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GUEST EDITORIAL

Hepatic Regenerative Medicine

Exploiting the Liver's Will to Live

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Hepatic Homeostasis

The liver is bestowed with the capacity to regenerate, which is a unique attribute for an adult organ. This regenerative capacity is essential perhaps due to its strategic location and its indispensable functions for host survival, including synthesis, metabolism, and detoxification.[1] The cellular and molecular basis of liver regeneration has been studied for decades using two major types of models. Partial hepatectomy entails surgically removing three of the five lobes in a mouse or three of the four lobes in a rat, amounting to removal of two-thirds of the entire liver mass.[2] This sets in motion a cascade of events that allows for the residual hepatocytes to undergo cell division and cell hypertrophy to make up for the lost mass within days. This has been the basis of successful partial hepatectomies and split liver transplantation[3] in patients. Toxicant-induced liver injury also promotes specific cell and molecular signaling that allows for a partially injured liver to recover via hepatocyte proliferation. A classical example is the use of a sublethal dose of acetaminophen that results in pericentral necrosis followed by proliferation of hepatocytes in spared zones to restore hepatic architecture.[4] In addition to these two models, yet another form of regeneration stems from the proliferation of resident hepatocytes by direct mitogens in the absence of any liver injury. These classes of direct mitogens include triiodothyronine (T_3), peroxisome proliferators, lead nitrate, and 9-*cis* retinoic acid, among others.[5]

Careful dissection of pathways using genetic approaches, inhibitors, and antisense strategies has yielded highly relevant information that demonstrates a certain degree of redundancy at the cellular and molecular levels that ensure hepatic health during the hours of insult. Such molecular redundancy is evident in a number of knockout mouse models for which absence of a single gene is rather easily compensated by activation of another pathway that then drives hepatocyte proliferation to restore liver mass in models such as partial hepatectomy.[6] A classic example is the delay in liver regeneration when hepatocyte-specific ß-catenin− or Met−knockout mice are subjected to partial hepatectomy.[7,8] Cellular redundancy also ensures proper liver regeneration. Although mature epithelial cells of the liver (ie, hepatocytes and cholangiocytes) have been convincingly shown to replicate and restore the lost hepatic mass, these cells can also transdifferentiate into one another to repair the lost cell type.[6] This is primarily driven by cell-selective injury that may induce a cell to crossover to the other cell type, which is developmentally reasonable because there is a common progenitor precursor of these two epithelial cell types. Yet another level of cellular redundancy comes from adult liver progenitors or oval cells that expand and differentiate into the cell of choice in response to an overwhelming hepatic insult or due to the inability of adult cells to divide in response to an adverse liver environment.[9] Among the last-resort cellular redundancies contributing to restoration of liver structure and function during duress may be the extrahepatic cell sources such as bone marrow−derived stem cells that contribute to hepatic repair through mechanisms, which are incompletely understood.[10]

With exhaustion or failure of redundant mechanisms to act such as during an overwhelming acute insult, the liver decompensates requiring a patient to seek medical attention. This may also occur when injury to the liver is acute and

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overwhelming. A significant subset of patients with end-stage liver disease requires liver transplantation as the only effective treatment.[3] This is severely limited, however, by the dearth of donor organs. Thus, progress toward defining therapies for end-stage liver disease includes identification of novel strategies that broadly encompass the discipline of hepatic regenerative medicine. In the current issue of *The American Journal of Pathology*, four timely topics relevant to this area are reviewed in depth.

Hepatic Metabolism and Regeneration

Stimulating liver regeneration by various modalities may be highly relevant as a therapy for end-stage liver disease and may have implications for liver transplantation. Although we understand the process of hepatic regeneration relatively well, what initiates it has continued to evade us. Since liver performs key metabolic, synthetic, and detoxification functions, recent studies have started to investigate if sensing of a temporal deficit in any of these constitutive hepatic functions after procedures like hepatectomy may in fact be the initiator signal for the regeneration process. Several reports have now shown changes in fat and glucose metabolism in the liver during the early hours after partial hepatectomy. In fact, modulation of these metabolic alterations after partial hepatectomy has led to an impairment of the regenerative process.[11] Lessons learned from these studies will have significant bearing on how to best induce regeneration in a setting of chronic or acute liver insufficiency by altering the metabolic load, and this may have both biological and clinical implications. Huang and Rudnick[12] overview the data supporting a metabolic model of liver regeneration and reflect on clinical implications and areas for further study.

