Review Article

Involvement of epithelial–mesenchymal transition in liver fibrosis

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Abstract

Fibrosis of the liver is an inherent wound healing response to chronic liver injury. Regeneration of liver epithelium and restoration of normal liver structure were generally involved in this process. Although the liver has a striking capacity to adapt to damage through tissue repair, excessive accumulation of extracellular matrix during this process often leads to scar tissue formation and subsequent fibrosis. Epithelial to mesenchymal transition (EMT) enables a polarized epithelial cell to undergo multiple changes biochemically and to bear a mesenchymal cell phenotype. EMT plays a critical role in tissue and organ development and embryogenesis. In the liver, it is proposed that epithelial cells can acquire fibroblastic phonotype via EMT and contribute to fibrogenesis. This made EMT a potential target for antifibrotic strategies. Following an original passion, many investigators devote themselves to exploring this mechanism in liver fibrosis. However, as research continues, this hypothesis became highly controversial. The exact contribution of EMT to fibrogenesis was challenged due to the contradictory results from related studies. In this review, we summarized the recent advances regarding EMT in hepatic fibrosis and discussed the potentially involved liver cell types and pathways in order to reach rational and helpful conclusions.

Keywords: Cholangiocyte, epithelial–mesenchymal transition, hepatocyte, hepatic stellate cell, liver fibrosis

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INTRODUCTION

The epithelial–mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell–cell adhesion, and acquire migratory and invasive properties to become mesenchymal cells.[1,2] EMT was first observed in embryogenesis and was critical for the development of tissues and organs.[3,4] EMT is not irreversible; together with its reverse process mesenchymal–epithelial transition (MET), EMT plays crucial roles in cancer progression,[2,5,6] wound healing,[2,7] and organ fibrosis.[1,2,8,9]

Liver fibrosis is a protective response to chronic liver injury from diverse etiologies.[10-12] The feature of liver fibrosis is the excessive accumulation of extracellular matrix (ECM) produced by myofibroblasts.[13] Activated hepatic stellate cells (HSCs) are believed to be the primary source of myofibroblasts.[14] Advanced liver fibrosis results in cirrhosis, portal hypertension, even liver failure, and other life-threatening complications and only liver transplantation will rescue the patients.[10,15] Recently, EMT was implicated in liver fibrosis.[16-18] As research continues, however, the notion becomes controversial.[19,20]

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CLASSIFICATION OF EMT

EMT was classified into three subtypes that carry different functional effects based on distinct settings they encountered.[21,22] The EMTs that contributed to implantation, embryogenesis, and organ development through transformation of various cell types are termed as Type 1 EMT.[22] These transformed cells usually share common mesenchymal phenotypes and have the potential to undergo a reverse process, namely MET, to generate epithelial cells.[22] Type 2 EMT was implicated in wound healing, tissue regeneration, and organ fibrosis.[22] This kind of EMT usually occurs following tissue injuries such as inflammation, and forms fibroblasts to repair injured tissue. Once inflammation receded, the transition ceased.[22] However, in fibrotic organs, the EMT continuously responded to inflammation, and result in organ destruction ultimately.[22] The third proposed subtype of EMT is Type 3 EMT, which occurs in cancer cells that have previously undergone genetic and epigenetic alterations.[22] Through this transition, neoplastic cells may invade and metastasize via the circulation and finally lead to cancer progression and metastasis.[22]

