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Stem Cell Therapy For Inherited Metabolic Disorders Of The Liver

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Abstract

Modern medicine has conquered an enormous spectrum of health concerns, from the neonatal to the geriatric, the chronically ill to the acutely injured. Among the unmet challenges remaining in modern medicine are inborn disorders of metabolism within the liver. Such inherited metabolic disorders (IMDs) often leave an otherwise healthy individual with a crippling imbalance. As the principal regulator of the bodies many metabolic pathways, mal-encoded hepatic enzymes can drastically disrupt homeostasis throughout the entire body. Severe phenotypes are usually detected within the first few days of life, and treatments range from palliative lifestyle modifications, to aggressive surgical procedures. While orthotopic liver transplant is the single last resort 'cure' for these conditions, research over the past few years has brought new therapeutic technologies ever closer to the clinic. Stem cells, therapeutic viral vectors, or a combination thereof are projected to be the next, best, and final cure for IMDs, which is well reflected by this generation's research initiatives.

Keywords

Inborn errors of metabolism; Stem cell therapy; Liver disease

Introduction to Inherited Metabolic Disorders

The liver is a remarkable organ in both form and function. Indeed, it stands alone in the mammalian world with a lasting capacity to self-regulate its mass in proportion to the needs of the body; a feat accomplished by a mechanism of compensatory hyperplasia (1). Post-natally, the liver is responsible for maintaining homeostasis through a myriad of processes; some involving detoxification and others, synthesis. The liver processes consumed substances, sustains reserves of iron and other vitamins and minerals, it detoxifies alcohol, drugs and other noxious chemicals, and removes inhaled poisons such as exhaust, smoke, and volatile chemicals which accumulate in the bloodstream. As a production plant, the liver makes bile for digestion of lipids, stores energy by stockpiling glycogen, and manufactures albumin, blood clotting factors and a host of other key plasma proteins (2). A single misprint in the genetic coding of a liver enzyme could completely destroy its capacity to carry out any one of these functions and, if left unchecked, often threatens the life of the afflicted patient.

Inherited metabolic disorders (IMDs) represent an array of conditions tied to one of the liver's many functions (3). Monogenic disorders are often inherited through an autosomal recessive mechanism, resulting from a single coincident misprint in each parents' genetic code (4–6).

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Both parents of an afflicted child are effectively proven carriers of the recessive gene, and together bear a 1 in 4 risk of passing on the disease to each future offspring. Individual IMDs are exceptionally rare but, taken together, they are suspected to account for up to 20% of illnesses amongst full-term neonates without prior-known susceptibility. In fact, an estimated 1:5,000 newborns are afflicted with one of over 70 IMDs, and a clinician is likely to see a number of such patients throughout the course of his career (7).

The average family is unlikely to have heard of any IMDs. Familiar or otherwise, it is an overwhelming situation for any family to deal with such a diagnosis in their own child. In addition to coming to terms with a decreased quality and sometimes quantity of life, parents are often faced with a daunting treatment regime. Generally, these treatment regimes must be employed immediately upon diagnosis in order to minimize any impact on early mental and physical development. Less severe IMDs may be managed by treating the symptoms of the disease. IMDs that involve more critical liver functions must be dealt with much more aggressively, and often necessitate liver transplant.

Palliative Treatment of IMDs

In cases where the disorder may be managed prior to or in lieu of risky procedures, a palliative approach involving diet or lifestyle modification is often the preferred course of action. Phenylketonurics, for instance, follow a strict dietary regime whereby they avoid foods rich in phenylalanine, thus minimizing intake of a molecule that is improperly metabolized, and avoiding buildup of the neurotoxic metabolite (8).

Other enzyme defects may be managed by supplementation with cofactors or vitamins that either enhance the residual activity of a partial defect, or bolster an alternate metabolic pathway. Infants suffering from carnitine deficiency usually present with hypoketotic hypoglycemia. While life threatening cardiac malfunction may result from neglect of the symptoms, this outcome is easily avoided by adding L-carnitine to the diet (9). Exogenous detoxifying agents are often prescribed to patients with deficient endogenous detoxification capacity. For example, urea cycle defects that lead to hyperammonemia may be treated with ammonium detoxicants that enhance the elimination of nitrogen. This treatment combined with arginine supplementation has proven to be quite effective in lowering ammonia levels in patients with carbamyl phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, and argininosuccinate lyase deficiencies (10,11).

