Gut

Leading article

Hepatic stem cells

For several decades investigators have been addressing the tantalising question, is there a hepatic stem cell?¹ Despite widespread attempts to identify and characterise such cells in the liver and a plethora of papers, reviews, and monographs on the subject,^{2–4} doubts as to their very existence have remained. It is only relatively recently that these doubts have been removed, at least in the eyes of most investigators, with convincing evidence from rodent studies and novel developments in the cell biology of the pathogenesis of human liver disease. Moreover, new exciting findings indicate that in tissues, including bone marrow and the brain, there reside cells with an innate ability to differentiate into divergent cell types. The stem cell field is currently a “hot” topic.

Although essentially a quiescent organ, the normal adult liver can fully regenerate following surgical resection or injury. Much of what we have learned about liver growth control derives from the classical two thirds partial hepatectomy model in the rat. The process begins with growth activation of mature hepatocytes; other cell types, including biliary epithelial cells (BEC) and sinusoidal cells, proliferate with a delayed response.⁵ However, if liver damage is so severe that hepatocytes are largely obliterated or for some reason are prevented from entering the growth cycle by exposure to hepatotoxins or carcinogens, then activation of a liver stem (progenitor) cell population is postulated,^{2,3} giving rise to so-called “oval” cells. These cells are thought to have both clonogenic and bipotential capacity—that is, the ability to proliferate and differentiate into cells of either hepatocyte or BEC lineage.^{6,7} There is also evidence that under certain conditions oval cells can be induced to differentiate into non-hepatic lineages including intestinal and pancreatic epithelium.³ The origin of oval cells and their precise location within the liver has remained enigmatic. However, recent evidence from a variety of animal models and indeed now human studies has begun to provide answers.

Experimental studies

Oval cells are activated following dosing of animals with a variety of toxins and carcinogens, alone or combined with other surgical or dietary regimens.^{2,3,8,9} In one of the most studied models, acetylaminofluorene treatment followed by partial hepatectomy, an array of cytokines and growth factors have been shown to be up regulated during the response.² Notably, some of the regulatory mechanisms are beginning to be delineated, for example the recent interesting observation that interferon γ is implicated in orchestrating the process.¹⁰ Oval cells themselves however probably represent the activated progeny of a dormant

stem cell compartment and while oval cells are readily identified in injured liver, one area of great controversy is the question of where these putative stem cells reside in the normal liver.

Because of the lack of specific markers of liver stem cells (even the so-called oval cell specific marker OV6 recognises bile duct epithelium in normal rat liver)¹¹ they have been notoriously difficult to localise other than in the injury models described above. That they are present is supported by the relative ease with which liver epithelial cell lines have been established from *normal* liver.⁹ Where however, do these putative stem cells reside? One suggestion is that they are present in the canals of Hering, that is the region where cells are transitional between the periportal hepatocytes and the biliary cells lining the smallest terminal bile ducts.⁸ Others suggest that there are cells which are found located in the portal tracts, in the periductular region, or even that periportal hepatocytes have stem cell or metaplastic properties.³

Further confusing terminology arises with the description of a population of cells isolated from rat liver called “small hepatocytes”¹² and recently extended to include clonal expansion of “small” human hepatocytes.¹³ These cells appear to have the capacity to clonally expand and yet retain hepatocyte phenotype *in vitro*. Again, the precise origin or location within the liver of these cells has not been defined. Independently, in a series of hepatocyte transplantation studies using a mouse model of hereditary tyrosinaemia, a population of adult mouse hepatocytes with apparent stem cell capacity—that is, multiple rounds of proliferation—demonstrated the ability to largely regenerate and repopulate the liver.¹⁴ In neither approach was there evidence to suggest that the proliferative cells in question gave rise to epithelium of ductal biliary phenotype. While not proved, it seems likely that the cell populations of Mitaka *et al*, Hino *et al*, and Overturf *et al* are the same or similar. Thus these small hepatocytes differ from oval cells and the term unipotential seems more applicable to this population.

Human liver stem cells

Identifying stem cells or their progeny in human liver has of course been even more of a challenge. In the developing human fetal liver, differentiation of hepatoblasts into biliary epithelium is regulated by signals from the portal mesenchyme¹⁵; hepatoblasts not in contact with portal mesenchyme mature into differentiated hepatocytes. Although much is known about the bipotential nature of

Abbreviations used in this paper: BEC, biliary epithelial cells.

hepatoblasts, present in fetal liver in large numbers, previous work has shown that while these cells can proliferate *in vitro*¹⁶ they are thought not to demonstrate clonogenic potential. Thus although hepatoblasts are considered by some as liver stem cells, to describe them as equivalent to oval cells or their progenitors is misleading.

The identity of stem cells in the adult human liver and their role in response to liver damage/injury has also been contentious. Numerous morphological studies have highlighted the presence of small cells present in diseased human liver that are suggested to be putative progenitor cell derivatives,¹⁷ located in or close to bile ducts or in periportal regions adjacent to hepatocyte margins.¹⁸ More convincingly, using markers originally shown to be expressed or up regulated by rat oval cells (including OV-6, *c-kit*, and CD34), several groups have identified oval cells in hepatoblastoma,¹⁹ hepatocellular carcinoma,²⁰ and cirrhotic liver.^{21–23} Using double immunolabelling techniques, some of these cells co-express hepatocytic or biliary phenotypic markers implying lineage progression.^{21–23} However, morphological immunocytochemical studies on tissue sections convey only a limited picture since they present a static single “snap shot” of what is undoubtedly a dynamic process and interpretation is difficult. Additionally, it is likely that some (or all) of the markers in question are expressed on cells transiently.

