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Commentary

Hepatic Stem Cells

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The existence of hepatic stem cells has been, and no doubt will continue to be, a matter of considerable controversy. This controversy is partly fueled by the fact that cell turnover in the liver is very slow and the two major types of hepatic epithelial cells, hepatocytes and biliary epithelia, are capable of proliferation and can, at least in a healthy liver, meet replacement demands of cellular loss from these two differentiated populations. The best example of the capacity of adult hepatocytes and bile epithelial cells to proliferate is seen after partial hepatectomy in rats and mice, in which the compensatory hyperplasia of these cells in the remaining lobes restore the liver mass. The increased use and success of liver transplantation in clinical medicine have shown that these animal models correctly reflect the capacity of the human liver to regenerate.[1] What then is the evidence that there exists a stem cell compartment in the liver? The existence of hepatic stem cells was first postulated by Wilson and Leduc[2] in 1958 based on experiments involving liver regeneration in the mouse after chronic injury induced with a methionine-rich basal diet mixed with an equal amount of bentonite. The authors concluded that "prolonged and severe injury to the liver may make direct restoration by division of pre-existing parenchymal cells impossible, and that, when this occurs, the new parenchyma is derived from the indifferent cholangiole cells." The major support for the existence of hepatic stem cells has, perhaps not surprisingly in light of the earlier work by Wilson and Leduc, come from extensive studies of hepatic carcinogenesis (for reviews, see refs. 3–5). The earliest cellular response in animals exposed to a variety of chemical hepatocarcinogens is the proliferation of small periportal cells with scant cytoplasm and ovoid nuclei that have been termed oval cells.[6–9] The existence of similar cells has been reported in human liver.[10,11] These oval cells are thought to represent a progeny of the hepatic stem cell compartment, and, at least in some instances, to be a precursor for the hepatic tumors.[4,5,12] The precise anatomical location of the hepatic stem cell compartment in normal liver is still unclear, but present data suggest that the terminal ductule cells connecting the canals of Hering with the bile canaliculi and/or a distinct population of periductal cells constitute the hepatic stem cell compartment.[2–5] Recent studies in the rat liver have shown that oval cells are capable of differentiating, in addition to bile epithelium, into at least two lineages *in vivo*, including hepatocytes[8] and intestinal type epithelium.[13] Furthermore, isolated oval cells in culture can be induced to differentiate into both hepatocyte-like cells and biliary type cells.[14–16] These data and other results not reviewed here have supported the notion that oval cells have similar lineage options as displayed by the hepatoblasts in early stages of liver development. As such, the oval cells can be regarded as "bipotential precursors" for the two hepatic parenchymal cell lineages. However, oval cells may, particularly when the hepatic microenvironment is drastically disrupted, exhibit a capacity to differentiate into non-hepatic lineages.

In addition to the *in vivo* data mentioned above, significant support for the existence of the hepatic stem cell has come from results obtained in studies on hepatic cell cultures. Several research groups have been able to isolate and establish long-term cultures of small, morphologically and functionally simple epithelial cells by enzymatic perfusion of fetal and adult rat liver and utilizing culture conditions that exclude differentiated hepatocytes (for review, see refs. 17–20). These rat liver–derived epithelial (RLE) cells share some phenotypic properties with both bile duct epithelial cells and hepatocytes but are phenotypically much closer to some of the oval cell lines.[14,21,22]

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The notion that oval cells are descendants of hepatic stem cells suggests that the RLE cells, because of the phenotypic similarities to oval cells, are also progeny from such a stem cell compartment. This suggestion is supported by data obtained from extensive use of RLE cells for *in vitro* transformation studies with both chemical carcinogens and oncogenes. These studies have demonstrated various transformation properties of RLE cells.[20,23,24] Most importantly, when the transformed RLE cells are transplanted into either syngeneic rats or nude mice, a spectrum of tumors are encountered that includes highly differentiated hepatocellular carcinomas, hepatoblastomas, cholangiocarcinoma, and mixed epithelial-mesenchymal tumors,[23] which suggests a blastic nature of the RLE cells and demonstrates the potential of the cells to differentiate via both the hepatocytic and biliary lineages. However, no *in vitro* culture system exists that conclusively demonstrates differentiation of the RLE cells into either hepatocytes or bile epithelial cells.

A notable contribution clarifying the nature of the RLE cells and the potential role of hepatic stem cells in liver biology is provided by Coleman and colleagues in this issue of *The American Journal of Pathology.* Coleman and his associates have succeeded in demonstrating, after intrahepatic transplantation of RLE cells carrying the *Escherichia coli* β-galactosidase reporter gene, that the RLE cells integrate into hepatic plates and acquire the size and nuclear structure of mature hepatocytes. In addition, and of no less importance, Coleman and associates show that intrahepatic transplantation of an aneuploid, neoplastically transformed derivative of the RLE cells, which produces aggressively growing tumor when transplanted subcutaneously, does not produce tumor in the liver but integrates into the hepatic plates and morphologically differentiates.

The results from this study raise several important issues for basic hepatic biology as well as clinical hepatology. Although differentiated hepatocytes isolated from enzymatically prepared suspensions of murine liver cells have been shown to integrate into the hepatic plate after transplantation into liver of congenic animals,[25,26] this is the first demonstration that primitive or "stem-like" RLE cells upon intrahepatic transplantation integrate into the hepatic plates and differentiate, based on morphological criteria, into hepatocytes. These results taken together with the large body of data, some of which have been cited above, on the blastic nature of the RLE cells, strongly support both the existence of a hepatic stem cell compartment and the notion that RLE cells are derived from this compartment. Furthermore, the present study also reinforces the notion that both oval cells and RLE cells represent a progeny from the hepatic stem cell compartment.

The demonstration that RLE cells can differentiate along the hepatocytic pathway in normal liver upon intrahepatic transplantation suggests that both humoral and extracellular matrix factors exist in normal adult liver that support this differentiation. Regeneration of the liver via the stem cell compartment occurs primarily under conditions in which the capacity of the adult hepatocytes to replicate is compromised. It would therefore be of interest to examine whether the intrahepatic transplantation of RLE cells under those conditions would lead to more efficient hepatocytic differentiation and possibly utilization of other lineage options. The critical issue for future research in this area is the identification of the hepatic factors responsible for inducing differentiation of the RLE cells.

The observation that the hepatic microenvironment can, at least in some instances, suppress the capacity of neoplastically transformed RLE cells to form tumors in the liver by inducing hepatocytic differentiation offers a unique opportunity to define both humoral and genetic factors involved in hepatic oncogenesis, in particular the factors responsible for malignant conversion. Also, it seems likely that the same hepatic factors involved in the differentiation of normal RLE cells are involved in suppressing the neoplastically transformed RLE cells. With the establishment of this experimental system, we can now look forward to exciting new insights into the mechanisms of hepatic oncogenesis.

The implications of the results presented by Coleman and his associates for clinical hepatology are obvious and need not to be extensively outlined here. It is sufficient to note that increased understanding of the cellular and molecular biology of the hepatic stem cell compartment will greatly impact areas of chronic liver diseases, hepatic transplantation, and gene therapy.

Finally, in spite of the exciting data presented by Coleman and co-workers, a word of caution is appropriate. All the evidence presented is based on morphology, and this is of course recognized by the investigators, without supporting evidence for functional hepatocytic differentiation. These data are now needed and no doubt will soon be provided. Coleman and colleagues have contributed important and exciting new results that should greatly enhance prospects for research on hepatic stem cells and lineages. Perhaps the research on hepatic stem cells has finally come of age.

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