1 are upregulated, while the expression of HSC-specific marker genes such as RGS5, IGFBP5, ADAMTS1, and GEM are downregulated. Activated myofibroblasts that are positive express high levels of PDGFRA and TNFRSF12A, and SAMs cells express their ligands PDGF and TNFSF12. Through co-culture experiments, it was found that TNFSF12 and PDGF can induce the proliferation of HSCs and the transition of HSCs into myofibroblasts. Furthermore, SAMs express epidermal growth factor receptor (EGFR) ligands that are known to regulate myofibroblast cell activation[2]. Additionally, a profibrotic THBS1+ macrophage subpopulation,similar to SAMs,was identified, which expands in the fibrotic livers of mice and humans, activating HSCs via the PI3K/AKT/mTOR signaling pathway[10].

5. Therapeutic targets associated SAMs

TGFβ, a potent cytokine instrumental in extracellular matrix (ECM) synthesis, secreted by Sams. While long-term inhibition of TGFβ is discouraged due to potential adverse effects, short-term targeted therapies using anti-TGFβ antibodies or ALK5 inhibitors have demonstrated efficacy in preclinical mouse studies. These interventions reduce liver fibrosis by impeding the transformation and multiplication of myofibroblasts[11].Beyond direct TGFβ inhibition, alternative strategies focus on molecules and pathways related to TGFβ. Notably, the pharmacological targeting of αV integrins, especially αVβ6, which is instrumental in activating latent TGFβ, has proven effective in mitigating liver disease and fibrogenesis in mice. Additionally, the inhibition of CD105 on activated hepatic stellate cells (HSCs), a receptor that amplifies TGFβ signaling, has shown to reduce inflammation and fibrogenic responses[12].

Moreover, the use of anti-CCR2 antibodies to block CCL2-CCR2 axis, led to a decrease recruitment of CCR+ Monocytes and SAMs [13]. Tyrosine kinase inhibitors, including sorafenib, imatinib, and nilotinib, which hinder the activation of PDGF and VEGF receptors on HSCs, have also displayed potential in curbing the migration, dispersion, proliferation,and activation of hepatic myofibroblasts and stromal cells in the context of liver injury[14].

6. Conclusion

Liver fibrosis, leading to cirrhosis and the development of hepatocellular carcinoma, is a major cause of death worldwide. Despite numerous clinical trials, effective drugs for the treatment of fibrosis are still lacking. Previous studies have focused on macrophages as a whole. With the discovery of the SAMs cell group, it is possible to target thecellular interactions between SAMs and HSCs, such as TGF-β, TNFSF12-TNFRS12A, PDGF-PDGFR, THBS1-ITGA3, and EGFR ligands. Drugs or inhibitors with greater specificity can be further developed based on these targets. Finally, the treatment of liver fibrosis is a complex process that also involves inflammation, cholangiocyte reactions, and tissue repair processes, which interact with each other. More in-depth and comprehensive research is essential to drive progress in the field of liver fibrosis management and treatment.

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