Solution-based treatment of IMDs

When such relatively simple measures are unavailable or insufficient to maintain health, physicians look to more direct treatment strategies. Exogenous supply of the deficient enzyme, known as enzyme replacement therapy (ERT) has proven quite effective for treating a handful of enzyme deficiencies. For example, patients with Gaucher's disease and other lysosomal storage disorders are able to benefit from this type of supplementation (12). Nonetheless, it has been less successful for the liver specific pathologies (13). Glycogen Storage Disease (GSD) results from nonfunctional Glucose-6-phosphate dehydrogenase (G6Pase). During the last step in gluconeogenesis, the energy intermediate glucose-6-phosphate is actively transported into the cytoplasm where the normal enzyme frees glucose for use in sustaining metabolism (14). Therefore by necessity, G6Pase activity is localized within hepatocyte endosomes, thus administering the enzyme systemically is ineffective for restoring its function.

Even when ERT is therapeutically effective, it does not provide a cure from the endogenous disorder, and the patient is left with lifelong dependence on pharmaceuticals. Disorders like Gaucher's exemplify the true potential of cell based therapies, as a bone marrow transplant

could directly and permanently eliminate the disease by restoring enzyme function to the monocyte population.

Many metabolic disorders are manageable for a period of time, but often bear a predictable need for more drastic future treatment. Patients with Crigler Najjar Syndrome (CNS) lack functional uridine diphospho-glucuronosyl transferase, an enzyme necessary for bilirubin metabolism (15). Afflicted individuals are unable to convert bilirubin to the excretable hydrophilic form, and it accumulates to toxic levels. As with GSD, the enzyme missing in CNS is also meant to function within cellular endosomes, rendering ERT ineffective. Instead, CNS patients are prescribed a unique therapy involving abundant sun exposure and nights spent unclothed beneath a bright blue light. In adjunct to the natural UV rays, this light produces a wavelength that can penetrate the skin and break down bilirubin as it flows through subdermal microcapillaries, effectively replacing the role of the missing enzyme. Though this treatment is often sufficient through childhood, it becomes less so as the patient's skin naturally thickens with age; eventually a threshold is reached whereupon a healthy donor liver is imperative to escape lethal bilirubin accumulation (16).

Liver Transplantation

The most insidious of metabolic syndromes do not always afford a patient time to mature, but instead require immediate intervention for life to be sustained. In the case of neonatal hemochromatosis, palliative care is insufficient and patients are generally transplanted as soon as a suitable donor organ becomes available (17). Since its inception in 1963, Orthotopic liver transplantation has, thus far, been the ultimate solution for most patients refractory to palliative or solution based management (18). A healthy donor liver will restore endogenous enzyme production, effectively repairing the metabolic disorder. This aggressive approach is not without pitfalls and, taken together, the scarcity of available organs, risk of surgical complications, expense, indefinite immunosuppression, and eventual rejection, lessen enthusiasm for this means to a cure.

Viral Based Gene Therapy

As new treatment options emerge, it becomes clear that the most effective therapies often bear the highest risks. This has driven research towards strategies that utilize minimally invasive techniques. Preclinical advances over the last decade have finally brought realistic hope for curing metabolic liver disorders without the complications inherent to orthotopic transplantation. Vector-based gene therapy has made headlines as a potential means for correcting imbalances at the genetic level.

Research on plasmid vectors as potential vehicles for gene replacement is also underway, but the current generation of such vectors produces only transient gene expression (19). Viral vectors such as AAV maintain their construct-bearing genome episomally for a variable period post-transduction, but also loose expression as cells replicate endogenous DNA. To the contrary, vectors such as lentivirus are able to integrate the transgene into the cellular genome, resulting in transduced cells that produce the encoded factor indefinitely (20). While this may eliminate the need for repeated administration of the vector, it also carries with it a series of very real risks. Genetic dosing and regulation of the transgene product are of foremost concern in cases where the enzyme activity levels should be restrained. The spread of vector and thus gene expression are also difficult to control when the virus is administered to the body directly, and despite the availability of tissue-specific promoters, the potential effects of systemic integration must also be considered.