MAIN LIVER CELL TYPES INVOLVED IN EMT

Hepatocyte and EMT

In fibrotic liver, the identified origin of collagen-producing cells includes activated HSCs, portal fibroblasts, and bone marrow-derived myofibroblasts.[10] However, evidence from research suggested that hepatocytes could also acquire a fibroblastic phenotype through EMT in liver fibrosis.[16,17,23,24] Zeisberg *et al.*[23] found that upon stimulation with transforming growth factor β-1 (TGF-β1), adult mouse hepatocytes underwent changes phenotypically as well as functionally. In addition, using lineage-tracing technique, they observed that hepatocytes-derived cells demonstrated with fibroblast-like morphology and with expression of fibroblast-specific protein 1 (FSP-1), and this report provides the first *in vivo* evidence for hepatocyte EMT.[23] Similarly, other research also revealed that hepatocytes actively participate in fibrogenesis through TGF-β-dependent EMT.[17,25,26] Dooley *et al.*[17] confirmed a coexpression of collagen and transferrin in liver samples from patients with HBV infection, indicating the possible occurrence of EMT. They also found that specific inhibition of TGF-β signaling in hepatocyte-derived cells can attenuate fibrogenic response. Lee *et al.*[27] demonstrated that apamin can inhibit TGF-β1-induced E-cadherin loss and vimentin increase *in vitro*, and prevent CCL4-induced liver fibrosis *in vivo*. This suggests that through suppressed TGF-β1-induced hepatocyte EMT, apamin can inhibit hepatic fibrogenesis. Kong *et al.*[28] found that cobalt chloride ($CoCl_2$) can upregulate mesenchymal markers, including vimentin, N-cadherin, and α-smooth muscle actin (α-SMA) and thus induce a mesenchymal cell phenotype in hepatocytes. They further confirmed that curcumin, a natural antifibrotic compound, can repress this process by decreasing TGF-β receptor expression and inhibiting Smad2/3 expression and phosphorylation. Other studies[29,30] suggest that geniposide and celecoxib can inhibit hepatocytes EMT in liver fibrosis as well. These findings provided potential strategies to prevent liver fibrosis by targeting hepatocytes EMT. However, a recent study reported that mouse hepatocytes do not undergo EMT in liver fibrosis.[31] Using transgenic mice, Taura *et al.*[31] found that hepatocytes do assume a TGF-β-induced fibroblast-like morphology, but failed to express mesenchymal markers including FSP-1, α-SMA, and vimentin.[31] They also confirmed that hepatocytes are not the origin of type I collagen-producing cells in liver fibrosis.[31] These results were quite different from the previous work performed by Zeisberg and his colleagues. Taura *et al.*[31] hold that β-Gal staining in Zeisberg study may yield false-positive results due to technical limitations. Although Taura and colleagues' work effectively challenges the existence of hepatocyte EMT, lineage tracing technique has its own pitfalls,[32] and it is still too early to exclude EMT in liver fibrosis completely.

Cholangiocyte and EMT

Cholangiocyte, another cell type which has been proposed, contributes to liver fibrosis through EMT.[24] Omenetti *et al.*[33] provided direct evidence of the contribution of cholangiocyte EMT to liver fibrosis. In their study, the investigators found that cholangiocytes isolated from rats with biliary fibrosis induced by bile duct ligation (BDL) expressed high level of FSP-1 and low level of aquaporin-1 and cytokeratin 7/9 (Krt7/9). In addition, they demonstrated that an immature cholangiocyte line cocultured with myofibroblastic HSCs (MF-HSC), or treated with activated HSC conditioned medium, was induced to undergo complete EMT by silencing epithelial gene expression, inducing mesenchymal gene expression and acquiring a migratory phenotype. Moreover, they confirmed that inhibiting Hedgehog (Hh) signaling pathway can block EMT in the cholangiocytes under MF-HSC conditioned medium treatment.[33,34] Several other studies found cholangiocytes from rats with biliary fibrosis or human tissues coexpressed epithelial and mesenchymal markers.[35-37] In biliary atresia, evidence also support that biliary epithelial cells may directly contribute to fibrogenesis via EMT.[38,39] Diaz *et al.*[40] provided histological evidence, suggesting that EMT occurs in biliary atresia. In

