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Expression of specific hepatocyte and cholangiocyte transcription factors in human liver disease and embryonic development

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Abstract

Transcription factors are major determinants of cell-specific gene expression in all cell types. Studies in rodent liver have shown that alterations in transcription factor expression determine lineage specification during fetal liver development and signify transdifferentiation of cells of the biliary compartment into 'oval' cells and eventually hepatocytes in adult liver. We examined the cellular localization of hepatocyte- or BEC-associated transcription factors in human fetal and adult liver and in diseases in which transdifferentiation between hepatocytes and biliary cells may play a role. In the normal adult human liver, hepatocyte nuclear factor (HNF)4α and HNF6 appeared exclusively in hepatocytes; HNF1β, HNF3α, and HNF3β were observed only in BEC. During fetal development both BEC and hepatocytes expressed HNF3α, HNF3β, and HNF6. HNF1α was expressed only in fetal hepatocytes. We further examined expression of transcription factors in massive hepatic necrosis and in specific types of chronic liver disease. Hepatocyte-associated transcription factors HNF4α and HNF6 also appeared in BEC in massive hepatic necrosis and chronic hepatitis C virus infection. Similarly, HNF3β that is expressed only in BEC in normal adult liver was also observed in hepatocytes in primary biliary cirrhosis and chronic biliary obstruction. These data mimic previous findings in rodents in which hepatocyte-associated transcription factors appear in biliary cells prior to emergence of oval cells, which function as progenitor cells for hepatocytes when the regenerative capacity of the latter is compromised.

Keywords

transcription factors; embryonic human liver; transdifferentiation; hepatocytes; biliary epithelial cells

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Hepatocytes and biliary epithelial cells (BEC) are distinctly different cell types in the adult liver. In addition to transcription factors expressed commonly in many cell types, there are transcription factors expressed (in the adult liver) only in hepatocytes or BEC. Expression of transcription factors is crucial in determining specificity of cell differentiation. Cellular specificity in gene expression is in large part controlled by nuclear protein complexes, which include transcription factors. The latter impart specificity to gene expression based on cell type. Thus, emergence of new transcription factors in fully differentiated cells transcends the

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appearance of mere single-gene markers and is interpreted to signify large-scale reprogramming in gene expression. [1,2] Whereas in normal adult liver, the expression of hepatocyte- or BEC-associated single genes is localized to the specific cell types, cells with promiscuous expression of both hepatocytic and BEC single-gene markers are often seen in chronic liver disease.[3–7] The appearance of 'marker genes' from the other (hepatocyte or BEC) cell type suggests that a reprogramming in gene expression is taking place, as an adaptation to a specific pathobiologic or physiological challenge. Although the appearance of single-gene markers of alternate specificity is well documented, changes in transcription factors controlling in human liver have not been characterized.

The regenerative capacities of hepatocytes and BEC have been tested in many experimental studies in rodents. In uncomplicated liver regeneration after partial hepatectomy or during BEC proliferation following bile duct ligation, hepatocytes or BEC respectively proliferate to generate more cells of their type.[8–10] If, however, the capacity of hepatocytes or BEC to regenerate more of their own is impaired, then (in rodent systems) hepatocytes and BEC can transdifferentiate into each other. BEC (from canals of Hering and/or portal ductules) proliferate transiently to generate 'oval' cells which undergo gradual morphologic and biochemical changes to become hepatocytes.[11–15] Oval cells express both biliary- and hepatocyte-associated genes. There are two pieces of evidence supporting the biliary origin of the oval cells. In the first piece of evidence, oval cells do not appear when BEC are subjected to lethal toxic injury prior to the induction of the oval cell protocol.[16] The second piece of evidence is the appearance of hepatocyte-associated transcription factors in BECs very soon after the initiation of the oval cell protocol.[17] We have also shown in rats that when the capacity of BEC to proliferate is impaired by delivering a toxic injury by DAPM, more BEC can be formed by transdifferentiation of periportal hepatocytes.[18]

Hepatocytic and BEC lineage is specified in part by a set of specific liver-enriched transcription factors.[1,2,19,20] Studies with knockout mice have shown that hepatocyte nuclear factor (HNF) 1α and HNF4α regulate transcription of genes essential for the hepatocytic cell lineage,[21–23] whereas HNF1β and HNF6 are involved in development of the gallbladder and bile ducts.[24–26] Previous studies with rodents have shown that the earliest step in the generation of the oval cells is the appearance of hepatocyte-associated transcription factors in BEC of portal ductules and canals of Hering.[17,27] This is direct evidence of initiation of large-scale gene reprogramming, as transcription factors affect expression of multiple genes associated with differentiation of specific cell types. In this model, appearance of hepatocyte-associated transcription factors in biliary cells suggests initiation of expression of genes associated with hepatocyte differentiation in biliary cell types. This is perfectly consistent with the large-scale expression of hepatocyte-associated genes in oval cells, as they gradually transform to small and eventually regular hepatocytes in the AAF-hepatectomy protocol.[11–13]

As most of the literature of expression of transcription factors in hepatic cell types derives from studies in rodents, we first assessed transcription factor expression in hepatocytes versus BEC in human fetal and normal adult liver, to provide a comparison standard with literature derived from rodent-based experimental studies. We also examined the presence of specific transcription factors in liver diseases in which hepatocytes or biliary cells are selectively affected. Liver diseases selected for this study were massive hepatic necrosis and hepatitis C virus (HCV) infection (associated with damage primarily to the hepatocytes); and late stages of primary biliary cirrhosis (PBC) and chronic biliary obstruction (primarily BEC injury). There are numerous studies documenting the appearance of cells with intermediate hepatocyte/BEC differentiation patterns in massive hepatic necrosis.[28–31] These cells, often called 'ductular hepatocytes'[27] are always thought as representing cells types with intermediate differentiation between hepatocytes and BEC. Structures containing hepatocytes, BEC and intermediate cell types were recently described in a 'bipolar' ductular reaction.[4–6,32] The

above studies have well documented the appearance of alternate single-gene markers in hepatic cells in different disease states. Expression of genes specific to cell types (markers) has always been used as basis for assessing differentiation status of a given cell. However, gene expression patterns (including cell-specific markers) in any cell type are determined in large part by the expression of specific transcription factors, which, in synergy with epigenetic changes, determine cell differentiation and define cell lineage. Thus we investigated expression of hepatocyte- or BEC-associated transcription factors in both hepatocytes and BEC in human liver, as a tool to determine initiation of promiscuous altered gene expression patterns between hepatocytes and biliary cells during chronic liver disease, comparable to the changes seen in impaired regeneration models in rodents.