The dogma that the vast majority of the human genome was unimportant has recently come under scrutiny. The discovery of several genetic control mechanisms sandwiched between the

coding regions of DNA has called into question the wisdom of allowing viral vectors to randomly integrate into the genome. Retrotransposons, regulatory micro RNAs and other control elements that had once flown beneath our radar are now gaining due recognition. These elements, as well as oncogenic sites, are important to consider while weighing the suitability of viral based gene therapy. Such a remedy relies on a degree of luck with regard to integration site. These are, of course, only of concern if the vector is able to successfully transduce cells. In the case of lentivirus for instance, target cells may produce a family of proteins known as apolipoprotein B mRNA-editing enzymes (21). These proteins may both limit transduction of cells, and trigger an immune response to viral antigens. With so many details regarding the safety and dynamics of systemic transduction yet to be mastered, cell based therapies do stand out as the imminent solution to IMDs.

Hepatocyte transplantation

While hepatocytes are generally quiescent, the ability to rapidly enter the cell cycle offers a means to respond to chemical and physical insults. In the absence of inhibition, acute liver injury induces a state of massive compensatory hyperplasia amongst resident hepatocytes (1). Indeed, transplantation studies in mice have shown a remarkable proliferative capacity over a span of several serial transplantations; a youth-sustaining feat which is suspected to originate from persistently high telomerase activity (22–24).

In the case of IMDs, compensatory hyperplasia yields a futile expansion of endogenous cells producing the mal-encoded enzyme. Fortunately, when the nature or extent of the dysfunction overtakes its capacity for self-repair, liver function may still be regained by procedures such as organ or cell transplantation. The amount of active enzyme needed to sustain health varies by metabolic function. Therefore the easiest disorders to correct are those which respond well to replacement of a relatively small portion of liver by cells bearing functional gene. In such cases, terminally differentiated hepatocytes may be useful for cell-based enzyme replacement

One of the earlier records of such an experimental treatment was detailed in the 1976 article by Matas *et al.* using the rat model of Crigler-Najjar Syndrome, (familial hyperbilirubinemia) (25). When immunosuppressed Gunn rats lacking the enzyme uridine diphosphate glucuronyltransferase were infused with normal hepatocytes via the portal vein, plasma bilirubin concentrations stably decreased, ameliorating clinical symptoms of the disease (25). The viability of this procedure was verified a year later when Groth *et al.* reproduced the curative effect of mature hepatocyte transplantation in the same animal model (26). Two decades later, Fox *et al.* also saw success using hepatocyte infusion to treat humans with CNS-1 (27). This reported achievement, supported by ongoing studies in the preclinical setting, would seem to validate this simple strategy.

In these models which employ partial-hepatectomy followed by donor cell infusion, endogenous hepatocytes still outnumber the healthy donor cells by two to three orders of magnitude. Following proportional expansion *in vivo*, the minor contribution of healthy donor cells would provide some functional enzyme, but the level of enzyme activity needed to produce a normal phenotype is different for each metabolic disorder. Indeed, most animal models have shown that a two-thirds partial-hepatectomy alone is not sufficient to promote engraftment of healthy donor hepatocytes in numbers that would be clinically useful (28). Therefore, a selective advantage must be intrinsic to the transplanted cells for this therapeutic approach to be effective for a majority of IMDs. The mouse model for hereditary tyrosinemia type 1 represents one disorder in which the transplanted hepatocytes possess a substantial proliferative disadvantage. Unable to produce fumaryl acetoacetate hydrolase (FAH), resident hepatocytes succumb to the toxicity of tyrosine catabolites. Upon withdrawal of the therapeutic

drug 2(2-nitro-4 trifluoromethylbenzoyl)-1,3 cyclohexane dione (NTBC), the genetically defective hepatocytes begin to die, while healthy transplanted cells proliferate freely (29).

While engraftment may be regarded as a rate limiting factor in the outcome of cell transplantation, simple collagenase pre-treatment of the liver can induce localized destabilization of the extracellular matrix, creating an environment in which transplanted cells can more easily take up residence (30). A majority of the animal cell transplantation models requires pretreatment of the recipient liver with an anti-proliferative agent such as retrorsine or monocrotaline prior to a partial hepatectomy and cell transplantation (28,31). Such pretreatment is not desirable in humans as the anti-proliferative agents are also carcinogenic.

