Basic and clinical aspects of liver growth: Prometheus revisited

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The phenomenon of liver regeneration is startling. In the healthy adult the rate of hepatocyte cell division is usually slow. In rats, for example, one cell in 300 may be in a state of DNA synthesis. But within a few hours of massive injury, over 30% of surviving cells may have entered DNA synthesis. After removal of 70% of the liver, normal liver mass is replaced in a few days. This phenomenon of rapid regeneration is not limited to experimental animals—or even to demigods like Prometheus whose liver was gnawed daily by vultures on the slopes of Mount Caucasus in retribution for having stolen fire from the gods for mankind. Rapid regrowth of the remnant liver also occurs after major hepatic resection in man (Fig 1).

The mechanisms that control the growth of the liver have been intensively investigated. Four general possibilities have been evoked to explain rapid regeneration.

- The greater metabolic load on the remaining liver cells might induce cell division.
- Positive growth factors might be released.
- Normally, liver growth might be actively restrained by an inhibitory factor (chalone) synthesised in the liver: reducing the liver mass would decrease synthesis and diminish the inhibitory drive until normal liver mass was restored.
- A neural drive to hepatic regeneration might occur after damage.

Cross-circulation experiments on dogs after partial hepatectomy demonstrated the presence in the circulation of substances that could initiate DNA synthesis in the liver of a parabiotic intact animal [1]. This demonstration of a positive growth promoter encouraged cell biologists to try to identify a single substance responsible for this action. It is now clear that more than one growth factor capable of initiating hepatocyte proliferation is generated at the time of partial hepatectomy. Nor is cessation of liver regeneration a passive process: it involves a set of negative growth factors that bring to an end the surge of hepatic DNA synthesis.

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The search for positive growth factors

By the mid-1980s, much evidence suggested that the growth factor involved in initiating hepatocyte DNA synthesis after partial hepatectomy was epidermal growth factor (EGF) [2]. This 6,000 mol wt polypeptide is indeed a powerful mitogen for many epithelial cells, and when added to cultures of isolated hepatocytes stimulates them to synthesise DNA [3]. EGF receptors are present on hepatocytes. Following experimental hepatic resection these receptors are down-regulated and phosphorylated [4,5], and radiolabelled EGF administered to an animal at that time is internalised and transported to the nucleus [2]. Anti-EGF antibodies administered at the time of partial hepatectomy reduce the amount of DNA in the liver five days later [6]. However, conspicuously missing from the jigsaw is any evidence of a significant increase in EGF either in the circulation or within the liver at the time of partial hepatectomy.

The paradox of EGF receptor down-regulation without the presence of additional EGF was resolved when it became known that the EGF receptor can also bind the growth factor TGFα. TGFα is a polypeptide growth factor with 35 to 40% homology with EGF, and is also a powerful mitogen for hepatocytes. Furthermore, unlike EGF, it is produced in the liver, and TGFα mRNA increases strikingly within 12 hours of partial hepatectomy [7]. Mead and Fausto proposed an autocrine loop as a mechanism for the onset of hepatic regeneration—TGFα is generated by the hepatocyte and then acts on the TGFα/EGF receptor of that and presumably also of adjacent cells [7]. The evidence for this appears convincing. However, by the time this role for TGFα had been demonstrated, evidence strongly implicating another growth factor had been accumulating.

Nakamura *et al* found a high mol wt hepatocyte growth factor when they assessed the bioactivity of fractions of rat sera taken 24 hours after partial hepatectomy [8]. Sequential molecular sieving and heparin affinity chromatography led to partial purification of what is now called 'hepatocyte growth factor' (HGF). We demonstrated similar activity in human serum taken from patients 24 hours after hepatic lobectomy for tumour [9]. The factor was eventually purified from human plasma (from patients with fulminant hepatic failure [10]), rat platelets [11], and rabbit

