Published in final edited form as:
J Gastroenterol Hepatol. 2011 January ; 26(Suppl 1): 203–212. doi:10.1111/j.1440-1746.2010.06539.x.

New Concepts in Liver Regeneration

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Abstract

The unique ability of the liver to regenerate itself has fascinated biologists for years and has made it the prototype for mammalian organ regeneration(1–3). Harnessing this process has great potential benefit in the treatment of liver failure and has been the focus of intense research over the past 50 years. Not only will detailed understanding of cell proliferation in response to injury be applicable to other dysfunction of organs, it may also shed light on how cancer develops in a cirrhotic liver, in which there is intense pressure on cells to regenerate. Advances in molecular techniques over the past few decades have led to the identification of many regulatory intermediates, and pushed us onto the verge of an explosive era in regenerative medicine. To date, more than 10 clinical trials have been reported in which augmented regeneration using progenitor cell therapy has been attempted in human patients(4). This review traces the path that has been taken over the last few decades in the study of liver regeneration, highlights new concepts in the field, and the challenges that still stand between us and clinical therapy.

The two layers of defense against liver injury

It is now well accepted that there are two physiological forms of regeneration in the liver as responses to different types of liver injury (Figure 1). At the frontline of defense are mature, normally quiescent adult hepatocytes, and in the majority of liver injuries due to drugs, toxins, resection, or acute viral diseases, hepatocytes are the main cell type to proliferate and regenerate the liver. The second layer of defense lies in the reserve progenitor cell population, which is also a quiescent compartment in the liver, but is activated when injury is severe, or when the mature hepatocytes can no longer regenerate the liver due to senescence or arrest.

First line of Defense: Regeneration by Hepatocytes

Regeneration of the liver after resection is actually compensatory hyperplasia rather than a true restoration of the liver's original gross anatomy and architecture(1,2). A particularly fascinating point about this process is that the degree of hyperplasia is precisely controlled by the metabolic needs of the organism, such that the process stops once an appropriate liver to body weight ratio is achieved. Two-thirds partial hepatectomy (PH) in rodents has been used extensively to study molecular and cellular mechanisms behind liver regeneration, with initial physiologic principles outlined in rats through the pioneering work of Nancy Bucher(5–7). Later, the advent of genetically modified mice has allowed the study of various specific molecules and dissection of pathways implicated in regeneration. More recently, studies of global gene expression profiling have returned our thoughts to the "big picture", as there are clearly multiple overlapping redundant pathways working in concert to achieve this impressive physiologic accomplishment.

PH is reproducible and leads to a proliferative stimulus that is initiated by an inflammatory stimulus, in the absence of significant cell death. Regeneration of the liver is critical to survival of mammals and is therefore evolutionarily conserved. Thus, pathways leading to its completion are (with few exceptions) redundant. The phenotype of most genetically modified mouse models studied using the PH model thus consists of a delay rather than a complete abrogation of regeneration.

Signaling networks activated after Partial Hepatectomy

Given the extent of cell proliferation needed to restore original mass after 2/3 PH, it is intuitive that virtually all cellular machinery be activated during regeneration, and that this could realistically entail hundreds of pathways (there are only 20,000 exons). It is proposed that there is an initial activation of the cytokine cascade in Kupffer cells, which then stimulates growth factor and metabolic pathways in hepatocytes. Other non-parenchymal cells (stellate cells, vascular and biliary endothelial cells) proliferate after hepatocytes, presumably responding to yet another set of signals.

