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Proliferation and Differentiation of Fetal Liver Epithelial Progenitor Cells after Transplantation into Adult Rat Liver

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To identify cells that have the ability to proliferate and differentiate into all epithelial components of the liver lobule, we isolated fetal liver epithelial cells (FLEC) from ED 14 Fischer (F) 344 rats and transplanted these cells in conjunction with two-thirds partial hepatectomy into the liver of normal and retrorsine (Rs) treated syngeneic dipeptidyl peptidase IV mutant (DPPIV$^-$) F344 rats. Using dual label immunohistochemistry/*in situ* hybridization, three subpopulations of FLEC were identified: cells expressing both α-fetoprotein (AFP) and albumin, but not CK-19; cells expressing CK-19, but not AFP or albumin, and cells expressing AFP, albumin, and cytokeratins-19 (CK-19). Proliferation, differentiation, and expansion of transplanted FLEC differed significantly in the two models. In normal liver, 1 to 2 weeks after transplantation, mainly cells with a single phenotype, hepatocytic (expressing AFP and albumin) or bile ductular (expressing only CK-19), had proliferated. In Rs-treated rats, in which the proliferative capacity of endogenous hepatocytes is impaired, transplanted cells showed mainly a dual phenotype (expressing both AFP/albumin and CK-19). One month after transplantation, DPPIV$^+$ FLEC engrafted into the parenchyma exhibited an hepatocytic phenotype and generated new hepatic cord structures. FLEC, localized in the vicinity of bile ducts, exhibited a biliary epithelial phenotype and formed new bile duct structures or were incorporated into pre-existing bile ducts. In the absence of a proliferative stimulus, ED 14 FLEC did not proliferate or differentiate. Our results demonstrate that 14-day fetal liver contains lineage committed (unipotential) and uncommitted (bipotential) progenitor cells exerting different repopulating capacities, which are affected by the proliferative status of the recipient liver and the host site within the liver where the transplanted cells become engrafted. These findings have important implications in future studies directed toward liver repopulation and *ex vivo* gene therapy. *(Am J Pathol 2000, 156:2017–2031)*

The liver originates from the gut endoderm. On embryonic day (ED) 8.5 in the mouse and 1 day later in the rat, primitive epithelial cells of the foregut contact the cardiac mesoderm and form the liver diverticulum.[1–6] These cells proliferate extensively, invade the septum transversum, begin to differentiate, and, on ED 9.5 in mice and ED 10.5 in rats, acquire the morphological appearance of immature liver epithelial cells (hepatoblasts), expressing first α-fetoprotein (AFP) and then albumin.[6,7] Following the expression of these and other hepatic markers, including also cytokeratins (CKs), most authors conclude that hepatoblasts are bipotential cells, capable of differentiating along the hepatocytic or bile duct epithelial cell lineage.[7–11] At ED 15–16, the rat liver already contains committed immature hepatocytes and bile duct epithelial cells.[7,8,12,13] In both rats and humans, embryonic hepatoblasts in large vascular spaces also form primitive ductal structures, which ultimately give rise to the intrahepatic bile ducts.[14–16]

A number of transcription, signaling, and growth factors have been identified that play an essential role in gut endoderm differentiation and fetal liver development. These include factors that bind to the GATA DNA sequence motif (GATA), signal transducers and activators of transcription (STATs), hepatocyte nuclear factors (HNF)-3-α and -β, HNF-4, HNF-6, and certain fibroblast growth factors (FGFs).[17–25] However, the mechanisms by which primitive pluripotential endodermal cells undergo hepatic specification and how bipotential hepatoblasts differentiate further into hepatocytes and bile duct epithelium remain largely unknown.

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Studies in the adult liver have also provided strong evidence for the existence of putative liver stem cells, ie, undifferentiated liver epithelial cells that can be activated to proliferate and differentiate into hepatocytes or bile duct epithelial cells.[26–28] These cells are thought to reside within or adjacent to the canals of Hering. Unlike stem cells in other tissues, such as bone marrow, skin, and intestine, which undergo continuous renewal, liver stem-like cells are facultative; they comprise a quiescent compartment of dormant cells that is activated only if the regenerative capacity of hepatocytes is impaired. Attempts have been made to identify their counterpart in fetal liver,[7,28–31] and it has been suggested that the dormant stem-like cells originate most probably from bipotential fetal liver epithelial progenitor cells.[28,32,33]

To explore the ability of fetal liver epithelial progenitor cells (FLEC) to proliferate and differentiate into hepatocytes (Hc) and bile duct epithelial cells (BDEC) and become incorporated into structural components of the liver lobule, we have used a cell transplantation approach to monitor the fate of these cells under different experimental conditions. Cells were transplanted into the liver of a syngeneic mutant Fischer 344 (F344) rat strain, deficient in the exopeptidase dipeptidyl-peptidase IV (DPPIV).[34] Because this enzyme is expressed in both Hc and BDEC, the genetically DPPIV-deficient F344 rat is an excellent model to follow the proliferation, lobular distribution, and morphological appearance of transplanted wild-type (DPPIV$^+$) hepatic cells.[34–38]

