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Short Communication

Evaluation of the Differentiation Potential of WB-F344 Rat Liver Epithelial Stem-Like Cells *in Vivo*

Differentiation to Hepatocytes after Transplantation into Dipeptidylpeptidase-IV-Deficient Rat Liver

William B. Coleman,* Karen D. McCullough,* Gwyn L. Esch,* Ronald A. Faris,† Douglas C. Hixson,‡ Gary J. Smith,* and Joe W. Grisham*

From the Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina, and the Departments of Pediatrics† Medical Oncology,‡ Rhode Island Hospital, Brown University, Providence, Rhode Island*

After intrahepatic transplantation into livers of adult syngeneic German-strain Fischer 344 rats that are deficient for the bile canalicular enzyme dipeptidyl peptidase IV (DPP-IV), cultured WB-F344 rat liver epithelial cells (without exogenous marker genes) integrate into hepatic plates and differentiate into hepatocyte-like cells that are morphologically and functionally indistinguishable from mature hepatocytes. In this model system, the differentiated progeny of transplanted WB-F344 cells are identified among the DPP-IV-negative host hepatocytes by their expression of bile canalicular DPP-IV enzyme activity. DPP-IV-positive hepatocyte-like cells also expressed other markers of hepatocytic differentiation, including albumin, transferrin, and α-1-antitrypsin, suggesting that the progeny of transplanted WB-F344 cells express a complete hepatocyte differentiation program. These results complement our previous studies indicating WB-F344 cells can serve as stem-like precursor cells for differentiated hepatocytes and strengthen the suggestion that WB-F344 rat liver epithelial cells represent the cultured counterpart of liver stem-like hepatocyte progenitor cells present in the normal adult rat liver. *(Am J Pathol 1997, 151:353–359)*

Although the existence of a stem-like progenitor cell compartment in the adult mammalian liver continues to be debated, considerable evidence has accumulated that supports the existence of such a cell type in the adult rodent liver.[1–3] Much of this evidence comes 1) from studies on the re-establishment of epithelial lineages by poorly differentiated oval cells that proliferate in various models of liver pathology[4–8] and 2) from studies on the isolation and culture of clonogenic epithelial cells derived from adult livers[9–11] that express markers of liver cell differentiation under the appropriate conditions *in vitro*[12–14] or after transplantation to appropriate sites *in vivo*.[15,16] Our studies have focused on the possibility that phenotypically simple epithelial cells isolated from the livers of adult rats represent the cultured counterpart of hepatocytic progenitor cells.[16] We have previously reported the establishment of the diploid WB-F344 rat liver epithelial cell line from the liver of an adult Fischer 344 rat under conditions that exclude mature hepatocytes as the cell of origin.[17] Although WB-F344 cells share some phenotypic properties with both hepatocytes and biliary epithelial cells, their overall phenotype differs distinctively from either of these differentiated cell types.[17,18] Insight into the differentiation potential of these cells emerged from examination of the histologies of tumors that arose in syngeneic animals after transplantation of neoplastically transformed derivatives of WB-F344.[18,19] These tumors included well differentiated hepatocellular carcinomas, adenocarcinomas (biliary and intestinal types), and hepatoblastomas (containing cartilage and osteoid),[20] demonstrating the capacity of these cells to differentiate

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Address reprint requests to Dr. William B. Coleman, Department of Pathology and Laboratory Medicine, CB 7525, University of North Carolina School of Medicine, Chapel Hill, NC 27599.

along both hepatic epithelial cell (and other) lineages at least when transformed. After transplantation of normal WB-F344 cells into the interscapular fat pads of syngeneic Fischer 344 rats, small clusters of cells morphologically resembling hepatocytes were identified,[16] suggesting that the transplanted WB-F344 cells could acquire characteristics of hepatocytes *in vivo*. However, to demonstrate a precursor-product relationship between transplanted cells and differentiated cell types of the liver, methods had to be established that would allow the definitive identification of the progeny of the transplanted cells among host cells in the adult liver. In our initial studies, WB-F344 rat liver epithelial cells were genetically modified by infection with the CRE BAG2 retrovirus, which encodes the *Escherichia coli* β-galactosidase gene and the Tn5 neomycin resistance gene.[21] The resulting BAG2-WB cells were transplanted into the livers of adult Fischer 344 rats, and the livers of these rats were examined for the presence of β-galactosidase-positive cells at various times after transplantation. In these studies, β-galactosidase-positive hepatocyte-like cells were detected in the hepatic plates of recipient rats among the host hepatocytes.[15] The size and morphological appearance of the β-galactosidase-positive cells was indistinguishable from that of host hepatocytes, and these cells were observed at all time points examined, up to greater than 1 year after transplantation.[16] Subsequent studies demonstrated that the β-galactosidase-positive hepatocyte-like cells express functional differentiation typical of hepatocytes, including expression of albumin, transferrin, α-1-antitrypsin and tyrosine aminotransferase.[16]

