Review

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Cell therapy for liver disease: From liver transplantation to cell factory

Stuart J. Forbes[1,*], Sanjeev Gupta[2], Anil Dhawan[3]

[1]*MRC Centre for Regenerative Medicine, Scottish Centre for Regenerative Medicine, 5 Little France Drive, Edinburgh EH16 4UU, United Kingdom;* [2]*Departments of Medicine and Pathology, Albert Einstein College of Medicine, Jack and Pearl Resnick Campus, 1300 Morris Park Avenue, Ullmann Building, Room 625, Bronx, NY 10461, United States;* [3]*Paediatric Liver GI and Nutrition Center and NIHR/Wellcome Cell Therapy Unit, King's College Hospital at King's College, London SE59RS, United Kingdom*

Summary

Work over several decades has laid solid foundations for the advancement of liver cell therapy. To date liver cell therapy in people has taken the form of hepatocyte transplantation for metabolic disorders with a hepatic basis, and for acute or chronic liver failure. Although clinical trials using various types of autologous cells have been implemented to promote liver regeneration or reduce liver fibrosis, clear evidence of therapeutic benefits have so far been lacking. Cell types that have shown efficacy in preclinical models include hepatocytes, liver sinusoidal endothelial cells, mesenchymal stem cells, endothelial progenitor cells, and macrophages. However, positive results in animal models have not always translated through to successful clinical therapies and more realistic preclinical models need to be developed. Studies defining the optimal repopulation by transplanted cells, including routes of cell transplantation, superior engraftment and proliferation of transplanted cells, as well as optimal immunosuppression regimens are required. Tissue engineering approaches to transplant cells in extrahepatic locations have also been proposed. The derivation of hepatocytes from pluripotent or reprogramed cells raises hope that donor organ and cell shortages could be overcome in the future. Critical hurdles to be overcome include the production of hepatocytes from pluripotent cells with equal functional capacity to primary hepatocytes and long-term phenotypic stability *in vivo*.

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* Corresponding author. Tel.: +44 (0) 131 651 9500; fax+44 (0) 131 651 9501.
E-mail address: stuart.forbes@ed.ac.uk (S.J. Forbes).
Abbreviations: ATM, ataxia telangiectasia mutant; APOLT, auxiliary partial orthotopic liver transplantation; ES Cells, embryonic stem cells; *FAH*$^{-/-}$, fumarylacetoacetate-hydrolase-deficient; GMP, Good Manufacturing Practice; HPCs, Hepatic progenitor cells; iHeps, induced hepatocytes; iMPCs, induced multipotent progenitor cells; iPSCs, induced pluripotent stem cells; LDLR, low-density lipoprotein receptor; LSEC, liver sinusoidal endothelial cells; MSCs, mesenchymal stem cells; NAR, Nagase analbuminemic rats; OLT, Orthotopic liver transplantation; VEGF, vascular endothelial growth factor; WHHL, Watanabe heritable hyperlipidemic cholesterolemic; HLC, hepatocyte-like cell.

Introduction

Orthotopic liver transplantation (OLT) is the standard of care for people with end-stage liver disease and for certain liver-based metabolic defects [1]. However, successful replacement of deficient liver functions by transplantation of healthy hepatocytes, e.g., in animal models and people with Crigler-Najjar syndrome due to UGT1 enzyme deficiency, familial hypercholesterolemia due to low-density lipoprotein receptor (LDLR) deficiency, or acute and chronic liver failure indicated that OLT could possibly be avoided [2–6]. This general concept has been emphasized by similar successes with auxiliary partial orthotopic liver transplantation (APOLT) for enzymatic deficiency states or acute liver failure [7]. In the latter case, discontinuation of immunosuppression when the native liver regenerates after APOLT may lead to spontaneous rejection and atrophy of the allogeneic liver graft [8,9] The clinical experience with APOLT gives credence to the hypothesis that the relevant functional unit of the liver – "the hepatocyte" could be used to correct discrete enzyme defects and support metabolic functions for the failing liver after injury whilst it regenerates. Similarly, successful correction of haemophilia by OLT, indicated that consideration of cell therapy will be appropriate for other classes of diseases. In principle, cell transplantation is far simpler than either OLT or APOLT, because 1) cells from a donor liver may be transplanted into multiple recipients; 2) cell transplantation is simpler using cell administration via intravascular catheters rather than complex surgery; 3) if cryopreserved cells are used, therapies could be undertaken in a prospective non-emergency setting; 4) cells may even be transplanted repeatedly, the procedure can be considered "reversible" since the native liver is not removed; and 5) the costs of transplanting cells should be considerably less than that of organ transplantation.

