Liver cells: biology to therapeutics*

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ABSTRACT – The liver has a remarkable capacity to regenerate after injury. Recent work has demonstrated that repair may call upon either the division of pre-existing mature cells, or the expansion of intrahepatic progenitor cells. Furthermore, progenitors may migrate into the liver from the bone marrow. Understanding and exploiting the cell biology of the liver provides the basis for innovational treatment, including the use of growth factors, transplantation of isolated cells, genetic manipulation of hepatocytes and liver cell progenitors, and the development of artificial liver support systems.

KEY WORDS: bio-artificial liver, cell transplantation, gene therapy, hepatocyte

Introduction

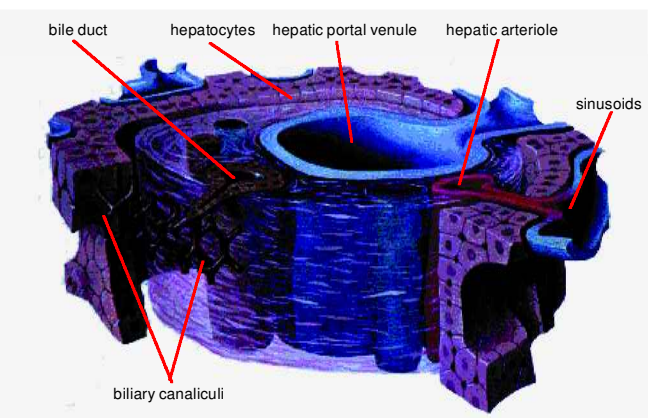
Despite its homogeneous external appearance, the liver is architecturally highly complex. Within the solid parenchyma of the liver are multiple structurally and functionally distinct cell populations. Most numerous are the hepatocytes or parenchymal cells (ca 60% of the total cell number, 90% of the volume), arranged in cords of cells extending from the portal tract, where most blood enters the liver, to the central hepatic venous tributary. Portal blood fills the sinusoids of the liver between the hepatic cords, and the sinusoids are lined with littoral cells (Kupffer and sinusoidal endothelial cells), providing both immune function and a modest blood-hepatocyte filter. Other cell populations, stellate cells responsible for matrix synthesis and degradation, and lymphocytes lie within the parenchyma. Between the hepatocytes, aligned along the hepatic cords and bounded by a specialised part of the hepatocyte apical membrane, are biliary canaliculi which drain into canals, lined initially with cholangiocytes and then with biliary epithelial cells (Fig. 1).

The liver was early recognised as possessing a uniquely developed capacity to regenerate after injury, illustrated in its most striking form by restoration of liver volume after resection of large segments. In rats, 70% partial hepatectomy is followed by regeneration to within 80% of previous volume by four days and to normal size by 10–12 days. In humans, liver mass can be restored from 50% to 100% of normal size within a few weeks. It is customary to refer to the legend of Prometheus, eternally chained to Mount Olympus for stealing fire, eternally losing tissue to his aquiline tormentor by day and regenerating it by night.[1] Knowledge of the processes involved in such regenerative growth has pointed the way to new therapeutic areas for liver disease. Excitingly in the past few years, the realisation of unrecognised pathways for liver regrowth has opened further therapeutic options.

Liver regrowth after partial hepatectomy

After major liver resection in adults (which has been extensively investigated in rodents), there is a rapid surge in DNA synthesis in all important liver cell populations. Hepatocytes, normally mitotically quiescent in adult liver, undergo a semisynchronous surge of DNA synthesis, commencing within 16 hours of surgery, with peaks of S-phase at 24 hours and of mitosis six hours later. Up to 40% of hepatocytes will undergo DNA synthesis at the peak time, and by four days over 70% will have undergone one or more rounds of DNA synthesis and division. This replicative round amongst hepatocytes is followed 24 hours later by a replicative peak amongst sinusoidal epithelial, Kupffer and biliary cells. The restored liver mass then represents the consequences of cell division of mature cells, allied with some degree of hypertrophy of individual cells.