MATERIALS AND METHODS

Case Selection

With approval from the IRB of the University of Pittsburgh (IRB no. 0501051), paraffin-embedded liver sections were obtained from the archives of the Department of Pathology, University of Pittsburgh Medical Center. Specimens were obtained from five cases each of first, second, and third trimester fetal liver tissue, and normal adult (NL). Data presented in this article were collected from four cases of end-stage cirrhosis from chronic HCV infection, five cases of massive hepatic necrosis (causes included HBV infection, autoimmune hepatitis, acetaminophen), five cases of chronic biliary obstruction (none of the cases included pancreatic cancer), and three cases of late-stage PBC. The specimens related to specific liver diseases all represent end-stage liver disease in their type. They were obtained from livers explanted for the purpose of orthotopic liver transplantation. The normal adult liver tissue samples were selected from liver specimens resected for metastatic colorectal carcinoma.

Immunohistochemistry

Immunohistochemical localization studies of HNF1α, HNF1β, HNF3α, HNF3β, HNF4α, HNF6 were conducted on formalin-fixed paraffin-embedded liver sections (4-μm thick). Corresponding staining for hepatocyte and BEC markers, HepPar1 and CK19, respectively was performed on each of the selected case. Antigen retrieval was achieved by steaming the slides for 60 min in Target Retrieval or Hi pH Target Retrieval solution (Dako, Carpinteria, CA, USA). The slides were bathed in 3% H_2O_2 solution for 5 min to quench endogenous peroxidase. Endogenous avidin and biotin were also blocked using the Avidin-Biotin blocking kit (Vector, Burlingame, CA, USA). Primary antibody was then applied in the appropriate concentration (Table 1), and the sections were incubated overnight at 4°C. Nonspecific binding sites were blocked with 10% serum of the appropriate host animal in protein block (Dako) with incubation for 10 min, and the biotinylated secondary antibody was applied with incubation for 30 min (Table 1). The sections were then incubated with Vectastain ABC Elite (Vector) at room temperature for 30 min. The sections were then visualized with chromogen for 10 min, counterstained with aqueous hematoxylin/blue Scott's solution in tap water, crystal mounted, and allowed to dry before a coverslip was placed. This procedure was modified for staining for HepPar 1 and CK19. Staining for CK19 was achieved by steaming the liver sections for 20 min in Hi pH Target Retrieval solution (Dako). Staining for HepPar1 did not require mechanical antigen retrieval. Both involved incubation with primary antibody for 1 h rather than over-night. Detail concentrations of antibodies used is shown in Table 1.

Transcription factor expression levels were determined by examining cells identified morphologically and immunophenotypically (HepPar1 positive and CK19 negative) as hepatocytes. BECs were considered those cells comprising a basement membrane-lined duct confined within the limiting plate of the portal tract (HepPar1 negative and CK19 positive). Positive cells were those with strong (3+–4+) nuclear positivity. A total of 200 hepatocytes

and BEC were counted in adult normal liver (NL), HCV infection, massive hepatic necrosis, biliary obstruction, and PBC.

RESULTS

Transcription Factor Expression Profile during Human Fetal Liver Development and in Adult Liver

We compared expression of various transcription factors associated with hepatic development in rodents, between fetal and adult human liver. HNF1α was unique in its expression only in fetal hepatocytes (Figures 1a–d). It was strongly expressed in hepatocytes till the second trimester (Figures 1a and b) however, sharply decreased in the third trimester (Figure 1c) and was completely lost in the adult hepatocytes (Figure 1d). HNF1α staining was not noticed in bile ducts or ductal plate during the entire fetal growth or in bile ducts of the adult liver. HNF4α was exclusively expressed by the fetal and adult hepatocytes (Figures 1e–h). No HNF4α expression is seen in the fetal ductal plate or in bile duct epithelium during development (Figures 1e–g) or in the normal adult liver (Figure 1h). HNF6 is also expressed by the fetal and adult hepatocytes however, it is expressed by fetal BEC as well till the second trimester (Figures 1i–l). HNF6 was completely lost from the BEC with maturation of the biliary phenotype. In addition, conventional hepatocyte marker HepPar1 staining was performed. Weak HepPar1 staining was observed in hepatoblasts in the first trimester (Figure 1m), which increased from second trimester to a diffuse strong staining in the hepatocytes (Figure 1n) till the third trimester (Figure 1o) and continued into the mature adult hepatocytes (Figure 1p). No biliary cells expressed HepPar1 during development or in the adulthood.

Transcription factors expressed in the fetal and adult BEC, among others, are HNF1β, HNF3α, and HNF3β. HNF1β is an exclusive BEC transcription factor expressed only in BEC during fetal development and in adult liver (Figures 2a and d). Its expression delineated the ductal plates in the second and third trimester (Figures 2b and c). In addition to the BEC, HNF3α (Figures 2e–g) and HNF3β (Figures 2i–k) were also present in the hepatocytes till the second trimester; however, were lost from the adult hepatocytes (Figures 3h and l). Identification of BEC was also pursued by immunohistochemistry for the conventional BEC marker CK19. During development, CK19 stains the ductal plate and biliary ductules in all three trimesters (Figures 2m–o). Hepatocytes stain positive in the first trimester, the CK19 expression disappearing by the third trimester. In the adult liver, only BECs stained positive for CK19 with no mature hepatocytes expressing this marker (Figure 2p).