Subsequent to the early demonstrations of whether transplanted cells may engraft and function in the liver and in a variety of extrahepatic sites [10], a large body of work in many small and large animal models supported studies of the potential of hepatocyte transplantation [11,12]. More recently, the therapeutic value of other liver cell types was elucidated. For instance, transplantation of liver sinusoidal endothelial cells (LSECs) cured haemophilia A in mice after LSECs were found to be the major

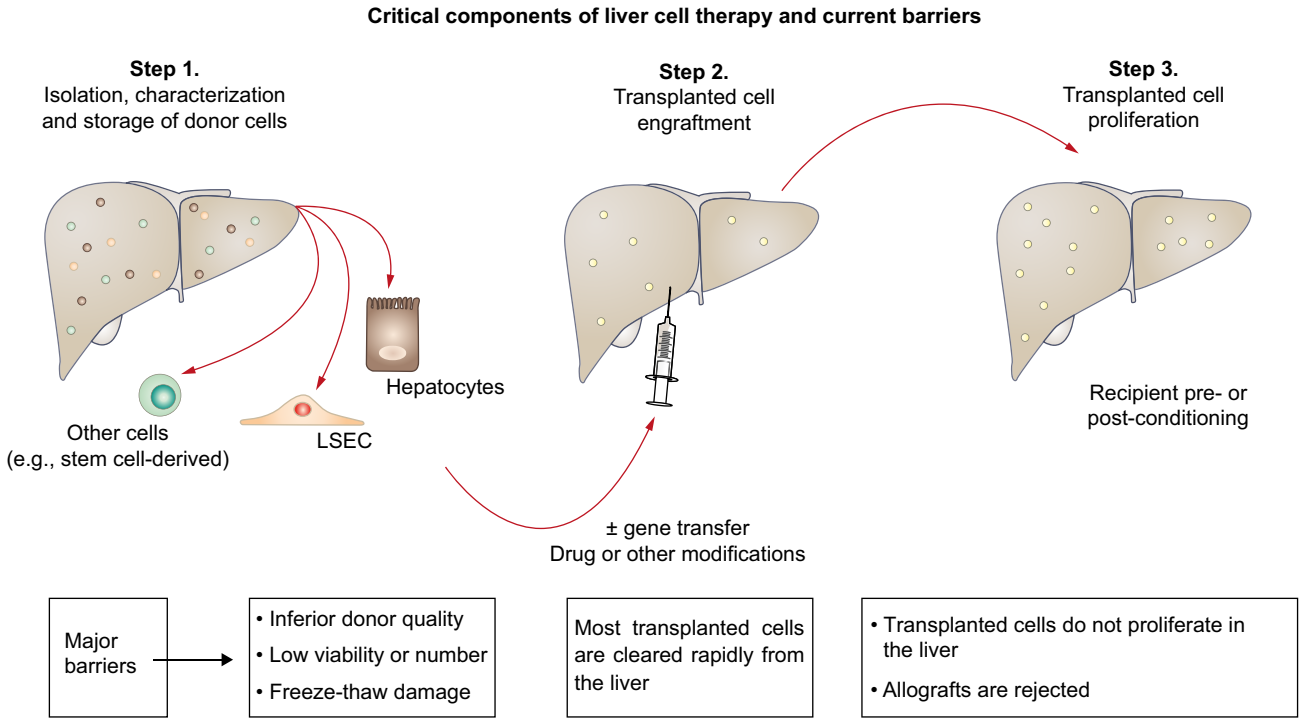

Fig. 1. Depiction of critical components in liver cell therapy and barriers in various steps. The first step in cell therapy requires isolation, characterization and storage of suitable donor cells. These steps are restricted by donor organ shortages or their inferior quality, procedural limitations in isolating cells of high viability and large numbers, as well as difficulties in cryopreservation of cells. The second critical step concerns engraftment of transplanted cells in the liver (or extrahepatic sites), which requires overcoming of early transplanted cell clearances. Cells may be modified by gene transfer vectors, drugs or other ways for improving cell viability, engraftment and proliferation. In the third and final step, transplanted cells must survive over the long-term and also proliferate to the necessary extents for imparting therapeutic benefits, which may require conditioning of recipients either before or after cell transplantation, as well as development of suitable regimens for controlling allograft rejection.

source of FVIII [13]. Applications of LSECs may extend to liver repair since these cells have been shown to be critical for liver regeneration in mice [14]. Pathophysiological processes that could be altered during chronic liver injury and fibrosis by the cell transplantation approach have also gained interest [15]. In some people with acute liver failure, cell transplantation has been successful for bridging to OLT, whereas in other instances, people with liver failure or enzymatic deficiency states had to be treated with OLT because cell therapy proved unsuccessful [6]. In part, this difficulty in achieving superior outcomes of cell therapy has been related to immunosuppression following allogeneic cell transplants, since optimal regimens for inducing tolerance to transplanted liver cells are to be established.