Although the previous studies provided evidence suggesting that transplanted WB-F344 cells incorporate into host hepatic plates, morphologically and functionally differentiate into hepatocytes, and remain a stable component of the hepatic parenchyma over long periods of time, the use of a retrovirally transfected β-galactosidase marker gene poses several potential problems with respect to lineage determination in liver. These potential problems include the possibility of production of infectious retrovirus by transplanted cells and subsequent infection of host hepatocytes and the ambiguity of marker enzyme systems (such as β-galactosidase) due to endogenous activity in host hepatocytes. These potential problems were stringently controlled in our previous studies.[15,16] Nonetheless, the present study was undertaken to examine the fate of transplanted WB-F344 cells in a transplantation model that did not require previous genetic modification of the cells and that was not hindered by staining of endogenous hepatocytes in the histochemical detection of transplanted cells. Wild-type WB-F344 cells (without exogenous marker genes) were transplanted into the livers of German-strain Fischer 344 rats that are deficient for dipeptidylpeptidase IV (DPP-IV) enzyme activity.[22,23] DPP-IV is an ectopeptidase enzyme that cleaves amino-terminal dipeptides from polypeptides for which the penultimate residue is proline.[24] It is expressed in a number of tissues in normal rats, including liver, where its expression is limited to the bile canalicular domain of the hepatocytes.[25,26] Examination of liver sections reacted histochemically for DPP-IV enzyme activity revealed DPP-IV-positive hepatocyte-like cells among the DPP-IV-negative host hepatocytes at various intervals after transplantation. Histochemical staining for bile canalicular ATPase activity demonstrated the continuity of bile canaliculi between host hepatocytes and the differentiated progeny of the transplanted WB-F344 cells. Furthermore, immunohistochemical detection of albumin, transferrin, and α-1-antitrypsin in DPP-IV-positive cells suggested the expression of a complete hepatocyte differentiation program in these cells. These results show that wild-type diploid WB-F344 rat liver epithelial cells incorporate into hepatic plates and morphologically and functionally differentiate after transplantation into the livers of syngeneic rat hosts. The results from these transplantation studies utilizing the DPP-IV-deficient rat model strengthens our previous suggestion that WB-F344 rat liver epithelial cells represent the cultured counterpart of the liver stem-like hepatocyte progenitor cell present in the normal adult rat liver.

Materials and Methods

Animals and Cell Lines

German-strain Fischer 344 rats were obtained from a colony maintained at Brown University. American-strain Fischer 344 rats were obtained from Charles River Laboratories (Wilmington, DE). The normal diploid nonhepatocytic WB-F344 rat liver epithelial cell line was isolated from the liver of an adult American-strain Fischer 344 rat by primary cloning.[17] WB-F344 cells were used at passages 7 to 10 and were routinely cultured in Richter's improved minimal essential medium with zinc option, supplemented with insulin (Irvine Scientific, Santa Ana, CA), as described.[27]

Cell Transplantation

WB-F344 cells intended for transplantation were harvested from cell culture by trypsinization, washed in Thilly's buffered salt solution to remove traces of serum, resuspended in Thilly's buffered salt solution (to 5×10^6 cells/0.2 ml), and held on ice until used. Cells in a volume of 200 μl (5×10^6 cells) were injected with a tuberculin syringe directly into the median liver lobe of young adult German-strain Fischer 344 rats (150 to 200 g) anesthetized lightly with ether. When required, hemostasis was maintained by the application of Gelfoam sponge to the site of injection. No loss of cell viability was evident upon culture of cells after their maintenance on ice during transplantation procedures (1 to 2 hours). All animal studies were carried out in accordance with federal and institutional guidelines put forth by the National Institutes of Health and the Institutional Animal Care and Use Committee of the University of North Carolina.

Enzyme Histochemistry

DPP-IV enzyme activity was detected using the histochemical staining reaction described by Lodja,[28] as

modified by M. Dabeva and D. A. Shafritz (personal communication). Briefly, liver cryosections were fixed in ice-cold acetone and then air dried. Fixed sections were washed in ice-cold 95% ethanol and air dried before the histochemical staining reaction. The DPP-IV enzyme substrate consisted of 0.5 mg/ml Gly-Pro-methoxy-β-naphthylamide (Sigma Chemical Co., St. Louis, MO) and 1 mg/ml Fast Blue BB, in a buffer containing 100 mmol/L Tris/maleate (pH 6.5) and 100 mmol/L sodium chloride. Staining was accomplished at 37°C for 30 to 60 minutes. All staining reactions included DPP-IV-positive control liver sections and DPP-IV-negative control liver sections to insure the quality of the enzyme substrate and histochemical reaction. Positive and negative control tissues were obtained from unmanipulated American-strain Fischer 344 rats and German-strain Fischer 344 rats, respectively.

