Review

JOURNAL OF HEPATOLOGY

Role of epithelial to mesenchymal transition in hepatocellular carcinoma

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Keywords: HCC; EMT; MET; Epithelial plasticity; Tumor heterogeneity; Cell invasion; Therapy; Patient survival.

Received 25 February 2016; received in revised form 20 April 2016; accepted 3 May 2016

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Summary

The epithelial to mesenchymal transition (EMT) is a multistep biological process whereby epithelial cells change in plasticity by transient de-differentiation into a mesenchymal phenotype. EMT and its reversal, mesenchymal to epithelial transition (MET), essentially occur during embryogenetic morphogenesis and have been increasingly described in fibrosis and cancer during the last decade. In carcinoma progression, EMT plays a crucial role in early steps of metastasis when cells lose cell-cell contacts due to ablation of E-cadherin and acquire increased motility to spread into surrounding or distant tissues. Epithelial plasticity has become a hot issue in hepatocellular carcinoma (HCC), as strong inducers of EMT such as transforming growth factor-β are able to orchestrate both fibrogenesis and carcinogenesis, showing rising cytokine levels in cirrhosis and late stage HCC. In this review, we consider the significance of EMT-MET in malignant hepatocytes as well as changes in the plasticity of hepatic stellate cells for cellular heterogeneity of HCC, and further aim at explaining the current limiting insights into EMT by snapshot analyses of HCC tissues. Recent advances in the identification of clinically relevant mechanisms that impinge on important EMT-transcription factors, as well as on miRNAs causing EMT signatures and HCC progression are highlighted. In addition, we draw particular attention to framing EMT in the context of potential clinical relevance for HCC patients. We conclude that some aspects of EMT are still elusive and further studies are required to better link the clinical management of HCC with biomarkers and targeted therapies related to EMT.

Abbreviations: BCLC, Barcelona Clinical Liver Cancer; CLDN, claudin; CTC, circulating tumor cell; CSC, cancer stem cells; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial to mesenchymal transition; FSP-1, fibroblast-specific protein; GANK, gankyrin; GFP, green fluorescent protein; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HNF, hepatocyte nuclear factor; HNRNP, heterogeneous ribonuclear protein; HIF, hypoxia-inducible factor; HSC, hepatic stellate cell; K, cytokeratin, Lcn, lipocalin; lncRNA, long non-coding RNA; MET, mesenchymal to epithelial transition; miR, microRNA; PI3K, phosphoinositide 3-kinase; PDGF, platelet-derived growth factor; RTK, receptor tyrosine kinase; SMA, smooth muscle actin; TACE, transarterial chemoembolization; TIP, Tat-interacting protein; TCF, T cell factor; TF, transcription factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; YFP, yellow fluorescent protein.

Introduction

Hepatocellular carcinoma (HCC) ranks sixth among the most common malignancies worldwide and shows the third highest mortality among cancer patients [1,2]. Long-term intoxication with alcohol or aflatoxin, chronic infection with hepatitis B or C virus (HBV, HCV) or fatty diets leading to obesity are well-defined etiological factors that can cause the development of HCC over decades [2]. Liver carcinogenesis develops in a sequential evolution from dysplastic lesions, harboring minor genetic variations, to advanced stages of HCC, showing a vast molecular heterogeneity [3]. Generally, the heterogeneity derives from differences in etiology, underlying chronic liver disease, genetic and epigenetic events, expression signatures and differentiation of carcinoma cells, as well as the impact of the tumor microenvironment [4]. HCC show heterogeneity at the cellular level by the neoplastic transformation of hepatocytes and progenitor cells which are both epithelial cell types. The complexity of the cellular heterogeneity is increased by changes in the plasticity of these epithelial cells, commonly described as epithelial to mesenchymal transition (EMT). Heterogeneity is further provided at the genetic level by the multifocal development of HCC that is caused by the synchronous formation of tumor nodules or by intrahepatic metastases of the primary cancer. While multiple HCC nodules show an accumulation of different sets of genetic and epigenetic changes, few genetic alterations have been observed between primary HCC, portal vein tumor thrombi and intrahepatic metastasis [5].

The subset of metastatic HCC patients has been identified by an imbalance of Th1/Th2 cytokines associated with an increased expression of colony stimulating factor in the tissue microenvironment, suggesting that the inflammatory milieu displays a key role in promoting metastasis and affecting the clinical outcome [6]. The extensive tumor heterogeneity at multiple stages of HCC development hampers the stratification of patients for effective therapy. In particular, the identification of those HCC patients that will develop disease recurrence after curative therapy is of outmost relevance. In a recent study, a metastatic gene signature combined with α-fetoprotein has been reported as a good predictor of HCC outcome independently of etiology and ethnicity [7].

