Published in final edited form as:

Curr Opin Organ Transplant. 2013 April ; 18(2): 197–202. doi:10.1097/MOT.0b013e32835f07e2.

Liver repopulation and regeneration: new approaches to old questions

Andrew W. Duncan^{a,b} and Alejandro Soto-Gutierrez^{a,b,c,d}

^aDepartment of Pathology, University of Pittsburgh

^bMcGowan Institute for Regenerative Medicine, University of Pittsburgh

^cThomas E. Starzl Transplantation Institute, University of Pittsburgh

^dTransplantation Section of Children's Hospital of Pittsburgh, Department of Surgery, Center for Innovative Regenerative Therapies, Pittsburgh, Pennsylvania, USA

Abstract

Purpose of review—Significant recent developments have occurred in the field of liver regeneration. Although the regenerative response to partial hepatectomy has been studied extensively, in recent years the use of new experimental approaches has incorporated a fresh look that may lead to a better understanding of hepatocyte dysfunction and regeneration.

Recent findings—Liver injury promotes the regenerative responses that are relatively rare in healthy livers. Current research efforts focus on the mechanisms of hepatocyte adaptation in response to liver injury. We will discuss how hepatic aneuploidy and polyploidy contributes to liver regeneration, as well as new modalities to study cellular interactions using the organ-specific microenvironment.

Summary—High mortality is generally limited to patients who develop terminal liver failure, which occurs when regenerative responses are unable to compensate for liver injury. Cellular adaptations and organ microenvironment changes are present during disease processes. This review aims to provide insights into the innovative approaches taken to investigate regeneration in liver diseases.

Keywords

aneuploidy; liver regeneration; liver repopulation; liver tissue engineering; polyploidy

INTRODUCTION

The liver has extraordinary regenerative capabilities, a phenomenon that has been known for centuries [1]. Historically, partial hepatectomy in rodents has been one of the most studied experimental models, revealing amazing molecular and cellular mechanisms behind liver regeneration [2]. Partial hepatectomy is highly reproducible and results in a proliferative stimulus of the residual liver mass that is mediated by tissue-specific mechanisms in the

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Correspondence to: Alejandro Soto-Gutierrez, MD, PhD, Department of Pathology, University of Pittsburgh, 3511 Rangos Research Building, 530 45th Street, Pittsburgh, PA 15201, USA. Tel: +1 412 692 5562; fax: +1 412 692 6599; sotogutierrez@upmc.edu.

Conflicts of interest

This work was supported by the grants to A.S.G. from the NIH (DK083556) and the American Society of Transplantation, as well as a grant to A.W.D. by the Commonwealth of Pennsylvania Department of Health (4100061184).

None declared.

absence of significant cell death. The use of genetically engineered mice has also facilitated the study of molecules and signaling pathways that participate in liver regeneration [3]. In recent years, global gene-expression profiling has been used to elucidate the complex cascade of molecular events that regulate liver regeneration.

Although partial hepatectomy has been used as an experimental model for decades, contributing to the understanding of physiologic principles of initiation and termination of liver regeneration, partial hepatectomy does not necessarily reflect the regenerative process during human disease. Liver regeneration in patients is influenced by numerous factors, including tissue necrosis, innate immunity, and varying degrees of acute or chronic inflammation. New approaches to investigating the liver seek to provide novel insights into regeneration, homeostasis, and disease. Herein, we will highlight three promising areas of investigation. First, although hepatic polyploidy has been extensively characterized, pervasive aneuploidy in normal liver tissue was recently described. New studies suggest that random hepatic aneuploidy promotes adaptation to chronic liver disease. Secondly, liver function is controlled by more than just hepatocytes. New research aims to elucidate how the different cell types within the liver coordinate to regulate liver function. Thirdly, liver function is influenced by the architecture of the organ. Current efforts utilizing organ bioengineering technology seek to elucidate how the three-dimensional architecture, extracellular matrix content, and different cellular interactions affect liver function.