In summary, HNF3α, HNF3β, and HNF6 are expressed by both hepatocytes and BEC during fetal growth, whereas in the normal adult liver HNF4α is expressed solely by hepatocytes, and HNF1β, HNF3α, and HNF3β are expressed only by the BEC indicating that these transcription factors are important in lineage specification. The results also demonstrate the beginning of the ductal plate in the first trimester (Figure 2i) and its presence in the second and the third trimester (Figure 2).

It should be noted that the above findings related to specific transcription factor expression relate to nuclear localization. Nucleus is the site in which transcription factors exert their effects on gene expression. Weak cytoplasmic staining is often seen without cell specificity (eg hepatocytes express weak cytoplasmic staining for HNF1β; Figures 2b–d). The significance of this often-seen weak cytoplasmic staining in different cell types is not clear and it may reflect intermediary processing forms, potentially ending in degradation in the absence of nuclear localization.

Expression of Biliary Transcription Factors in Hepatocytes in Chronic Biliary Disease

Expression of HNF3β in BEC remained consistent in all disease conditions examined in this study. However, in PBC and biliary obstruction in addition to BEC, a substantial number of hepatocytes became positive for HNF3β (Figure 3). The percent of hepatocyte nuclei expressing HNF3β was 73% in PBC and 99% in biliary obstruction. The atypical ductules appearing in the PBC and biliary obstruction also stained positively for CK19, as expected (Figures 3e and f).

Expression of Hepatocyte-Associated Transcription Factors in Biliary Cells, in Liver Diseases Associated with Hepatocytic Injury

HNF4α expression was seen in nuclei of BEC in both massive hepatic necrosis (25.9%). and severe chronic HCV (12%). The results are shown in Figure 4. In addition, the bile ducts in the massive hepatic necrosis and HCV also expressed HNF6. The percent of nuclei of BEC expressing HNF6 was 22.8% in PBC, 16.50% in massive hepatic necrosis, and 25.5% in end-stage HCV infection. Expression of HNF6 in the hepatocytes (also seen minimally (1.5%) in the adult liver) was also elevated in PBC (13%), massive hepatic necrosis (12.75%), and end-stage HCV infection (6%). The expression of hepatocyte-associated transcription factors in BEC apparently also affected expression of specific gene patterns, as evidenced by the expression of the classic hepatocyte marker HepPar1 in many biliary cells arranged in sheets or ductules (Figures 4j and k).

Proliferation Indices of Hepatocytes and Biliary Epithelial Cells in Different Disease States in Which Promiscuous Expression of Transcription Factors is Noted

Studies with rodents have indicated that promiscuous expression of transcription factors between hepatocytes and BEC occurs in situations in which either of the two cell types needs to regenerate more of its own but the regenerative capacity is blocked. We employed immunohistochemistry for the commonly used protein Ki67, which is expressed in nuclei engaged in DNA synthesis. The results are shown in Figure 5. Hepatocytes had comparable low (<1.5% Ki67-positive nuclei) proliferation rate in normal liver as well as in PBC, biliary obstruction and end-stage cirrhosis from hepatitis C. There was no detectable proliferation of BEC in normal liver; there was a small increase in BEC proliferation in biliary obstruction (0.7%) and HCV cirrhosis (0.38%). Extensive proliferation of both hepatocytes (17.2%) and BEC (5.3%) was noted in massive hepatic necrosis (five cases).

DISCUSSION

The results of our study document that similar transcription factors are expressed and probably govern rodent and human embryonic development, further strengthening the importance of studies of hepatic embryogenesis in rodent models as a means to understand hepatic embryogenesis in the human. The unique phenotype of hepatocytes or BEC arises in part from the expression of different transcription factors, combining in a cell-specific fashion. Such transcription factors include family members of HNF1, HNF3, HNF4, and HNF6. None of these transcription factors are limited to the liver, but their expression during development and adult liver has characteristic patterns within hepatocytes and BECs. In our study, immunohistochemical analyses of the liver sections indicate that hepatocytes and BEC during fetal growth express some common and some specific transcription factors and they maintain expression of distinct transcription factor patterns in the adult life. During fetal development, HNF3α, HNF3β, and HNF6 are expressed by both hepatocytes and BEC, whereas later in the adult life HNF1β, HNF3α, and HNF3β are exclusively expressed by BEC, and HNF4α and HNF6 are expressed only by hepatocytes. HNF1α was unique in its expression only in fetal hepatocytes. This has also been shown in murine studies where HNF1β is expressed early on during embryonic development, in the endoderm of the foregut, whereas HNF1α is activated

later, upon condensation of the hepatic parenchyma, and its expression decreases in the adult liver.[2] Overall, variations seen between the results of this study and studies of transcription factor expression during rodent embryogenesis reflect only different time-dependent patterns of variations in expression, with the identity of transcription factors being essentially the same between human and rodent.

