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Epithelial to Mesenchymal Transition in Liver Fibrosis: Here Today, Gone Tomorrow?

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The concept of the epithelial to mesenchymal transition (EMT) has taken the fibrosis world by storm. It is perhaps the most intriguing and controversial of recent hypotheses on the mechanism of fibrosis that injured epithelial cells, via an EMT, contribute directly to matrix deposition and repair. Originally invoked as a source of collagen-producing cells in the kidney (1, 2), EMT is now thought to occur in fibrosis of the lung and, through the transition of both hepatocytes and cholangiocytes, the liver (3–5). This has important theoretical and practical implications for studying fibrosis: EMT provides a potential mechanism for the rapid mobilization of large numbers of fibrogenic cells after injury, and it proceeds by unique signaling programs that may prove to be viable therapeutic targets.

EMT occurs when epithelial cells lose key epithelial characteristics, including apical-basal polarity, intercellular adhesion complexes, and adherence to a basal basement membrane, while simultaneously becoming motile, invasive, and, in some cases, fibrogenic (6, 7). One recently proposed scheme divides EMT into three distinct categories, type 1 occurring in development, type 2 in fibrosis, and type 3 in cancer and metastasis (6, 8). Type 1 EMT yields mesenchymal cells while type 2 yields fibroblasts which produce collagen although they may or may not later become myofibroblasts (8). This is an important point when considering fibrosis in the liver and other organs, since there is an abundance of data implicating α-smooth muscle actin (α-SMA) positive myofibroblasts in matrix deposition (9, 10). As discussed below, many studies on EMT in fibrosis have failed to rigorously define EMT or to reconcile evidence of EMT with previous observations about the central role of myofibroblasts in fibrosis. Additionally, high-level collagen expression is not synonymous with a mesenchymal or fibroblast phenotype, although it is unquestionably the characteristic most relevant to fibrosis. EMT in fibrosis, although poorly defined in the literature, should incorporate two key elements: that cells lose their epithelial identity, and that in this new state they deposit relevant amounts of collagen. In the absence of any suggestion that non-fibrogenic transitioned cells have a significant role in fibrosis, convincing studies of EMT need to address both points.

The identification of EMT in vivo is at the heart of the controversy over its role in fibrosis. Demonstrating motility, loss of cell-cell adhesion, and basement membrane breakdown in tissue samples is difficult given current methods, and many investigators have turned to surrogate markers of the epithelial and mesenchymal states as a means of defining EMT. Many of those in common use, however, are problematic because of a lack of specificity (e.g. vimentin) or because it is technically challenging to assess potentially subtle differences in localization or expression (e.g. epithelial markers like E-cadherin). The expression of fibroblast-specific protein 1 (FSP1, also referred to as S100A4) in cells with

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epithelial markers has been widely used to define EMT in vivo. This protein is reported to be specific for fibroblasts and to play a causal role in EMT (2). Significant data are emerging, however, questioning its specificity. In the kidney, carefully performed work suggests that FSP1 is a marker not of fibroblasts but rather of leukocytes and other, non-fibroblastic cell types (9, 11). This raises questions about studies postulating EMT on the basis of FSP1 staining, which includes most studies of EMT in liver fibrosis.

Against this backdrop, Taura and colleagues used definitive marker analyses to readdress the question of whether hepatocytes undergo EMT and deposit collagen in the injured liver (12). In their paper in this issue of HEPATOLOGY, they first investigated convincing reports that EMT occurs in hepatocytes in vitro. Several groups previously demonstrated that primary mouse hepatocytes, when treated with the classical EMT inducer transforming growth factor-β (TGF-β) or isolated from cirrhotic livers and cultured, adopt a fibroblast-like morphology, with decreased membrane-bound E-cadherin and increased expression of vimentin and type I collagen (although not the myofibroblast marker α-SMA) (13–15). Because precursor cells may lose epithelial markers during EMT, one group used primary hepatocytes carrying a permanent β-galactosidase tag to show that TGF-β treatment resulted in increased motility and FSP1 expression of cells clearly identified as hepatocytes (14).

