Pluripotent stem cells for regenerative medicine

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Pluripotent Stem Cells for Regenerative Medicine

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Table of Contents

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PLURIPOTENT STEM CELLS FOR REGENERATIVE MEDICINE: EMMA GRACE ORESKOVIC.

Keywords: (Pluripotent stem cells, Regenerative medicine, Reprogramming, Technology)

1. Summary

Pluripotent stem cells are cells that can differentiate into any type of cell, making them promising candidates for cell replacement therapies and tissue/organ engineering in regenerative medicine. Because of their ability to self-renew and differentiate into multiple lineages, pluripotent stem cells are ideal for regenerative medicine, tissue repair, and gene therapy for incurable diseases such as cardiac and spinal cord injuries, as well as neurological and endocrine disorders. There are two types of pluripotent stem cells, cells isolated from the inner cell mass of the blastocyst, referred to as embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs), derived by reprogramming adult differentiated cells. iPSCs are a useful resource for personalized regenerative medicine since they may be created from any patient in need for replacement of diseased or damaged tissues. Furthermore, reprogramming technology has become a powerful tool for studying cell fate and modeling human diseases, significantly increasing the prospect of discovering new medications and strategies for treating life-threatening diseases. As human iPSCs can be made from a patient' s own somatic cells, their production does not necessitate the destruction of human embryos, which avoids ethical issues associated with embryonic stem cells. There is a growing demand for stem cell banking and standardization of the process of obtaining pluripotent stem cells. A global goal of research in the field of regenerative medicine is to increase the quality of the reprogramming process required for induced pluripotent stem cells and to increase their yield. Roadblocks to successful translation of such technology into clinical therapy must still be eradicated, and a great deal of effort is being put into making grafts prepared from pluripotent stem cells clinically safe for patients, especially in regard of their unwanted tumorigenicity potential.

PLURIPOTENTNE MATIČNE STANICE U REGENERATIVNOJ MEDICINI: EMMA GRACE ORESKOVIC

Ključne riječi: (pluripotenske matične stanice, regenerativna medicina, reprogramiranje, tehnologija)

2. Sažetak

Pluripotentne matične stanice mogu se diferencirati u bilo koju stanicu, što ih čini obećavajućim opcijama za regenerativnu medicinu, uključujući nadomjesnu terapiju stanicama, tkivno inženjerstvo i proizvodnju organa. Zbog svoje sposobnosti samoobnavljanja i diferencijacije u sve stanične loze, pluripotentna matična stanica obećava nove terapije u liječenju teško izlječivih bolesti kao što su kardiološke bolesti, bolesti leđne moždine, neurološke bolesti i bolesti endokrinog sustava. Postoje dvije vrste pluripotentnih matičnih stanica, stanice izolirane iz embrioblasta blastociste, koje se nazivaju embrionalne matične stanice (ESC), i inducirane pluripotentne matične stanice (iPSC), izvedene reprogramiranjem diferenciranih stanica odraslih. iPSC su koristan resurs za personaliziranu regenerativnu medicinu jer se mogu proizvesti od stanica bilo kojeg pacijenta kojem je potrebna zamjena oboljelih ili oštećenih tkiva. Nadalje, tehnologija reprogramiranja postala je moćan alat za proučavanje sudbine stanica i modeliranje ljudskih bolesti, značajno povećavajući mogućnost otkrivanja novih lijekova i strategija za liječenje bolesti opasnih po život. Sve je veća potreba za bankarstvom matičnih stanica i standardizacijom procesa dobivanja pluripotentnih matičnih stanica. Globalni cilj istraživanja u području regenerativne medicine je povećanje kvalitete procesa reprogramiranja potrebnog za inducirane pluripotentne matične stanice i povećanje njihovog prinosa. Budući da se ljudske iPSC mogu izraditi iz vlastitih somatskih stanica pacijenta, njihova proizvodnja ne zahtijeva uništenje ljudskih embrija, čime se izbjegavaju etička pitanja povezana s embrionalnim matičnim stanicama. Prepreke uspješnoj translaciji takve tehnologije u kliničku terapiju još uvijek se moraju preći, pa se ulažu veliki napori u izradu presadaka pripremljenih od pluripotentnih matičnih stanica, klinički sigurnih za pacijente, a posebno u dijelu koji se odnosi na njihov neželjeni tumorigeni potencijal.