ANEUPLOIDY AND POLYPLOIDY IN LIVER REGENERATION

The composition of the liver changes dramatically with age. Whereas hepatocytes in young individuals are relatively small and uniform in size, adult hepatocytes vary considerably in cell and nuclear size, number of nuclei per cell, and DNA content per nucleus. Many of the striking morphological changes associated with liver aging involve hepatic polyploidy (i.e., a numerical change in the entire complement of chromosomes). Hepatocytes are either mononucleate or binucleate, and ploidy is determined by the number of nuclei per cell as well as the ploidy of each nucleus [4]. Polyploid hepatocytes can be tetraploid (e.g., binucleate with two diploid nuclei or mononucleate with a single tetraploid nucleus), octaploid (e.g., binucleate with two tetraploid nuclei or mononucleate with a single octaploid nucleus), and so on. Hepatocytes are almost exclusively diploid in young individuals, and the degree of polyploidy increases with age. Polyploidy characterizes up to 90% of adult hepatocytes in mice [5] and approximately 50% in humans [6¹¹]. The primary mechanism for polyploidization involves failed cytokinesis and is related to alterations in insulin/AKT signaling [4,7,8]. Although polyploid hepatocytes were documented over a century ago [9], the specialized role played by these cells in liver homeostasis, regeneration, and disease is poorly understood.

Polyploid hepatocytes are historically thought to have limited mitotic capacity [10], but it has become apparent that polyploid hepatocytes are actually highly proliferative [5,11–13]. Octaploid mouse hepatocytes, isolated by fluorescence activated cell sorting and transplanted into mice undergoing liver failure, proliferate extensively to repopulate the entire host liver [5]. During regeneration, proliferating polyploid hepatocytes produce daughters of equal or greater ploidy, depending on the outcome of cytokinesis. Surprisingly, they also generate progeny with reduced ploidy. For example, transplanted octaploid hepatocytes produced octaploid and hexadecaploid daughters, as expected, but they generated neartetraploid and near-diploid progeny as well. Timelapse imaging shows that proliferating polyploids establish multiple spindles early in mitosis [5]. In most cases, spindles reorient into a bipolar arrangement, but in 5% of divisions the spindles never coalesce, and multipolar nuclear segregation ensues. Multipolar cell divisions generate three

or more nuclei in a single division; a subset of daughter nuclei inherit reduced nuclear content compared to the parental cell.

The formation of multipolar spindles by polyploid hepatocytes affects the fidelity of nuclear segregation [5]. Chromosomes can distribute unevenly during segregation toward multiple poles. Additionally, even in the majority of cases in which transient multipolar spindles resolve into a bipolar orientation, chromosomes are frequently excluded from the daughter nuclei. These ‘lagging’ chromosomes arise from merotelic chromosome attachments. As hepatocytes become polyploid in the postnatal period when growth and regeneration are ongoing, the overall result is that most hepatocytes are aneuploid, a condition defined by the gain or loss of whole chromosomes. Aneuploidy is seen in approximately 70% of adult mouse hepatocytes [5,14] and 30–90% of adult hepatocytes in humans [6[■]]; chromosome rearrangements are rarely detected. This high-degree aneuploidy is random, affecting all chromosomes equally, and occurs in healthy individuals lacking evidence of tumorigenesis. The process by which hepatocytes polyploidize, become aneuploid, and undergo ploidy reversal is called the ‘ploidy conveyor’ [5]. Aneuploidy is seen in many different healthy tissues, including blood, skin, brain, and placenta [15–17], albeit at much lower levels compared to the liver. Thus, the high incidence of random aneuploidy in the healthy liver is striking and suggests that this process could play some sort of physiological role.