As an alternative to chemical inhibition, Chowdhury's group replaced pharmacological pretreatment of the liver with site-directed irradiation. This combination of insults also conveyed a selective advantage to the cell transplant. Neither partial hepatectomy nor irradiation alone was able to support engraftment at therapeutic levels (32). Laconi's group published one of the few accounts suggesting that, given enough time, donor rat hepatocytes are able to substantially repopulate the retrorsine treated liver (33). Should this strategy be replicable, clinical application would still be unlikely due to the carcinogenic potential of retrorsine mentioned previously.

Additionally, obtaining hepatocytes for transplant face many of the challenges associated with finding a donor organ. Both live and cadaveric sources are scarce, and the requirement for MHC-matched tissue limits options still further. Autologous cell transplantation appears to be the most viable therapeutic strategy. However, without *ex vivo* modification, the patient's own cells are of little use. The replicative capacity of mature hepatocytes is greatly diminished *in vitro*, requiring a significant amount of cells for transplant. Cultured hepatocytes also lose metabolic function rather quickly (34). Taken in combination these obstacles lessen enthusiasm for hepatocyte cell therapy.

Liver Development

Liver stem cell based therapies will likely require *in vitro* recapitulation of at least a portion of the normal liver development process. Embryological studies have mapped the differentiation of early endodermal stem cells to both the hepatocytic and bile ductular cell lineages. Proliferation of undifferentiated endodermal cells associated with the developing ventral foregut followed by migration to the *septum transversum*, results in contact with pre-cardiac mesoderm which affects lineage determination of the liver stem cell population. Recent studies suggest that cells analogous to these very early epithelial progenitor cells may persist in the bone marrow of adults. (35–37) For the purpose of cell based therapies of IMDs, a bone marrow derived cell would hold the most promise from the standpoint of accessibility, though complicated *in vitro* processing would likely be required to move these cells to a differentiation state appropriate for transplant.

Alpha-fetoprotein (AFP) is the first, lineage specific, marker expressed by developing foregut cells. AFP expression is soon followed by the expression of classical hepatoblast markers including albumin. It is generally accepted that at least a portion of the bipotent liver progenitor cell population is AFP⁺ so it is likely that cells suitable for therapies will derive from cells at this differentiation state. As development proceeds, further lineage commitment is evidenced by expression of more mature markers such as transferrin receptor, c-CAM, H.2 and H.4. While it is difficult, though not impossible, to alter the terminal lineage of cells expressing these markers, they still possess a robust proliferation capacity (38). This fact leaves open the possibility to use cells already committed to the hepatocyte lineage for cell therapy.

As organogenesis advances, the biliary tree and hepatic plates are formed, each adopting a marker expression profile associated with the terminally differentiated phenotype (39). Proliferation capacity becomes more limited and suitability for use in cell therapy is diminished.

Bone Marrow Derived Hepatocytes

Literature of the late 20th century contained reports that bone marrow derived stem cells could develop down hematopoietic, endothelial and epithelial lineages. Their participation in liver regeneration, however, had not been shown. In 1999 Petersen *et. al.* demonstrated that bone marrow derived cells could give rise to hepatocytes following liver injury in lethally irradiated rats that had received a bone marrow transplant (35). This finding was confirmed when Y-chromosome positive hepatocytes were detected in the livers of female patients who had received bone marrow transplants from male donors (36,40–42). The results of these studies broadened the possibility of cell based therapies for the treatment of IMDs of the liver, as the bone marrow would represent a readily available source of liver progenitor cells.

In support of this notion, Grompe's research group began the millennium by with a report that challenged several paradigms of cell mediated liver regeneration. His team employed the FAH mouse model of tyrosinemia to investigate the potential for cell mediated rescue from an inherited enzyme deficit. They were able to demonstrate restoration of a normal phenotype by intravenous infusion of bone marrow cells (43). These findings, that healthy, syngenic, BM-derived cells were able to remediate a metabolic deficiency, were not new; rather, what stirred controversy was the claim that only the rigorously purified sca-1⁺ Hematopoietic Stem Cells gave rise to hematopoietic and hepatic regeneration (44). This completely debunked the notion that the three germ layers were set early in embryogenesis, and that transdifferentiation among them was not possible.