consistence with this, Xiao *et al.*[39] showed that Krt7 and α-SMA colocalized to the intrahepatic biliary epithelial cells in patients with biliary atresia. Besides, they demonstrated that EMT in primary human intrahepatic biliary epithelial cells was induced by TGF-β and confirmed that the process can be inhibited significantly by miR-200b.[39] Seemingly, there is solid evidence that cholangiocytes can contribute to fibrosis via EMT. However, the authenticity of cholangiocyte EMT was seriously challenged recently. Using cell fate tracing technique, Scholten *et al.*[41] revealed that no EMT of cholangiocytes was identified by genetic labeling that contributes to liver fibrosis in mice. Actually, the investigators detected no coexpression of myofibroblast marker and cholangiocyte marker in both biliary and panlobular fibrosis.[41] In addition, they also showed that no epithelial or liver progenitor marker was coexpressed by genetically labeled HSCs in response to liver injury.[41] Consistent with this, Chu *et al.*[42] also found no cholangiocytes undergone EMT in murine models of hepatic fibrosis. Although they observed that cultured primary cholangiocytes can undergo EMT (i.e., loss of cell–cell contacts and acquisition of fibroblast-like morphology) after TGF-β1 treatment, but in the mouse BDL and CCL4 models, the investigators found that cholangiocytes do not undergo EMT *in vivo*.[42] Moreover, they further demonstrated that EMT does not occur in cholangiocyte precursors (oval cells). However, further studies are still needed to confirm whether cholangiocyte EMT contributes to liver fibrosis.

Hepatic stellate cell and MET

HSC is the best studied fibrogenic mesenchymal cell in the liver. Now HSCs, as the main source of ECM, have been corroborated to be the dominant contributors to liver fibrosis independent of its etiology.[10,43,44] The concept that HSCs are able to undergo MET is intriguing. Sicklick *et al.*[45] analyzed the expression profile of primary HSC, HSC cell lines, and hepatic epithelial progenitors and found that epithelial progenitors express HSC markers. Furthermore, epithelial progenitor microRNAs were also expressed by HSC cell lines.[45] In addition, HSCs that express progenitor cell markers were confirmed with the potential to differentiate into hepatocytes when cultured under certain condition.[46] Yovchev *et al.*[47] revealed that oval cells coexpressed epithelial and mesenchymal markers, and transplantation of these hepatic progenitor cells could repopulate injured livers. Choi *et al.*[48] even found that transition of quiescent HSCs between epithelial and mesenchymal fates were regulated by Hh signaling pathway. Loss of E-cadherin is a characteristic behavior of EMT.[49] Cho *et al.*[50] reported that E-cadherin is capable of inhibiting TGF-β1 gene induction in HSCs by suppressing RHoA-dependent Smad3 phosphorylation and preventing liver fibrosis. But Scholten *et al.*,[41] using cell fate tracing technique, found no epithelial markers coexpressed by HSCs in response to fibrogenic liver injury in mice. However, Conigliaro *et al.*[51] lately reported that hepatocytes and HSCs may arise from common progenitor isolated from embryonic livers. Their study also showed that these progenitor cells were able to transdifferentiate into both hepatocytes and HSCs *in vitro* and *in vivo*.[51] Interestingly, Yang *et al.*[52] found that HSCs can secrete type I collagen to trigger EMT of hepatoma cells. Zhao *et al.*[53] found that microRNA-21 (miR-21) can simultaneously promote HSC activation and hepatocyte EMT in liver fibrosis. They also confirmed that miR-155 can modulate similar process.[54] Collectively, there seems to be plenty of evidence indicating that HSCs can undergo MET during hepatic fibrogenesis. But more recently, Lua *et al.*[55] demonstrated that HSCs are not capable of differentiating into either hepatocytes or cholangiocytes in mouse. Using cell lineage tracing technique, they found that mesodermal mesenchymal cells, including HSCs and portal fibroblasts, comprise a major source of MFs and do not undergo MET during fibrogenesis.[55] In addition, they even found that no HSCs contributed to oval cells via MET.[55] This was supported by research from Troeger *et al.*[56] Troeger *et al.* employed single-cell polymerase chain reaction and genetic cell fate tracking to investigate whether HSC deactivation represents an alternative mechanism for liver fibrosis resolution. They found that HSC activation gradually decreased during fibrosis reversal and no HSC contributed to hepatocytes and cholangiocytes via MET.[56] Together, these data provided fairly good evidence that refutes the notion that HSCs undergo MET to yield either hepatocytes or cholangiocytes. The contradictory conclusion from Yang and colleagues' report may result from the inappropriate engagement of HSC marker.