Our results also demonstrate that whereas in normal adult liver the expression of hepatocyte- or biliary-associated transcription factors follows standard patterns, the expression becomes promiscuous in disease states. Hepatocyte-associated transcription factors appear in BEC in diseases with acute massive or chronic hepatocyte damage, such as end-stage HCV and massive hepatic necrosis. The latter condition has been associated with the appearance of ductular hepatocytes,[28,29,33,34] cells of intermediate phenotype between hepatocytes and BEC. The appearance of HNF4α in BEC in these situations mimics the reported increase in HNF4α and other hepatocyte-associated transcription factors in biliary cells (portal ductules and canals of Hering) in rodents, when hepatocytes are blocked from proliferation by AAF following partial hepatectomy.[11–13] In that situation, oval cells emerge from the biliary compartment and transdifferentiate into hepatocytes, rescuing the regeneration of the whole organ. The situation in massive hepatic necrosis however appears more complex, because there does not appear to be any inhibition of proliferation of either hepatocytes or biliary cells (Figure 5). These findings are consistent with earlier studies in which we showed that hepatocytes have a high percent of their nuclei positive for PCNA in fulminant hepatic failure.[28] It is conceivable that though proliferation of hepatocytes occurs (Figure 5), it is much less than what it should be, thus generating a ‘gap’. This may trigger stimuli comparable to those operating in rodents in the AAF/hepatectomy model, which induce promiscuous expression of transcription factors and reprogramming of gene expression in BEC. As these considerations cannot be fully explored in the human, this interpretation unavoidably remains speculative. Similar considerations may also apply in end-stage HCV, in which expression of HNF4α is also seen in biliary cells. As pointed out in Figures 4g–i, the appearance of hepatocyte-associated transcription factors in BEC is also accompanied by the appearance of hepatocyte-associated genes, such as that of the protein HepPar1.

Similarly, expression of BEC-associated HNF3β in hepatocytes during chronic biliary disease (PBC and biliary obstruction) suggests that the opposite pathway (gene reprogramming of hepatocytes to BCE) is also operative in the human liver. Similar changes are seen in rodents, in situations in which BECs forced to proliferate (following bile duct ligation) are also simultaneously damaged by exposure to the biliary toxin DAPM.[18] In that situation, periportal hepatocytes undergo transdifferentiation to BECs and participate in the formation of biliary ductules derived from hepatocytes.[18] Similarly, the expression of biliary-associated transcription factors in periportal hepatocytes in chronic biliary disease suggests that these cells may have a role to play in repair of biliary epithelium in chronic biliary disease, when the capacity of BEC to proliferate may be impaired for a variety of reasons. The data in Figure 5 do not resolve the issue of the capacity of BEC to proliferate in PBC, as the proliferative index of BEC remains very low and comparable to that of normal liver. There is increased proliferation of BEC in the biliary obstruction, however, in which there is striking increase in expression of HNF3β in hepatocytes (Figure 3). It should be noted that, despite the treatment with DAPM, BEC cells in the rodent model also demonstrate extensive proliferation and hepatocytes are recruited to become BEC nonetheless. In the absence of clear cell-tracking methodology, it is tempting to speculate that such conversion of hepatocytes to BCE also occurs in humans, but proof cannot be derived only from histologic observations. As mentioned above, in addition to our observations on transcription factors in this study, previous studies have documented the appearance of single-gene markers associated with BEC in periportal hepatocytes during obstructive cholangiopathy and PBC.[3,5–7]

The expression of the transcription factors was evaluated only on the basis of immunohistochemistry. Though more sensitive methods (eg western blot) could be applied, they are not easily amenable to material from paraffin blocks. In addition, methods requiring homogenization of liver tissue would not yield information related to cellular localization. The main aim of this study was cellular localization of the different transcription factors. There is considerable variation in percent of different cell types in different disease states, especially in massive hepatic necrosis. Thus, techniques relying on tissue homogenates would be difficult to control in terms of variations in cell populations and heterogeneity of the degree of damage within the whole organ.

Overall, our studies suggest that, as in rodents, human hepatocytes and biliary cells can enter into extensive gene reprogramming. In rodents, such changes are associated with transformation of hepatocytes and BEC into transiently amplifying cell populations (oval cells, from BEC to hepatocytes), which can function as progenitor cells for each other and assist in organ repair in response to massive liver damage or chronic disease. The similar observed changes in humans regarding expression of transcription factors in a promiscuous manner between hepatocytes and BEC suggests that similar changes may also occur in the human liver in the context of chronic liver disease. The full capacity of human hepatocytes and BEC to function as facultative stem cells for each other, however, can only be determined by experimental conditions.

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References

1. Duncan SA. Transcriptional regulation of liver development. Dev Dyn 2000;219:131–142. [PubMed: 11002334]
2. Lee CS, Friedman JR, Fulmer JT, et al. The initiation of liver development is dependent on Foxa transcription factors. Nature 2005;435:944–947. [PubMed: 15959514]
3. Nijjar SS, Wallace L, Crosby HA, et al. Altered Notch ligand expression in human liver disease: further evidence for a role of the Notch signaling pathway in hepatic neovascularization and biliary ductular defects. Am J Pathol 2002;160:1695–1703. [PubMed: 12000721]
4. Nijjar SS, Crosby HA, Wallace L, et al. Notch receptor expression in adult human liver: a possible role in bile duct formation and hepatic neovascularization. Hepatology 2001;34:1184–1192. [PubMed: 11732008]
5. Crosby HA, Hubscher SG, Joplin RE, et al. Immunolocalization of OV-6, a putative progenitor cell marker in human fetal and diseased pediatric liver. Hepatology 1998;28:980–985. [PubMed: 9755234]
6. Crosby HA, Hubscher S, Fabris L, et al. Immunolocalization of putative human liver progenitor cells in livers from patients with end-stage primary biliary cirrhosis and sclerosing cholangitis using the monoclonal antibody OV-6. Am J Pathol 1998;152:771–779. [PubMed: 9502419]
7. Strain AJ, Crosby HA, Nijjar S, et al. Human liver-derived stem cells. Semin Liver Dis 2003;23:373–384. [PubMed: 14722814]
8. Michalopoulos GK. Liver regeneration. J Cell Physiol 2007;213:286–300. [PubMed: 17559071]
9. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology 2006;43:S45–S53. [PubMed: 16447274]
10. Michalopoulos GK, DeFrances MC. Liver regeneration. Science 1997;276:60–66. [PubMed: 9082986]
11. Evarts RP, Hu Z, Omori N, et al. Precursor-product relationship between oval cells and hepatocytes: comparison between tritiated thymidine and bromodeoxyuridine as tracers. Carcinogenesis 1996;17:2143–2151. [PubMed: 8895481]