While conflict grew amongst research groups trying to identify which sub-population actually bore true therapeutic potential, enthusiasm for an extrahepatic source of liver stem cells was transiently diminished for another reason entirely. Shortly following these publications, Grompe and Vassilopoulos each reported that the genetically wildtype hepatocytes which replaced the diseased cells in the tyrosinemia model were not the product of bone marrow cell transdifferentiation, but rather the product of cell fusion (45,46). Extensive karyotyping revealed severe anuploidy consistent with the combining of genomes between donor and recipient cells. The following years were witness to a slew of reports supporting either the transdifferentiation or fusion theory of bone marrow derived liver cells.

Undoubtedly, a complete understanding of post-transplantation events is required to maximize the effectiveness of therapies based on bone marrow derived cell transplant. However, scientists with a more clinically-driven mentality will quickly point out that the most important aspects of developing a clinical therapy using bone marrow derived cells is the evaluation of safety and the effectiveness in treating disease. Several studies over the past few years have clearly demonstrated beneficial participation of bone marrow derived cells in the repair of liver injury (47–50). Thus, bone marrow remains a promising source of material for cell based therapies regardless of the mechanism by which the restoration of normal liver function is achieved.

To date, only one other study implicates bone marrow as a source of liver progenitor cells shown to correct an IMD *in vivo*; nonetheless, interest in the research arena is now gathering momentum. In 2006, Mishawa *et. al.* used desialylated bone marrow cells for the treatment of Wilson's disease in a rodent model. The major finding of this study was that the interaction between the asialoglycoprotein receptor on hepatocytes and the desialylated bone marrow cells increased the rate of parenchymal engraftment. Large nodules of bone marrow derived

hepatocytes were identified within recipient livers, and survival was improved in the transplanted groups (51).

In 2007, Yan *et al.* published the results of a chinese study which demonstrated that peripheral blood mononuclear cells mobilized from the bone marrow of hepatitis B infected patients could be enriched for early hepatocyte markers and transplanted back into patients (52). This autologous transplant resulted in functional hepatocytes that sustainably improved liver function in transplant recipients. Similar studies have also shown improved liver function in patients following autologous bone marrow cell therapy (53–55). While these studies do not deal directly with IMDs, they do verify that bone marrow derived cells are capable of engrafting into the liver, and giving rise to functional hepatocytes.

Future studies will undoubtedly focus on specific bone marrow cell sub-populations which show enriched potential for engraftment and hepatocyte differentiation within the recipient liver. Ratajczak and colleagues have been at the forefront of this field for the past half-decade. In 2004, the Ratajczak lab identified a sub-population of CXCR4⁺ bone marrow cells which are mobilized by granulocyte colony stimulating factor and migrate in response to an SDF-1 gradient. Circulating amongst pool of peripheral blood mononuclear cells, this particular subpopulation expressed markers for muscle (Myf-5, Myo-D), neural (GFAP, nestin) and liver origins (CK19, alpha-fetoprotein)(37) Further studies by Ratajczak *et al.* have identified an extraordinary cell population within the bone marrow (56). Termed VSELs (very small embryonic like), these cells are extremely small (2 um) with almost no cytoplasm. They express CXCR4 along with several embryonic stem cell markers including Oct-4. Ratajczak hypothesizes that these cells remain dormant in the bone marrow following embryogenesis and may persist into adulthood as epithelial progenitors. The suitability of these cells as transplantable elements for the treatment of liver IMDs remains to be determined.

Of course, the propensity of stem cells for *ex vivo* expansion provides for the replenishment of cell stocks available for transplant. Indeed, in contrast to donor hepatocytes, stem cells are not nearly so limited by tissue origin, nor numbers available at harvest. Verfaillie *et al.* published a 2002 report in which multipotent adult progenitor cells (MAPCs) from a variety of murine tissues maintained multilineage capacity through more than 70 passages *in vitro* (57). A 2006 article by Tamama's group highlights the benefits of EGF-induced cell expansion for this purpose, reporting that proliferation was bolstered in MAPCs derived from human bone marrow (58).

Berger *et al.* critiques a wide assortment of studies on mesenchymal stem cells (MSC), which have produced an array of collective outcomes (59). In his evaluation, Berger urges scientists to look beyond the differences in study observations, and suggests that they first scrutinize more fundamental questions; foremost, of which is whether or not expanded MSC populations truly offer a proportionally enhanced therapeutic capacity, and whether there is notable benefit to using human serum in place of the standard FCS for purification and/or amplification of MSCs.

