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Review

Stem Cells and Liver Disease

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ABSTRACT: Liver transplantation is the primary treatment for various end-stage hepatic diseases but is hindered by the lack of donor organs, complications associated with rejection and immunosuppression. An increasingly unbridgeable gap exists between the supply and demand of transplantable organs. Hence stem cell research and regenerative medicine have the potential to revolutionize the future of medicine with the ability to regenerate damaged and diseased organs. Stem cells serving as a repair system for the body, can theoretically divide without limit to replenish other cells. These cells could relieve the symptoms of liver disease or the genetic error could potentially be corrected by gene therapy. In cases of acute liver failure in adults, stem cell therapies might be used to support the liver, allowing it time to recover.

KEY WORDS: *Stem cells; Liver disease; Therapies*

INTRODUCTION

The global health burden from liver disease is immense with over 450 million individuals infected with viral hepatitis and hepatocellular carcinoma (HCC)¹. Cirrhosis kills 27,000 Americans each year, and over 25 million Americans have liver-related diseases. The World Health Organization estimates that there are at least 21.3 million hepatitis C virus (HCV) carriers in the Eastern Mediterranean countries, which is close to the number of carriers estimated in the Americas and Europe combined². It is estimated that of the world's six billion people, one third (two billion) have been infected with Hepatitis B virus (HBV)³. HBV is thought to be responsible for more than 350 million HBV carriers in the world, with an annual mortality rate of one million patients⁴. Studies in Saudi Arabia have shown HBV is the most predominant type of hepatitis, accounting for 49.3% of the cases, followed by HCV (40.7%) and HAV (10%). HBV predominates in young adults (31-50 years), and HCV in older adults (51-70 years)⁵. Approximately 20% of Hepatitis C infected individuals progress to end stage liver

disease and there are currently nearly 17,000 people waiting for a liver transplant in the United States. This shortage of donor livers exists worldwide and has prompted the search for alternative cell therapies for intractable liver disease⁶.

The hope that many diseases can someday be treated with stem cell therapy is inspired by the historical success of bone marrow transplants in improving the long-term survival of patients with leukemia and other cancers, inherited blood disorders, and diseases of the immune system. Nearly 40 years ago, the cell type responsible for those successes was identified as the hematopoietic stem cell⁷. The ability of hematopoietic stem cells (HSCs) to self-renew continuously in the marrow and to differentiate into the full complement of cell types found in blood qualifies them as the premier adult stem cells.

BACKGROUND

The possibility that the human body contains cells that can repair and regenerate damaged and diseased tissue is rapidly developing into a new clinical 'tool' with huge potential. Stem cells are basic cells of all multicellular organisms having the potential to differentiate into a wide range of adult cells (**Table 1**). Stem cells can be classified into three broad categories, based on their ability to

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differentiate. Totipotent stem cells are found only in early embryos. Each cell can form a complete organism (e.g., identical twins). Pluripotent stem cells exist in the undifferentiated inner cell mass of the blastocyst and can form any of the over 200 different cell types found in the body. Multipotent stem cells are derived from fetal tissue, cord blood, and adult stem cells. Although their ability to differentiate is more limited than pluripotent stem cells, they already have a track record of success in

cell-based therapies⁸. The term, “plasticity” has been coined to emphasize the process in which pluripotent stem cells are able to differentiate into multiple cell lineages, and is supported by the finding of intrahepatic chimerism in transplant recipients as well as the apparent repopulation of injured livers with bone marrow derived cells. The term, “transdifferentiation”, is used to focus on differentiation of cells from one organ specific lineage to those of another organ⁹.

