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Hypoimmunogenic Stem Cells and Their Impact on Liver Disorders

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ABSTRACT

Induced pluripotent stem cells (iPSCs) have enormous potential in a wide range of medical fields, from disease modeling to regenerative therapy. As the number of clinical trials using iPSCs grows, there is a growing emphasis on liver problems. Diseases caused by genetic abnormalities, such as Wilson's Disease, Alpha-1 Antitrypsin Deficiency, hemochromatosis, and Glycogen Storage Diseases, provide complex therapeutic issues due to immunological barriers and transplantation challenges. Hypoimmunogenic stem cells represent a possible approach to avoiding immunological rejection by allowing these cells to develop into functional liver cells such as hepatocytes and endothelial cells. To develop iPSCs with a low immunogenic profile, strategies including gene deletions and immunomodulatory substances are being investigated, and the use of CRISPR/Cas9 technology assists in the generation of hypoimmunogenic iPSCs. These cells have a remarkable capacity for self-renewal and differentiation, which opens up new avenues for in vitro liver disease modeling and therapeutic approaches. Wilson's Disease, which is characterized by copper buildup, may benefit from iPSC-derived hepatocytes as a viable alternative to immunosuppressive-laden liver transplants. Similarly, Glycogen Storage Diseases and hereditary hemochromatosis, which are caused by glycogen and iron imbalances, provide opportunities for iPSC-based therapeutic techniques that might change therapy paradigms.

Introduction

Induced pluripotent stem cells (iPSCs) are an important resource in many areas of medical research, including disease modeling, drug development, and regenerative medicine. Scientists have created patient-specific pluripotent stem cells for both research and therapeutic uses due to their unique characteristics such as self-renewal and the ability to become any cell type. Clinical studies involving iPSCs are developing quickly, with the majority of them focusing on eye illnesses, malignancies, neurological degenerative disorders, and cardiovascular diseases [1]. Various liver illnesses caused by genetic mutations, such as Wilson's Disease, Alpha-1 Antitrypsin Deficiency, hemochromatosis, and Glycogen Storage Diseases, have recently been treated. Following that, differentiated cells from hPSCs can be used as a cell source for patient therapy. However, immunological barriers to the therapeutic use of hPSCs restrict allogeneic cell transplantation. Immunological barriers are created when human leukocyte antigens (HLAs) on the cell surface match between patients and allogeneic donated cells. Curiosity drove scientists to use allogeneic cells in their efforts to address the immunological rejection problem caused by HLA mismatch [2].

The promise of Hypoimmunogenic Stem cells:

Utilizing hypoimmunogenic stem cells offers a primary advantage: apart from preventing immunological rejection, these cells can develop into functional liver cells including hepatocytes, nonparenchymal cells like endothelium, and hepatic stellate cells. Examples of such cells include TNF, 21-hydroxylase, heat shock protein, and tumor necrosis factors. Various methods have been employed to create iPSCs with a limited immunogenic profile. These methods encompass the direct removal of HLA-I genes or targeting specific genes necessary for their production alongside HLA-Il-associated reduction. Additionally, diverse strategies have been explored to address the susceptibility of HLAI-deficient cells to natural killer (NK) cell-based lysis, involving approaches like boosting the presence of immunomodulatory agents such as Programmed Death Ligand-1 (PD-L1), HLA-G, and integrin-associated protein (CD47) [3-4]. Furthermore, increasing HLA-E in conjunction with removing the PVR cell adhesion molecule (CD155), and eliminating immunogenic HLAA/HLA-B genes while retaining HLA-C gene, which possesses the capability to suppress NK cells, have been investigated (g vulnerability of HLAI-deficient cells to natural killer (NK) cell-based lysis) [5-6].

Generation of Hypoimmunogenic Stem cells:

Using the CRISPR/Cas9 system, we can create hypoimmunogenic iPSCs by specifically targeting the B2M gene, responsible for encoding the 2M protein crucial in the stable surface expression and folding of HLA-I molecules, along with the CIITA gene, a master regulator controlling HLA-II gene expression. Remarkably, these cells retain their self-renewal capacity and can differentiate into all three germ layers even in the context of double knockout (dKO) clones. Notably, from these hypoimmunogenic iPSC lines, it becomes feasible to successfully generate endothelial cells, hepatocytes, and hepatocellular cells. These derived populations from hypoimmunogenic iPSCs display regular morphology and function effectively in vitro. They offer a promising avenue for serving as in vitro models that replicate the intricate structure and functions of the liver [7].

Diseases caused due to gene mutation:

Wilsons Disease:

Wilson's disease (WD) is a liver disorder stemming from the dysfunction of the ATPase copper transporting beta (ATP7B). This condition occurs in approximately one in every 30,000 live births globally, with about one in every 90 healthy individuals carrying an abnormal version of the ATP7B gene. ATP7B plays a crucial role in facilitating the transportation of copper into the bloodstream for tissue utilization, as well as the elimination of excess copper into bile to maintain proper copper balance. Genetic mutations in ATP7B, whether homozygous or compound heterozygous, lead to disrupted copper regulation within cells and the accumulation of excess copper in the liver, brain, and other organs. This accumulation results in a variety of clinical symptoms. Without appropriate treatment, individuals affected by this condition will eventually experience liver failure and/or neurological issues, which can ultimately lead to premature death.