Clearly, many details involved in BMC to liver differentiation remain to be described. While there is strong evidence that BMCs can aid hepatic reconstitution, intra-hepatic progenitors remain the most extensively studied stem cells for the treatment of IMDs.

Hepatic Oval Cells

Distinct from hepatocytes and non-parenchymal cells, the liver is able to deploy a population of intra-hepatic progenitors when hepatocyte proliferation is inhibited. The bipotential hepatic 'oval' cell has been characterized both in rodent models, and in human subjects (60,61). Investigators have recently begun to include these cells in transplant studies (31).

While a liver specific stem cell may seem like the most likely candidate for cell based therapies for IMDs, several issues lessen their clinical value. The largest obstacle that would need to be overcome is accessibility. Oval cells are extremely rare in healthy liver, most often associated with chronic hepatic injury in humans. In rodents, oval cell proliferation is induced either with chronic injury or by blocking endogenous hepatocyte proliferation prior to chemical or physical injury (31). Whether donor derived or autologous, obtaining oval cells in sufficient quantity for transplant would require a significant volume of chronically injured liver, precluding live humans as a source.

Given this serious clinical drawback, the oval cell stands to be the greatest beneficiary of the abovementioned *ex vivo* expansion strategy. Hepatic oval cells have been characterized and maintained in culture systems. Clonal subpopulations are morphologically homogeneous, being small polygonal cells with a large ovoid nucleus and scant cytoplasm (61). They propagate with ease under certain culture conditions, and can maintain a unique combination of markers including AFP, CK19, and albumin (62).

The ability to culture cells between isolation and transplant provides a host of advantages. Culturing allows for the amplification of a small number of isolated cells to a volume sufficient for transplant. It also provides an opportunity to control the differentiation state of the cells, providing either highly proliferative bipotential progenitors or lineage committed progenitors with less proliferative potential.

In 2002 Dr. Sell's team reported an elegant strategy for modulating the lineage development of rodent oval cells in culture. They had suspected that non-transformed oval cell lines might exhibit a malleable differentiation state. Indeed, they found that oval cells grown in standard media on a feeder-layer of embryonic fibroblasts steadfastly maintained an immature phenotype; however, those grown without the feeder layer committed to the hepatocyte lineage. In contrast, oval cells cultured on matrigel adopted a biliary-epithelial phenotype. Significantly, because these cells were derived without using carcinogens, such bipotential LPC lines would be suitable for cell based therapies (63).

Three-dimensional culture systems have also been used for the *ex vivo* expansion of oval cell populations. Hwal *et al.* developed an oval cell culture system using a 3-D collagen scaffold impregnated with hormone factors and cytokines (64). Three weeks of tracking albumin secretion and rates of ammonia detoxification showed that scaffold-cultured oval cells achieve higher levels of hepatocyte function than those grown on two-dimensional tissue culture plates (QQ). Such innovations in biomaterial engineering are setting the foundation upon which clinicians will test the feasibility of *ex vivo* expansion. For oval cells specifically, systems that benefit expansion, genetic manipulation and genotypic stability *ex vivo* may lessen the risks associated with their use in the correction of IMDs.

Liver Progenitor Cells from Bone Marrow

With an enormous body of literature illustrating their capacities, our understanding of oval cell potential is currently more agreed upon than their origin. Some suggest that oval cells arise by dedifferentiation of resident hepatocytes (65). A majority of experts still find that a bone marrow origin of oval cells is, at least, conceivable. In support of this theory, *in vitro* studies have revealed that bone marrow cells may express hepatic oval cell markers (66). *In vivo* studies have often employed female animals, lethally irradiated and rescued with allogenic male BM cells detected by simple survey for the Y-chromosome. As mentioned previously, Petersen *et al.* used a mitotic inhibitor to block proliferation of endogenous hepatocytes in female rats that had been transplanted with male bone marrow carrying a genetic marker. Physical injury was subsequently used to induce a proliferative response in the oval cell population. The resulting hepatic repair did, in fact, include hepatocytes that displayed both Y chromosome as well as

the donor marker (35). The laboratories of Lagassee, Theise, Petersen and others have repeatedly observed donor hepatocytes in the livers of BM transplant recipients, reinforcing the notion that bone marrow may act as a functional reservoir for liver progenitors (35,36,67, 68). Whether or not these bone marrow derived hepatocytes transitioned through an oval cell phase was not addressed by a majority of these studies.