Taura et al. provide clear evidence that these examples of hepatocyte EMT in vitro are artifacts of cell culture (12). The group generated triple transgenic mice (Rosa26-stop-βGal; Albumin-Cre; Col I-GFP) which permanently and heritably express β-galactosidase in hepatocytes and activate green fluorescent protein (GFP) in cells expressing type I collagen. In their first experiment, they isolated hepatocytes from the livers of untreated transgenic animals and cultured these cells in the presence of TGF-β for 48 hours (Fig. 1). Consistent with previous reports, the hepatocytes assumed a fibroblast-like morphology and expressed collagen, as determined by co-expression of β-galactosidase and GFP, although they did not express either α-SMA or FSP1. The key in vitro experiment, however, was the second, in which the investigators isolated both parenchymal and non-parenchymal cells from acutely and chronically CCL_4-treated livers and showed that not a single freshly isolated cell – of hundreds of thousands examined by fluorescence-activated cell sorting and direct microscopy – expressed both markers. Similarly, no hepatocyte, as identified by β-galactosidase staining, expressed the mesenchymal markers α-SMA, FSP1, or vimentin. This showed clearly that hepatocyte EMT in vitro, although undeniable, is a function of the combination of TGF-β treatment and culture, and that hepatocytes isolated from diseased livers do not produce type I collagen.

The in vivo evidence for hepatocyte EMT comes primarily from the study by Zeisberg et al. (14). This group used Albumin-Cre; Rosa26-stop-β-Gal mice (in which all hepatocytes and their descendents, regardless of phenotypic changes, are irreversibly tagged with β-galactosidase) to carry out lineage tracing studies in the setting of CCl_4-induced fibrosis. They observed a significant population of hepatocyte-derived cells expressing FSP1 and concluded that these cells were the product of an EMT. Note, however, that the investigators did not examine the potentially transitioned hepatocytes for other mesenchymal markers or for collagen production, and that α-SMA expression was absent. Taura et al. readdressed the conclusions from the Zeisberg study using the triple transgenic animals described above. They did not observe *any* co-expression of hepatocyte and collagen markers in CCl_4-treated animals, regardless of the degree of fibrosis. Similarly, no heritably labeled hepatocyte expressed α-SMA or FSP1 as determined by immunohistochemistry. Although staining for additional mesenchymal markers would have strengthened the conclusions, the data are nonetheless compelling in demonstrating in the CCl_4 model that hepatocytes and their derivatives do not express FSP1, type I collagen, or α-SMA and thus do not undergo EMT.

Why do the authors of the two lineage tracing papers on hepatocyte EMT reach such different conclusions? Taura et al. propose that technical limitations associated with β-galactosidase staining yielded false positive results in the Zeisberg study. This hypothesis is supported by the observation that detection of β-galactosidase expression by immunostaining does not coincide with detection of β-galactosidase activity by X-gal (12). I would suggest that failure to rigorously define EMT is another reason for the divergent findings. Zeisberg et al. define EMT through expression of the controversial and potentially non-specific marker FSP1 but do not examine collagen synthesis, the feature ultimately most relevant to fibrosis. Taura et al., although focused on collagen synthesis as a primary marker of EMT, also demonstrate that hepatocytes in the fibrotic liver fail to express α-SMA, a finding of key importance given the many demonstrations (including in their study) that α-SMA positive cells make up a large percentage of fibrogenic cells.

Does the work of Taura et al. lay to rest the concept of hepatocyte EMT? The answer is a qualified yes. There are caveats, including the reality that neither the genetic background of the mice nor the injury model (CCl_4) accurately models human disease. Regardless, this study effectively refutes the published data that support hepatocyte EMT. Although it is still theoretically possible that hepatocyte EMT occurs in human disease, new lines of evidence will be required for this to reemerge as a viable concept.

Interestingly, an exhaustive study has recently been published calling into question EMT in the kidney. Using two different epithelial cell-specific drivers, two different reporters, and two different models of renal fibrosis, Humphreys et al. find no evidence that epithelial cells of the kidney contribute to the myofibroblast population in vivo (or express FSP1) (16). Like Taura and colleagues, this group suggests that non-specific methods to detect the β-galactosidase reporter could have contributed to discordant findings in the literature. Thus, there is now convincing evidence that neither hepatocyte nor renal epithelial cell EMT occurs in fibrosis.