A great deal of recent work has focused on how pattern recognition receptors and a variety of inflammatory molecules are activated and initiate the cytokine signaling cascade after PH. As they have been extensively discussed elsewhere(2), we will not go into great detail about these pathways in this review. In brief, involved pathways include (at least) the activation of nuclear factor-kappa B (NF-κB) in Kupffer cells via tumor necrosis factor (TNF)(8), lymphotoxin (from T cells)(9,10), MyD88(11,12), and/or complement components(13), with downstream secretion of interleukin-6 (IL6)(14). In turn, IL-6 binds its receptor on hepatocytes and leads to activation of the transcription factor signal transducer and activator of transcription 3 (STAT3)(15). Fascinating newer work in mice with a hepatocyte-specific deletion of inhibitor-of-kappaB-kinase 2 (IKK2), which normally acts to activate NF-κB, demonstrated earlier and increased NF-κB activation in Kupffer cells, which had intact IKK2, with a concomitant decrease in NF-κB activation in hepatocytes(16). These animals had more rapid hepatocyte proliferation than control littermates, perhaps via prolonged JNK activation, highlighting both the cross talk between different cell types during liver regeneration and the critical importance of inflammatory stimuli in priming hepatocytes for replication.

After cytokines have triggered the G_0 to G_1 transition, several secondary signals then stimulate progression through the cell cycle. These growth factors are numerous and redundant to a great extent, again highlighting the physiologic importance of liver regeneration to the survival of the animal. Ligands of the epidermal growth factor (EGF) receptor have been extensively studied, including EGF itself(17,18), transforming growth factor alpha (TGFα)(19,20), amphiregulin(21), and heparin binding EGF-like growth factor (HB-EGF)(22,23). HB-EGF appears to be particularly required for a robust proliferative response, as it is differentially regulated after 2/3 vs 1/3 PH (the latter leads to minimal DNA replication)(23). More recently, genetic loss of the EGFR itself has been investigated, either by RNA interference or constituitive deletion in mice, confirming a critical role of the signaling pathway in regeneration(24,25).

Hepatocyte growth factor (HGF) is another key hepatic mitogen active following PH. It is released from the extracellular matrix following PH to bind its receptor, c-Met, on the surface of hepatocytes. Conditional deletion of c-Met in the livers of mice was initially shown to cause either a significant delay in cell cycle entry after PH(26), or an inability to survive the procedure(27). Studies using RNAi against HGF or c-Met in rats supported the former study, showing a suppression of cell proliferation with successful knockdown of this pathway (28). Newer work has demonstrated that the mitogenic pathways activated via the

EGFR and HGF/Met pathways might compensate for one another, as further characterization of the regenerative defect in hepatocyte-specific Met KO mice demonstrated that this defect could be partially reversed in culture by treatment of the cells with EGF (29). Similarly, in a study in Michelopoulos and colleagues using rats treated with RNAi against the EGFR, the resultant defect cell proliferation after PH was associated with a compensatory up-regulation of Met(24).

A family of proteins that appears to function across signaling networks is the matrix metalloproteinase (MMP) family. Through studies of animals genetically modified to lack inhibitors of MMPs (tissue inhibitors of MMPs, or TIMPs), MMPs have been shown to be important in the cleavage and release of growth factors from the extracellular matrix. Specifically, TIMP1 loss of function leads to increased MMP activity after PH, with increases in HGF activity and accelerated cell proliferation. Accordingly, a gain of Timp1 function lead to a delay in cell proliferation(30). Loss of Timp3 leads to a particularly interesting phenotype, with sustained TNF activity and ultimate hepatocyte death and liver failure. The remarkable finding was attributed to Timp3's function in inhibiting TACE(31). Thus, it is not just signaling pathways within the hepatocyte that are critical to regeneration; the surrounding environment is also important.

The metabolic challenges facing the regenerating liver are quite impressive.The liver must continue to regulate systemic energy levels while meeting its own demands for significant nucleotide and protein synthesis needed for cell division. In fact, some of the most profound phenotypes seen in genetically-modified mice after PH have been demonstrated in those with defects in the phosphoinositide-3 kinase (PI3K) pathway. For instance, liver-specific deletion of phosphoinositide dependent protein kinase 1 (Pdk1) leads to a near-complete failure of regeneration after PH in mice(32). Important downstream effectors of this pathway include Akt, which activates mTOR and appears to affect cell size specifically(33,34), and p70 S6 kinase, which regulates the 40S ribosomal protein S6 to regulate protein synthesis and cell proliferation. Additionally, deletion of a downstream effector of mTOR, S6 protein itself, lead to a profound deficit in DNA replication after PH with specific effects on cyclin E induction(35). While mTOR may play a critical role in regulating cell size in response to the metabolic demands of the remaining functional hepatocytes, further characterization of how this interplay leads to initiation and termination of liver restoration after PH is warranted.