Table 1: Differential potential ranges of Different Stem Cells

Differentiation	Number of cell types	Example of stem cell	Cell types resulting from differentiation
Totipotent	All	Zygote, blastomere	All cell types
Pluripotent	All except cells of the embryonic membranes	Embryonic Stem cells	Cells from all three germ layers
Multipotent	Many	Hematopoietic cells	skeletal muscle, cardiac muscle, liver cells, all blood cells
Oligopotent	Few	Myeloid precursor	5 types of blood cells (Monocytes, macrophages, eosinophils, neutrophils, erythrocytes)
Quadripotent	4	Mesenchymal progenitor cell	Cartilage cells, fat cells, stromal cells, bone-forming cells
Tripotent	3	Glial-restricted precursor	2 types of astrocytes, oligodendrocytes
Bipotent	2	Bipotential precursor from murine fetal liver	B cells, macrophages
Unipotent	1	Mast cell precursor	Mast cells
Nullipotent	None	Terminally differentiated cell e.g. Red blood cell	No cell division

ETHICAL CONTROVERSY

Basic work on human stem cells and their possible applications require an ample supply of the cells from a variety of genetically diverse viable cell lines. To date, pluripotent stem cells, from embryos or fetuses, have seemed potentially the most promising for medical applications, but obtaining them entails the destruction of an embryo or fetus, which touches on ethical issues concerning abortion¹⁰. Additional issues are cloning of humans, a technology that conceivably could be used to produce embryos to supply stem cells. Efforts are being directed to the use of adult stem cells, obtained from developed tissue (regardless of the age of the organism). These appear to be multipotent and give rise to specialized cells needed by the body for tissue regeneration after the

organism develops beyond the embryonic stage, and they generally produce cell types found in the tissue in which they reside^{11,12}.

CELL CULTURE

An essential prerequisite to the successful development of stem cell-based therapies is the development of techniques to propagate pure populations of stem cells on a large scale. Mesenchymal stem cells are very rare, existing at an estimated frequency of about 1 in 100,000 bone marrow cells¹³. To obtain sufficient numbers, cells are encouraged to multiply indefinitely in the laboratory. Briefly, the process entails isolating cells from a source such as umbilical cord and growing them in a cell culture facility whereby the cells are expanded over several population

doublings. Mesenchymal stem cells are traditionally grown on a fibroblast feeder layer that provides them with the necessary chemical signals to remain undifferentiated and to continue dividing over and over. After expansion these cells retain their ability to differentiate into a variety of mature cell types¹⁴. It had been thought previously that stem cells are directly influenced by cells in the local environment or 'niche', but Canadian researchers have recently demonstrated that human embryonic stem cells can actually produce distinctive niche cells in vitro, which then release stem cell nourishing proteins to help maintain the 'parent' cells¹⁵. The niche represents a route for modifying stem cell behaviour. If human stem cells can be reliably guided down a particular pathway, then they can be more readily used for future clinical therapy to regenerate damaged tissue¹⁶.

LIVER DISEASE

Liver cirrhosis in humans represents the end stage of chronic liver injury and is characterized by the disorganized proliferation of hepatocytes and biliary cells, excessive scarring, and loss of the three-dimensional architecture of the hepatic lobule, which leads to chronic liver failure eventually requiring transplantation. The risk of developing hepatocellular carcinoma is significantly increased in patients with hepatitis virus infection, alcoholic liver disease, or genetic hemochromatosis¹⁷.

CELL REPAIR

Liver regeneration is a complex phenomenon involving the proliferation of different cell lineages in response to damage such as after liver resection; it is the hepatocytes themselves which act as the stem cell reserve, proliferating to replace the lost liver mass. But in situations where the hepatocytes are prevented from dividing, and in some disease states, other cells derived from the terminal bile ductules and called oval cells, appear, and these are capable of providing both biliary epithelium and hepatocytes^{18,19}.

The extent of progenitor cell activation and the direction of differentiation are correlated with the severity of the disease and the type of mature epithelial cell (hepatocyte or bile duct epithelial cell), respectively, that is damaged. Analogous to findings in animal models of hepatocarcinogenesis, human hepatic progenitor cells most likely can give rise to hepatocellular carcinoma²⁰.

Oval cells appear to reside in the smallest units of the bile duct epithelium within the periportal region, termed the canals of Hering and/or periductular region, from which they can migrate into the liver parenchyma. Oval cells, which express immature hepatocyte markers, such as α -fetoprotein, γ -glutamyl-transferase, albumin, K19,

OV-6, and OC.2, as well as several hematopoietic cell markers, such as CD34, Thy1.1, Flt3-receptor, and Kit, can give rise to both hepatocytes and bile ductular cells in vitro and in vivo. In particular, the signaling networks involving HGF, EGF/TGF- α , vascular endothelial growth factor (VEGF), SCF, Wnt/ β -catenin, TGF- β , and stromal-derived factor-1 (SDF-1) appear to assume a critical role in the regulation of the proliferation, survival, and differentiation of oval cells into hepatocytes during hepatic regeneration²¹.