Fig 1. Diagram showing the composition of the liver (reproduced courtesy of UC Davis).



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The molecular signals for this replicative growth surge are complex, but key issues appear to include tumour necrosis factor-alpha priming of hepatocytes leading to upregulation of transcription factors such as NFκB, associated with phosphorylation of tyrosine kinase growth factor receptors (notably c-met, the hepatocyte growth factor (HGF) receptor), in turn leading to cyclin D activation and initiation of DNA synthesis.[2]

Hepatocyte growth factor

HGF was first described as hepatotrophin, originally in the plasma of rats and then in human liver 24 hours after major resectional surgery.[3] Intriguingly, this heparin-binding growth factor is stored within the liver substance bound to extracellular matrix. An early event that trips the cascade of growth factor-induced intracellular signalling is the cleavage of matrix-bound HGF by plasmin activated by damage.[4]

Although defined by researchers as the long-sought liver growth factor, HGF was also described as a substance (scatter factor) that induced motility in cultured cells. Subsequently, HGF has emerged as a pluripotential factor, affecting proliferation, cell migration and development in multiple cell types (mitogen, motogen and morphogen). Different effects have been reported in animal models:

- enhancement of wound and gastric ulcer healing
- reduction of stroke size
- enhancement of renal regeneration, and
- effects on liver growth.

Growth factors in therapy for liver disease

Experiments involving HGF in the context of liver growth and remodelling have demonstrated enhanced survival when HGF was supplemented in animal models of acute hepatic failure. It is debatable whether this offers a therapeutic avenue in man as elevated levels of HGF are already present in liver failure – indeed such patients provided the starting material for the eventual isolation of the factor. The higher the HGF level in acute liver failure, the poorer the prognosis.[5] It seems likely that hepatocytes are incapable of responding in fatal acute liver failure.

More intriguingly, in experimental rat fibrotic liver disease and cirrhosis, exogenous HGF can increase liver cell mass, reduce fibrosis and restore architecture to normal.[6] However, it is questionable whether the recent (typically 12-week-old) cirrhosis of experimental rats induced by drugs such as dimethylnitrosamine or injection of porcine serum is an appropriate model for the years-old fibrosis of most cases of established cirrhosis in man. It is interesting, though, that there seem to be some examples of reversible cirrhosis, such as successfully treated autoimmune hepatitis.

A more intriguing question of potential relevance is whether such growth factor approaches could usefully be applied in man in the surgical setting, either to enhance normal liver mass prior to resection of an abnormal segment or to increase total size prior to donation of a lobe of the liver in living related transplantation. At least in experimental animals, an opportunity is provided by tri-iodothyronine which has proved to be a modulator of liver cell mass, acting as a primary mitogen for the liver (ie able to initiate hepatocyte proliferation in the intact liver). This was initially explored as a means of initiating DNA synthesis and rendering cells susceptible to retroviral gene transfer[7,8] (as discussed below). However, it has the interesting potential of increasing total liver cell mass, total DNA and total protein by inducing proliferation of cells, acting predominantly on hepatocytes in the mid-zonal area of the liver lobule. This stimulus is also effective, and indeed additive if applied at the same time as the conventional maximal stimulus to liver growth in experimental animals (70% partial hepatectomy), suggesting that this or similar approaches might be able to enhance recovery after liver resection in man.

Cell transplantation

Further study of the fate of livers enlarged by administration of exogenous primary mitogens demonstrated, probably predictably, that after reaching a greater than normal mass (at 10 days) random apoptosis of cells returns liver mass to normal – a reminder of the well-explored conundrum, how does the liver know what size to be? This question was raised over 30 years ago in experiments by Mito *et al*[9] who identified the fascinating phenomenon of the survival of extra, indeed 'unnecessary', hepatocytes in normal animals. Isolated hepatocytes transplanted into the spleen of syngeneic animals would survive, proliferate and function over months to years. Using endogenous markers to distinguish transplanted from native hepatocytes, it was later shown that many of the cells initially implanted into the spleen transmigrate through the portal circulation and enter existing hepatic cords, where they survive and function long term.

