MINI REVIEW

Epithelial-mesenchymal transition in the liver

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Available online 7 July 2010

Abstract  Epithelial-mesenchymal transition (EMT) is a physiological process occurring in the embryo. In adult organisms, EMT could be involved in disease development. In the liver, the possibility that EMT of liver epithelial cells participate to liver fibrosis is increasingly discussed. Furthermore, the involvement of hepatocyte EMT to liver cancer biology has also been documented over the past few years. In this review, we will first describe how EMT participates to embryological development. We will then discuss the involvement of hepatocytes and biliary epithelial cells in liver fibrosis. Finally, we will describe how EMT may impact the metastatic process and resistance to therapy in hepatocellular carcinoma.

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The epithelial-mesenchymal transition (EMT) plays a key role in the developing embryo. In adults, EMT also participates to physiological processes, such as tissue repair. Increasing evidence indicates that EMT could be involved in chronic diseases, such as organ fibrosis and cancer. In humans, EMT has been documented in fibrotic liver diseases with a ductular-proliferation component. However, cell-tracing methods in animals argue against the possibility that EMT of liver epithelial cells participates to liver fibrosis. Yet, liver epithelial cells can undergo EMT in other pathological settings as evidenced in liver cancer.

EMT in embryonic development and physiology

Mesenchymal and epithelial cells are the two main cell types present in organs and tissues. Mesenchymal cells are non-polarized cells that are loosely organized in the extracellular matrix. Mesenchymal cells do not display any intercellular interactions and can thus move individually through the extracellular matrix. By contrast, epithelial cells are defined as polarized cells that adhere to a basal lamina. Epithelial cells form cohesive cell layers through intercellular protein complexes, such as adherens junctions, desmosomes and tight junctions. Epithelial cells are thus able to form entities that can function as protective barriers and/or absorptive epithelia. The coordinate regulation of cells within an epithelium is enabled by the ability of epithelial cells to communicate with one another through gap junctions. Furthermore, the disposition of cellular interactions creates an apicobasal polarity that allows epithelial cells to compartmentalize their cellular activities. These cellular interactions limit the motility of epithelial cells to lateral movement within the epithelial cell layer. This feature implies that epithelial cells cannot detach from the cell layer in adult tissue under basal conditions. Epithelial cells may however acquire motility by converting to mesenchymal cells, a process referred to as EMT.
Figure 1  Schematic representation of epithelial-mesenchymal transition (EMT).

Epithelial cells can convert to mesenchymal cells by a process termed EMT. EMT is characterized by the loss of epithelial markers and the acquisition of mesenchymal properties.

EMT involves three major phenotypic changes:

- the loss of apicobasal polarity;
- the disruption of cell-cell junction and modification of cytoskeleton protein expression;
- the acquisition of migratory and invasive properties (Fig. 1).

Among these changes, the ability to invade and migrate through extracellular matrix is considered as the functional hallmark of EMT. EMT is not a final process and cells may secondarily go through mesenchymal to epithelial transition (MET). This ability underlines cellular embryonic plasticity, a phenomenon essential to germ layer formation and cell differentiation processes that will generate the different adult cell types.

Primary EMT processes give rise to two main early developmental events: gastrulation and neural crest formation [2]. Gastrulation is a fundamental process that leads to the formation of the three embryonic germ layers (i.e. ectoderm, mesoderm, endoderm). Gastrulation begins with a phenotypical change of epiblast cells that allows them to detach from the epithelial layer. These cells will then differentiate into mesenchymal cells and will migrate through the basal lamina to form the mesoderm. In vertebrates, a specific population of epithelial cells, termed the neural crest cells, which are located between the neural tube and the ectoderm, will also undergo primary EMT. Individual neural crest cells delaminate from the dorsal neural epithelium and migrate throughout the embryo to give rise to most of the peripheral nervous system, the craniofacial structures and glial or satellite cells. Primary EMT events are thus followed by differentiation processes that will generate the different adult cell types.