Mito and Kusano²² were the first to attempt hepatocyte transplantation in cirrhotic patients. Hepatocytes were isolated from the segments of the cirrhotic livers of the patients and transplanted by injection into the splenic pulp, splenic artery, splenic vein, or portal vein. Although the injections were tolerated well and there was some evidence of improvement in encephalopathy, protein synthesis, and renal function, the ultimate clinical outcome was not altered significantly. This study was a landmark for taking hepatocyte transplantation into clinics²³.

Many other growth factors and cytokines, such as EGF, TGF, IL-6, and TNF stimulate mitogenesis. Co-mitogenic factors such as estrogen, glucagon, and insulin upregulate the activity of mitogenic factors and can accelerate the liver regeneration process. Prolonged stimulation by some hepatotrophic factors can lead to hypertrophy or development of neoplasms. Prolonged intake of estrogen, mainly in the form of high-dose oral contraceptives or anabolic steroids, is associated with the development of hepatocellular adenomas and an increased risk for the development of hepatocellular carcinoma²⁴.

Isolation of hepatic progenitors from a human source is a major challenge for the clinical application of this therapy. Intrahepatic sources include cadaver livers that are considered of insufficient quality for organ transplantation. Hepatocytes isolated from aborted human fetuses are another potential source²⁵. Extra-hepatic sources include autologous bone marrow, umbilical cord blood, Wharton's jelly, peripheral blood monocytes^{26,27}. Programmable cells of monocytic origin are capable of differentiating into neohepatocytes, which closely resemble primary human hepatocytes with respect to morphology, expression of hepatocyte markers, and specific metabolic functions. After transplantation into the liver of severe combined immunodeficiency disease/non-obese diabetic mice, neohepatocytes integrated well into the liver tissue and showed a morphology and albumin expression similar to that of primary human hepatocytes transplanted under identical conditions. Programmable cells of monocytic origin-derived pancreatic neoislets expressed β cell-specific transcription factors, secreted insulin and C peptide in a glucose-

dependent manner, and normalized blood glucose levels when xenotransplanted into immunocompetent, streptozotocin-treated diabetic mice. The ability to reprogram, expand, and differentiate peripheral blood monocytes in large quantities opens the real possibility of the clinical application of programmable cells of monocytic origin in tissue repair and organ regeneration^{28,29}. Liver stem cells can be transplanted through several routes: Intraperitoneal, and percutaneous intrahepatic artery catheterization in acute liver failure, and umbilical vein catheterization, percutaneous intrahepatic route, and portal vein or intrahepatic artery catheterization in metabolic liver diseases³⁰. Intrasplenic artery, hepatic artery and portal vein catheterization in chronic liver diseases³¹. Attempts have been made to infuse cells from autologous bone marrow along with granulocyte stimulating factor. The preferred route is hepatic artery catheterization³².

STEM CELL THERAPY

The bone marrow is a potential source of multipotential stem cells. Bone marrow stem cells expressing hematopoietic antigens have been proven to have the capacity to differentiate into hepatocytes³³. Previous attempts to isolate hepatocyte progenitors from bone marrow have resulted in a mixture of liver and hematopoietic progenitor cells. These cells share common cell surface receptors and antigens³⁴.

Hematopoietic stem cells (HSCs) and mesenchymal stem cell (MSCs) are two main subtypes of bone marrow stem cells. The diseased liver may recruit migratory stem cells, particularly from the bone marrow, to generate hepatocyte-like cells either by transdifferentiation or cell fusion. Transplantation of bone marrow stem cells (BMSCs) has therapeutic effects of restoration of liver mass and function, alleviation of fibrosis and correction of inherited liver diseases³⁵. There are still controversial results over the potential effects of BMSCs on liver diseases, and some of the discrepancies are thought to lie in the differences of experimental protocols, differences in individual research laboratories, and the uncertainties of the techniques employed. Several potential approaches for BMSCs delivery in liver diseases have been proposed in animal studies and human trials. The optimal stem cells delivery route should be easy to perform, less invasive and traumatic, minimum side effects, and with high cells survival rate³⁶.