Bile canalicular ATPase was detected using the histochemical staining reaction described by Wachstein and Meisel.[29] Briefly, liver cryosections were incubated for 30 minutes at 37°C in a substrate consisting of 0.5 mg/ml ATP (sodium salt), 100 mmol/L magnesium sulfate, and 2% lead (II) nitrate, in 200 mmol/L Tris/maleate buffer (pH 7.2). ATPase activity was visualized by briefly incubating sections in a 0.22% solution of ammonium sulfide.

Co-localization of DPP-IV and bile canalicular ATPase was accomplished by first performing the DPP-IV staining reaction, washing the reacted tissue sections in phosphate-buffered saline, and then immediately processing the tissue for ATPase activity. All tissue sections were counterstained in 0.1% methyl green and mounted in glycerol.

Immunohistochemistry

Immunohistochemical staining was performed on liver tissue sections after the histochemical detection of DPP-IV enzyme activity. Antibodies directed against rat albumin (Cappel/Organon Teknika, Durham, NC), rat transferrin (Cappel/Organon Teknika), and human α-1-antitrypsin (Sigma Chemical Co.) were used in these studies. Tissue sections were incubated in a blocking buffer consisting of 10% bovine serum, 150 mmol/L sodium chloride, and 100 mmol/L sodium phosphate buffer (pH 7.4) before incubation with primary antibodies and, subsequently, horseradish-peroxidase-conjugated secondary antibodies, diluted in the same buffer. Color development was accomplished with diaminobenzidine/NiCl horseradish peroxidase enzyme histochemistry substrate (Vector Laboratories, Burlingame, CA). Control immunostaining reactions were performed without inclusion of a primary antibody or using conditioned medium from mouse myeloma cells that do not produce a specific IgG.

Results

Identification of DPP-IV-Positive Hepatocytes in Hepatic Plates of DPP-IV-Deficient Rats

Histochemical staining of liver cryosections from DPP-IV-deficient German-strain Fischer 344 control rats revealed no DPP-IV-positive bile canaliculi between host hepatocytes (Figure 1). Nonetheless, intact bile canaliculi could be demonstrated in German-strain Fischer 344 rat livers through histochemical staining for bile canalicular ATPase activity (Figure 1). Liver cryosections from American-strain Fischer 344 control rats showed abundantly staining DPP-IV-positive bile canaliculi that were also positive for bile canalicular ATPase activity (Figure 1).

Wild-type WB-F344 cells were transplanted into the livers of DPP-IV-deficient syngeneic rats to examine the fate of these cells in a transplantation model that does not depend upon the use of exogenous marker enzymes. Cultured WB-F344 cells do not express histochemically detectable DPP-IV enzyme activity but do express DPP-IV mRNA that is detectable by reverse transcriptase polymerase chain reaction (unpublished results). The detection of DPP-IV mRNA transcripts in cultured WB-F344 cells suggested that DPP-IV enzyme activity would be useful in the detection of transplanted cells. Rats were sacrificed at various intervals after transplantation of WB-F344 cells (30 to 60 days), and livers were examined for the presence of DPP-IV-positive hepatocytes. This analysis revealed DPP-IV-positive hepatocyte-like cells among the DPP-IV-negative host hepatocytes in all rats receiving transplanted WB-F344 cells (Figure 2). In some cases, individual DPP-IV-positive hepatocytes were identified, but in many instances, small groups of DPP-IV-positive cells were observed (Figure 3). The occurrence of clusters of DPP-IV-positive hepatocytes suggests the possibility that transplanted WB-F344 cells proliferated before their differentiation into hepatocyte-like cells within the host livers. However, we did not directly examine this possibility in the current study. The DPP-IV-positive hepatocytes in hepatic plates were comparable to adjacent host hepatocytes in size and morphology and contained large spherical nuclei (Figures 2 and 3). Close physical contact between the differentiated progeny of the transplanted cells and host hepatocytes was verified through the co-localization of DPP-IV staining and ATPase staining in hybrid bile canaliculi (Figure 2). In addition, the localization of DPP-IV staining to bile canaliculi shows that the surface membranes of differentiating WB-F344 cells have acquired the polarization characteristic of fully differentiated hepatocytes.