MAIN SIGNALING PATHWAYS IMPLICATED IN EMT

Hedgehog Signaling Pathway and EMT

Signaling pathways involved in EMT have been explored substantially, and one of the well-documented pathways is Hh signaling. The Hh pathway plays crucial role in organogenesis and tissue remodeling.[57,58] Recent studies suggest that activation of Hh pathway appears to be implicated in fibrogenesis through regulation of EMT.[33-35,48,59-61] Omenetti *et al.*[35] showed that in rodent model induced by BDL, Hh signaling was activated to guide remodeling of the biliary epithelia and stroma after cholestatic injury. They further revealed that enhanced EMT responses to BDL were related to excessive

activation of Hh pathway, which promotes biliary fibrosis progression.[33] Syn *et al.*[59] found that in nonalcoholic fatty liver disease (NAFLD), sonic Hh suppressed expression of epithelial genes and EMT inhibitors but induced mesenchymal genes in cultured progenitors of ductular cell. In mouse models of NAFLD, they also found that activation of Hh pathway was followed by EMT, expansion of myofibroblastic populations, and liver fibrosis.[59] In addition, researchers found that Hh pathway functions critically in transition of quiescent HSCs into myofibroblastic HSCs, and enables quiescent HSC to transit between epithelial and mesenchymal fates.[48] Omenetti *et al.*[34] demonstrated that Hh signaling was excessively activated in biliary atresia and resulted in biliary EMT, which may lead to biliary dysmorphogenesis and finally fibrosis. Interestingly, Yu *et al.*[62] recently reported that patched1, a negative regulator of Hh pathway, was downregulated during liver fibrosis. They further confirmed that decreased expression of patched1 was associated with its DNA hypermethylation. Slvianolic acid B can induce miR-152 to target DNA methyltransferase 1 and demethylate patched1; thus prevent liver fibrosis by inhibiting Hh signaling-induced EMT.[62] Although these data provide evidence that Hh signaling pathway can regulate EMT, given the questioned existence of EMT of hepatocytes or cholangiocytes, its contribution to liver fibrosis remains a subject of some debate. However, it is indisputable that Hh signaling can coordinate epithelial–mesenchymal interactions to regulate repair and regeneration and maintain tissue homeostasis.[63]

TGF-β Signaling Pathway and EMT

TGF-β has been commonly recognized as a critical factor stimulating collagen and ECM production in HSCs during hepatic fibrogenesis.[64] Kaimori *et al.*[25] reported that TGF-β1 is capable of mediating EMT in hepatocytes *in vitro*. They found that administration of TGF-β1 significantly increased α1 collagen mRNA expression and type I collagen deposition, which were defined as the characteristic of EMT state.[25] They also showed that in the EMT state, TGF-β1 induced snail-1 and activated Smad2/3 pathway in hepatocytes, while silencing Smad4 inhibited EMT.[25] Similarly, Kojima *et al.*[26] reported that in mature hepatocytes, EMTs were induced by TGF-β-mediated downregulation of claudin-1. Furthermore, studies revealed that hepatocytes actively participated in fibrogenesis after EMT induced by TGF-β, whereas specific ablation of TGF-β signaling by Smad7 in hepatocytes effectively slacked the fibrogenic response.[17] Rygiel *et al.*[36] also showed that EMT in response to TGF-β contributes to portal tract fibrogenesis. Recently, Kong *et al.*[28] confirmed that curcumin can inhibit EMT in hepatocytes by interfering with TGF-β/Smad signaling. Schizandrin and propolis have also been shown to inhibit fibrosis and EMT induced by TGF-β.[65,66] Transmembrane 4 L6 family member 5 (TM4SF5) is a transmembrane glycoprotein which can induce EMT and is highly expressed in hepatocellular carcinoma.[67] Investigators found that expression of TM4SF5 in hepatocytes can be induced by TGF-β1 and epidermal growth factor receptor (EGFR) signaling pathways.[67] Increased TM4SF5 expression was found in CCl4-mediated liver fibrosis mouse model and correlated with α-SMA expression, collagen I deposition, and TGF-β1 and EGFR signaling activation.[68] Interestingly, as a kinase inhibitor drug approved for the treatment of cancer, sorafenib was confirmed to be capable of inhibiting TGF-β-mediated EMT in hepatocytes and fibrosis.[67,69] Collectively, TGF-β and its related proteins are major inducers of EMT. However, contrary to the popular notion that TGF-β is a main contributor to liver fibrosis,[70] Mu *et al.*[71] showed that epithelial TGF-β signaling does not promote liver fibrosis, but inhibits cholangiocytes proliferation to prevent cholangiocarcinoma development.[71] Therefore, further studies are needed to clarify whether TGF-β-induced EMT plays a role in liver fibrosis.