REGENERATIVE MEDICINE

Regenerative medicine is a rapidly growing field of biomedicine that seeks to create substitute tissues and organs for the human body, to repair or replace those whose function is lost through illness, injury,

aging or congenital anomaly. It is widely accepted that regenerative medicine is at the forefront of 21st century medical research and represents a significant evolution in medical treatment³⁷.

Cell-therapies in hepatology have numerous advantages when compared to organ liver transplant (OLT): the cells can be expanded in vitro, genetically manipulated, cryopreserved, obtained from the same patient and infused without major surgery. Possible cell-based treatments consist of hepatocyte transplantation and the development of bio-artificial liver systems (BALs). BALs have been mainly applied as supportive devices in patients excluded from or waiting for OLT and hepatocyte transplantation has limited overall success, related to the large amount of cells required to achieve acceptable function³⁸. Therefore, stem cell-based therapies are emerging as new alternatives to OLT for end-stage liver pathologies. The most promising source for stem cell-based therapies is currently represented by BMSCs and/or by mobilizing/proliferating agents, such as granulocyte-colony stimulating factor (G-CSF), which is able to both enhance the BMSC mobilization into the peripheral blood and facilitate the endogenous liver stem cell activation. BMSCs seem to be physiologically involved in the processes of liver repair in humans. The possible therapeutic potential of these cells has been investigated by intraportal autologous transplantation of BMSCs, which achieved some clinical improvement. However, some authors reported negative results regarding BMSC-therapies for end-stage liver disorders. Other clinical approaches have been based upon the administration of G-CSF alone or in combination with the reinfusion of the mobilized BMSCs. The feasibility, safety, and pattern of BMSC mobilization following G-CSF treatment in patients affected by cirrhosis has been evaluated in a few clinical trials¹.

Overall, the use of adult stem cells (ASCs) for the treatment of gastrointestinal and hepatic disorders holds several advantages, such as easy accessibility, unlimited supply (given the possibility to expand the collected cells in vitro) and no risks of rejection or need for immunosuppressive therapies when autologous cells are employed. Nonetheless, some conceptual issues still limit the diffusion of such treatments into clinical practice. Firstly, on the basis of preclinical data, BMSCs seem to facilitate gastrointestinal and hepatic regeneration mainly by a microenvironment modulation, which is likely to be transitory. In such a case, multiple treatments would presumably be required to achieve significant and lasting clinical results. Moreover, it has been observed that in some models of apparent transdifferentiation, stem cells may actually be fusing with cells in the host tissue. Fusion

phenomena between BMSCs and other cells (Purkinje cells, cardiomyocytes and hepatocytes) have been shown both *in vitro* and *in vivo*³⁹. The implications of this discovery are notable: fusion and transdifferentiation are not synonymous, since transdifferentiation requires that a specific stem cell program be activated on the basis of extracellular signals, whereas in the case of fusion, the plasticity is triggered by endogenous factors upon mixing of the cytoplasm and joining of the nuclei. It must also be noted that the fused cells are aneuploid and potentially unstable. Consequently, the possibility of cell fusion and the risk of malignant transformation of the transplanted cells, especially those pre-expanded *in vitro* before re-infusion, cannot be excluded and impose a need for careful evaluation and longer follow-up periods for assessing the safety and efficacy of these stem cell-based treatments⁴⁰.