Expression of Hepatocyte-Specific Proteins in DPP-IV-Positive Hepatocytes

The functional differentiation of transplanted WB-F344 cells was evaluated by the immunohistochemical detection of hepatocyte-specific protein expression in DPP-IV-positive hepatocytes. DPP-IV-positive hepatocytes in hepatic plates were positive for expression of albumin (Figure 2), transferrin (not shown), and α-1-antitrypsin (not shown), consistent with our previous observations using retrovirally tagged WB-F344 cells.[16] Antibodies to each of these proteins decorated the majority of both DPP-IV-positive (progeny of transplanted WB-F344 cells) and DPP-IV-negative (host) hepatocytes. Control immunostaining reactions (minus primary antibody or contain-

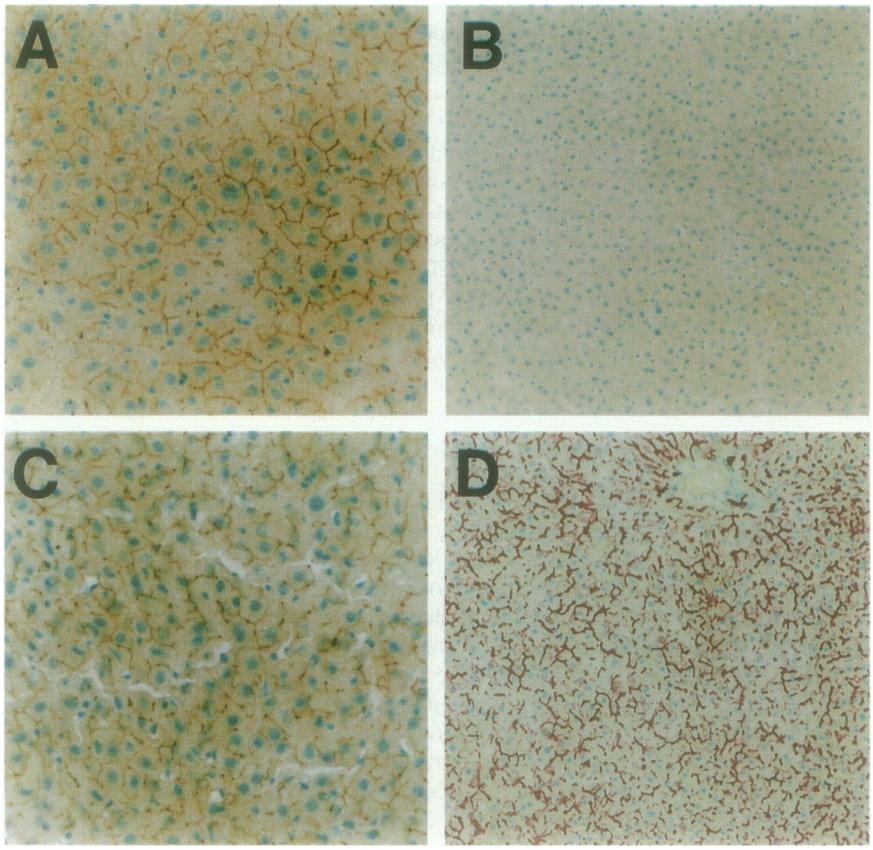

Figure 1. Expression of bile canalicular ATPase and DPP-IV enzyme activities in liver cryosections from American-strain Fischer 344 rats and German-strain Fischer 344 rats. **A** and **B:** Liver cryosections from German-strain Fischer 344 rats. **C** and **D:** Liver cryosections from American-strain Fischer 344 rats. **A** and **C** show histochemical staining for bile canalicular ATPase (brown staining). **B** and **D** show histochemical staining for DPP-IV enzyme activity (red staining). Magnification, ×170 (**A** and **C**) and ×80 (**B** and **D**).

ing nonspecific IgG) did not result in the deposition of discernible horseradish peroxidase reaction product on liver sections (not shown). These results confirm our earlier observations that transplanted WB-F344 cells insert into hepatic plates and morphologically and functionally differentiate after transplantation into the microenvironment of the hepatic parenchyma.

Discussion

The current study provides direct evidence that WB-F344 cells can integrate into hepatic plates and undergo hepatocytic differentiation after transplantation into the adult rat liver in a transplantation model that does not require previous genetic manipulation of the cells to introduce marker enzymes. Therefore, this transplantation model does not suffer from the potential problems associated with the use of retrovirally transfected marker enzymes in liver: 1) the potential for production of infectious retrovirus and subsequent infection of host hepatocytes and 2) the ambiguity of marker enzymes systems (such as β-galactosidase) due to endogenous activity in host hepatocytes. Nonetheless, the results of the present study using the DPP-IV-deficient rat model are absolutely consistent