Hepatocyte transplantation has wide potential therapeutic scope, ranging from possible use in hepatic failure and the treatment of inborn errors of metabolism to the enhancement of function in cirrhosis.

Liver failure

Massive infusion of hepatocytes in experimental systems has supplemented function and increased survival in liver failure, but providing an adequate number of hepatocytes in the hostile milieu of liver failure will always be formidable. Additionally, experiments are difficult to interpret, as cytosolic extracts of hepatocytes given in these circumstances seem to enhance the regeneration of normal liver.

Cirrhosis

In cirrhosis, the liver would seem to be an inhospitable site for implantation as its micro-architecture has been disturbed and extracellular matrix has already interfered with cell function.

Key Points

- After mild or moderate acute liver damage, liver repair depends on division of pre-existing mature cells
- With more severe damage, hepatic progenitor cells may become activated. The bone marrow is one source of such progenitors
- Isolated liver cells transplanted into a new home can fulfil their normal metabolic range, and in disease may have a similar advantage
- In pilot studies, cell transplantation, gene therapy and bioartificial liver support have all proved feasible in man

Inborn errors of metabolism

There is a greater likelihood of success with cell transplantation for inborn errors of metabolism, particularly if only a single secretory or enzymatic function is missing. We have investigated the mouse model of histidinaemia, a single amino acid substitution in a hepatic cytosolic enzyme that leads to elevated serum and urine histidine.[10] There is a similar condition in man, usually symptomless although some problems with speech and higher mental function have been associated. The biochemical hallmark of the disease, elevated urinary histidine, was returned to normal by intraperitoneal injection of less than 5% equivalent of normal hepatocytes. Wild-type cells implanted into the peritoneal cavities survived in islands of hepatocytes expressing histidase.

In developing cell transplantation for treating disease in man, the difficulties become more formidable, largely reflecting problems in obtaining functioning human hepatocytes. If an intact donor liver of good quality is available, it is generally, and appropriately, used for orthotopic transplantation. As discussed later, although primary hepatocytes in man can be cultured, it is extremely difficult significantly to enhance their numbers. In the first human cell transplantation for metabolic disease, in a patient with severe congenital hyperbilirubinaemia of Crigler-Najjar type 1, an injection of approximately 5% of liver mass was partially effective in reversing the elevated bilirubin characteristic of this condition, but the improvement remained partial with no progressive improvement over time.[11]

Perhaps this should not be surprising. Substrate induction might be expected to maximise specific substituted function in a bilirubin transport defect, but there is little pressure within that system for selective expansion of the fully functioning cells at the expense of the abnormal. This is different in other conditions. For example, in the uroplasminogen activator-deficient transgenic mouse, spontaneous mutation of some cells back to normality is followed by clonal expansion of the successful mutants and extensive replacement of abnormal areas of the liver by normal. Most strikingly, in the mouse model of tyrosinaemia (the FAH– mouse), infusion of a few hundred fully functional hepatocytes leads to progressive repopulation of the abnormal liver by normal hepatocytes.[12,13]

In addition to pointing the way to new therapeutic avenues, such experiments can be adapted to identify how many times a single adult hepatocyte can divide. By serial transplantation through different tyrosinaemic livers, an expansion potential of 7×10^{20} has been achieved.[14] Such expansion is perhaps merely an extension of the recognised ability of mature hepatocytes to divide rapidly and restore liver mass. However, one of the most astonishing areas of insight in liver cell biology, with clear therapeutic potential, has been the evidence that, in addition to division of pre-existing hepatocytes, numerous other pathways can lead to the generation of new liver cells even in adults.[15–17]