In the setting of metabolic liver disease and hepatic injury, e.g., hereditary tyrosinemia type-1 or Wilson's disease, animal studies established that disease correction can be achieved because even modest numbers of healthy transplanted hepatocytes can proliferate and repopulate the liver [16,17]. This process of liver repopulation has been shown in rodents to be accelerated by recipient organ preconditioning [18]. By contrast, in the setting of metabolic diseases where the native liver is unaffected and remains totally healthy, as in Crigler-Najjar syndrome or familial hypercholesterolemia, transplanted hepatocytes engraft but do not proliferate in the liver because this is not physiologically required. Therefore, in achieving therapeutic levels of repopulation further manipulation is required by either: a) preconditioning of the recipient's liver using techniques such as DNA-adduct forming chemicals, radiation, oxidative stress or by b) modification of donor cells by altering liver growth or cell cycle controls, such that transplanted cells receive survival and/or proliferation advantages over native cells [18,19]. In this way, the concept of "liver transplantation to cell factory" may be gained if one considers that successive generations of daughter cells may emanate in the recipient liver from transplanted hepatocytes, as was elegantly established using serial hepatocyte transplants in the fumarylacetoacetate-hydrolase-deficient ($FAH^{-/-}$) mouse model [20]. If these concepts regarding liver repopulation are reduced to drug-based approaches then barriers in transplanted cell engraftment and proliferation will be overcome for more effective clinical trials.

For many reasons, the clinical application of liver cell therapy has proceeded at a gradual pace in people compared to the successes in preclinical animal studies. Some of the obstacles concern limited availability of donor livers, difficulties in isolating good-quality cells from often suboptimal donor livers, mechanistic restrictions in cryopreserving human liver cells without losing viability, low levels of engraftment and proliferation in transplanted liver cells, as well as the general lack of therapeutic benefits over the long-term due to allograft rejection (Fig. 1). Another important point is that the animal models used often translate poorly to the clinic. Liver damage may have accumulated over decades in patients with severe distortion of liver architecture and impairment of function. The models of liver injury developed in mice and rats typically occur over days or weeks and are often milder than the human diseases they seek to model. An important message is that more realistic models of these liver injuries are required.