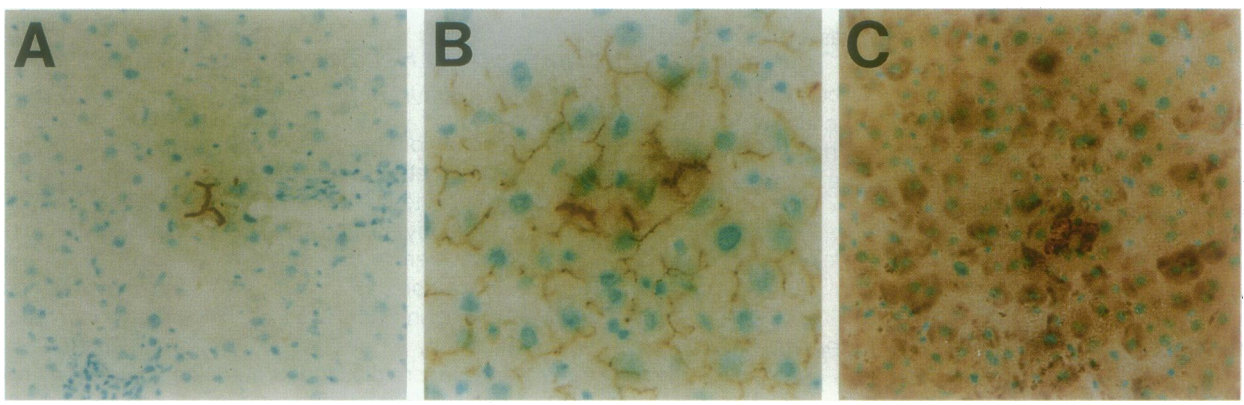

Figure 2. Co-localization of DPP-IV enzyme activity and bile canalicular ATPase or hepatocyte-specific markers. **A:** Detection of DPP-IV-positive hepatocytes (red staining) among host DPP-IV-deficient hepatocytes. **B:** Co-localization of DPP-IV (red staining) and bile canalicular ATPase (brown staining). **C:** Albumin immunostaining of DPP-IV-positive hepatocytes. Magnification, ×170 (**A** and **C**) and ×335 (**B** and **D**).

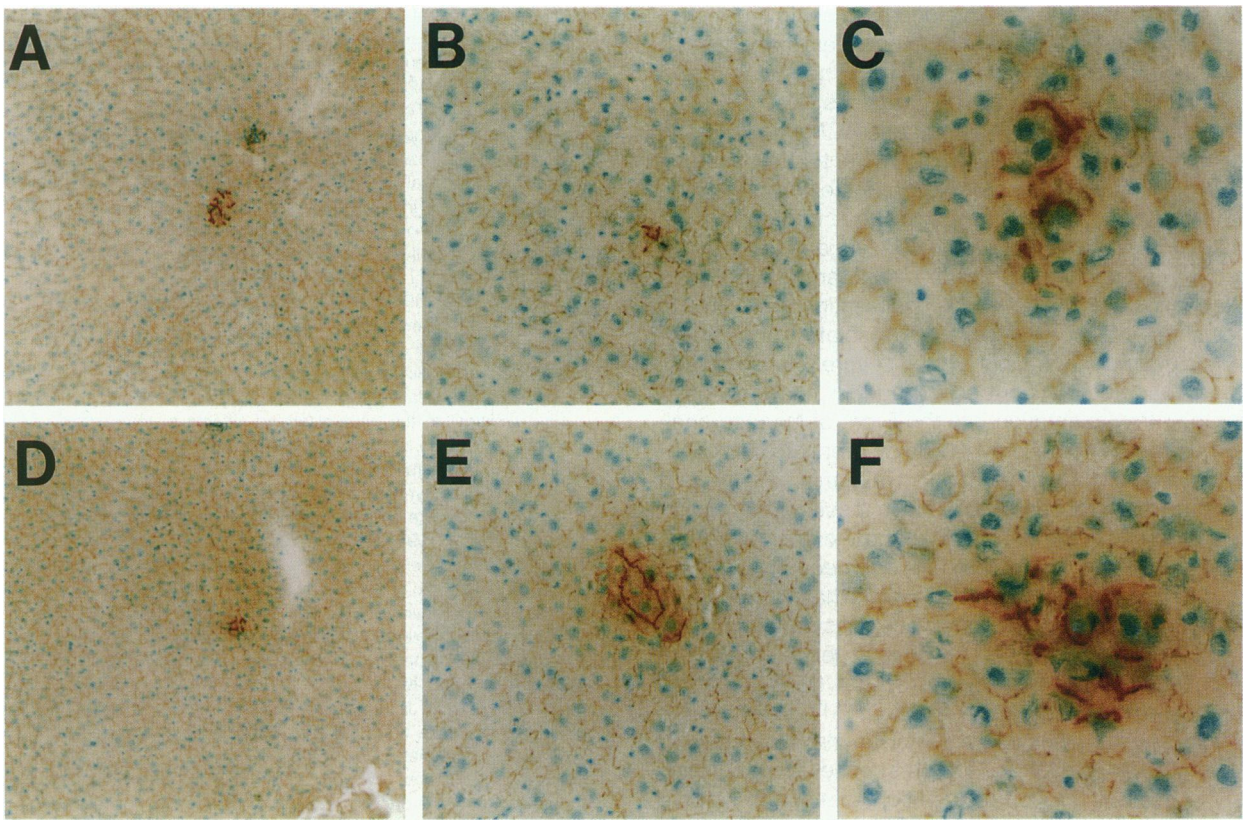

Figure 3. Representative liver cryosections from DPP-IV-deficient rats demonstrating DPP-IV-positive differentiated progeny of transplanted WB-F344 rat liver epithelial stem-like cells. Each panel shows a representative liver section that has been histochemically stained for DPP-IV enzyme activity (red staining) and bile canalicular ATPase (brown staining). **A** to **C**: Liver sections from a rat 30 days after transplantation of WB-F344 cells. **D** to **F**: Liver sections from a rat 60 days after transplantation of cells. Magnification, ×80 (**A** and **D**), ×170 (**B** and **E**), and ×335 (**C** and **F**).