One means of solving this problem is to develop a defined cell culture based model where the cell fractions in question can be isolated, the regulatory events carefully investigated, and agents responsible for induction of growth and differentiation determined. To this end, using antibodies against *c-kit* and CD34 which recognise surface determinants, cells have been specifically immuno isolated and cultured from fetal, paediatric, and adult human liver^{24–25} and lineage progression followed using well characterised phenotypic markers of hepatocyte or biliary specificity. This work is ongoing and it is hoped will help resolve the questions which remain concerning the identity and potential use of liver derived stem cells.

Haematopoiesis and liver stem cells

While the debate on the source and location of hepatic stem cells is ongoing, two recent papers add a new dimension and offer a challenging alternative hypothesis to explain the origin of oval cells. As already discussed, we know that a number of surface determinants are shared between haematopoietic derived progenitor cells and oval cells, including *c-kit*,²⁶ CD34,²⁷ and Thy-1²⁸ in rodents, and *c-kit*²³ and CD34²⁴ in humans. These observations have been brought sharply into focus with the demonstration that a population of haematopoietic stem cells originating in the bone marrow may give rise to oval cells in the liver and have the potential to further differentiate into hepatocytes and/or ductal cells.²⁹ By transplanting rat bone marrow into lethally irradiated recipients and following the fate of syngeneic cells using various markers, they clearly showed striking changes in the livers of animals induced to regenerate following 2-acetylaminofluorene and CCl₄ treatment. Male donor marrow cells were visualised in female recipients and in a second model, marrow from dipeptidyl peptidase IV positive animals was transplanted into dipeptidyl peptidase IV deficient recipients. In both cases evidence was presented to suggest that the donor cells migrated into the livers of recipient animals and subsequently underwent differentiation to become hepatocytes although it was less clear whether ductular cells of biliary phenotype developed.²⁹

A second recently published study describes a similar approach comprising a mouse marrow transplant model but which interestingly did not include a liver injury step.³⁰

This new report provides important confirmatory evidence that bone marrow derived haematopoietic stem cells can indeed give rise to hepatocytes. In both studies, the number of cells which undergo the transition appears to be relatively small. Therefore, one is left unsure as to whether the response represents a true physiological or pathophysiological phenomenon or simply a quirk of nature uncovered by chance experimental observations. Following bone marrow transplantation, the immune cell chimaerism which ensues will result in cells in the liver with the donor genotype. Therefore, to prove the hypothesis unequivocally, it is vital that convincing marker co-expression work be used to confirm the phenotype of the cells purported to differentiate from haematopoietic derived cells into cells of hepatic epithelial (hepatocyte) lineage. Such studies are presently underway in a number of centres.

Parallels between liver and brain

Fascinating recent findings in neural cell biology studies add a further dimension to this emerging stem cell field. Neural cells found in a post-mitotic epithelial cell layer, the ependyma, overlying the ventricular layer in the brain appear to have stem cell properties.³¹ Rather like the putative hepatic stem cells discussed above, they appear to remain dormant in normal adult tissue but when activated, they proliferate, migrate, and then differentiate. They divide asymmetrically, one daughter cell staying as an undifferentiated stem cell while the other migrates and gives rise to neuronal or glial cells.³¹ It is not known whether hepatic stem cells demonstrate this property. Thus two organs, the liver and brain, where normally little cell turnover occurs, harbour cells with surprising differentiation potential. In other studies,³² putative neural stem cells transplanted into bone marrow have been shown to differentiate into blood cells. As an interesting caveat, this transdifferentiation response occurred only *in vivo*,³² raising the problem of designing culture models with which to investigate the regulatory features of the (neural or hepatic) stem cell differentiation process.

Clinical implications

The ability to identify and exploit a human hepatic clonal stem cell could have important clinical implications, as generating large numbers of differentiated and therefore fully functional human hepatocytes has enormous potential. Primary hepatocytes remain the ultimate choice for use in bioartificial liver support devices.³³ Reliance on primary hepatocytes from pigs or other species remains problematical due to the sensitive issue, now under the wider public debate, of xenotransplantation and the perceived inherent risks (possible cross over retroviral infection) of approaches which involve the use of cells or tissue from foreign species. Other important areas where progress has been limited due to lack of sufficient numbers of good quality primary human hepatocytes include hepatocyte transplantation for the treatment of metabolic disorders or fulminant liver failure,³⁴ and evaluation of drug toxicology and pharmacokinetics so vital today for the development of safe new therapeutic drugs.³⁵ Despite widespread efforts, no one has yet achieved the goal of generating a safe, fully functional yet clonal, immortalised, or genetically engineered human cell model that can be substituted for primary hepatocytes in these various applications. This clearly adds further impetus to the search for the definitive human hepatic stem cell.

Conclusions

In summary, the consensus that there are cells in the liver with stem cell potential has achieved acceptance. Together with the findings from other fields of cell biology, the plas-

tivity of certain cell types is clearly more extensive than previously realised, at least when dealing with organs or tissues traditionally regarded as cell quiescent. The potential exploitability of these recent developments in hepatic stem cell biology awaits further investigation.

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