In search of solid evidence, Shafritz, Dabeva and colleagues designed a study in which lethally irradiated female rats were transplanted with BM from males carrying a genetic marker, and then subjected to three models of oval cell activation and expansion. Results from this model suggested that it was not the BM derived oval cells, but rather the endogenous liver progenitors that provided the injured liver with the vast majority of expanding oval cells (69). Using a serial transplant model, Grompe's research group deduced that hepatic oval cells do not originate from bone marrow but from the liver itself. In this two-step experiment using the FAH mouse model, the team reported that primary recipients of *fah* (+/+) hepatocytes become almost entirely reconstituted with donor derived hepatocytes reflective of their strong proliferative advantage. However, chemically induced oval cells did not express the donor marker. Secondary transplant recipients of bone marrow also displayed the standard oval cell response to injury, but genetic analysis revealed that very few oval cells bore the original donor marker. With both dedifferentiation and bone-marrow origin ruled out, the group concluded that non-parenchymal cells of the liver were in fact the source of emergent oval cells. While in total these findings reinforced the value of oval cells for therapeutic liver repopulation, it contested a bone-marrow origin for the progenitors detected (70).

In opposition to the studies conducted by Dabeva and Grompe, the Petersen laboratory conducted a study wherein rats were pretreated with a chemical agent that inhibited the liver resident oval cell reaction prior to BM transplant (31). Following establishment of the chimeric BM, these rats were subjected to an oval cell inducing injury. Up to 20% of the resulting oval cells carried the BM donor marker. It does appear that BM may give rise to oval cells, though whether or not this happens under normal circumstances remains to be determined.

The abovementioned are just a sampling of liver stem cell studies. While the debate rages on over the true origin of these cells, opinions loose relevance without proof that the dynamics in animal models are, in fact, shared by potential human beneficiaries. Early investigations suggest that if the human liver does respond in a similar manner as rodent models, isolation of such a progenitor should be translatable to the clinic. Rather than join the speculation, an Italian group recently tackled this central question by isolating and characterizing a hepatocyte progenitor population from human liver. This cell type was found to display hepatic, osteogenic, and endothelial potential, which indeed surpasses the plasticity of the rodent analog. Most importantly, the human progenitor proved its clinical usefulness by successfully reconstituting the SCID mouse liver post-xenotransplantation (71). The reaffirmation of such a progenitor in man, capable of isolation and hepatic reconstitution, opens all possible doors for their use in treating metabolic liver disease.

Conclusion

Where once the fate of those with hepatic IMDs was inevitable, there are now emerging options which stand to provide a means to a true cure without the need for orthotopic transplant. Researchers in the fields of gene therapy work feverishly to produce suitable vectors that safely and sustainably transfer the desired gene to the correct tissues at the right dosage. Unfortunately, a 'perfect vector' is quite a ways down the road. In a different court, parallel research on cell transplantation has begun to provide a glimpse of the true capabilities of hepatic repopulation. The potential for cellular infusion, be they autologous or allogenic, progenitor

or committed, will be realized when methods are optimized for administering an appropriate cell population to a niche that permits engraftment.

Perhaps, the most useful studies are those that address the basic tenets of the natural mechanisms of liver repair. Ultimately, our capacity to correct IMDs will most benefit from a detailed characterization of pathways that mediate transdifferentiation and hepatic regeneration. This level of understanding will ease the transition from complex animal models to clinical application. While the earliest years of the 21st century witnessed great advances in research towards therapeutic potential, controversies remain. Indeed, it is likely that the best therapeutic approach will vary for each IMD, and specific strategies will need to be tailor fitted depending on requirements for each disorder.

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Figure 1. Potential material for liver cell therapy

Transplantable cells for the correction of liver metabolic disorders may be considered from among several differentiation states. These cell populations may be considered analogous to well characterized stages of liver development. Accessibility, expandability and suitability for genetic manipulation must be considered when selecting cells for therapeutic strategies.

Figure 2. The curative approach to IMDs may evolve in many forms

Gene therapy could offer healthy copies of the corrupted gene directly to the patient via systemic infusion of a tissue-specific vector. Stem cell research is closing in on means to repopulate tissues from which a disease originates. For IMDs, transplantable stem cells will derive from a healthy allogenic donor, or from the patient, provided the cells are genetically corrected *ex vivo*.