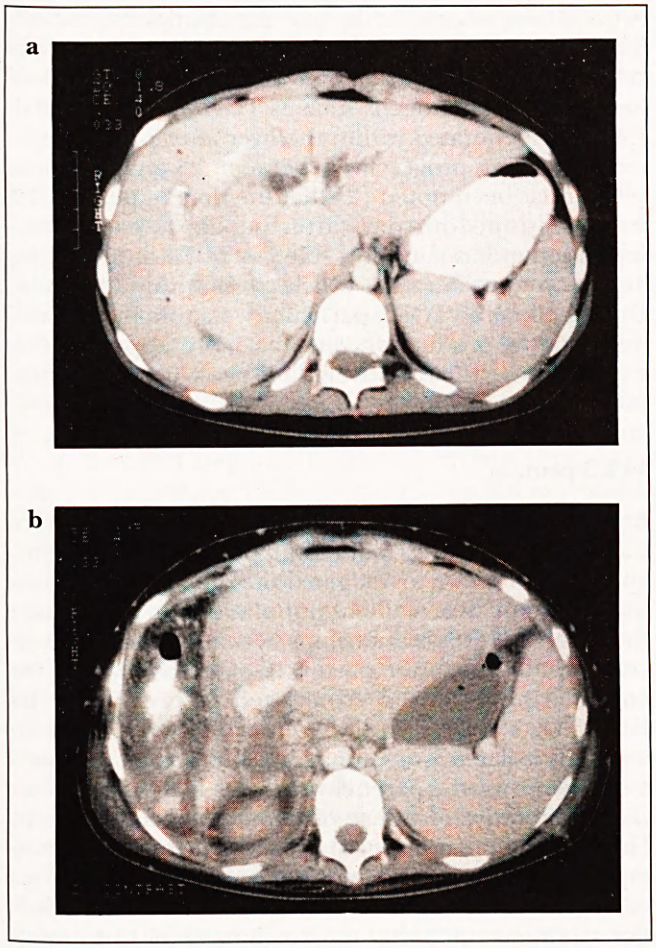

Fig 1. *CT appearances (a) before (b) 20 days after extensive right hepatic resection for tumour.* Note the striking increase in the size of the left lobe of the liver

plasma [12], all these isolations being reported between 1988 and 1989. Previous names under which the substance had been studied—hepatopoietin A [13]—and hepatotropin [8] were abandoned and replaced with the term 'hepatocyte growth factor'. The predicted cDNA sequence for HGF was published in 1989 [14,15]. The molecule has one α and β chain, forming a shape reminiscent of an old-fashioned carpet sweeper, with mol wt of 70,000. It has some homology with plasminogen.

HGF is produced in the liver within a few hours of damage. Our experiments, using a cDNA constructed to the β chain of HGF, demonstrated peak mRNA production in the rat liver 10 hours after partial hepatectomy [16]. When recombinant HGF became available, experiments on human hepatocytes demonstrated that on a molar basis HGF is a more powerful hepatocyte mitogen than TGFα [17]. Its role in liver growth is unlikely to be limited merely to the response to damage, because we also found high levels of HGF mRNA in human fetal liver [15]. We demonstrated that the gene coding for human HGF is situated on the long arm of chromosome 7 [18].

The full role of HGF remains to be defined. Recombinant HGF acts on a spectrum of cell types: for example, kidney epithelial cells and melanocytes, although not fibroblasts [19]. But hepatocytes still appear to be the most sensitive cell line. Within the liver, HGF is generated in non-parenchymal cells, particularly the lipocytes [20], but it is also found demonstrable in other tissues, including the placenta, brain, skin, and gastrointestinal tract [21]. Furthermore, it now appears that HGF has another set of properties, appreciated when it was realised it has the same structure as the previously described substance 'scatter factor', which influences intercellular organisation and can affect cell movement and tissue architecture [22]. In other cell lines, however, HGF displays antiproliferative activity [23]. The phrase 'mitogen, motogen, morphogen' has been used to summarise the wide range of potential actions of HGF-scatter factor [24].

The HGF receptor is a tyrosine kinase. It had previously been described as a product of the *c-met* oncogene, and until early 1991 was a tyrosine kinase in search of a function [25]. The liver expresses a considerable number of mRNA transcripts of the *c-met* gene, but their role is as yet uncertain [26]. Following hepatic resection, the expression of mRNA for the HGF receptor increases in the remaining liver, suggesting renewal of surface receptors that have been occupied by HGF [27].

Clinical relevance of HGF

Apart from its role in repair after damage, and in fetal development, a role for HGF in liver growth might be sought in the benign hyperplasia of the liver that is characteristic of chronic liver disease, or in tumour development. There is yet no direct evidence of its involvement in hyperplasia and chronic liver disease, although HGF serum levels are higher in patients with active cirrhosis than in patients with inactive cirrhosis [28]. They are much higher in patients with fulminant hepatic failure [28]. While this supports the putative role of HGF as the growth factor involved in regenerative repair, the presence of a high circulating level in fulminant hepatitis dampens enthusiasm for the concept that it might usefully be given to patients to enhance regeneration in severe liver disease. It may well be that, at least in this form of severe hepatitis, the cells are damaged beyond repair and cannot respond to this regenerative stimulus. There are, however, types of subacute hepatitis in which progressive severe liver disease emerges over a long time course, for which one synonym is 'regeneration defective hepatitis'. If in this subgroup there was a relative deficiency of HGF, HGF might play a therapeutic role.