Secondary EMT occurs when epithelial cells from newly formed structures undergo a new EMT event. In the liver diverticulum, hepatoblasts that originate from the endoderm proliferate to form the hepatic bud. During this process, hepatoblasts will invade the septum transversum, a mesodermal tissue, by migrating through the basement membrane of the hepatic bud. This process may be seen as a secondary EMT, because hepatoblasts lose both epithelial morphology and E-cadherin expression (see [3] for an in-depth review on liver development).

Tertiary EMT occurs mainly in cardiac embryonic development. After a secondary EMT, endocardial progenitors migrate into extracellular matrix and undergo MET to form the endocardium. At this developmental stage, the two epithelial layers of the heart, the external myocardium and internal endocardium, are separated by the cardiac jelly to form a primitive heart tube. Some cells of the endocardium may then undergo a tertiary EMT and migrate through the cardiac jelly to form the endocardial cushion and later the primitive valves and cardiac septa [4].

In adult tissues, EMT can also be observed in physiological settings. Cells from the ovarian surface epithelium are able to escape apoptosis and undergo EMT after each ovulation. Thus, they contribute to the scaring process of the ovarian surface through EMT [5]. Keratinocytes may as well undergo a partial EMT during skin wound healing and reepithelialization [6].

EMT in liver fibrosis

EMT is an emerging concept in adult organ fibrosis speculating that this process protects epithelial cells against injury-associated apoptosis and favors tissue repair by contributing to the production of extracellular matrix (Fig. 2). Evidence of EMT in fibrosis was first demonstrated in the kidney. In vitro, adult renal tubular epithelial cells were shown to undergo EMT [7,8]. Thereafter, induction of renal fibrosis in mice by unilateral ureteral obstruction (UUO) showed that epithelial marker expression (i.e. E-cadherin) was lost in tubular epithelial cells, while the mesenchymal marker α-smooth muscle actin (α-SMA) expression was increased [9]. A cell tracing method later established that mice submitted to UUO display EMT-derived fibroblasts that contribute to the fibroelastic population [10].

Because the liver is an organ prone to fibrosis and because the origin of fibroelastic cells in fibrotic liver is still debated, the possibility that liver epithelial cells participate to fibrosis by EMT is appealing. Such hypothesis was strengthened by the observation that hepatic stellate cell lines express E-cadherin, while hepatic epithelial progenitor cells are positive for α-SMA [11]. Kaimori et al. then demonstrated that freshly isolated hepatocytes were able to convert to mesenchymal cells in vitro. Hepatocyte EMT, characterized by a decrease in E-cadherin expression and concomitant acquisition of mesenchymal markers (vimentin and type I collagen), was observed when cells were incubated with the profibrogenic cytokine, TGF-β [12]. Zeisberg et al. confirmed these in vitro observations and further demonstrated that hepatocyte EMT was observed in carbon tetrachloride-induced liver fibrosis (CCL4) by developing transgenic mice specifically expressing a molecular tag in hepatocytes (i.e. β-galactosidase). In these mice challenged with CCL4, 45% of the cells expressing the mesenchymal marker S100A4 were also positive for β-galactosidase expression. Furthermore, the inhibition of the TGF-β pathway limited the
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Figure 2  Physiological and pathological epithelial-mesenchymal transition (EMT). EMT is involved in embryonic development (green arrow) and in pathological settings (red arrows). In organ fibrosis, EMT-derived fibroblasts may contribute to the production of fibrous tissue. In cancer, EMT promotes cell migration, tumor invasiveness and metastatic dissemination. (Adapted from Derynck R, Akhurst RJ. Nat Cell Biol 2007).