with our previous studies using β-galactosidase as a marker enzyme that suggested the ability of WB-F344 cells to give rise to hepatocyte progeny.[15,16] After transplantation into the livers of DPP-IV-deficient rats, WB-F344 cells integrate into hepatic plates among host hepatocytes and undergo hepatocytic differentiation. The resulting differentiated progeny are morphologically indistinguishable from host hepatocytes based upon location in the hepatic plates, cell size, and the size and morphology of their nuclei. Functionally, the progeny of the transplanted WB-F344 cells express a number of proteins characteristic of mature hepatocytes, including albumin, transferrin, and α-1-antitrypsin, in addition to DPP-IV polarized to the plasma membrane surfaces of bile canaliculi. Furthermore, the bile canaliculi of hepatocyte progeny of WB-F344 cells are connected with the canaliculi of the host hepatocytes. These results combine to suggest strongly that, after transplantation into the livers of adult rats, phenotypically simple undifferentiated WB-F344 cells can give rise to differentiated progeny that express a complete hepatocyte differentiation program.

Multiple lines of evidence now support the hypothesis that WB-F344 rat liver epithelial cells represent the cultured counterpart of the stem-like hepatocyte progenitor cells of the adult rat liver. However, the observation that WB-F344 rat liver epithelial cells can differentiate into hepatocytes after transplantation into the livers of syngeneic rats may not represent a generalized phenomenon applicable to all cultured rat liver epithelial cells. Gupta et al[30] have transplanted differentiated hepatocytes and FNRL rat liver epithelial cells[31] into the livers of DPP-IV-deficient rats. Although the authors routinely detected transplanted hepatocytes that express DPP-IV activity in the parenchyma of recipient rats, no DPP-IV-positive cells were detected in the livers of rats receiving FNRL cells.[30] This observation suggests that FNRL rat liver epithelial cells either did not migrate into hepatic plates or did not differentiate after their possible incorporation into the hepatic microenvironment and raises the possibility that not all propagable rat liver epithelial cell lines possess identical stem-like characteristics. Several lines of epithelial cells have been established from the livers of normal adult rats,[3] and comparison of their phenotypic traits suggests a remarkable degree of correspondence among them.[2] Unfortunately, other than the FNRL line, WB-F344 cells represent the only one of these several rat liver epithelial cell lines derived from normal liver that has been evaluated for its ability to differentiate into hepatocytes after transplantation into the adult rat liver. However, some studies have evaluated the potential for transplantation-induced hepatocytic differentiation of oval cells isolated from livers in which they have proliferated after severe liver damage.[32–34] Oval cells are phenotypically simple epithelial cells that may be related to liver stem-like cells.[1–3,35] In other studies, enriched oval cell preparations obtained from the pathological livers of ACI/

Vsp rats that had been maintained on a choline-deficient diet containing 2-acetylaminofluorene were transplanted into the remnant livers of partially hepatectomized LExACI F1 progeny.[36] Transplanted cells were identified by their immunoreactivity with LE anti-ACI antiserum and their lack of immunoreactivity to ACI anti-LE antiserum. The progeny of the transplanted oval cells morphologically resembled hepatocytes, and these hepatocyte-like cells expressed the hepatocyte-specific antigens recognized by monoclonal antibodies H.2 and HBD.1 and lacked expression of the oval cell antigen OC.2.[36] In other studies, liver oval cells were isolated from Long-Evans cinnamon rats[37] that exhibit severe liver pathology related to defective copper storage in hepatocytes.[38] After their transplantation into the livers of Nagase analbuminemic rats,[39] these oval cells differentiate into hepatocytes and initiate production of albumin, resulting in increased levels of serum albumin in these mutant rats.[37] In similar studies, the possibility that pancreatic oval cells can serve as liver progenitor cells has been investigated using proliferating pancreatic oval cells that were isolated from rats maintained on a copper-deficient diet[40] and introduced into the livers of DPP-IV-deficient rats via transplantation into the spleen.[41] After transplantation, hepatocyte-like cells that express DPP-IV activity were observed in the livers of recipient animals, suggesting that oval cells proliferating in response to pancreatic injury caused by the copper-deficient diet can also serve as hepatocyte progenitor cells.[41] The results from the current investigation combine with these studies from the literature to indicate that some lines of simple epithelial cells isolated from the livers of normal rats (WB-F344) and some lines of oval cells isolated from pathological liver or pancreas can give rise to differentiated hepatocyte progeny after their transplantation into the adult rat liver. Collectively, these observations support the idea that oval cells are related to normal stem-like cells of the liver[1–3,38] and the proposal that there may be a common stem-like progenitor cell for several tissues of the gastrointestinal tract.[42]