The Wnt/beta-catenin pathway has been extensively studied in a myriad of developmental processes in a variety of organs; liver regeneration is no exception. Using reporter mice, some investigators have demonstrated activation of this pathway after PH(36), while others have suggested that the canonical Wnt pathway is preferentially activated during the proliferation of oval cells (a type of progenitor cell) (37,38). Hepatocyte specific beta-catenin KO mice regenerate in a delayed fashion after PH, however, perhaps via decreased activation of the EGFR(39). Of additional interest is the finding that constituitive over-expression of beta-catenin via an activating mutation at serine 45 lead to an acceleration of regeneration after PH and earlier development of HCC after diethylnitrosamine (DEN) injection(40).

While the cytokine, growth factor, and metabolic signaling networks are each vital to normal liver regeneration, significant cross talk between networks adds another level of complexity to this process. Suppressors of cytokine signaling (SOCS) are important mediators of this type of interaction, as their expression is induced by cytokines and their function is to act in a negative feedback loop to inhibit signaling through a whole host of receptors, including those of insulin and several growth factors(41). Specifically in hepatocytes, SOCS3 is highly induced after PH(42), is critical to shutting down cytokine signaling after PH. and

hepatocytes without SOCS3 were hyper-proliferative in response to growth factors in culture(43). Mice without SOCS3 in hepatocytes demonstrated enhanced regeneration after PH, and an earlier development of HCC after DEN injection, suggesting that this protein is critical in controlling normal and abnormal proliferative responses in the liver

Global Regulation of Transcription during Liver Regeneration

Given the simultaneous activation of multiple diverse pathways that occurs after PH, one might expect significant changes in global gene expression during this process. In evaluating gene expression profiles during early G1, late G1, and the S phase of the cell cycle after PH, Greenbaum and colleagues described an initial decrease in the expression of genes involved in steroid and lipid metabolism and hormone biosynthesis, i.e. normal activities of the quiescent liver(44). As expected, later in G1 genes involved in protein synthesis and cytoskeletal organization were up-regulated, a pattern which continued through S phase, when expression of nucleotide metabolism genes became more prominent. Gene expression profiling was recently used to examine the differential proliferative response that occurs after 1/3 (minimal proliferation) vs. 2/3 PH (robust proliferation). It was found that even 1/3 PH leads to significant changes in gene expression(45). Interestingly though, between 4 and 12 hours after the two operations, a transcriptional shift seemed to occur, committing hepatocytes toward replication. This transcriptional shift consisted of the activation of genes enriched in transcription regulatory elements for FOXD3, FOXI1, CUX1, ER and E2F-1 at 4h after 2/3 PH, and their replacement at 12h by genes enriched in TREs for c-jun, CCAAT box, Myb, Ets-1, Elk-1 and USF, which are associated with DNA replication. These data demonstrate that the liver initially responds to PH with massive changes in gene expression, even if the operation does not result in DNA replication, and suggest that genomic and epigenomic changes function as a “wake up” call for quiescent hepatocytes to prepare them for the decision to replicate, which occurs 12h after PH or later.

Micro RNAs appear to serve as an additional layer of regulation during liver regeneration. These small non-coding RNAs modulate translation by binding to specific mRNAs and either directly inhibit their translation, or inducing degradation of those same mRNAs(46). While this is a relatively new area of study, initial investigations demonstrated that mice with deficient microRNA processing had a delay in the G1 to S transition after PH(47). In particular, miR21 is induced after PH, with repression of miR378(47,48), though the precise mRNAs that are modulated by these miRNAs have not been clearly defined.