Liver cell progenitors

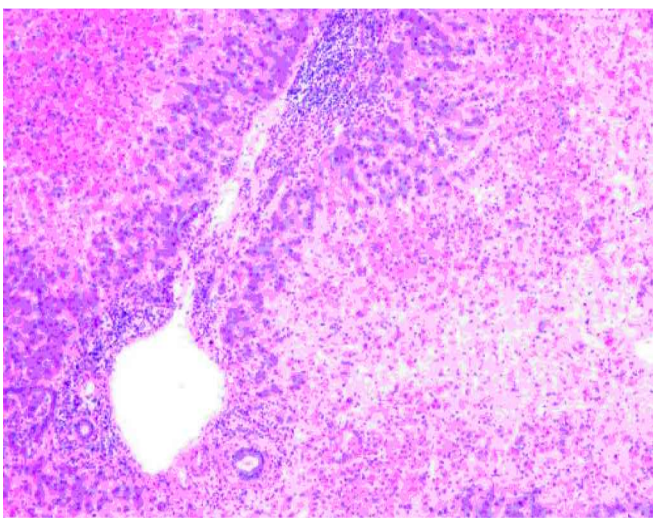
Intrahepatic progenitors

The first of these populations, the progenitor cells in the periportal area of the liver, consisting of cells in the canal of Hering,[18] were first recognised experimentally after they had been stimulated to proliferate when liver growth was required but mature hepatocyte proliferation was prevented: for example, partial hepatectomy performed in the presence of acetoaminofluorine which prevents hepatocyte division. The resulting 'oval' cells in the periportal area have the potential to mature into either hepatocytes or biliary lineage cells. They proliferate in man after severe paracetamol poisoning, for example, when hepatocytes have been seriously damaged (Fig. 2).

Extrahepatic precursors

Even more strikingly, there is evidence in both animals and man for extrahepatic progenitors of hepatic cells persisting in adults. It was initially demonstrated that if liver regeneration was stimulated but hepatocyte regeneration prevented, and

Fig 2. Liver regeneration in man in the presence of massive hepatic necrosis. Cells with mixed hepatocyte-biliary characteristics morphologically have proliferated from intrahepatic progenitors (reproduced courtesy of Professor A Dhillon).



simultaneously a bone marrow graft was performed, hepatocytes bearing genetic markers of the transplanted bone marrow donor could be identified after an interval.[19] As few as 200 bone marrow cells infused could result in colonisation of the recipient with cells of hepatocyte morphology and function of bone marrow origin.[20] This was demonstrated in humans in the new livers of men who had received female liver grafts and in women who were recipients of male bone marrow transplants.[21,22] In each case, male liver cells, both hepatocyte and biliary, which could only have been of extrahepatic origin, were demonstrated by the presence of Y chromosome-positive liver cells where there should have been only XX female liver cells. The frequency and functional importance of this phenomenon are currently being unravelled, but it raises some intriguing questions:

1 How much of the week-to-week turnover of an organ such as the liver reflects replacement of cells from within the organ and how much supplementation from bone marrow precursors?
2 Can hepatic stem cells be isolated from bone marrow, manipulated (eg by replacement of a defective gene) and then re-implanted to mature and express normal function within the liver? If achievable, the potential for treating inborn errors of liver metabolism is formidable.
3 Can hepatic progenitor cells be isolated from bone marrow and *in vitro* yield proliferating cell lines which would provide large numbers of cells for implantation for extrahepatic support?

In considering the potential sources of such proliferating hepatocyte precursors, consideration does not end with bone marrow but extends to fetal liver, and indeed to embryonic stem cells. From both the latter, including human cells, progenitors with a potential to proliferate and mature along both hepatocyte and biliary lineages have been identified.

Gene transfer

Liver gene therapy has other potential paradigms in addition to its potential of using stem cells:

- *in vivo* therapy to transduce existing cells
- *ex vivo* gene therapy to mature hepatocytes using cells disaggregated from a surgical specimen and re-implantation into the portal vein of the liver.