extent of liver fibrosis in the CCL4-injected mice [13]. In vitro TGF-β treatment induced higher vimentin expression in cirrhotic liver-derived hepatocytes than in normal liver-derived hepatocytes [14]. Taken together, these results suggest that hepatocyte EMT is triggered by TGF-β and contributes to liver fibrosis. In patients with chronic HBV infection, the activation of the TGF-β pathway was also shown by the accumulation of phosphorylated Smad2 in hepatocyte nuclei. Furthermore, the induction of Snail, a transcription factor known to repress E-cadherin expression, and the co-expression of type I collagen and transferrin in HBV livers, indicated that hepatocyte EMT was a feature of human liver fibrosis [15] (Fig. 3A). However, the hypothesis of hepatocyte EMT contributing to liver fibrosis has been challenged by a cell lineage strategy in mice. Triple transgenic mice with permanent cell labeling were produced to track hepatocyte-derived cells and type I collagen-expressing cells. Hepatocytes isolated from these triple transgenic mice were able to undergo EMT in culture when incubated with TGF-β. However, in mice challenged by CCl4, no cells exhibited a double labeling specific for both hepatocytes and collagen expressing cells [16]. These observations suggest that hepatocytes may not undergo EMT in vivo, while the observed transition in vitro might be an experimental artefact.

Because liver fibrosis is a timely regulated process [26], liver epithelial cells could undergo rounds of EMT and MET, thus making their detection critical without serial analysis. This assumption is further supported by the transient and reversible EMT that occurs in hepatic cancers.

EMT in liver cancer

In cancer, EMT has been proposed as a crucial step in promoting cell migration, tumor invasiveness and metastasis (Fig. 2). EMT in cancer is a dynamic and reversible process representing the first step of the invasive and metastatic process. In hepatocellular carcinoma (HCC), EMT has been
Figure 3  Epithelial-mesenchymal transition in human liver.
A. Liver sections from healthy and HBV-infected patients were examined by confocal microscopy after immunostaining for collagen type I (green) and transferrin (red). Costaining of collagen type I and transferrin suggests that hepatocytes undergo EMT in the liver of HBV-infected patients. (Reprinted from [15] with permission from Elsevier). B. Liver sections from patients with PBC were examined for evidence of EMT by staining with antibodies against CK19 (brown) and FSP1 (blue). Colocalization of CK19 and FSP1 is seen in BECs in PBC (yellow arrowheads), but not in normal liver (purple arrowhead), suggesting that BECs undergo EMT in biliary-type liver diseases. (Reprinted from [22] with permission from Elsevier). C. Liver sections from patients with HCC were immunostained for E-cadherin, β-catenin, Snail, and Twist. Case 1 is representative of HCC with preserved E-cadherin expression, while case 2 is representative of HCC cases with E-cadherin expression modifications. Yellow arrows indicate membranous expression of E-cadherin or β-catenin, the blue arrows indicate the cytoplasmic translocation of β-catenin, and the red arrows indicate the nuclear expression of β-catenin, Snail, or Twist. These observations suggest that hepatocytes undergo EMT in HCC. (Reprinted from [28] with permission from John Wiley and Sons).

linked to the ability of hepatic tumor cells to invade the capsule or the portal vein.

EMT of neoplastic hepatocytes is considered a central event in intrahepatic dissemination and distal metastasis formation. Increased expression of the E-cadherin transcriptional repressors Snail and Twist emerges as a critical step driving EMT in HCC, while loss of E-cadherin is frequently associated with capsular invasion, intrahepatic metastasis and poor prognosis in HCC [27]. Yang et al. have reported that Snail and Twist overexpression was associated with
a decreased expression of E-cadherin and concomitant nuclear translocation of β-catenin (Fig. 3C). Interestingly, HCC patients with the shorter cancer-free interval and worst prognosis were positive for both Snail and Twist expression [28]. Moreover, in human HCC tissues, the overexpression of Snail and Twist were associated with tumor invasiveness and metastasis [29—31]. In vitro, an inverse correlation between Snail and E-cadherin expression has been reported in various HCC cell lines. Differentiated HCC cells expressed E-cadherin but not Snail, while undifferentiated HCC cells expressed Snail but not E-cadherin [32]. Ectopic expression of Snail or Twist in HCC differentiated cells promoted morphologic changes from epithelial to fibroblastoid appearance, which was accompanied by a gain of mesenchymal markers and a loss of epithelial markers, by increased invasion and upregulation of matrix metalloproteinase, including MT1-MMP [29,33]. Taken together, these observations indicate that Snail and Twist might be crucial molecules governing acceleration of cancer invasion and dedifferentiation during HCC progression through the induction of EMT.