Polyploidy and Liver

Another hallmark of a normal adult liver is the existence of polyploidy. In fact, more than half of the hepatocytes in adult human liver and up to 90% of hepatocytes in rodent liver display polyploidy. Most of the polyploidy in hepatocytes has been shown to be a function of failed cytokinesis. Intriguingly, a major pathway implicated in this process is insulin-Akt signaling.[13] Although liver is normally composed of polyploid hepatocytes, specific situations can lead to additional changes in ploidy, including iron overload, altered redox state of a cell, and partial hepatectomy.[14] The existence of polyploidy in the liver has been known for more than a century, but the features unique to polyploid hepatocytes and their function are a timely concept. Two major theoretical advantages of polyploid hepatocytes include a functional hepatocyte and a more robust or resistant cell to genotoxic or environmental stress. Both of these related functions represent a gene-dosage effect due to higher copies of specific synthetic or metabolic genes present in a cell with higher ploidy. Thus, understanding the regulation of ploidy can have far-reaching consequences in hepatic regenerative medicine, specifically in repair and restoration of hepatic function after surgical or toxicant-induced liver injury, as well as in tissue engineering and cell therapy. Gentric and Desdouets[15] explore the mechanisms that lead to the development of polyploid cells, our current state of understanding of how polyploidization is regulated during liver growth, and its consequence on liver function.

Human iPS Cell-Derived Hepatocytes

With the advent of technologies enabling reprogramming of adult somatic cells to a pluripotent state, the field has gained much momentum.[16] For the first time, it is now theoretically possible to model a disease from every patient through generation of induced pluripotent stem (iPS) cells and inducing their differentiation to the affected tissue. This is particularly interesting with monogenetic diseases that remain poorly characterized from a biological standpoint. However, these iPS cells also represent an innovative cell source for regenerative medicine, as these cells can differentiate into any mature cell such as a hepatocyte for applications in cell therapy, tissue engineering, and bioartificial liver devices.[17] Although this is an attractive concept due to a host immunological tolerance, the field still faces challenges such as limited efficiency to induce pluripotency, lack of an ideal cell source for generating an iPS cell due to its retained memory, and inability to generate fully functional mature hepatocytes from iPS cells. Several relevant studies are now examining normal prenatal development of the liver to identify key cellular and molecular interactions that in turn can be temporally applied to iPS cells in culture. This strategy is an effort to mimic ontogeny and effectively generate fully differentiated functional hepatocytes from these stem cells. This highly innovative advance may have a major impact on the way we study and treat specific diseases of the liver and other organs. Si-Tayeb and colleagues[18] reviewed the choice of somatic cells to be reprogrammed by emergent new and nonintegrative strategies, as well as the application of differentiated human-induced pluripotent stem cells in hepatology.

Decellularized Hepatic Matrix and Hepatic Tissue Engineering

Due to the shortage of organs for transplant, research on alternate modalities, such as hepatic tissue engineering has gained momentum. Additionally, there is a need to engineer whole organs in a three-dimensional configuration to better understand cell−cell interactions from a biological and functional perspective. The applications of such engineered organs could be envisioned not just in the setting of transplantation, but also in areas such as toxicology, disease modeling, and drug development. However, there have been major hurdles to engineering the liver, such as lack of optimal cell source, maintenance of function, and longevity of these engineered

tissues. With the advent of iPS cells, an autologous cell source may no longer be an issue, although its differentiation to mature hepatocyte still remains a challenge. An innovative advance in the arena to further the maturation of a hepatocyte from iPS cells has been the realization of an important role of extracellular matrix in maintenance of differentiated hepatocyte phenotype. Rather than using combinations of various matrices to facilitate hepatocyte differentiation, a unique strategy recently used was to derive intact extracellular matrix from a liver using a decellularization process. Using the whole organ acellular matrix as a three-dimensional scaffold for seeding hepatocyte-like cells derived from iPS cells, a fully functional hepatocyte may become a reality. In addition, use of nonparenchymal cells of the liver in such a tissue-engineering strategy may provide an intricate spatiotemporal environment that may be ideal for generating a functional and optimal organ for any number of applications.[19,20] This is truly an evolving and timely field with much ongoing research focused at optimizing engineering of an organ for experimental and translational purposes. Soto-Gutierrez and colleagues[21] highlight the most recent advances in organ assembly regarding the development of liver tissue *in vitro*.

Concluding Remarks

The liver's will to live is effectively supported by a host of cellular and molecular means that provide a tiered restoration mechanism ensuring hepatic homeostasis. We hope that this series of reviews will help illuminate these pathways for our readers and provide a clearer picture of the state of the art of hepatic regenerative medicine.

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