How do aneuploid hepatocytes affect liver function? Taking a cue from the yeast literature [18–21], a compelling new idea suggests that aneuploidy is beneficial and promotes adaptation to chronic injury via a multistep process [22[■]]. First, extensive aneuploidy creates a high level of genetic diversity within the liver. Chromosome gains and losses impact the overall level of gene expression. Moreover, up to 10% of genes are monoallelically expressed, leading to complete loss-of-function of a large number of genes in cells with monosomies [23]. In the healthy adult, heterogeneous hepatocytes collaborate to maintain normal liver function. Secondly, although continuous exposure to liver-specific toxins kills the vast majority of hepatocytes, a subset of aneuploid hepatocytes emerges that is refractory to liver damage. Cells with injury-resistant karyotypes either preexist in the liver or they arise by multipolar divisions by polyploid hepatocytes. Finally, injury-resistant aneuploid hepatocytes respond to regenerative signals and proliferate to restore liver mass and homeostasis. The notion of proadaptive aneuploidy in the liver was recently validated in a mouse model of hereditary tyrosinemia [22[■]]. In response to tyrosinemia, which ordinarily leads to liver failure and death, most of the hepatocytes in the liver quickly became pale and sickly. However, a subset of hepatocytes dispersed randomly throughout the liver remained healthy and expanded tremendously. Tyrosinemia-resistant hepatocytes protected the liver and prevented death of the animal. Karyotype analysis demonstrated that tyrosinemia-resistant cells share a common chromosome abnormality, monosomy of chromosome 16. Thus, these observations prove that hepatic aneuploidy can greatly promote liver regeneration, especially in the context of liver disease.

The extent to which hepatic aneuploidy affects health, regeneration, and disease in humans is a mystery. Preexisting aneuploidy could serve as ‘first hit’ en route to tumor formation. However, considering the high degree of hepatic aneuploidy and the paucity of spontaneous liver cancer, it is clear that aneuploidy is not always a predisposition for liver cancer. Alternatively, and perhaps more importantly, hepatic aneuploidy could promote stress-induced liver regeneration, similar to what was seen in mice. In the case of hepatitis C infection, for example, it was shown that up to half of noncancerous cirrhotic nodules were clonal [24,25]. It is therefore possible that such clonal nodules were derived from aneuploid hepatocytes resistant to hepatitis C; and rigorous testing of this hypothesis remains to be performed. Currently, it is tantalizing to consider how naturally occurring hepatic aneuploidy or induced aneuploidy might facilitate liver regeneration in the context of other

diseases. New therapies or diagnostic tools centered on hepatic aneuploidy may become viable strategies in the future.

LIVER MICROENVIRONMENT AND REGENERATION

The function and morphogenesis of tissues, including the liver, is heavily influenced by the biophysical configuration and biochemical properties of the cellular microenvironment. Emerging evidence indicates that organ-specific factors (e.g., chemokines, growth factors, and extracellular matrix) and cell types (e.g., macrophages, stellate cells, sinusoidal endothelial cells, and bile duct cells) influence the myriad functions of hepatic cells during homeostasis and pathological conditions.

It is well established that liver progenitor cells become activated when hepatocyte functional/ regenerative capacity is impaired [3,26]. In a recent important work, Boulter *et al.* [27[■]] showed that the fate of bipotential progenitors is influenced by injury-specific changes in the progenitor niche. This elegant study demonstrated a role for Kupffer cells in the expansion of hepatic progenitor cells. In response to the phagocytosis of biological debris, which occurs during hepatocellular regeneration induced by choline-deficient ethionine (CDE) supplemented diet in mice, macrophages express Wnt3a. Secretion of Wnt3a by Kupffer cells induces neighboring progenitor cells to express Numb, which then suppresses Notch transcriptional targets, including cholangiocyte-associated genes. Differentiation of progenitor cells preferentially along the hepatocytic lineage promotes replacement of the damaged hepatocytes. Consistent with the idea that Kupffer cell activity attenuates progenitor cell differentiation, systemic ablation of macrophages using clodronate liposomes caused an increase in ductular structures in livers of mice undergoing hepatocellular injury. Whether this effect can be achieved by depletion of exclusively Kupffer cells will have to be elucidated.