Whether cholangiocyte EMT contributes to fibrosis in the liver is still an open question. Several groups, making use of both animal models and human tissue, have reported that cholangiocytes in fibrotic livers (from bile duct ligated mice as well as humans with primary biliary cirrhosis, biliary atresia, and several other diseases) co-express multiple epithelial and mesenchymal markers by immunostaining and are therefore likely to be undergoing EMT (17–20). Two studies demonstrate cholangiocyte shape changes and loss of basement membrane integrity, which are also consistent with EMT (17, 18). Taura et al. do not examine the issue of cholangiocyte EMT. Members of their group, however, reported in abstract format at the 2009 AASLD Annual Meeting that mouse cholangiocytes, analyzed by robust lineage tracing techniques with the CK19 promoter, show no evidence of EMT in bile duct ligation or CCl_4 fibrosis models (21). Our group has obtained similarly negative results in AFP-Cre; Rosa26-YFP mice, in which both hepatocytes and cholangiocytes are tagged. These studies now require the screen of peer review, but the coincident results are hard to ignore, and it appears that lineage tracing may debunk the concept of cholangiocyte EMT in the same way hepatocyte EMT was addressed in the paper in this issue.

For cholangiocytes, however, it is hard to dismiss the observation that bile duct basement membranes undergo degradation in fibrosis, and that cholangiocytes assume fibroblast-like, non-cuboidal shapes. How can these convincing findings from histological analyses be reconciled with the negative data from lineage tracing experiments? As detailed above, most of the initial data in favor of hepatocyte EMT were derived from animal models, making these models an appropriate way to study this phenomenon. Evidence in favor of cholangiocyte EMT, however, is for the most part derived from human samples – and there are significant differences between human diseases and the bile duct ligation and CCl_4

rodent models, in particular in the extent of progenitor cell activation and the ductular reaction (22). It is therefore critical to identify reliable surrogate markers of EMT for use in human tissue staining, regardless of the organ under study. Some progress in this area may come with the development of panels of specific markers based on recently described global regulators of EMT programs (23). The existence of reliable biomarkers might have called hepatocyte (and renal epithelial) EMT into question earlier. These will be essential to investigating EMT in cholangiocytes, other cells of the liver, and other organs as the study of fibrosis moves forward.

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The abbreviations used are

EMT	epithelial to mesenchymal transition
α-SMA	α-smooth muscle actin
FSP1	fibroblast-specific protein 1
TGF-β	transforming growth factor-β
GFP	green fluorescent protein

References

1. Iwano M, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest. 2002; 110:341–350. [PubMed: 12163453]
2. Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neilson EG. Identification and characterization of a fibroblast marker: FSP1. J Cell Biol. 1995; 130:393–405. [PubMed: 7615639]
3. Choi SS, Diehl AM. Epithelial-to-mesenchymal transitions in the liver. Hepatology. 2009; 50:2007–2013. [PubMed: 19824076]
4. Parola M, Pinzani M. Hepatic wound repair. Fibrogenesis Tissue Repair. 2009; 2:4. [PubMed: 19781064]
5. Willis BC, Borok Z. TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Lung Cell Mol Physiol. 2007; 293:L525–534. [PubMed: 17631612]
6. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009; 119:1420–1428. [PubMed: 19487818]
7. Acloque H, Adams MS, Fishwick K, Bronner-Fraser M, Nieto MA. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest. 2009; 119:1438–1449. [PubMed: 19487820]
8. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest. 2009; 119:1429–1437. [PubMed: 19487819]
9. Lin SL, Kisseleva T, Brenner DA, Duffield JS. Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. Am J Pathol. 2008; 173:1617–1627. [PubMed: 19008372]
10. Yamaoka K, Nouchi T, Marumo F, Sato C. Alpha-smooth-muscle actin expression in normal and fibrotic human livers. Dig Dis Sci. 1993; 38:1473–1479. [PubMed: 8344103]
11. Le Hir M, Hegyi I, Cueni-Loffing D, Loffing J, Kaissling B. Characterization of renal interstitial fibroblast-specific protein 1/S100A4-positive cells in healthy and inflamed rodent kidneys. Histochem Cell Biol. 2005; 123:335–346. [PubMed: 15856273]
12. Taura K, Miura K, Iwaisako K, Osterreicher CH, Kodama Y, Penz-Osterreicher M, Brenner DA. Hepatocytes do not undergo epithelial-mesenchymal transition in liver fibrosis in mice. Hepatology. 2010 in press.