3. Introduction

Stem cells can be defined as the fundamental building blocks of the human organism- the cells that give rise to other cells with specialized roles. When a stem cell divides into two daughter cells, one of them usually becomes a more specialized form of cell. The other daughter cell continues to be a stem cell needed to replenish the pool of stem cells in a tissue. Although differentiated cells, such as liver cells, can divide to produce more cells that are identical to themselves, it is only stem cells that have plasticity(1). Daughter cells are formed when stem cells divide under the correct conditions in the body or *in vitro* in the laboratory(2). Stem cells, such as bone marrow stem cells, have already been used for clinical applications (transplantation), while other types offer the potential to produce therapies for replacing defective or damaged cells caused by several diseases and injuries, including neurological diseases, heart disease, and diabetes (3).

Stem cells are classified into four categories based on their differentiation potential: (1) unipotent, (2) multipotent, (3) pluripotent, and (4) totipotent. The totipotent stem cells in the human body such as the zygote or the blastomere from the cleavage stage embryo, can give rise to an entire organism. On the other hand, cells from the embryo's inner cells mass (ICM) are pluripotent in nature and can differentiate into all cells of an embryo apart from trophoblast cells that will produce placenta (4). Stem cells can be divided into pluripotent and adult stem cells, classified according to the cell types they can differentiate into(5). Adult stem cells follow a specific fate, differentiating only into specific tissue types, while pluripotent stem cells can differentiate into any tissue derived from the three germ layers (i.e.: ectoderm, mesoderm, endoderm)(6). Pluripotent stem cells can be further subdivided into the embryonic stem cells (ESC), harvested from the inner cell mass of a blastocyst, and induced-pluripotent stem cells (iPSCs), which, by reprogramming differentiated cells, acquire characteristics of their former counterpart, the ESC (7).

Human pluripotent stem cells can also be divided based on their sources, which include inner cell mass-derived embryonic stem cells from the 5–6-day old blastocyst (human embryonic stem cells), those derived by the advent of somatic cell nuclear transfer (SCNT), and those

obtained by nuclear reprogramming of somatic cells which generates the “induced” pluripotent stem cell(8). These pluripotent stem cell states are useful in the field of regenerative medicine, a developing discipline of medical science, which focuses on the functional restoration of specific tissue and/or organs in patients with serious injuries or chronic disease states (4).

Several categories of pluripotent or undifferentiated stem cells exist including embryonic stem cells (pluripotent stem cells derived from the inner cell mass of a blastocyst); very small embryonic-like stem cells (pluripotent stem cells derived from adult tissues); nuclear transfer stem cells where one new single cell-reconstituted zygote is produced by the transplantation of the nucleus from a differentiated cell into an enucleated oocyte of a donor egg and where reprogramming of the nucleus occurs allowing formation of the blastocyst; RSC-reprogrammed stem cells (pluripotent stem cells generated by reprogramming adult cells) that are derived by the advent of various laboratory methods(9). There are also adult stem cells (a type of cell in proximity to nutrient-rich microenvironments such as vessels, bone marrow, or organs (heart and brain, etc.)) in the mature or adult organism that can respond to tissue-specific stimulation to produce differentiated cells. It is the pluripotent stem cell which shows great promise in tissue and organ regeneration(10).

A complicated mechanism including genetic and epigenetic components maintains pluripotency(11). Recent research has revealed that transcription factors, signal pathways, and microRNAs interact closely with the enzymes and other specialized proteins involved in chromatin structure assembly(11). Cell identity reflects a cell type-specific gene expression profile and cell type-specific transcription factor networks are thought to be at the heart of a cellular phenotype. Although cell identity is usually stable, it can be reprogrammed in vitro by forced alterations to the transcriptional network, as demonstrated by the generation of pluripotency in somatic cells by ectopic expression of specific transcription factors (12).