12. Evarts RP, Nagy P, Nakatsukasa H, et al. In vivo differentiation of rat liver oval cells into hepatocytes. Cancer Res 1989;49:1541–1547. [PubMed: 2466557]
13. Evarts RP, Nagy P, Marsden E, et al. A precursor-product relationship exists between oval cells and hepatocytes in rat liver. Carcinogenesis 1987;8:1737–1740. [PubMed: 3664968]
14. Alison M, Golding M, Lalani EN, et al. Wholesale hepatocytic differentiation in the rat from ductular oval cells, the progeny of biliary stem cells. J Hepatol 1997;26:343–352. [PubMed: 9059956]
15. Golding M, Sarraf CE, Lalani EN, et al. Oval cell differentiation into hepatocytes in the acetylaminofluorene- treated regenerating rat liver. Hepatology 1995;22(4 Part 1):1243–1253. [PubMed: 7557877]
16. Petersen BE, Zajac VF, Michalopoulos GK. Bile ductular damage induced by methylene dianiline inhibits oval cell activation. Am J Pathol 1997;151:905–909. [PubMed: 9327722]
17. Nagy P, Bisgaard HC, Thorgeirsson SS. Expression of hepatic transcription factors during liver development and oval cell differentiation. J Cell Biol 1994;126:223–233. [PubMed: 8027180]
18. Michalopoulos GK, Barua L, Bowen WC. Transdifferentiation of rat hepatocytes into biliary cells after bile duct ligation and toxic biliary injury. Hepatology 2005;41:535–544. [PubMed: 15726663]
19. Zaret KS. Liver specification and early morphogenesis. Mech Dev 2000;92:83–88. [PubMed: 10704889]
20. Lemaigre FP. Development of the biliary tract. Mech Dev 2003;120:81–87. [PubMed: 12490298]
21. Pontoglio M, Barra J, Hadchouel M, et al. Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell 1996;84:575–585. [PubMed: 8598044]
22. Li J, Ning G, Duncan SA. Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. Genes Dev 2000;14:464–474. [PubMed: 10691738]
23. Hayhurst GP, Lee YH, Lambert G, et al. Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. Mol Cell Biol 2001;21:1393–1403. [PubMed: 11158324]
24. Coffinier C, Gresh L, Fiette L, et al. Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1beta. Development 2002;129:1829–1838. [PubMed: 11934849]
25. Clotman F, Lannoy VJ, Reber M, et al. The onecut transcription factor HNF6 is required for normal development of the biliary tract. Development 2002;129:1819–1828. [PubMed: 11934848]
26. Clotman F, Lemaigre FP. Control of hepatic differentiation by activin/ TGFbeta signaling. Cell Cycle 2006;5:168–171. [PubMed: 16357531]
27. Bisgaard HC, Nagy P, Santoni-Rugiu E, et al. Proliferation, apoptosis, and induction of hepatic transcription factors are characteristics of the early response of biliary epithelial (oval) cells to chemical carcinogens. Hepatology 1996;23:62–70. [PubMed: 8550050]
28. Vandersteenhoven AM, Burchette J, Michalopoulos G. Characterization of ductular hepatocytes in end-stage cirrhosis. Arch Pathol Lab Med 1990;114:403–406. [PubMed: 2322100]
29. Demetris AJ, Seaberg EC, Wennerberg A, et al. Ductular reaction after submassive necrosis in humans. Special emphasis on analysis of ductular hepatocytes. Am J Pathol 1996;149:439–448. [PubMed: 8701983]
30. Desmet V, Roskams T, Van Eyken P. Ductular reaction in the liver. Pathol Res Pract 1995;191:513–524. [PubMed: 7479372]
31. Roskams T, De Vos R, Van Eyken P, et al. Hepatic OV-6 expression in human liver disease and rat experiments: evidence for hepatic progenitor cells in man. J Hepatol 1998;29:455–463. [PubMed: 9764994]
32. Zhou H, Rogler LE, Teperman L, et al. Identification of hepatocytic and bile ductular cell lineages and candidate stem cells in bipolar ductular reactions in cirrhotic human liver. Hepatology (Baltimore, MD) 2007;45:716–724.
33. Sirica AE. Ductular hepatocytes. Histol Histopathol 1995;10:433–456. [PubMed: 7599440]
34. Fiel MI, Antonio LB, Nalesnik MA, et al. Characterization of ductular hepatocytes in primary liver allograft failure. Mod Pathol 1997;10:348–353. [PubMed: 9110297]