References

1. Fausto N: Liver stem cells. The Liver: Biology and Pathobiology. Edited by IM Arias, JL Boyer, N Fausto, WB Jakoby, D Schachter, DA Shafritz. New York, Raven Press, 1994, pp 1501–1518
2. Grisham JW, Thorgeirsson SS: Liver stem cells. Stem Cells. Edited by CS Potten. New York, Academic Press, 1997, pp 233–282
3. Coleman WB, Grisham JW: Epithelial stem-like cells of the rodent liver. Liver Growth and Repair: From Basic Science to Clinical Practice. Edited by AJ Strain, AM Diehl. New York, Chapman and Hall, 1997 (in press)
4. Evarts RP, Nagy P, Marsden E, Thorgeirsson SS: A precursor-product relationship exists between oval cells and hepatocytes in rat liver. Carcinogenesis 1987, 8:1737–1740
5. Evarts RP, Nagy P, Nakatsukasa H, Marsden E, Thorgeirsson SS: In vivo differentiation of rat liver oval cells into hepatocytes. Cancer Res 1989, 49:1541–1547
6. Lemire JM, Shiojiri N, Fausto N: Oval cell proliferation and the origin of small hepatocytes in liver injury induced by D-galactosamine. Am J Pathol 1991, 139:535–552
7. Dabeva MD, Shafritz DA: Activation, proliferation, and differentiation of progenitor cells into hepatocytes in the D-galactosamine model of liver regeneration. Am J Pathol 1993, 143:1606–1620
8. Golding M, Sarraf CE, Lalani E-N, Anilkumar TV, Edwards RJ, Nagy P, Thorgeirsson SS, Alison MR: Oval cell differentiation into hepatocytes in the acetylaminofluorene-treated regenerating rat liver. Hepatology 1995, 22:1243–1253
9. Coon HG: Clonal culture of differentiated cells from mammals: rat liver cell culture. Carnegie Inst Washington Yearbook 1968, 68:419–421
10. Grisham JW, Thal SB, Nagel A: Cellular derivation of continuously cultured epithelial cells from normal rat liver. Gene Expression and Carcinogenesis in Cultured Liver. Edited by LE Gerschenson, EB Thompson. New York, Academic Press, 1975, pp 1–23
11. Grisham JW: Cell types in long-term propagable cultures of rat liver. Ann NY Acad Sci 1980, 349:128–137
12. Nagy P, Evarts RP, McMahan JB, Thorgeirsson SS: Role of TGF-β in normal differentiation and oncogenesis in rat liver. Mol Carcinog 1989, 2:345–354
13. Chapekar MS, Huggett AC, Cheng CC, Hampton LL, Lin K-H, Thorgeirsson SS: Isolation and characterization of a rat liver epithelial cell line resistant to the antiproliferative effects of transforming growth factor β (type 1). Cancer Res 1990, 50:3600–3604
14. Coleman WB, Smith GJ, Grisham JW: Development of dexamethasone-inducible tyrosine aminotransferase activity in WB-F344 rat liver epithelial stemlike cells cultured in the presence of sodium butyrate. J Cell Physiol 1994, 161:463–469
15. Coleman WB, Wennerberg AE, Smith GJ, Grisham JW: Regulation of diploid and some aneuploid rat liver epithelial (stem-like) cells by the hepatic microenvironment. Am J Pathol 1993, 142:1373–1382
16. Grisham JW, Coleman WB, Smith GJ: Isolation, culture, and transplantation of rat hepatocytic precursor (stem-like) cells. Proc Soc Exp Biol Med 1993, 204:270–279
17. Tsao M-S, Smith JD, Nelson KG, Grisham JW: A diploid epithelial cell line from normal adult rat liver with phenotypic properties of 'oval' cells. Exp Cell Res 1984, 154:38–52
18. Tsao M-S, Grisham JW, Nelson KG, Smith JD: Phenotypic and karyotypic changes induced in cultured rat hepatic epithelial cells that express the "oval" cell phenotype by exposure to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Am J Pathol 1985, 118:306–315
19. Tsao M-S, Grisham JW, Chou BB, Smith JD: Clonal isolation of populations of γ-glutamyl transpeptidase-positive and -negative cells from rat liver epithelial cells chemically transformed in vitro. Cancer Res 1985, 45:5134–5138
20. Tsao M-S, Grisham JW: Hepatocarcinomas, cholangiocarcinomas, and hepatoblastomas produced by chemically transformed cultured rat liver epithelial cells: a light- and electron-microscopic analysis. Am J Pathol 1987, 127:168–181
21. Price J, Turner D, Cepko C: Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. Proc Natl Acad Sci USA 1987, 84:156–160
22. Watanabe Y, Kojima T, Fujimoto Y: Deficiency of membrane-bound dipeptidyl aminopeptidase IV in a certain rat strain. Experientia 1987, 43:400–401
23. Thompson NL, Hixson DC, Callanan H, Panzica M, Flanagan D, Faris RA, Hong W, Hartel-Schenk S, Doyle D: A Fischer rat substrain deficient in dipeptidyl peptidase IV activity makes normal steady-state RNA levels and an altered protein. Biochem J 1991, 273:497–502
24. Walter R, Simmons, WH, Yoshimoto T: Proline-specific endo- and ectopeptidases. Mol Cell Biochem 1980, 30:111–127
25. Hartel S, Gossrau R, Hanski C, Reutter W: Dipeptidyl peptidase (DPP) IV in rat organs: comparison of immunohistochemistry and activity histochemistry. Histochemistry 1988, 89:151–161
26. Fukui Y, Yamamoto A, Kyoden T, Kato K, Tashiro Y: Quantitative immunogold localization of dipeptidylpeptidase IV (DPP-IV) in rat liver cells. Cell Struct Funct 1990, 15:117–125
27. Lee LW, Raymond VW, Tsao M-S, Lee DC, Earp HS, Grisham JW: Clonal cosegregation of tumorigenicity with overexpression of c-*myc* and transforming growth factor α genes in chemically transformed rat liver epithelial cells. Cancer Res 1991, 51:5238–5244
28. Lodja Z: Studies on dipeptidyl(amino)peptidase IV (glycyl-proline naphthylamidase). Histochemistry 1979, 59:153–166
29. Wachstein M, Meisel E: Histochemistry of hepatic phosphatases at a physiological pH. Am J Clin Pathol 1955, 27:13–23
30. Gupta S, Rajvanshi P, Lee C-D: Integration of transplanted hepatocytes into host liver plates demonstrated with dipeptidyl peptidase IV-deficient rats. Proc Natl Acad Sci USA 1995, 92:5860–5864