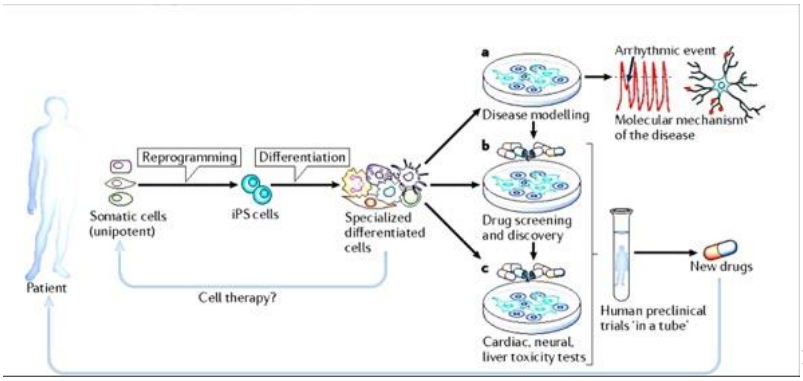
4. Pluripotent Stem Cells

The story of stem cells began at the University of Toronto, where James A. Till and Ernest A. McCulloch determined that stem cells derived from mouse bone marrow cells have the ability to differentiate into several specialized cell types(13). A major goal of stem cell research is to use stem cells to treat patients. However, technical and ethical issues plague both human pluripotent ES cells and the process of somatic cell nuclear transfer (SCNT), making therapeutic usage of such cells in humans especially problematic(14).

4.1. Embryonic Stem Cells

Stem cell advancements reached new heights in 1998, when James Thomson isolated the first human embryonic stem cells (hESCs)(13). Now there are many hESC lines. In vitro, embryonic stem cells form embryoid bodies, three-dimensional aggregates of pluripotent stem cells which spontaneously differentiate into various cell types including mural cells, cardiomyocytes, and endothelial cells after interacting with proximal cells(10).

Pluripotency is seen only in early embryos and can be maintained in vitro in cultivated ES cells extracted from blastocyst's inner cell mass (15). Isolated ES cells can differentiate into any somatic cell lineage and can maintain their population by multiplying and self-renewing. Under certain settings, self-renewal allows ES cells in culture to undergo multiple cell cycles without losing pluripotency(16,17). For this to occur, however, co-culturing mouse ES cells with a feeder layer of cells that contain crucial factors is required. To avoid differentiation, this culture media must additionally contain leukemia inhibitory factor (LIF) for mouse ES cells or fibroblast growth factors (FGFs) for human ES cells(18). ES cells spontaneously differentiate and lose their pluripotency in the absence of feeders or cytokines (19).



[1]

Figure 1: Derivation, differentiation, and uses of human iPS cells. Unipotent adult somatic cells can be reprogrammed into induced pluripotent stem (iPS) cells from any patient. Human iPS cells can differentiate into specialized cells after being differentiated in vitro, which can be used in a variety of way(9).

4.2 Somatic Cell Nuclear Transfer (SCNT)/ Therapeutic Cloning

[1] Bellin M, Marchetto MC, Gage FH, Mummery CL. Induced pluripotent stem cells: the new patient? Nature Reviews Molecular Cell Biology. 2012 Nov 4;13

Briggs and King performed the first somatic cell nuclear-transfer (SCNT) studies more than 50 years ago, proving that by injecting a nucleus from a mature blastula cell into an enucleated frog egg, a tadpole could be produced(14). Thereafter, John Gurdon demonstrated that nuclei from comparatively more differentiated frog intestinal cells can be reprogrammed to produce adult animals, though with low efficiency(14).