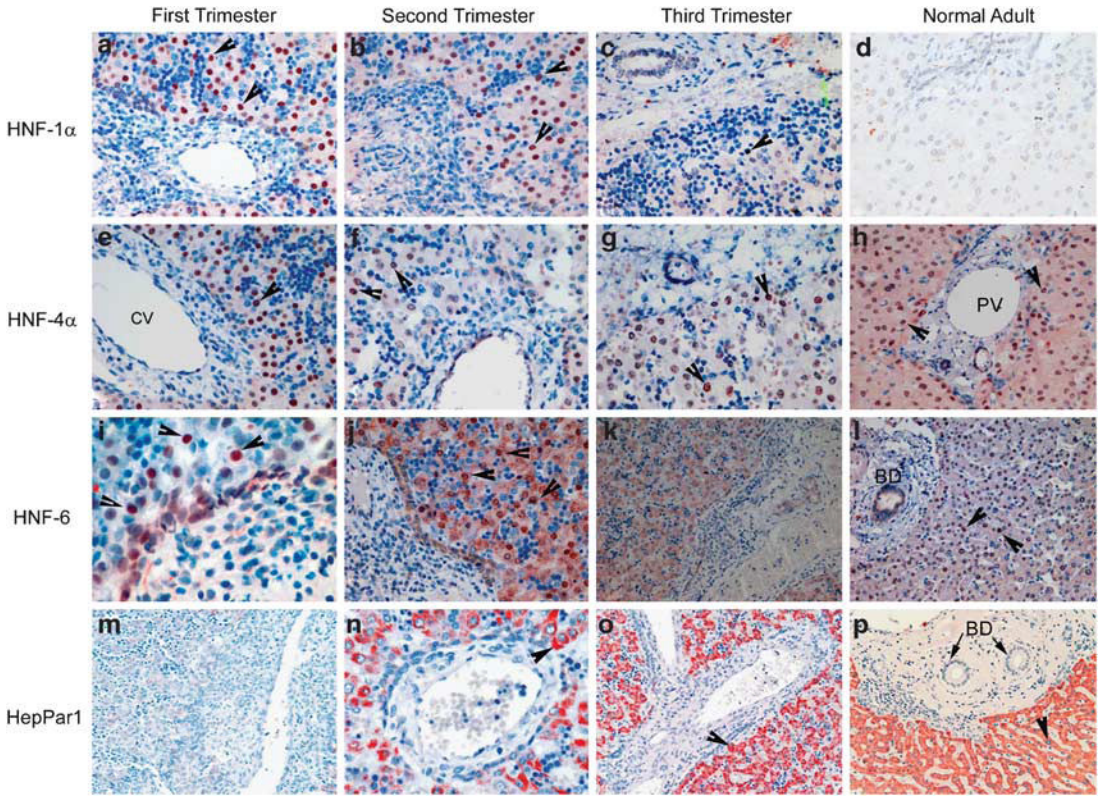

Figure 1. Immunohistochemical staining of hepatocyte-specific transcription factors in the developing human fetal liver and in normal human adult liver

Nuclear staining of HNF1α (**a–d**) appears only in fetal hepatocytes in the first and second trimester. HNF4α (**e–h**) is seen only in hepatocyte nuclei at all stages of fetal and adult liver. HNF6 (**i–l**) is expressed in both hepatocytes and biliary cells in the first two trimesters, following which its expression is limited to hepatocytes. Cytoplasmic staining of HepPar1 was seen only in hepatocytes. It was weakly positive in the first trimester and strongly positive in the second and third trimester and adult liver (**m–p**). Arrows indicate positive staining. CV, central vein; PV, portal vein; BD, bile duct.

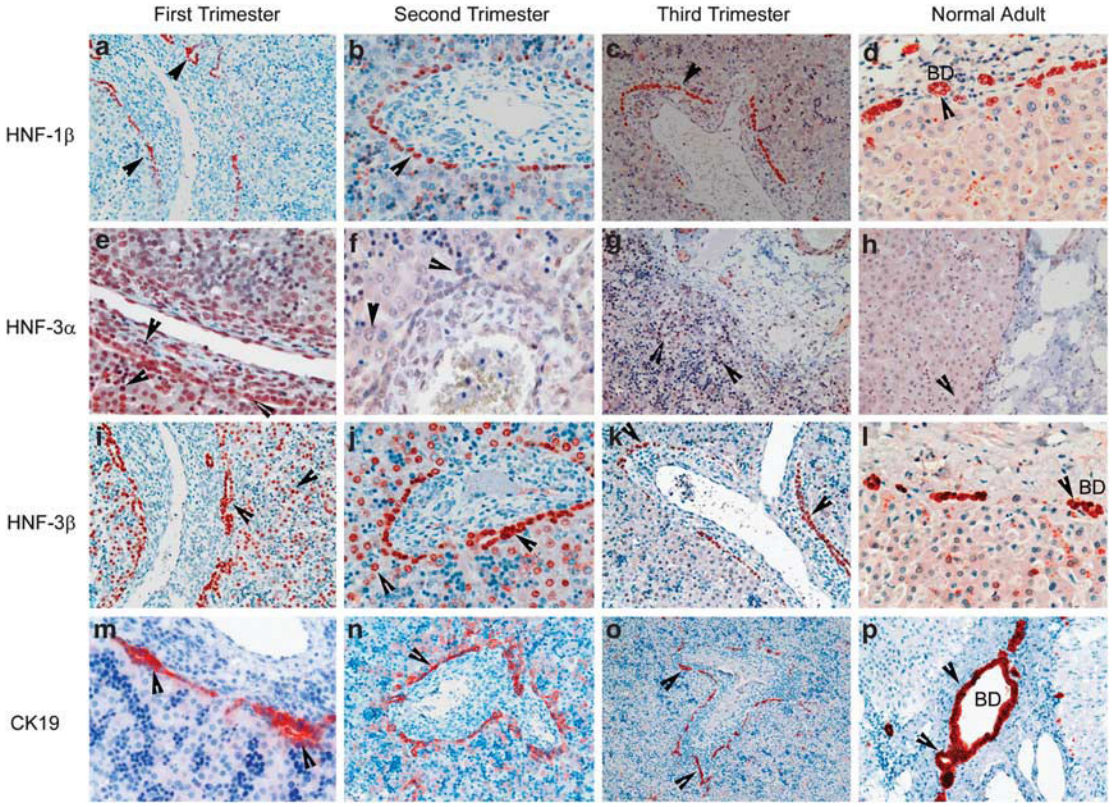

Figure 2. Immunohistochemical staining of biliary-specific transcription factors in the developing fetal liver and in normal adult liver

Nuclear staining of HNF1β (**a–d**) and HNF3β (*i–l*) is seen in biliary cells of all stages. HNF3α (**e–h**) is expressed in biliary cells only in the first trimester. Cytoplasmic staining of CK19 in BEC in fetal and adult liver (**m–p**). The photomicrographs also illustrate the formation of ductal plate during the different trimesters. Arrows indicate positive staining. BD, bile duct; CV, central vein; PV, portal vein.