When normal liver is subjected to partial hepatectomy (PH), liver regeneration occurs through proliferation of pre-existing mature hepatocytes.[39–40] However, when rats are treated with retrorsine (Rs), this pyrrolizidine alkaloid is taken up by hepatocytes and metabolized to a bioactive form, which alkylates cellular DNA. This interferes with cell cycle progression and leads to inability of hepatocytes to proliferate.[41–43] In the present study, we used both normal and Rs-treated DPPIV$^-$ rats to follow the proliferation, lineage progression, and differentiation of transplanted ED 14 FLEC cells. This was evaluated by their morphological appearance, histochemical expression of DPPIV, and expression of markers specific for Hc or BDEC, using dual label immunohistochemistry and *in situ* hybridization (ISH). Our results demonstrate that FLEC are a heterogeneous population of cells with a single or dual phenotype (unipotential or bipotential) and that their lineage commitment and proliferative activity varies depending on the engraftment site and functional status of the host liver.

Materials and Methods

Materials

Rs and diaminobenzidine (DAB) were purchased from Sigma Chemical (St. Louis, MO). The Vectastain Elite ABC kit was from Vector Laboratories (Burlingame, CA). Rabbit anti-rat red blood cells IgG was from Rockland (Gilbertsville, PA). Radioactive ^{35}S-UTP (SJ603) and CK-19 antibody (RPN 1165) were obtained from Amersham Life Science Products (Arlington Heights, IL). CK-14 antibody (NCL-LL002) was from Novocastra Laboratories (United States distributor, Vector Laboratories). OV-6 monoclonal antibody was a generous gift from Dr. S. Sell (Albany Medical College, Albany, NY). Digoxigenin RNA labeling mix and anti-digoxigenin-POD, Fab fragments were from Boehringer Mannheim (Indianapolis, IN). Autoradiographic emulsion, type NBT2, was purchased from Eastman Kodak Company (New Haven, CT). Dr. N. Fausto (University of Washington, Seattle, WA) kindly provided plasmid BAF700, used for synthesis of the fetal form of AFP mRNA riboprobe.

Animals and Animal Treatment

Normal Fischer rats (F344) were purchased from Charles River Laboratories (Wilmington, MA). Mutant DPPIV-deficient (DPPIV$^-$) F344 rats were obtained from the Special Animal Core Facility of the Liver Research Center, Albert Einstein College of Medicine. All studies with animals were conducted under protocols approved by the Animal Care Use Committee of the Albert Einstein College of Medicine and were in accordance with National Institutes of Health guidelines. Rs treatment of the animals was as described previously.[38] In all experiments, cell transplantation recipients were female DPPIV$^-$ F344 rats. For experiments in which cell transplantation recipients were treated with retrorsine, rats weighing 90 to 100 g were given two intraperitoneal injections of Rs, 2 weeks apart, each of 30 mg/kg body weight. One month after the second injection, animals were subjected either to two-thirds PH and transplantation or to transplantation without PH. For stimulation of cell proliferation with triiodothyronine (T3), 1 day before cell transplantation and every week thereafter, animals received subcutaneous injections of T3 (Sigma) at a dose of 400 μg/100 g body wt, for a total of four T3 injections.

Isolation of FLEC

Fourteen-day FLEC from DPPIV$^+$ animals were isolated by a modification of the procedure of Sigal et al.[12] In brief, fetal livers were placed in ice-cold modified Hanks' balanced salt solution (HBSS; Gibco BRL, Grand Island, NY) without Ca^{2+}, containing 0.8 mmol/L $MgCl_2$ and 20 mmol/L HEPES, pH 7.4, and then triturated gently several times in modified HBSS containing 1 mmol/L EGTA. After centrifugation for 5 minutes at 450 $\times$ g at 4°C, the pellet was suspended in modified HBSS containing 0.2% collagenase, 0.07% DNase, and 1 mmol/L $CaCl_2$. Digestion was carried out for 15 minutes at 37°C, with gentle trituration every 5 minutes. The reaction was stopped by adding an equal volume of modified HBSS containing 1 mmol/L EGTA and fetal bovine serum at a final concentration of 10%. The cell suspension was filtered through a 45-μm nylon mesh, and cells were collected by centrifugation as above. The cell pellet was washed twice with modified HBSS/0.1% bovine serum albumin, centrifuged, and suspended at a concentration of 10^7 cells/ml. The cell suspension was subjected to two rounds of panning

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