Recent Additions

Despite the wide array of studies of the ‘classical’ signaling pathways governing regeneration, investigators continue to use the PH model to add to the greater knowledge of cellular biology. For example, using PH in conjunction with a transplantation model and *in vitro* work, Grompe and colleagues discovered that hepatocytes undergo multiple changes in ploidy during this physiologic process, perhaps predisposing to oncogenesis if aneuploid cells are allowed to further proliferate(49). Additionally, further work in genetically modified mouse models has lead to the discovery of novel and at times unexpected factors that drive hepatocyte proliferation after resection. One such development was the description of the critical role of platelets and platelet-derived serotonin in liver regeneration(50). In particular, these investigations demonstrated that thrombocytopenic mice (or mice with a variety of functional platelet defects) had a significant impairment in hepatocyte proliferation after PH. This deficit could be corrected by reconstituting the organism's supply of serotonin, a hormone typically carried by platelets.

Mice with hepatocyte-specific over-expression of glypican 3 exhibit decreased cell proliferation and restoration of liver weight after PH(51). Other recent work has focused on

the role of the extracellular matrix in determining the appropriate size of the liver at the completion of regeneration, i.e. regulating the termination phase of regeneration. Mice with a hepatocyte-specific loss of integrin-linked kinase subjected to PH, were left with livers an average of 58% larger than their original weights(52). The proposed mechanism was sustained activation of the HGF and beta-catenin pathways.

Second Line of Defense- Regeneration by Liver Progenitor cells

As mentioned at the outset of this review, when hepatocytes are prevented from proliferating, liver progenitor cells serve as the second line of defense against liver failure. Farber first described the presence of a liver progenitor cell population in 1956 when he noted the presence of small cells with high nuclear-cytoplasmic ratio and called them “oval cells”(53). Work by Fausto(54), Sell(55) and others demonstrated that these cells were activated in animal models of liver injury and had bipotential ability to differentiate into hepatocytes and bile duct cells. Most of the data on this cell population has come from animal models that use toxins to inhibit native hepatocytes, in conjunction with a trigger to stimulate liver regeneration.

The adult human equivalent of these progenitor cells have been localized to the terminal bile ductules, known as the canals of Hering(56). This quiescent cell population acts as reserve population to be activated only when the adult hepatocytes are not able to repair and regenerate the injured liver, either due to senescence or cell cycle arrest due to liver toxins such as alcohol(57). Upon activation, these progenitor cells proliferate in the portal zone and are seen as a collection of progenitor cells and cells of intermediate differentiation(58). The “streaming liver hypothesis”(59) proposes that these cells then migrate toward the central vein in the liver lobules as progressively differentiated daughter hepatocytes. Using mitochondrial DNA mutation tracking, this was demonstrable in the normal human liver(60) as well as in regenerative nodules of liver cirrhosis(61).

While the above is the most widely accepted concept, work by Kuwahara *et al*(62) suggests that it may be an oversimplification, and that the liver has a multi-tiered system of regeneration. There maybe up to four potential stem cell niches, in the canal of Hering, intralobular bile ducts, periductal mononuclear cells and peribiliary hepatocytes, respectively.

Identifying the intrinsic liver stem cell

One of the key challenges facing the liver progenitor field is that many of the reported progenitor cell populations have different and variable immunomarkers. While rat oval cells are OV-6 positive and appear to express albumin, alpha-fetoprotein (AFP) and CK19 markers(63,64), there are a paucity of epitopes to detect mouse oval cells, with the exception of A6(65,66). Using a systematic screen, Grompe’s group has identified several novel antibodies that define subpopulations of these progenitor cells(67). These include MIC1-1C3; OC2-1D11; OC2-2F3 (ductular oval cells) and OC2-1C6; OC2-2A6; OC2-6E10 (periductular oval cells). This work promises new tools that will reliably isolate and characterize each oval cell subset. Other markers, such as CD34, c-kit, and CD90, have been less consistent(64,68). For example, CD90, a widely reported stem cell marker, was recently shown to be detect myofibroblasts rather than in progenitor cells in the liver(69). It is likely that the liver progenitor population is a heterogeneous group of cells, which, depending on the model from which these progenitor cells are derived(62,70), specific culture techniques, and whether the cultures are clonal, may have a different cell signature.