These new data raise the exciting possibility that Kupffer cells (through Wnt pathway) can change the balance of ductular reactions and direct the differentiation abilities of progenitor cells following toxin-mediated injury. This observation could aid in the development of new diagnostic tools, as well as treatment modalities to improve liver regeneration.

Resident natural killer (NK) cells, the major lymphocyte population in the liver sinusoids, have also been implicated in promoting liver regeneration [28[■]]. Release of extracellular ATP by stressed cells or necrotic cells functions as a potent 'find-me' signal for recruitment of phagocytes [29]. The release of endogenous nucleotides, like ATP, also functions to modulate cellular crosstalk, injury, and proliferation during liver regeneration [30]. In a recent work, Graubardt *et al.* [31] showed that NK cells express markers of activation and cytotoxicity, P2 type receptors, after partial hepatectomy in response to ATP release by necrotic cells. To demonstrate a direct correlation between NK cells and extracellular ATP release, ATP phosphohydrolase, an enzyme that induces clearance of extracellular ATP, was administered and markers of cell replication assessed. Surprisingly, animals that received ATP phosphohydrolase after partial hepatectomy showed elevation of cellular replication. This work suggests the possibility that manipulation of the immune system could accelerate the restoration of liver tissue following injury.

Chronic tissue injury caused by ischemia, autoimmunity, or numerous other processes results in changes in the composition of the extracellular matrix, leading to hepatic dysfunction and liver failure. Our group recently demonstrated the importance of the liver microenvironment during advanced cirrhosis [32[■]]. Liu *et al.* showed that cells derived from cirrhotic livers with decompensated function had severe alterations in gene expression, as well as defects in proliferative capacity. Repopulation experiments proved that hepatocytes from rats with decompensated cirrhosis could engraft in livers of noncirrhotic

recipients. Initially, the donor cells failed to expand or produce albumin, but after 2 months engrafted hepatocytes were able to nearly completely repopulate livers of retrorsine-treated analbuminemic rats. This report shows for the first time that hepatocytes from end-stage cirrhotic livers could recover secretory and replicative functionality when placed in a noncirrhotic liver environment. It would be fascinating to determine how the cirrhotic microenvironment impairs hepatocyte-specific metabolic, biosynthetic, and excretory functions. Such approaches are very exciting as they offer the promise of improving hepatocyte function outcomes, even when the specific cause of liver injury cannot be cured.

A major milestone in liver regeneration and cell–cell communication was the demonstration of the role of gap junctions in the prevention of fulminant hepatic failure and acetaminophen-induced hepatotoxicity. Elegant experiments by Patel *et al.* addressed the role of connexin 32 (Cx32) [33], a key hepatic gap junction protein, during drug-induced liver injury. Studies showed that liver-specific gap junction inhibitors could limit liver injury caused by hepatotoxic drugs by affecting cell-cell communication [34[■]]. Cx32-deficient (Cx32^{-/-}) and wildtype mice were treated with either thioacetamide or acetaminophen to induce liver injury. Interestingly, Cx32^{-/-} mice were protected from liver injury. Moreover, treatment of wildtype mice with a small molecule inhibitor of Cx32 gap junctions produced a similar hepatic protective effect. These results implicate a role for gap junctions in mediating the damage response to liver injury. Thus, cellular communications between parenchymal cells of the liver may represent an interesting mechanism for enabling amplification of injury, making it a promising therapeutic target for hepatoprotection.

In recent years, a significant amount of attention has been drawn to the concept of the microenvironment and liver regeneration in an effort to better describe and predict the phenotypic characteristics of tissue repair. Improved understanding of the unique interplay between the various aspects of parenchymal cells, nonparenchymal cells, and the microenvironment will be useful in the search for novel therapeutic targets.