31. Herring AS, Raychaudhuri R, Kelley SP, Iype PT: Repeated establishment of diploid epithelial cell cultures from normal and partially hepatectomized rats. In Vitro 1983, 19:576–588
32. Dabeva MD, Alpini G, Hurston E, Shafritz DA: Models for hepatic progenitor cell activation. Proc Soc Exp Biol Med 1993, 204:242–252
33. Thorgeirsson SS: Stem cells and hepatocarcinogenesis. Liver Regeneration and Carcinogenesis. Edited by R Jirtle. New York, Academic Press, 1995, pp 99–112
34. Alison MR, Golding M, Sarraf CE, Edwards RJ, Lalani E-N: Liver damage in the rat induces hepatocyte stem cells from biliary epithelial cells. Gastroenterology 1996, 110:1182–1190
35. Golding M, Sarraf C, Lalani E-N, Alison MR: Reactive biliary epithelium: the product of a pluripotential stem cell compartment? Hum Pathol 1996, 27:872–884
36. Faris RA, Hixson DC: Selective proliferation of chemically altered rat liver epithelial cells following hepatic transplantation. Transplantation 1989, 48:87–92
37. Yasui O, Miura N, Terada K, Kawada Y, Koyama K, Sugiyama T: Isolation of oval cells from Long-Evans cinnamon rats and their transformation into hepatocytes *in vivo* in the rat liver. Hepatology 1997, 25:329–334
38. Li Y, Togashi Y, Sato S, Emoto T, Kang J-H, Takeichi N, Kobayashi H, Kojima Y, Une Y, Uchino J: Spontaneous hepatic copper accumulation in Long-Evans cinnamon rats with hereditary hepatitis. J Clin Invest 1991, 87:1858–1861
39. Nagase S, Shimamura K, Shumiya S: Albumin-deficient rat mutant. Science 1979, 205:590–591
40. Rao MS, Dwivedi RS, Yeldandi AV, Subbarao V, Tan X, Usman MI, Thangada S, Nemali MR, Kumar S, Scarpelli DG, Reddy JK: Role of periductal and ductular epithelial cells of the adult rat pancreas in pancreatic hepatocyte lineage: a change in the differentiation commitment. Am J Pathol 1989, 134:1069–1086
41. Hwang SG, Dabeva M, Nehra V, Gupta S, Shafritz DA: The potential of pancreatic duct-like cells in rats as facultative hepatocyte progenitors. Hepatology 1994, 20:225a
42. Bisgaard HC, Thorgeirsson SS: Evidence for a common cell of origin for primitive epithelial cells isolated from liver and pancreas. J Cell Physiol 1991, 147:333–343