In humans, recent reports from several labs have identified a seemingly common progenitor cell population defined by expression of EPCAM, CK19 and CD44(64,71–73)(Figure 2).

These cells have been extensively detailed by Reid and are positive for CD133, claudin, and NCAM, but negative for albumin and AFP(73,74). In acute and chronically injured livers, as well as in developing fetal livers, these cells give rise to transit amplifying cells analogous to fetal hepatoblasts, which mature to form hepatocytes and bile duct cells(75). Collectively, they comprise the most well characterized entity representing the facultative human liver progenitor cell.

The origin of Liver Progenitor cells

The issue of whether liver progenitor cells may be a continuous population with bone marrow stem cells was first raised by the observation that female recipients of male bone marrow transplants had hepatocytes with XY chromosomes(56,76). This was followed by a flurry of reports on the ability of transplanted bone marrow or cord blood progenitors to repopulate animal models of liver injury(77–80). The most notable of these used the mouse model of tyrosinemia(79), and demonstrated that the liver could be completely regenerated by bone marrow stem cells. This phenomenon was subsequently found to be predominantly due to fusion(81). While there continues to be controversy) regarding whether bone marrow cells can transdifferentiate into hepatocyte- like cells under certain conditions(82, the weight of evidence suggests that the contribution of bone marrow to normal liver regeneration is insignificant(83,84).

The observation that liver progenitor cells have mixed epithelial and mesenchymal markers(72,73,85) and the ease by which mesenchymal stem cells can be converted to hepatocyte-like cells(86–88) raised the possibility that they may arise from mesenchymal lineage via mesenchymal to epithelial transition. Sicklick *et al*(89) further proposed that progenitor cells may be derived from hepatic stellate cells, and that the sonic hedgehog pathway regulated this process. In a follow up study, Yang *et al*(90) used cell fate mapping to show that stellate cells could became oval cells when activated in liver injury, and that these cells participate in ductular proliferation. The notion that there is a common schema within the stellate cell driving both fibrosis and regeneration by fluxing between epithelial and mesenchymal phenotypes(91,92) is an attractive one, but has not been borne out by other investigations. Careful fate mapping studies failed to show any evidence of mesenchymal to epithelial transition or vice versa during liver injury (93,94).

In light of conflicting evidence, the role of epithelial-mesenchymal transition and vice versa in liver injury and repair remains highly controversial(95,96). Nevertheless, taken in context with current evidence, it is likely that the majority of liver progenitor cells are *in situ* cells that are descendants of the fetal ductal plate(75). The main strategy in attempting to augment regeneration in the clinical setting thus lies in increasing the numbers of these progenitor cells following liver injury, either by stimulating the stem cell niche to proliferate, or simply by transplanting more progenitor cells into the injured liver.

The physiological role of regeneration by progenitor cells

The role of progenitor cell regeneration in normal liver physiology is still debated. These cells likely have no significant role in day-to-day liver turnover(97). The progenitor compartment is activated only in severe liver injury, and the belief that it plays an important role in regenerating the injured liver comes from three lines of evidence.