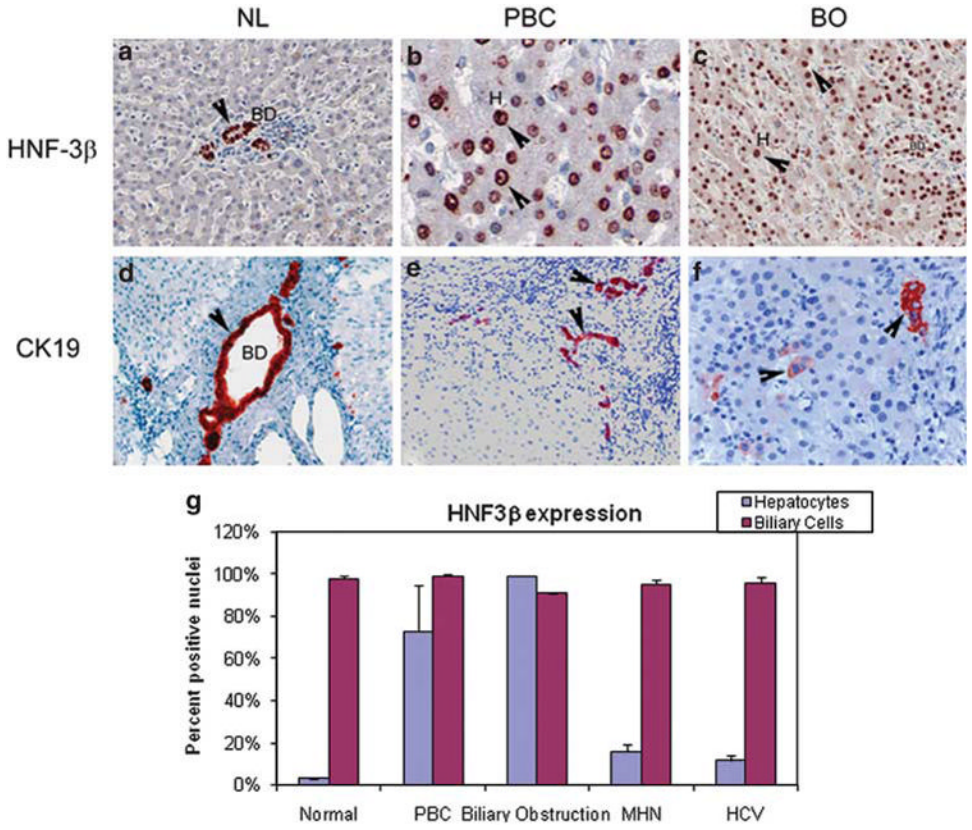

Figure 3. Expression of biliary-associated transcription factor HNF3β in hepatocytes, in cases of late-stage primary biliary cirrhosis (PBC) and chronic biliary obstruction (BO)

(**a–f**) Immunohistochemical staining of HNF3β and CK19 in normal *vs* PBC and BO. Hepatocytes are negative whereas bile ducts are positive for HNF3β in normal adult liver (**a**) however, hepatocytes stain positive for HNF3β in PBC (**b**) and BO (**c**). In normal adult liver, hepatocytes are negative (**d**) whereas bile ducts are positive for CK19 in PBC (**e**) and BO (**f**). Arrows indicate positive staining. BD, bile duct; CV, central vein; PV, portal vein. (**g**) Quantitative analysis of HNF3β immunostaining in the liver diseases in hepatocytes *vs* BEC. The bars indicate the mean and standard error from cell counts of separate cases, each disease from a number of separate specimens as described in 'Materials and Methods'. Blue, hepatocytes; red, biliary cells.