ORGAN ASSEMBLY AND REGENERATION

Despite the advantages of performing experiments on live animals, elucidation of signaling pathways and specific cell interactions in live animals and whole tissues has fundamental limitations. Whole organs are comprised of diverse cell types, and the data obtained from tissues does not represent parenchymal cells only. The techniques of liver cell isolation and hepatocyte culture, which were pioneered in the 1970s, were instrumental in helping to dissect the role of discrete populations of liver cells. One of the primary limitations of culturing primary cells in petri dishes, however, is that cell-cell interactions, as well as interactions between cells and the extracellular matrix are disrupted. New approaches seek to separate the cellular content of an organ from its stromal structure, a process called decellularization, which could facilitate the study of cell-cell and cell-matrix interactions in an organlike environment.

The concept of perfusion decellularization of an organ was proposed and realized within the last decade [35[■]]. This approach utilizes the vascular bed as an efficient means to decellularize the intact organ by reducing the diffusion distance required for decellularization agents to reach the cells and facilitating the removal of the cellular material from the tissue by convective transport [36[■]]. This technique has allowed for the creation of acellular scaffolds that retain their natural vascular channels, which can be recellularized by infusion with defined populations of cells. Tissue engineered hearts, livers, and lungs have been decellularized using this strategy [35[■],37,38[■],39,40]. The objective of the decellularization process is to maximize the removal of cellular material from the tissue, while minimizing damage and loss of target extracellular matrix components. To date, the

criteria that define successful whole-organ decellularization are poorly understood, and techniques for optimized decellularization are not well established.

It is hypothesized that biological scaffolds derived from decellularized livers could provide a rich environment (in terms of three-dimensional architecture and distribution of extracellular matrix components) for liver cell culture [37,38[■],39]. The native liver matrix presents an attractive model to study processes such as liver development and hepatic maturation, as well as complex interactions between parenchymal and nonparenchymal liver cells. For example, cell–cell interactions play an important role in regulating cell fate in numerous tissues [41,42], including the liver [36[■],43]. The use of liver decellularization approaches followed by liver ‘reassembly’ with defined populations of liver cells would allow investigators to probe cell–cell interactions in an organ-like microenvironment. Additionally, from a structural point of view, the three-dimensional configuration and the unique stiffness provided in the decellularized organs have been shown to dramatically affect integrin-binding and cell fate decisions [44].

Currently, a number of groups (including our own) are working to overcome the hurdles during each step of the decellularization and recellularization processes. For instance, because standardized procedures are essential to generate high-quality scaffolds, optimized protocols with measurable parameters for liver decellularization are under development. Additionally, the organ assembly process requires the use of multiple cell types that will eventually form an appropriate tissue structure. Real-time imaging techniques are needed to evaluate cell delivery and mobilization of different cell types during tissue assembly. Thus, liver assembly systems hold a great deal of promise, especially as we learn more about decellularizing existing livers and reconstructing them with defined populations of cells. In the future, this new technology could be used to construct artificial organs with similar architecture and functionality to normal organs that can be cultured, grown, and even transplanted.

CONCLUSION

Liver regeneration has been appreciated for centuries, and in the last few decades we have begun to understand how the process works. Recent efforts have focused on understanding the liver regeneration in the context of disease processes such as hepatocellular carcinoma and acute/chronic liver failure. A better understanding of the liver microenvironment and the processes by which all the liver cells coordinately respond to injury will likely translate into new therapies and diagnostic procedures for the detection and treatment of liver disease. Moreover, the use of innovative approaches, such as those outlined above, could also translate into strategies to prevent liver failure in at-risk patients. The ability to control liver regeneration has numerous potential applications. First, in liver cancer, aggressive hepatic resections are not practical because of the likelihood of developing hepatic failure as a consequence of insufficient hepatic mass. The use of pro-regenerative therapy should permit the removal of even greater amounts of liver tissue with reduced risk of hepatic failure. Secondly, in patients with inborn errors of metabolism, regenerative therapy could promote the repopulation of diseased livers by healthydonor hepatocytes. Finally, in the context of living-donor liver transplantation, regenerative therapy could revolutionize living-donor liver transplantation. For instance, left-lateral grafts are currently considered insufficient for transplantation into adult recipients, but this approach may become a viable surgical option if performed in tandem with therapies that boost liver regeneration. In addition, liver transplantation procedures induce ischemia–reperfusion injury, an insult that likely induces some degree of liver regeneration. It will be interesting to rigorously determine how ischemia–reperfusion affects repopulation and regeneration, and, importantly, how these parameters can be manipulated to improve current liver therapies. In conclusion, new