First, progenitor cells are present in advanced stages of many human liver diseases in which native hepatocytes are believed to be senescent or inhibited from proliferating, such as alcoholic and non-alcoholic cirrhosis, chronic viral hepatitis, and primary biliary cirrhosis(57,97–101). The presence of these cells directly correlates with both inflammation and the degree of liver injury(102); patients with higher MELD scores appear to have more

progenitor cell activation(103). Second, studies of chronic viral hepatitis in human patients showed that these progenitor cells are indeed surrounded by hepatocyte-like cells of intermediate differentiation, suggesting ongoing regeneration(75,102). Tracing of thymidine labeling in animal models(62,104) shows that progenitor cells differentiate into both hepatocytes and cholangiocytes. Lastly, transplantation of ex-vivo progenitor cells in animal models of liver injury has been convincingly shown to engraft and repopulate the liver(105,106), further underlining the capacity for these cells to regenerate.

Interestingly, although ductular proliferation is also seen after bile duct ligation and in primary biliary cirrhosis, the response in these systems is believed to come from cholangiocytes rather than progenitor cells. In advanced primary biliary cirrhosis, when cholangiocyte proliferation is arrested, proliferating ductal cells lean towards an undifferentiated pre-cholangiocytic phenotype, suggesting that the progenitor response is tailored and specific to the injury process(98,99,107).

In acute liver failure, progenitor cell proliferation has also been noted as a response mechanism, which fits with the understanding that progenitor proliferation kicks in when the liver is in “dire straits”(103). A threshold of loss of 50% of hepatocytes in conjunction with reduced proliferative activity of remaining mature hepatocytes triggers the progenitor population within the first week, with appearance of intermediate hepatocytes only after that week. The degree of progenitor cell activation correlates positively with clinical outcomes.

Despite the accumulating evidence of progenitor cell proliferation in liver injury, the extent to which progenitor cell regeneration contributes to repair and the natural history of human liver disease is not known. The triggers that activate this reserve component are also not well understood. Recent evidence using mitochondrial mutation tracking suggests that some of the regenerative nodules in liver cirrhosis are clonal and are likely to have arisen from a related facultative progenitor cell from a neighboring ductular reaction(61). It is likely that this regenerative process keeps the patient compensated and delays the onset of liver insufficiency, with clinical disease occurring only when the regeneration of these cells can no longer keep up with the injury process. Yet the fact that these cells are activated to a large degree only in end stage cirrhosis or fulminant liver failure, once liver injury is not reversible, suggests that manifestation of clinical disease may be more complex than just hepatocyte insufficiency alone. If this were the case, it would limit the ability of a progenitor cell transplant to reverse clinical outcomes in such late stage disease.

Understanding the liver stem cell niche

A stem cell environment, or “niche”, is believed to maintain the liver progenitor cell in its native state, and allows for regulatory signals to activate it when required(108). The companion supportive cells in this niche have long been suspected to be mesenchymal cells, such as portal fibroblasts, hepatic stellate cells or vascular endothelial cells(75). Yovchev *et al* reported that these cells are CD90 positive, explaining the previous misinterpretation of CD90 as a stem cell marker(64). More recent *in vitro* work suggests that angioblasts, CD133 or CD117 cells co-expressing vascular endothelial growth factor receptor 2 (VEGF R2), maintain and encourage the proliferation of progenitor cells in their native state. Other cell types, such as endothelial and hepatic stellate cells, support their differentiation into different lineages(109).

Multiple autocrine and paracrine factors have been reported to activate liver progenitor cells, and have been discussed in detail in excellent recent reviews(110,111). These include inflammatory cytokines, which are similar to those that stimulate mature hepatocyte proliferation and include the IL6 family, IL18, TNFα, interferon α and γ, stem cell factor, stromal derived factor (SDF-1), lymphotoxin beta, TNF-like weak inducer of apoptosis

(TWEAK)(112) and even the sympathetic nervous system. More recent discoveries include regulatory proteins such as MERLIN(113), which acts on the EGFR to regulate progenitor cell proliferation; Foxl1(114), a mesenchymal forkhead winged helix factor that may come from surrounding portal fibroblasts, and the Wnt/sonic hedgehog pathways that trigger ductal proliferation in alcoholic steatohepatitis(115,116). Other paracrine messengers from neighboring mesenchymal cells include HGF, FGF, and TGFα and β(111). Interestingly, these factors appear to have opposite effects on hepatocytes and progenitors, which may explain the regulatory mechanisms that transfer regeneration from one compartment to the other(116). Extracellular matrix arrives from surrounding cells is also thought to be important(117). Nevertheless, while there have been a wealth of studies on the mechanisms that regulate activation, proliferation, migration and differentiation of progenitor cells, translation into clinical intervention has not been forthcoming, underlying the complexities of manipulating network regulation.