13. Kaimori A, Potter J, Kaimori JY, Wang C, Mezey E, Koteish A. Transforming growth factor-beta1 induces an epithelial-to-mesenchymal transition state in mouse hepatocytes in vitro. J Biol Chem. 2007; 282:22089–22101. [PubMed: 17513865]

14. Zeisberg M, Yang C, Martino M, Duncan MB, Rieder F, Tanjore H, Kalluri R. Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. J Biol Chem. 2007; 282:23337–23347. [PubMed: 17562716]

15. Nitta T, Kim JS, Mohuczy D, Behrns KE. Murine cirrhosis induces hepatocyte epithelial mesenchymal transition and alterations in survival signaling pathways. Hepatology. 2008; 48:909–919. [PubMed: 18712785]

16. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. Am J Pathol. 2010; 176:85–97. [PubMed: 20008127]

17. Xia JL, Dai C, Michalopoulos GK, Liu Y. Hepatocyte growth factor attenuates liver fibrosis induced by bile duct ligation. Am J Pathol. 2006; 168:1500–1512. [PubMed: 16651617]

18. Diaz R, Kim JW, Hui JJ, Li Z, Swain GP, Fong KS, Csiszar K, et al. Evidence for the epithelial to mesenchymal transition in biliary atresia fibrosis. Hum Pathol. 2008; 39:102–115. [PubMed: 17900655]

19. Omenetti A, Porrello A, Jung Y, Yang L, Popov Y, Choi SS, Witek RP, et al. Hedgehog signaling regulates epithelial-mesenchymal transition during biliary fibrosis in rodents and humans. J Clin Invest. 2008; 118:3331–3342. [PubMed: 18802480]

20. Rygiel KA, Robertson H, Marshall HL, Pekalski M, Zhao L, Booth TA, Jones DE, et al. Epithelial-mesenchymal transition contributes to portal tract fibrogenesis during human chronic liver disease. Lab Invest. 2008; 88:112–123. [PubMed: 18059363]

21. Scholten D, Scholten A, Brenner DA, Kisseleva T. Epithelial-to-mesenchymal transition (EMT) in cholangiocytes does not contribute to liver fibrosis. Hepatology. 2009; 50:818A.

22. Santoni-Rugiu E, Jelnes P, Thorgeirsson SS, Bisgaard HC. Progenitor cells in liver regeneration: molecular responses controlling their activation and expansion. APMIS. 2005; 113:876–902. [PubMed: 16480456]

23. Warzecha CC, Sato TK, Nabet B, Hogenesch JB, Carstens RP. ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing. Mol Cell. 2009; 33:591–601. [PubMed: 19285943]

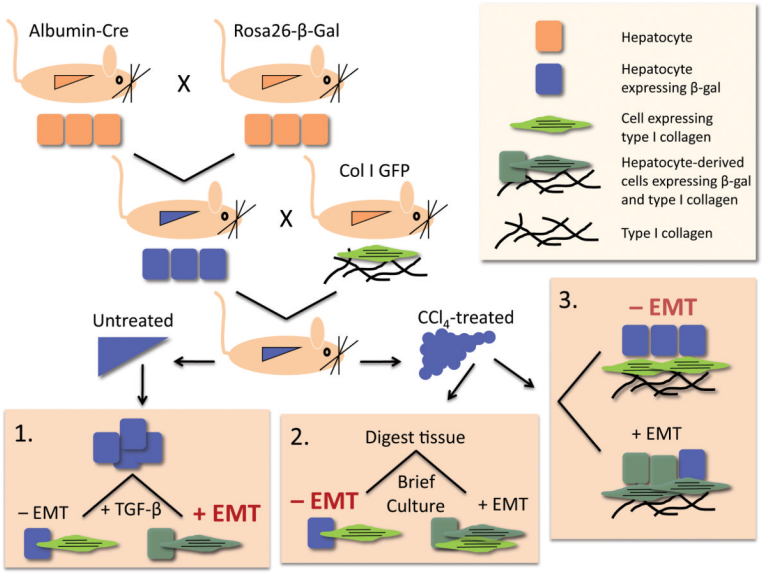

Fig. 1.

Schematic showing the use of lineage tracing to study hepatocyte EMT. The upper left shows the two crosses required to generate the triple transgenic mice used by Taura et al. (12). The group carried out three experiments using these animals, #1 with cells isolated from normal livers and #s 2 and 3 with cells and tissues, respectively, from fibrotic livers. The predicted findings in the absence or presence of EMT are shown; the actual findings of Taura et al. are indicated in red, illustrating that there was no EMT observed in the fibrosis model. Aqua indicates cells co-expressing β-galactosidase and GFP, consistent with EMT having occurred. Not shown is the heterogeneity in hepatocyte labeling with β-galactosidase