These investigations revealed that when differentiated adult cells are placed in the right environment, their nuclei retain nuclear plasticity, a characteristic of early embryonic nuclei (20). A notable moment on the timeline of stem cell technology, in 1996, Wilmut and colleagues created "Dolly," a sheep whose claim to fame is being the first cloned mammal. Dolly was produced via the nuclear transfer from adult cell into an enucleated egg (21). This discovery corroborated previous data that demonstrated that the epigenetic status of differentiated cell nuclei may be reversed and that nuclei can be reprogrammed by factors in oocytes or embryonic stem cells(11).

4.3 Induced Pluripotent Stem Cells

The ideal method for creating patient-specific pluripotent stem cells for use in regenerative medicine is iPS cell technology, which avoids ethical issues of human embryo destruction. The process of creating induced pluripotent stem cells by nuclear reprogramming, is the inverse of differentiation, in which differentiated cells revert to pluripotent cells(22). Based on previous nuclear-transfer and cell-culture experiments, scientists began to experiment directly with cells' genetic information, aiming to create developmental plasticity in mature, differentiated cells(23).

The first successful generation of iPS cells from somatic cells was accomplished by ectopic overexpression of pluripotency-related transcription factors(10). Takahashi and Yamanaka first created a mini library of 24 potential reprogramming factors known to be expressed in ES cells. Finally, only several pluripotency-associated genes were used in mouse embryonic fibroblasts to reprogram them to a pluripotent state (11). In 2012, Shinya Yamanaka merited the Nobel Prize in Medicine and Physiology after reprogramming adult somatic cells into a pluripotent state(24).

In the first Yamanaka experiment, drug-resistant colonies with ES cell–like proliferation, gene expression, and morphology formed when mouse embryonic fibroblasts (MEFs) were transduced with four out of 24 genes expressed in pluripotent stem cells(15). To narrow down the genes required for reprogramming, researchers tried various combinations until four were identified: *Oct3/4*, *Sox2*, *Klf4*, and *c-Myc* (17). The resulting cells were named induced pluripotent stem cells because they had pluripotent characteristics that were indistinguishable from those of ES cells. Yamanaka proved iPSC's pluripotency by obtaining teratomas after their subcutaneous transplantation to the adult mouse and by injection into blastocyst producing chimeras. In honor of Shinya Yamanaka, the method's creator, these four factors are sometimes referred to as “Yamanaka factors” (25). A 'gold standard' trilaminar teratoma experiment was used to examine the pluripotency of reprogrammed human cells in immunocompromised mice in vivo(26).

Early embryonic tissues and ESCs or iPSCs may form teratomas or even teratocarcinomas after transplantation in vivo. To avoid this, such cells should be first differentiated in vitro to the desired cell type. Therefore, derivatives of human pluripotent stem cells i.e., differentiated lines should be tested in a long-term animal teratoma assay to rule out malignancy(26). After human therapy with neural stem cells in 2007 was discovered to lead

to malignancy in the brain, it was determined that the standardized teratoma assay should be geared more toward the assessment of EC/malignant cell traits than when manipulating with differentiated tissues(26).