While liver transplantation holds promise as a potential solution for VP, its feasibility is constrained by donor scarcity, the potential for immunological rejection with allografts, and the drawbacks associated with prolonged immunosuppressive therapy [8]. An alternative or interim strategy for WD treatment is hepatocyte transplantation. This approach has been proposed as a viable option and continues to be explored. Previous studies have shown the effectiveness of allogenic hepatocyte transplantation in animal models of WD, and individuals afflicted with various hereditary liver metabolic disorders [9-10]. Notably, hepatocyte transplantation offers enhanced flexibility and less invasive surgery compared to standard liver transplantation. Additionally, it requires fewer hepatocytes to combat excessive copper buildup and mitigate disease impact. It's important to note, however, that immunosuppression remains essential to prevent the immune system from rejecting transplanted allogeneic hepatocytes from healthy donors [11]. Recent technological advancements, such as human induced pluripotent stem cells (iPSCs), precise genome editing utilizing CRISPR/Cas9, and innovative strategies for hepatic differentiation from pluripotent stem cells, have ushered in a new technological era. These breakthroughs enable the generation of iPSC-derived hepatocytes (iHeps) with corrected genetic profiles from patient-specific iPSCs [12]. This discovery lays the groundwork for autologous cell transplantation, an intriguing avenue that eliminates the necessity for immunosuppressive medications entirely. Autologous iHeps derived from patient-specific iPSCs with corrected genes emerge as a promising and potentially transformative cellular resource for effective cell-based treatment. In this manner, they surmount the challenges associated with acquiring allogenic human hepatocytes, which are constrained by availability and the risk of immunological rejection.

Glycogen Storage Diseases:

Glycogen storage disorders (GSDs) are rare genetic defects that are inherited in an autosomal recessive manner and are caused by mutations in genes that

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encode particular enzymes in the glycogen metabolism pathway [13]. One specific case, glycogen storage disease type III (GSDIII), is believed to affect one in every 100,000 people. This disease is caused by mutations in the AGL gene, which codes for the glycogen debranching enzyme (GDE). The GDE enzyme is essential in the breakdown of glycogen. Insufficiency of this enzyme causes aberrant glycogen buildup in a variety of organs, including the liver, skeletal muscles, and heart. Individuals with GSDIII experience difficult symptoms such as severe fasting-induced low blood sugar, visible enlargement of the liver (hepatomegaly), and development limits during childhood. As they reach maturity, they may develop more complicated liver disorders such as cirrhosis, as well as hepatic adenomas and hepatocellular carcinomas [14]. As GSDIII patients reach adulthood, they face a huge health issue in the form of a gradual and severe muscular condition characterized by widespread weakening in skeletal muscles, restricted capacity to tolerate exercise and eventual loss of walking ability. Glycogen accumulates in multiple focal sites within the sarcoplasm of muscular tissues, compromising appropriate muscle function and performance [15]. GSDIII frequently extends its influence beyond skeletal muscles to cardiac muscles, resulting in cardiomyopathy in around 15% of afflicted patients. GSDIII currently has no definite cure. To prevent recurring bouts of low blood sugar, patients require a precisely regulated dietary regimen that includes frequent meals and the addition of uncooked cornflour or continuous enteral feeding. Certain high-protein diets, such as the ketogenic and modified Atkins diets, have lately shown promise in reducing or stabilizing muscle and cardiac problems in some individuals [16]. GSDIII, particularly muscle involvement, remains an unmet medical need.

Hemochroatosis:

Iron, an essential trace element, exerts its effect by effortlessly switching between electron donor and acceptor positions, orchestrating a range of physiological activities [17]. However, while dualistic nature is necessary, it also creates a paradoxical problem. In instances of iron overload, the very redox potential that supports iron's physiological relevance becomes a source of toxicity. Iron buildup, caused by mutations in the hepcidin/ferroportin regulatory axis or owing to illnesses associated with inefficient erythropoiesis, poses a threat to organs such as the liver, heart, pancreas, gonads, and bone. To avoid iron excess and shortage, the body must actively control iron levels within a narrow range. In this delicate equilibrium, the liver plays a critical role in maintaining iron homeostasis. It not only stores iron, functioning as a reservoir to counterbalance shifts produced by fasting or dietary changes, but it also directs a larger strategy for systemic iron balance. The liver demonstrates its regulatory prowess by monitoring tissue and plasma iron concentrations and producing hepcidin, a key iron-regulating hormone. Hepcidin, which is produced by hepatocytes, exerts its impact by reducing iron inflow from duodenal enterocytes and reticuloendothelial macrophages via the breakdown of ferroportin, the iron exporter [18]. Any interruption in hepcidin synthesis or regulation results in systemic iron overload, which is a characteristic of 'hereditary hemochromatosis' (HH) [19]. Genetic inconsistencies, whether in the hepcidin-encoding HAMP gene or in other key players such as hemochromatosis gene (HFE), transferrin receptor 2 (TFR2), hemojuvelin (HJV), ferroprotein (FPN), or BMP6 propeptide, point to the intricate dance of molecules that orchestrates this finely tuned symphony of iron regulation [20].

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