In a small series of patients with hepatocellular cancers (HCC) we were unable to demonstrate higher HGF mRNA levels within the tumour than in adjacent

normal liver [29]. It may be that in liver tumours the expression of the receptor is of greater relevance, and we have defined both increased and decreased expression of a variety of *c-met* transcripts in this context [30].

Other growth factors

HGF and TGFα are both generated at the time of partial hepatectomy and each is a powerful mitogen able to initiate hepatocyte regeneration. It seems naive, however, to believe that production of these two substances in the liver explains all the phenomena of hepatic regeneration. Other growth factors have been implicated, acting either alone (as complete mitogens), or in an association with others [31]. They include acidic fibroblast growth factor [32], a low mol wt growth factor hepatopoietin B [13], and an incompletely characterised hepatic stimulatory substance, partially purified from remnant liver after partial hepatectomy [33]. An albumin-bilirubin complex—which might be expected to be present in greater amounts in the circulation in hepatic failure—can also act as a hepatomitogen [34]. Factors such as insulin and glucagon can enhance the action of complete mitogens, although *in vitro* studies indicate that they cannot act alone. Adrenaline, released from the local hepatic nerve supply, can also enhance regeneration [35].

The precise stimuli to the generation of even the best characterised hepatomitogens, HGF and TGFα, remain unclear. Evidence is emerging for a spleen-derived HGF stimulator [36]. Extra-hepatic sources of HGF may contribute to the very early increase in circulating HGF, within a few hours of partial hepatectomy [37], although matrix-bound HGF already sequestrated in the liver may contribute to an early surge, being liberated by alterations in extracellular adhesion following trauma [38].

What turns it off?

It seems likely that a similar complexity will involve the processes that lead to the ending of hepatic regeneration, dictating that it takes place when normal liver mass has been restored. One possibility is that hepatocytes that enter DNA synthesis during regeneration are programmed to go through only one or two cell cycles and then return to the quiescent state, but the evidence for a more active control system involving the generation of negative growth factors seems compelling. At least three factors putatively fulfil this role: all have been identified in the non-parenchymal cell population of the liver, predominantly Kupffer cells and sinusoidal endothelial cells.

TGFβ

TGFβ is a widely distributed 28,000 mol wt polypeptide, with a wide range of biological properties that include stimulation of angiogenesis, enhancement of extracellular matrix formation, modulation of cell differentiation, and inhibition of cell growth in various cell types. *In vitro,* TGFβ can inhibit the response of hepatocytes to mitogens such as TGFα, EGF and HGF [39]. It is generated within the liver after partial hepatectomy, rising progressively between 28 and 96 hours [40]. It has been noted [2] that the time of peak TGFβ transcription, four days after partial hepatectomy, seems somewhat late for a role for TGFβ in inhibiting hepatocyte proliferation which peaks within 24 hours. Other actions of TGFβ, particularly its role in extracellular matrix production, may be more appropriately explained by this relatively late peak of production [40].

14 kD protein

Another inhibitor transiently generated in the liver between 24 and 72 hours after partial hepatectomy, appeared when we investigated the effects of co-culturing different liver cell subpopulations to reproduce the complex interrelationships between different liver-cell populations after partial hepatectomy [41]. We cultured hepatocytes, stimulated to proliferate by HGF, TGFα or EGF, in the presence of non-parenchymal cells isolated from either normal liver or regenerating liver. Normal parenchymal cells had no effect on the proliferative response of hepatocytes to mitogens. However, non-parenchymal cells taken from regenerating liver strikingly inhibited the proliferative responses of hepatocytes to all these mitogens. Further investigations demonstrated that the mechanism of this inhibition was the secretion of a soluble, heat-labile polypeptide, mol wt between 14 and 17 kD, from the non-parenchymal cells. Appropriate antibody inhibition studies indicated that this was not TGFβ or other candidate cytokines such as TBF, IL6 or IL1α or β. The 14 kD protein inhibits the response of both normal and regenerating hepatocytes to hepatomitogen, as would be required for a role in controlling regeneration. It has some similarities to an inhibitory factor derived in small quantities from intact normal liver, but which to date has been only incompletely characterised [42].