Increasing progenitor cell populations

Repopulating the damaged liver is the key goal of progenitor cell therapy for liver failure. Multiple candidate cells of origin have been explored and several cell types have been shown to be able to differentiate *in vitro* into hepatocyte-like cells and repopulate animal models of liver injury(110,118). In general, these candidates progenitor cells classified into the upstream progenitors: fetal liver progenitors, embryonic stem cells (ESC) and induced pluripotent cells (IPSC)(119–121). Of these, IPSC may be most attractive, as they can be taken autologously from the patient, do not carry the ethical concerns of ES cells, and recently have been produced without viral vectors and are able to reconstitute the liver after 2/3 PH in FAH mice(122). Other approaches have looked at transdifferentiation of hepatocytes from other progenitor cell sources. Mesenchymal stem cells, whether from bone marrow, cord blood, cord lining cells, cord matrix cells, amniotic cells, adipose progenitor and muscle progenitors have been demonstrated to be capable of differentiation into hepatocytes *in vitro*(110,118).

Clinical Studies: (Table 1)

While it would be exhaustive to describe all human transplantation studies to date, a review on liver regeneration would be incomplete if studies relating to the original aims of understanding regeneration are not covered. To date, some 20 human studies have been undertakin, in which attempts were made to enhance liver regeneration. These can be grouped into adult hepatocyte transplantation, fetal hepatocyte transplantation and bone marrow stem cell transplantation.

As mature hepatocytes are the main cells that regenerate the injured liver, roughly 25 patients with acute liver failure have been transplanted with ten to a thousand million hepatocytes in an attempt to salvage the failing liver(123). Instead of adult hepatocytes, Habibullah *et al* (124) transplanted 6 patients with acute liver failure with 10^7 fetal hepatocytes. In these studies, there were transient clinical improvements in encephalopathy and ammonia levels, but there was no overall transplant-free survival benefit. It is likely that the quantity of cells (up to 5% of liver mass) transplanted for each patient may have been too low to register a clinical benefit, and that the window period was too narrow for these cells to regenerate.

Although the use of bone marrow stem cells as candidates for liver regeneration is controversial, the availability of these cells and ease by which they can be harvested has lead to the transplantation of bone marrow stem cells or peripheral blood stem cells in more than 100 patients with cirrhosis. Of note, one small study employed the infusion of AC133+ cells mobilized from the bone marrow after one lobe of the liver has been deliberately embolised,

and showed that regeneration in the remaining lobe was augmented(125). Most of these studies are uncontrolled, but clinical improvement in measurable parameters has been claimed(126). The mechanisms by which improvement has occurred are still not known, but studies have shown that remodeling in cirrhotic liver can occur by paracrine signals (metalloproteinases) from bone marrow mesenchymal cells, without actual transdifferentiation into hepatocytes. Whether this work represents true progenitor cell regeneration or the modulation of local environment for native hepatocytes to regenerate, this strategy may yet be promising as long as liver regeneration occurs and clinical outcome is improved.

Conclusion

The pursuit of understanding liver regeneration has yielded great progress over the last few decades. Technology has allowed us to decipher regulatory networks that control regenerative mechanisms, and has opened up options for therapeutic manipulation. This work has tremendous implications for clinical applications in acute liver failure, small for size transplantation, extensive liver resection, and delay of morbidity and mortality for cirrhotic patients. Regardless of whether this can be achieved by transplantation of progenitor cells to regenerate the liver, or supportive cells to enhance native regeneration, or by drugs to augment hepatocyte regeneration, a clear understanding of these mechanisms is needed to avoid tragic clinical complications that may set the field back. In tandem with other diseases, the world is poised to leap into human studies with stem cell therapies, representing the amalgamation of knowledge, hopes and public expectation. The drive to understand liver regeneration so as to be able to make a difference to our patients has never been more intense.