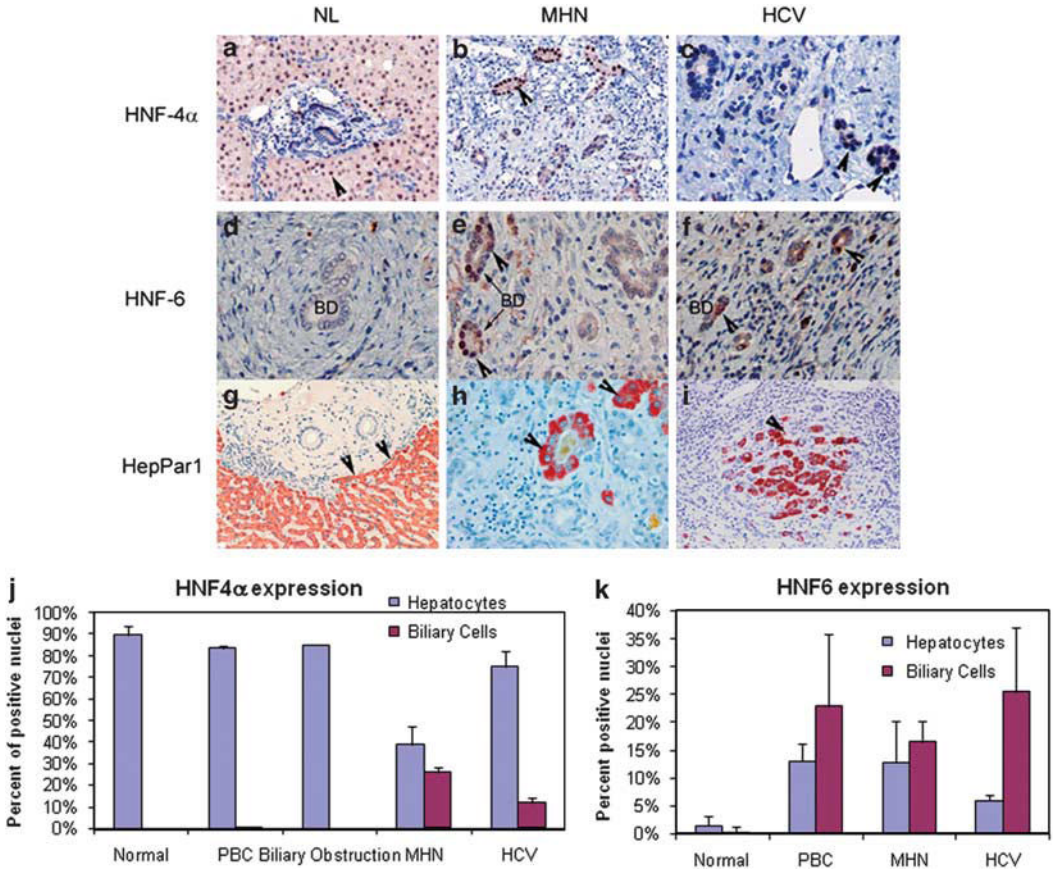

Figure 4. Expression of hepatocyte-associated transcription factors in biliary epithelial cell (BEC) in different hepatic diseases

(**a–i**) Immunohistochemical staining of HNF4α, HNF6, and HepPar1 in normal *vs* massive hepatic necrosis and end-stage HCV cirrhotic liver. BEC in normal adult liver are negative for HNF4α (**a**) and HNF6 (**d**). BEC in massive hepatic necrosis (MHN) express HNF4α (**b**) and HNF6 (**e**). BEC also expresses HNF6 in MHN (**c**) and HCV (**f**). The appearance of the single-gene hepatocyte marker HEPAR is also prominent is many ductular cells. Arrows indicate positive staining. BD, bile duct; CV, central vein; PV, portal vein. (**j**) Quantitative analysis of HNF4α immunostaining in liver disease in hepatocytes *vs* BEC. (**k**) Quantitative analysis of HNF6 immunostaining in liver disease in hepatocytes *vs* BEC. Both HNF4α and HNF6 emerge in BECs in diseases associated with chronic hepatocyte injury. In both (**j** and **k**), the bars indicate the mean and standard error from cell counts of separate cases, each disease from a number of separate specimens as described in ‘Materials and Methods’. Blue, hepatocytes; red, biliary cells.

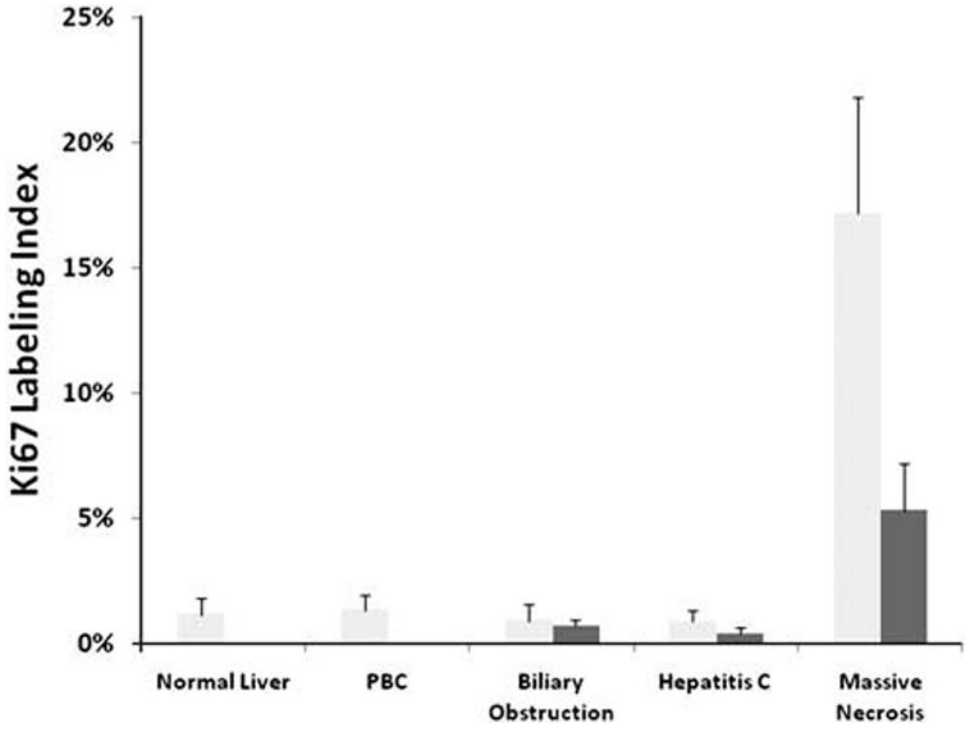

Figure 5.

Proliferation rates of hepatocytes and BEC in different hepatic diseases, as measured by the nuclear expression of Ki67, a marker of nuclear DNA synthesis. The bars indicate the mean and standard error from cell counts of separate cases, each disease from a number of separate specimens as described in ‘Materials and methods’. Light gray, hepatocytes; dark gray, biliary cells.

Table 1

Details of the primary antibodies

Antibody	Catalog no.	Concentration
HepPar 1	M7158[a]	1:50
CK19	M0772[a]	1:100
HNF1α	Sc-6547[b]	1:50
HNF1β	Sc-7411[b]	1:100
HNF3α	Sc-22841[b]	1:50
HNF3β	Sc-6554[b]	1:50
HNF4α	Sc-8987[b]	1:50
HNF6	Sc-13050[b]	1:50

[a] From Dako.

[b] From Santa Cruz Biotechnology.