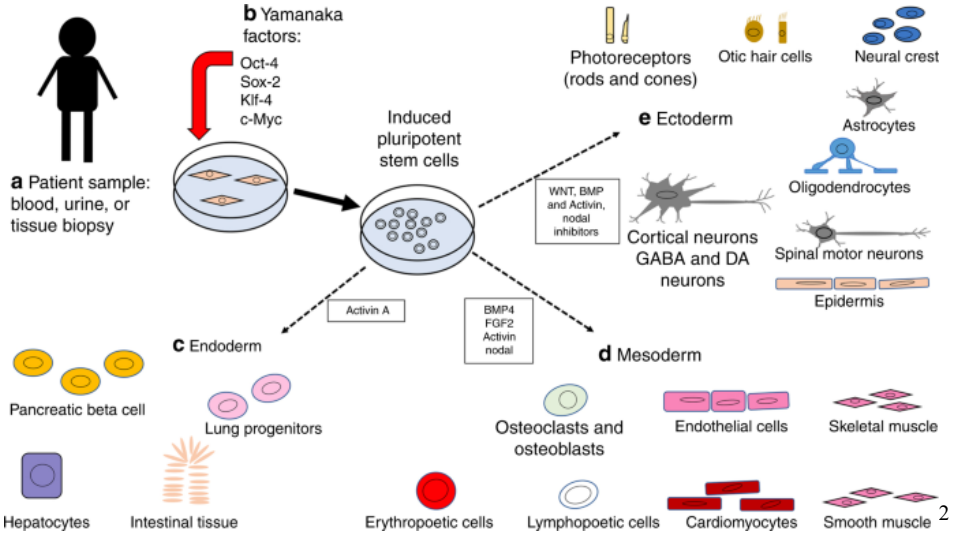


Figure 2: Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc) are highly expressed in embryonic stem cells and can induce pluripotency in both mouse and human somatic cells, suggesting that these factors modulate the developmental signaling network required for ES cell pluripotency(27).

5. The Pluripotent Stem Cell Microenvironment

[2] Durbin MD, Cadar AG, Chun YW, Hong CC. Investigating pediatric disorders with induced pluripotent stem cells. Pediatric Research. 2018 Oct 30;84

A fundamental component of pluripotent stem cells is their ability to differentiate into a designated cell type based on the microenvironment they are exposed to. It is this intricate environment that is necessary to produce specific cell lines(28). Broadly, we can classify this environment as one that requires both biophysical and biochemical cues(8). Several mechanisms are involved in programing stem cell fate. The cell responds to external cues by mechanosensory, mechanotransductory, and mechanoresponsive means(19). The immature cell employs these mechanisms of communication via signaling through cell surface receptors. Such receptors include integrins, for example, which assist in the reconfiguration of the microenvironment that allows the stem cells to integrate into the underlying cytoskeleton(8). For the hPSC to achieve a pluripotent state, a diverse set of proteins are required, including RhoA, E-cadherin, and kinases belonging to the Src family, all of which contribute to the mechanotransduction involved in attaining pluripotency(29). Loss of these proteins destabilizes the attainment of a pluripotent state through several means. For example, absent RhoA signaling would result in decreased E-cadherin mediated cell-cell contact(30). Likewise, this loss of E-cadherin results in impairment of the pluripotency transcriptional network(30). It is these transcriptional networks that are necessary in regulating the pluripotent state of the cell(23).

Moreover, the influence of growth factors in directing stem cell fate is undeniable, one that is necessary to take the stem cell at hand to a definitive tissue state, as exemplified by the role of transforming growth factor β (TGF-β), which is required to activate RhoA, driving stem cells to a more specific cell line(8). Equally important are the biophysical signals required for hPSC pluripotency. Signaling is sensed by the cell surface receptors, from which they are transduced via mechanosensitive ion channels, various peptide sequences, enzymatic alterations, and receptor-ligand interactions (29). By these means, signals, which include information pertaining to substrate composition, for example, are conveyed on a molecular level(8). Ultimately, it becomes the interplay of the biochemical and mechanosensory signaling that is necessary for hPSCs to follow a fate that consists of either self-renewal or differentiation into a specific cell lineage(8).

6. Pluripotent Stem Cell Banking

The International Stem Cell Banking Initiative (ISCBI) was founded in 2007 with the intention of merging leading stem cell banks that distribute human pluripotent stem cell (hPSC) lines for research and development to discuss best practices on a variety of topics, including donor consent, cell delivery, and cell-based medicines(31). Biobanking has been broadly defined as the practice of collecting health and genetic information, as well as a plethora of biological materials, and storing them in "banks"(32). Biobanks have become increasingly popular and essential as resources for genetic research in recent years, combining the preservation of tissue and DNA samples with genome sequencing data for genetics research and genome-wide association studies (GWAS)(33). The benefit of biobanking is that it allows stem cells to be preserved in a controlled and regulated manner for future scientific research or therapy. The National Institutes of Health (NIH) has made a concentrated effort to support the establishment of public biobanks to house stem cell samples that can later be employed for research purposes, and ultimately establish themselves as a leader in scientific research(33).