Abbreviations

PH	partial hepatectomy
TNF	tumor necrosis factor
NF-κB	nuclear factor-kappaB
IL6	interleukin 6
STAT 3	signal transducer and activator of transcription 3
IKK2	inhibitor of kappaB kinase 2
EGF	epidermal growth factor
TGFα	transforming growth factor alpha
HB-EGF	heparin binding EGF-like growth factor
HGF	hepatocyte growth factor
PI3K	phosphoinositide 3 kinase
Pdk1	phosphoinositide-dependent protein kinase 1
SOCS	suppressor of cytokine signaling
SDF	stromal cell-derived factor
TWEAK	tumor necrosis factor-like weak inducer of apoptosis

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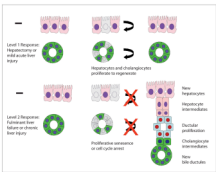

Figure 1. The Two Levels of Liver Regeneration

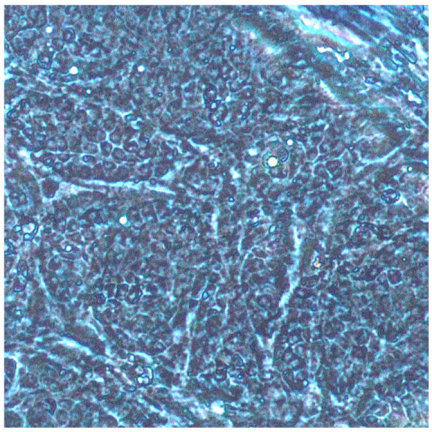

Figure 2. Cluster of Fetal Liver Progenitors

These cells have high nuclear-cytoplasmic ratio and are positive for EPCAM, CD44, CK19 and NCAM but negative for albumin, AFP and CK7. They are maintained on laminin extracellular matrix, mouse embryonic fibroblast feeder layers and kept in cultures with high dose FGF (unpublished data)

Table 1

Potential Strategies and Targets for Augmenting Regeneration (110,122,125)

	Potential targets	**Current Status**	**Human Studies**	**Potential**
Manipulation of signaling pathways	Inflammatory cytokines Growth Factors Regulatory Proteins	Wealth of knowledge GCSF efficacy in animal studies	Reported off label use but no conclusive clinical benefit yet.	Selective delivery to liver attractive option.
Hepatocyte Replacement	Hepatocytes from unused liver graft	Able to harvest and cryopreserve but unable to expand ex vivo	Transplanted in acute liver failure with limited success	Limited graft availability unless successful ex vivo expansion
Progenitor Replacement	Facultative Progenitors Precursors: (ESC/ IPSC/ Fetal) MSC (Bone marrow/Cord/ Amniotic / Adipose) Germ Cells	Good ex vivo evidence of differentiation and efficacy in rodent studies. Large # of	Only Bone marrow and PBSC MSC transplanted in 11 human studies – Differentiation into hepatocytes not proven	Needs large # of functional cells Safety and ethical concerns in some sources Potential for autologous transplants
Modulate repair and regeneration	? MSC, ? Endothelial Progenitor ? Cell culture Supernatant ?Metalloproteinase	Mechanism still not elucidated. Animal studies underway	BM or PBSC in 11 human studies show possible benefit	Bone marrow or PBSC sources readily available.

BM, bone marrow; GCSF, granulocyte colony stimulating factor; ESC, embryonic stem cells; IPSC, induced pluripotent cells; MSC, mesenchymal stem cells; PBSC, peripheral blood stem cells