Characteristics of EMT

EMT is the mechanism that drives a transient and reversible de-differentiation of epithelial cells to a mesenchymal-like or a mesenchymal phenotype, depending on how the completion of de-differentiation is. EMT-induced changes in epithelial plasticity are evidenced by the loss of epithelial markers, such as the adherence junction component E-cadherin and cytokeratins of the intermediate filament system (K8, K18, K19). In addition, the expression of the mesenchymal proteins such as N-cadherin, α-smooth muscle actin (α-SMA), fibroblast-specific protein (FSP-1) and the EMT-transcription factors (EMT-TFs) Snail (SNA1), Slug (SNA2), Twist and ZEB is increased. Some markers, such as FSP-1, lack specificity for the EMT of parenchymal liver cells as they are not expressed in myofibroblasts [8] or fail to show specificity, like vimentin, which is expressed in injured hepatocytes [9]. Epithelial cells with an induced but not complete EMT are referred to as "partial" EMT, and cells at this stage of de-differentiation co-express epithelial as well as mesenchymal markers. Both partial and complete EMT can be reversed by a mesenchymal to epithelial transition (MET), allowing the recovery of epithelial traits. Notably, the EMT-MET is essentially required for the metastatic colonization as demonstrated in breast and colon carcinoma progression [10,11], and may play a crucial role in intra-and extrahepatic metastasis. On the other hand, the EMT is crucial for tumor chemosensitivity, as in the case of pancreatic ductal adenocarcinoma, in which the deletion of Snail or Twist does not prevent invasion and metastasis but does sensitize pancreatic cancer cells to gemcitabine treatment, leading to an increased survival of mice [12]. However, genetic evidence by lineage tracing transgenes showing the relevance of EMT in the dissemination of HCC cells is lacking.

By evaluating the various EMT phenotypes of malignant epithelial liver cells using partial and complete EMT and the reversal to MET, we can estimate the complexity and cellular heterogeneity of HCC. This is difficult to assess by cell imaging *in vivo*. In this scenario, neoplastic hepatocytes are either (i) epithelial or (ii) mesenchymal-like after induction to partial EMT or (iii) mesenchymal after completion of EMT and indistinguishable from hepatic stellate cell (HSC)-derived myofibroblasts in fibrotic tissue. These phenotypes can be reversed at any stage by MET (Fig. 1). Thus, snapshots of EMT markers by expression analyses in transitional tissues – in the absence or presence of cell tracking – reveal only partial insights into the EMT process and provide a small window of the EMT-MET status at a given time. In addition, the complexity of epithelial cell plasticity in the liver is reinforced by the chimeric epithelial/mesenchymal phenotype of HSCs, which are derived from mesothelial cells of the epiblast during embryonic development. They express both epithelial and mesenchymal markers. HSCs undergo "EMT-like" processes via (de)-differentiation to either more mesenchymal cells which still express epithelial markers, or activation to a "MET-like" transformation by canonical Hedgehog signalling without complete ablation of mesenchymal markers [13–15].

EMT in fibrosis and HCC

Injured epithelial cells have been suggested to be an important source of fibroblasts which are essentially involved in tissue fibrosis of lung and kidney [16,17]. Similarly, the contribution of parenchymal cells to liver fibrosis by EMT has stimulated animated debate during the past decade. While primary hepatocytes and cholangiocytes can be induced to EMT *in vitro* by transforming growth factor (TGF)-β or Hedgehog [18–20], the role of EMT *in vivo* during hepatic fibrosis is controversial. Initially, FSP-1-positive myofibroblasts were detected in an albumin promoter-driven *LacZ* transgene during CCl_4-induced fibrosis, suggesting changes in epithelial plasticity of hepatocytes. Yet, this study showed incomplete Cre-dependent recombination of the *Rosa26-floxstop-LacZ* cassette in hepatocytes, and importantly, co-expression of *β-gal/FSP-1* failed to detect myofibroblasts [21]. However, multiple studies further addressed this issue by lineage tracing transgenes using markers which more faithfully monitor the hepatocyte-dependent transformation to myofibroblasts. In this context, the expression of α-SMA and deposition of fibrillar collagen were considered as relevant markers of hepatic myofibroblasts. Therefore, the collagen1α1-f/f-green fluorescent protein (GFP) reporter strain was crossed with albumin

Key point

A large body of evidence shows that hepatocellular EMT is a de-differentiation of malignant hepatocytes, hepatic progenitor cells or HSCs that can be reversed to MET; a genetic proof for EMT-MET in HCC by lineage tracing transgenes is lacking.

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