methodologies to studying the liver hold a great deal of promise; it will be exciting to see how these approaches contribute to improved strategies for the treatment and detection of disease.

Acknowledgments

None.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- ■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science*. 1997; 276:60–66. [PubMed: 9082986]
2. Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. *Am J Pathol*. 2010; 176:2–13. [PubMed: 20019184]
3. Riehle KJ, Dan YY, Campbell JS, Fausto N. New concepts in liver regeneration. *J Gastroenterol Hepatol*. 2011; 26(Suppl. 1):203–212. [PubMed: 21199532]
4. Guidotti JE, Bregerie O, Robert A, et al. Liver cell polyploidization: a pivotal role for binuclear hepatocytes. *J Biol Chem*. 2003; 278:19095–19101. [PubMed: 12626502]
5. Duncan AW, Taylor MH, Hickey RD, et al. The ploidy conveyor of mature hepatocytes as a source of genetic variation. *Nature*. 2010; 467:707–710. [PubMed: 20861837]
6. Duncan AW, Hanlon Newell AE, Smith L, et al. Frequent aneuploidy among normal human hepatocytes. *Gastroenterology*. 2012; 142:25–28. [PubMed: 22057114] This study characterizes aneuploidy in hepatocyte samples from patients. Random aneuploidy is found in 30–90% of adult hepatocytes. Importantly, aneuploidy is found in hepatocytes from healthy liver tissue, lacking evidence of tumorigenesis. The data suggest that random aneuploidy is a normal feature of the liver.
7. Celton-Morizur S, Merlen G, Couton D, et al. The insulin/Akt pathway controls a specific cell division program that leads to generation of binucleated tetraploid liver cells in rodents. *J Clin Invest*. 2009; 119:1880–1887. [PubMed: 19603546]
8. Margall-Ducos G, Celton-Morizur S, Couton D, et al. Liver tetraploidization is controlled by a new process of incomplete cytokinesis. *J Cell Sci*. 2007; 120(Pt 20):3633–3639. [PubMed: 17895361]
9. Milne LS. The histology of liver tissue regeneration. *J Pathol Bacteriol*. 1909; 13:127–158.
10. Hoare M, Das T, Alexander G. Ageing, telomeres, senescence, and liver injury. *J Hepatol*. 2010; 53:950–961. [PubMed: 20739078]
11. Overturf K, Al-Dhalimy M, Finegold M, Grompe M. The repopulation potential of hepatocyte populations differing in size and prior mitotic expansion. *Am J Pathol*. 1999; 155:2135–2143. [PubMed: 10595942]
12. Sigal SH, Rajvanshi P, Gorla GR, et al. Partial hepatectomy-induced polyploidy attenuates hepatocyte replication and activates cell aging events. *Am J Physiol*. 1999. 276(5 Pt 1):G1260–G1272. [PubMed: 10330018]
13. Weglarz TC, Degen JL, Sandgren EP. Hepatocyte transplantation into diseased mouse liver. Kinetics of parenchymal repopulation and identification of the proliferative capacity of tetraploid and octaploid hepatocytes. *Am J Pathol*. 2000; 157:1963–1974. [PubMed: 11106569]
14. Faggioli F, Vezzoni P, Montagna C. Single-cell analysis of ploidy and centrosomes underscores the peculiarity of normal hepatocytes. *PLoS One*. 2011; 6