RISKS OF STEM CELL THERAPY

Most problematic among the risks of stem cell-based therapies, in addition to the possible rejection or loss of function of the infused cells, is their potential neoplastic transformation. Indeed, stem cells may be used to cure devastating diseases, but their specific properties of self-renewal and clonogenicity may render them prone to generate cancers. Tumor-initiating cells mimic stem cell properties to sustain the growth and spread of the tumor, while eluding the intrinsic and extrinsic controls that regulate homeostasis within stem cell populations. The connection between normal stem cells and cancer has emerged in many tissues, particularly, blood, brain, mammary gland, gut and skin. Indeed, that cancer tissues resemble developing tissues is a long-standing observation, which has suggested a close connection between stem cells and cancer. The definitive demonstration of cancer stem cells in human neoplasia was first made in 1994 in leukaemia. Injection of a small subpopulation of acutemyelogenous leukaemia cells, identified by a cell surface phenotype also found in normal haemopoietic stem cells, caused a leukaemia in immunodeficient mice. Importantly, the cellular heterogeneity of the resulting leukaemia matched that of the patient's leukaemia. The principle of cancer stem cells was then extended to solid tumours, first in breast cancer and then in brain tumours. In breast cancer, a rare fraction of CD44C and CD24lo/K expressing human breast cancer cells uniquely caused tumours when small numbers of cells were engrafted into the mammary fat pads of non-obese diabetic severe combined immunodeficient (NOD/SCID) mice⁴¹⁻⁴³. Potential targets for cancer stem cell-based therapies in oncology might be found by comparing stem cells and cancer stem cell properties. i.e. it is well known that cancer stem cells share molecular

pathways involved in the maintenance of stemness (such as Wnt, Sonic Hedgehog, and Notch signalling) with stem cells and that they are responsive to similar morphogens involved in both stem cell migration and cancer metastasis. The development of drugs antagonizing these signals may be helpful in inhibiting cancer stem cell proliferation and mobilization, therefore blocking cancer growth and metastasis⁴⁴. For example, Chronic myelogenous leukaemia (CML) is a deadly form of leukaemia that is associated with chromosome rearrangements that result in the expression of the BCR-ABL oncoprotein. Treatment of CML with the BCL-ABL inhibitor imatinib mesylate (IM, Gleevec) has emerged as the first-line treatment for patients with CML. However, although most CML patients initially respond well to IM treatment, there is evidence that primitive quiescent leukaemia stem cells are retained in patients achieving remission after IM treatment and that these stem cells are responsible for disease recurrence. Treatment with histone deacetylase inhibitors in combination with imatinib mesylate (HDACis) combined with IM effectively induced apoptosis in quiescent CML progenitors resistant to elimination by IM alone, and eliminated CML stem cells capable of engrafting immunodeficient mice *in vivo*⁴⁵.

SUMMARY

Advances in stem cell biology and the discovery of pluripotent stem cells have made the prospect of cell therapy and tissue regeneration a clinical reality. Cell therapies hold great promise to repair, restore, replace or regenerate affected organs and may perform better than any pharmacological or mechanical device. There is an accumulating body of evidence supporting the contribution of adult stem cells, in particular those of bone marrow origin, to liver and pancreatic islet cell regeneration.

Human embryonic stem cell technology could solve the organ shortage problem by restoring diseased or damaged tissue across a range of common conditions. There is an argument in favor of encouraging contribution or intentional creation of embryos from which widely immunocompatible stem cell lines could be derived, towards a global human embryonic stem cell bank⁴⁶.

For the next few decades, the global eradication of viral hepatitis will be on the agenda. For the treatment of inherited and acquired metabolic, toxic and immune liver disease, targeted drugs, genes and antisense oligonucleotides will be added to our therapeutic repertoire. The widespread use of prenatal diagnosis, using DNA chip technology, may be expected to cause a dramatic decrease in the incidence of inherited diseases. Liver cirrhosis, hepatocellular carcinoma and inborn errors of

metabolism may be treated by gene transfer or gene repair therapy. Although eventually these developments may decrease the need for organ transplantation, this by no means is the case yet and no solution is available for an increased demand and a decreased supply of organs. In the long run, diseases caused by multi-drug-resistant infectious agents and diseases associated with the abuse of alcohol and drugs are expected to become major problems. Stem cell research poses many challenges but breakthroughs in this field may yield not only new forms of treatment, but provide a greater understanding of pathological mechanisms. The combination of stem cell transplantation and gene therapy is a particularly exciting one. Stem cell therapy is yet in the experimental stage; a lot of data needs to be defined and the clinical application has to be carefully monitored.

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