TGF-β1 may also play a key role in triggering EMT in HCC through its cooperation with laminin-5 [34]. Notably, laminin-5 was abundantly expressed at the invasive front along with the nuclear translocation of β-catenin, an increased expression of Snail and a reduced expression of E-cadherin. Loss of E-cadherin expression is frequently associated with a cadherin switch leading to overexpression of mesenchymal cadherins. Consistently, T-cadherin was overexpressed in tumor cells from tumoral areas of HCC exhibiting abnormal expression of E-cadherin. Furthermore, silencing T-cadherin in the hepatocarcinoma cell line Malhavu led to a decrease in cell invasive and motile ability [35]. Thus, T-cadherin expression in tumor cells may reflect a novel cadherin switch during EMT that participates to HCC metastasis by enhancing the motility of tumor cells.

Deregulation of microRNAs (miR) may also represent a central event in the intrahepatic metastasis of HCC. In particular, the expression of the liver-specific miR-122 was significantly decreased in liver cancers with intrahepatic metastasis. Accordingly, in vitro restoration of miR-122 in metastatic HCC cells decreased their ability to invade and migrate by reversing the cell mesenchymal profile [36]. Thus, miR-122 appears to be a novel regulator of EMT in HCC.

The involvement of viral proteins of the hepatitis B and C virus has also been implicated in HCC progression. Hepatitis C virus core proteins and hepatitis B virus-encoded HBX protein collaborate with TGF-β or STAT5b, respectively, to induce EMT and invasion of HCC [37,38]. In primary mouse hepatocytes isolated from HCC transgenic animals expressing the hepatitis C virus core proteins, TGF-β induced EMT as evidenced by reduced E-cadherin and increased α-SMA expression [37].

Finally, EMT may worsen HCC prognosis by modulating the sensitivity of HCC to antitumoral therapies, in particular to EGFR-targeted therapies. In a study using a panel of 12 human hepatoma cell lines classified as epithelial or mesenchymal, HCC mesenchymal cells were shown to be less susceptible to EGFR inhibition than HCC epithelial cells [39].

Conclusion

EMT in embryo development is an established process. Discussions now arise on the involvement of EMT in organ fibrosis. In liver fibrosis, EMT could occur in hepatocytes or biliary epithelial cells to increase the number of extracellular matrix producing cells and thus fibrous tissue deposition. The presence of cells expressing both epithelial and mesenchymal markers suggests that EMT is a feature of liver fibrosis, however the ability of these cells to produce extracellular matrix in vivo has not yet been documented. Nonetheless, EMT could as well be partial and thus participate to liver diseases by other means. For example, partial EMT of biliary epithelial cells could be involved in the disruption of small ducts during obstructive cholestasis. Liver epithelial cells could also undergo rounds of EMT that could have a pathophysiological impact while being extremely difficult to evidence. This assumption is supported by the epithelial plasticity of neoplastic hepatocytes that is associated with tumor cell invasion and metastasis in HCC. Moreover, hepatocyte EMT may be associated with both tumor invasiveness and resistance to cancer therapies. Thus, EMT in HCC may be a critical event worsening patient prognosis. Taken together, these observations ask for the development of studies assessing the role of liver epithelial cell EMT in hepatic pathophysiology.

Conflict of interest

The authors disclose no conflicts.

Acknowledgements

This work was supported by the French Association for the Study of the Liver (AFEF).

References