15. Conlin LK, Thiel BD, Bonnemann CG, et al. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum Mol Genet.* 2010; 19:1263–1275. [PubMed: 20053666]
16. Weier JF, Weier HU, Jung CJ, et al. Human cytotrophoblasts acquire aneu-ploidies as they differentiate to an invasive phenotype. *Dev Biol.* 2005; 279:420–432. [PubMed: 15733669]
17. Westra JW, Peterson SE, Yung YC, et al. Aneuploid mosaicism in the developing and adult cerebellar cortex. *J Comp Neurol.* 2008; 507:1944–1951. [PubMed: 18273885]
18. Chen G, Bradford WD, Seidel CW, Li R. Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy. *Nature.* 2012; 482:246–250. [PubMed: 22286062]
19. Pavelka N, Rancati G, Zhu J, et al. Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast. *Nature.* 2010; 321:250–325.
20. Rancati G, Pavelka N, Fleharty B, et al. Aneuploidy underlies rapid adaptive evolution of yeast cells deprived of a conserved cytokinesis motor. *Cell.* 2008; 135:879–893. [PubMed: 19041751]
21. Zhu J, Pavelka N, Bradford WD, et al. Karyotypic determinants of chromosome instability in aneuploid budding yeast. *PLoS Genet.* 2012; 8:e1002719. [PubMed: 22615582]
22. Duncan AW, Hanlon Newell AE, Bi W, et al. Aneuploidy as a mechanism for stress-induced liver adaptation. *J Clin Invest.* 2012; 122:3307–3315. [PubMed: 22863619] This proof-of-concept study demonstrates that hepatic aneuploidy performs beneficial role. In response to chronic liver stress, which is toxic to the liver, disease-resistant aneuploid hepatocytes maintain hepatic function and proliferate to restore liver mass.
23. Gimelbrant A, Hutchinson JN, Thompson BR, Chess A. Widespread monoallelic expression on human autosomes. *Science.* 2007; 318:1136–1140. [PubMed: 18006746]
24. Paradis V, Dargere D, Bonvoust F, et al. Clonal analysis of micronodules in virus C-induced liver cirrhosis using laser capture microdissection (LCM) and HUMARA assay. *Lab Invest.* 2000; 80:1553–1559. [PubMed: 11045572]
25. Paradis V, Laurendeau I, Vidaud M, Bedossa P. Clonal analysis of macronodules in cirrhosis. *Hepatology.* 1998; 28:953–958. [PubMed: 9755231]
26. Espanol-Suner R, Carpentier R, Van Hul N, et al. Liver progenitor cells yield functional hepatocytes in response to chronic liver injury in mice. *Gastroenterology.* 2012; 143 1564.e7–1575.e7.
27. Boulter L, Govaere O, Bird TG, et al. Macrophage-derived Wnt opposes notch signaling to specify hepatic progenitor cell fate in chronic liver disease. *Nat Med.* 2012; 18:572–579. [PubMed: 22388089] This study demonstrates the role of Kupffer cells to control the fate of bipotent progenitors.
28. Graubardt N, Fahrner R, Trochsler M, et al. Promotion of liver regeneration by natural killer cells in mice is dependent on extracellular ATP phosphohydrolysis. *Hepatology.* 2012 This study shows that limiting extracellular nucleotides after partial hepatectomy can affect NK cell activation and, hence, liver regeneration.
29. Elliott MR, Cheleni FB, Trampont PC, et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. *Nature.* 2009; 461:282–286. [PubMed: 19741708]
30. Gonzales E, Julien B, Serriere-Lanneau V, et al. ATP release after partial hepatectomy regulates liver regeneration in the rat. *J Hepatol.* 2010; 52:54–62. [PubMed: 19914731]
31. Hou X, Zhou R, Wei H, et al. NKG2D-retinoic acid early inducible-1 recognition between natural killer cells and Kupffer cells in a novel murine natural killer cell-dependent fulminant hepatitis. *Hepatology.* 2009; 49:940–949. [PubMed: 19177594]
32. Liu L, Yannam GR, Nishikawa T, et al. The microenvironment in hepatocyte regeneration and function in rats with advanced cirrhosis. *Hepatology.* 2012; 55:1529–1539. [PubMed: 22109844] This study demonstrates that hepatocytes from end-stage cirrhotic livers can recover their metabolic, secretory, and replicative function when placed in a noncirrhotic liver environment.
33. Naiki-Ito A, Asamoto M, Naiki T, et al. Gap junction dysfunction reduces acetaminophen hepatotoxicity with impact on apoptotic signaling and connexin 43 protein induction in rat. *Toxicol Pathol.* 2010; 38:280–286. [PubMed: 20097795]
34. Patel SJ, Milwid JM, King KR, et al. Gap junction inhibition prevents druginduced liver toxicity and fulminant hepatic failure. *Nat Biotechnol.* 2012; 30:179–183. [PubMed: 22252509] This study