Extracellular Signal-Regulated Kinase Signaling Pathway and EMT

Extracellular signal-regulated kinases (ERKs), namely the classical mitogen-activated protein (MAP) kinases, are serine/threonine kinases that play crucial roles in the modulation of cell growth and differentiation.[72] Evidence suggests that ERK signaling contributes to repression of EMT.[73,74] Arnoux *et al.*[7] reported that Erk5 controls the expression of slug, which involves the basal keratinocyte activation, spreading, and migration and contributes to re-epithelialization during cutaneous wound healing. Thum *et al.*[75] showed that increased ERK–MAP kinase activity promotes interstitial fibrosis and cardiac function and this process can be inhibited by miR-21. In liver fibrosis, Zhong *et al.*[76] found that reduced ERK1 expression suppressed HSCs proliferation and their expression of fibrosis-related genes *in vitro*; specific inhibition of ERK1 significantly weakens ECM deposition in fibrotic liver. They also found that myofibroblasts derived from hepatocytes and cholangiocytes were reduced markedly by selective inhibition of ERK1.[76] Dai *et al.*[53] reported that miRNA-21 was significantly higher in cirrhotic patients and rats, and sprouty 2 (SPRY2) and hepatocyte nuclear factor 4α (HNF4α) were identified as effective targets of miR-21. By targeting SPRY2 and HNF4α, miR-21 simultaneously stimulates ERK1 signaling in HSCs and induces hepatocytes EMT.[53] In another report, they found that miR-155, on the contrary, simultaneously suppresses EMT process and

ERK1 signaling, attenuates HSC activation, and prevents hepatic fibrosis.[54] These studies showed that inhibition of ERK signaling contributes to prevention of liver fibrosis, and EMT may be involved in this process.

Future perspectives

As a solid organ, the liver has a striking ability to adapt to damage through tissue repair, but excessive accumulation of ECM proteins during this wound healing response will lead to liver fibrosis; this highlights the significance of the balance of this process. Nevertheless, the complicated mechanisms underlying hepatic fibrogenesis have not been fully elucidated. The past decades have witnessed enormous progress in our understanding of hepatic fibrosis, and the discovery of EMT/MET provides us with new insights into its pathogenesis. However, in the light of conflicting evidence that refutes the role of EMT/MET in liver fibrosis, perhaps our enthusiasm should be curbed. Yet despite this, the role of EMT in hepatocellular carcinoma (HCC) has been identified recently. Although EMT has been proved not indispensable for breast cancer and pancreatic cancer metastasis,[77,78] its contribution to HCC metastasis and invasion has been confirmed and thus may serve as a prognosis predictor.[79-82] In addition, studies have revealed that EMT could induce chemoresistance in some types of cancer.[77,78] Therefore, a comprehensive understanding of EMT is urgently needed and will enable the development of novel diagnostic and effective therapeutic strategies to prevent HCC progression and improve patients' prognosis.

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Conflicts of interest

There are no conflicts of interest.

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