IL1β

The Kupffer cell-derived cytokine IL1β also inhibits the hepatocyte response to mitogens *in vitro* [43], but it is not clear whether it exerts this role during the off phase of the proliferative response to partial hepatectomy.

One explanation for the presence of at least three potentially inhibitory factors during the off phase of partial hepatectomy is that this reflects other functions of these molecules. In addition, attention has thus far concentrated exclusively on the parenchymal cells of the liver. The role of all these factors, both inhibitory and excitatory, in modulating the proliferation of the

non-parenchymal cell population after hepatocyte proliferation is relatively ill understood; one may anticipate substantial progress in this area in the next few years.

Further horizons

Our understanding of the processes controlling both benign and malignant cell growth has clear, although futuristic, applications in enhancing liver growth when liver-cell function is deficient, or inhibiting growth when malignant transformation occurs. Another field in which an understanding of the cell biology of liver growth may produce clinical dividends in the not-too-distant future is the field of isolated cell transplantation. Isolated hepatocyte transplantation has been explored since the 1970s, initially intended as a means of treating inborn errors of metabolism, or of providing acute rescue in hepatic failure [44]. Work in the 1970s established that isolated hepatocytes could be implanted either into the original donor or a syngeneic animal in a variety of sites. Hepatocytes would survive in fat pads, in skin, but most dramatically within the spleen [45]. It is probable that the reticuloendothelial matrix of the spleen, reminiscent of that in the liver, provides a particularly appropriate medium [46]; other sites include the pancreas and the liver itself [47], or the peritoneum which will support cells, particularly if they are implanted on a matrix [48]. Growth of implanted hepatocytes is slow, but proliferation does occur in some sites, and one can speculate on the potential of exogenous growth factors to enhance this. Evidence that this is a promising approach comes from the demonstration that the proliferation of isolated hepatocytes in the spleen increases when partial hepatectomy is performed [49]. Initial hopes, based on the relatively low expression of HLA antigens on hepatocytes, that cells would survive long term without immunosuppression when transplanted outside syngeneic systems, turned out to be ill founded; without immunosuppression there was a rapid rejection of isolated hepatocytes transplanted between strains [50]. Continuous, but not short-term, cyclosporin administration can prevent this.

A number of studies in the 1980s demonstrated that metabolic defects could be treated by this means in experimental animals. The biochemical abnormalities improved in rats with bilirubin transport defects [44, 51], and serum albumin increased in rats with analbuminaemia [48]. But as a means of treating acute experimental hepatic failure, enthusiasm was temporarily curbed when it was demonstrated that in some systems dead hepatocytes, or a cytosolic hepatocyte extract, seemed as effective in enhancing survival as did liver cells [52]. Progress in molecular genetics, however, now opens new applications for isolated hepatocyte transplantation with the potential development of somatic gene therapy.

The approach is, in principle at least, theoretically straightforward. Transfection techniques, involving either physical means such as calcium or cationic liposomes, or biological means such as adenoviruses or retroviruses, are available for introducing defective or missing genes into cells. In combination with a liver-specific promoter such as the albumin or transferrin promoter, *in vitro* expression of genes transfected into hepatocytes has been achieved [53, 54]. Current evidence indicates that for successful transfection to occur, with integration of DNA into the host genome to allow long-term expression, a degree of hepatocyte dedifferentiation is required, most readily achieved by inducing hepatocyte proliferation [55]. Treatment of genetic disorders of the hepatocyte might therefore involve harvesting cells by a partial hepatectomy, growing them in cell culture to allow dedifferentiation during which cell transfection would be performed, followed by reimplantation of the transfected cells. The list of inborn errors of metabolism that might be treated in this way is long—glycogen storage diseases, urea cycle defects, lipoprotein receptor defects, and so on. Indeed, this is an area where the future is already with us: the hypercholesterolaemic Watanabe rabbit has been treated by reimplantation of hepatocytes in which the missing LDL receptor gene has been transfected using a retroviral vector [56]. The result was a prolonged three-to-four months reduction in serum cholesterol levels, although it is too soon to say how long this improvement will be maintained, and in particular whether the newly integrated DNA will remain effective for the rest of the animal's life. At present it is known that one patient has been similarly treated, although full details remain unpublished.

Prometheus, contemplating with anguish the nightly return of his hepatophagous vulture, would indeed have been amazed.

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