In the histidinaemic mouse we developed a retroviral vector expressing the mouse histidase gene. The temporary surge of DNA synthesis induced by partial hepatectomy was used to facilitate retroviral gene transfer, allowing gene expression and improvement in histidine metabolism. In man, *ex vivo* gene therapy for inborn errors of lipid metabolism was pioneered in the mid-1990s, although the necessity for surgical resection of the liver to allow hepatocyte transduction was severely criticised.[23]

More recently, the propensity of adenoviral vectors (at least in rodents) to be rapidly taken up by the liver led to clinical trial of this technique for gene transfer in the treatment of urea cycle defects.[24] The tragic consequences in one patient (who died as a consequence of a major inflammatory reaction) highlighted the caution necessary in pursuing this approach. However, this should not stop future work which could lead to the successful application of gene therapy for a wide variety of metabolic and infectious diseases of the liver.

Extracorporeal liver support

The other major way in which liver cell biology could impact on therapeutics is in the provision of extracorporeal liver support. A liver machine, temporarily replacing liver function until normal regenerative and repair processes can be completed, remains a holy grail in liver research.[25] Whilst not of universal application, it would be of critical value in patients whose liver was sufficiently damaged that life could not be sustained for long enough for repair to take place, yet whose liver retained the potential to recover. Lesser, but useful, applications would be:

- to prolong survival whilst an appropriate organ could be found, and
- to allow restoration, at least to previous health, after acute decompensation in cirrhosis.

The problems are formidable, but the complexity and range of function to be provided mandate, in most opinions, a biological component to such a machine. It seems inconceivable that without living cells the full necessary complement of function, particularly detoxification, could be provided.

Normal human hepatocytes are scarce outside their natural habitat. The inability to proliferate readily in culture, shortage of donor organs, and competition (entirely justified) from transplant surgeons, all render primary cells from whole organs an unsatisfactory basis on which to develop bio-artificial liver machines. The options currently being explored are the use of:

- genetically engineered cells, for example transducing cells to express the SV40 large T antigen which binds to P53 to allow cell cycling
- well differentiated hepatocyte derived cell lines, such as HepG2 cells derived from a hepatoblastoma[26]
- primary xenogeneic cells[27]
- hepatocytes grown from stem cells.

Genetic manipulation

Most attempts to develop cycling cells by genetic manipulation have produced cell lines whose function is less than that of primary cells.

Differentiated hepatocyte derived cell lines

HepG2 cells have been used as the basis of a clinically utilised machine, although in its first form there was no beneficial effect in a clinical trial.[26] However, cell numbers were relatively small and, in particular, oxygenation of the cells in the extracorporeal chamber was apparently inadequate. A further generation of machine is under trial.

Our experiments with HepG2 cells confirm that manipulation of the extracellular component can strikingly enhance cell function *in vitro*.[28] For example, *per cell* synthetic values for liver proteins are equivalent to those *in vivo* when cells are cultured in alginate capsules which impose a three-dimensional growth pattern on the cells. However, there are significant deficiencies in liver-specific functions in such cells lines, particularly in detoxification, which are likely to demand genetic manipulation if the full range of specific functions is to be provided. Using these approaches, HepG2 cell-based systems have improved survival in animal models, and there is certainly hope for this approach.

Primary xenogeneic cells

Xenogeneic systems, based on primary porcine cells, also appear capable of efficacy in clinical practice. Extracorporeal machines using such cells have not yet provided a definite survival advantage, but short-term changes are reported (eg alterations in intracranial pressure and neurological status).[27] The problems with porcine systems include the potential for zoonoses, particularly for transmission of endogenous porcine retroviruses, so the future of such systems remains uncertain.

Hepatocytes grown from stem cells

Whilst hepatocytes grown from stem cells have future potential, adequate techniques for identification and culture to make this a reality are not yet available.

The future

Progress is slow, but the application of cell and molecular biology of hepatocytes has already shown the possibilities for new ways to treat disease, involving at least pilot applications of gene transfer, cell transplantation and the use of liver cells in extracorporeal circuits. Certainly, the potential is there.

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