describes a role for connexin 32 as an essential molecule in communication between liver parenchymal cells.

35. Ott HC, Matthiesen TS, Goh SK, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med.* 2008; 14:213–221. [PubMed: 18193059] This is the first report of perfused-organ decellularization.
36. Soto-Gutierrez A, Wertheim JA, Ott HC, Gilbert TW. Perspectives on whole-organ assembly: moving toward transplantation on demand. *J Clin Invest.* 2012; 122:3817–3823. [PubMed: 23114604] This work is a comprehensive review about the accomplishments and future applications of organ engineering technologies.
37. Soto-Gutierrez A, Zhang L, Medberry C, et al. A whole-organ regenerative medicine approach for liver replacement. *Tissue Eng Part C Methods.* 2011; 17:677–686. [PubMed: 21375407]
38. Uygun BE, Soto-Gutierrez A, Yagi H, et al. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. *Nat Med.* 2010; 16:814–820. [PubMed: 20543851] This is the first report for whole-organ liver engineering.
39. Baptista PM, Siddiqui MM, Lozier G, et al. The use of whole organ decellularization for the generation of a vascularized liver organoid. *Hepatology.* 2011; 53:604–617. [PubMed: 21274881]
40. Ott HC, Clippinger B, Conrad C, et al. Regeneration and orthotopic transplantation of a bioartificial lung. *Nat Med.* 2010; 16:927–933. [PubMed: 20628374]
41. Cortiella J, Niles J, Cantu A, et al. Influence of acellular natural lung matrix on murine embryonic stem cell differentiation and tissue formation. *Tissue Eng Part A.* 2010; 16:2565–2580. [PubMed: 20408765]
42. Tschumperlin DJ, Dai G, Maly IV, et al. Mechanotransduction through growthfactor shedding into the extracellular space. *Nature.* 2004; 429:83–86. [PubMed: 15103386]
43. Wang Y, Cui CB, Yamauchi M, et al. Lineage restriction of human hepatic stem cells to mature fates is made efficient by tissue-specific biomatrix scaffolds. *Hepatology.* 2011; 53:293–305. [PubMed: 21254177]
44. Huebsch N, Arany PR, Mao AS, et al. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. *Nat Mater.* 2010; 9:518–526. [PubMed: 20418863]

KEY POINTS

- Polyploidy and aneuploidy are common in normal livers. Hepatic aneuploidy can promote adaptation to chronic liver injury.
- Liver nonparenchymal cells can modulate hepatic regenerative responses.
- Cell–cell contacts are important in limiting liver failure and regeneration.
- Hepatocytes from end-stage cirrhotic livers can recover their secretory and metabolic function.
- Organ engineering technology can facilitate the study of the complex cellular interactions in the liver.