For various reasons, banking iPS cells differs from banking hES cells. The most crucial advantage is that the tissue utilized to create autologous, as well as heterologous iPS cells may be easily collected and banked in liquid nitrogen from a variety of publicly available sources(34). As demonstrated by recent endeavors, several elements of iPS cells make them particularly difficult to acquire consent for from an ethical standpoint. Because an iPS cell line can produce a range of cell types, the number of possible applications that need to be covered by a consent form is likewise extensive(32). Practically, any tissue type and disease phenotype are possible, and research applications of these cells range from very basic to translational, including potentially sensitive issues like whole genome sequencing (and genetic privacy/discrimination), human reproduction (especially since it became clear that functional gametes and embryos can be developed from iPS cells), and commercialization of iPS cells(33).

7. Applications of Pluripotent Stem Cells for Organ Regeneration

7.1 Pluripotent Stem Cells for Neural Regeneration

The first lineages to be successfully produced from hPSC sources were neural cell types (35). In an animal model, recent research has shown that induced neural stem cells (iNSCs) have substantial potential regarding regenerative and cell replacement treatment, offering more insight into the cells' potential as a treatment for humans. Intraparenchymal transplantation of human iNSCs in a stroke-damaged pig brain has proved efficacious in promoting tissue healing(36). After iNSC treatment, non-invasive longitudinal magnetic resonance imaging (MRI) of stroked rats revealed improvements in brain metabolism, white matter integrity, and cerebral blood perfusion. iNSC treatment also resulted in neuronal protection, reduced microglial activation, and increased endogenous neurogenesis, according to histologic analysis(36). The benefit of harvesting and transplanting neuronal cells derived from pluripotent stem cells is highlighted in discussing various prevalent neurodegenerative disorders.

7.1.1 Parkinson's Disease

Parkinson's disease is the second most prevalent neurodegenerative disease, and it is marked by the gradual death of various neural cell types in the CNS and PNS(37). Although the origins of Parkinson's disease are unclear, loss of midbrain dopamine neurons is responsible for most the illness's motor symptoms (37).

Current therapeutic objectives aimed at diminishing the debilitating Parkinsonian symptoms include restoring the functionality of dopaminergic neurons. Several studies have shown that cells with midbrain dopamine neuron-like features have been derived for nearly a decade now. These neurons, however, lacked several characteristics, including expression of the DNA-binding forkhead box protein A2 (FOXA2), and could not engraft well(37). Recent developments have resulted in a new methodology of neuronal generation from hESCs based on more accurate cell developmental patterning(35). The passage of the cells through a floor plate intermediate stage rather than the neuroepithelial intermediate stage employed in previous efforts is a critical component of this approach. The resulting floor plate-derived dopamine neurons have been successfully evaluated in mouse, rat, and rhesus monkey models of Parkinson's disease, and they have genetic, biochemical, and physiological properties of authentic midbrain dopamine neurons(35).

7.1.2 Huntington's Disease

Huntington's disease is an incurable autosomal dominant neurological illness marked by aberrant motions, cognitive deterioration, and psychological issues(38). An amplification of CAG repeats within the huntingtin gene (HTT) is the genetic etiology of the disorder(39). In patients with Huntington's disease, medium spiny striatal neurons are the most severely damaged cell type(40). Because there is still no established medicinal therapy for this genetic condition, symptom management is the primary treatment for HD. In HD, stem cells can be used in cell therapy therapeutic techniques to replace malfunctioning or dying cells(41).

7.1.3 Spinal Cord Injury

The first hESC-based product to reach clinical trials for treating spinal cord damage was produced from early glial progenitors(42). Following long-term in vitro cultivation and early exposure to retinoic acid and SHH agonists, more committed cells from the oligodendrocyte lineage were produced (43). A recent study using glial precursors obtained from long-term in vitro development of human induced pluripotent stem cells (iPSCs), which were then implanted into the newborn CNS of myelin-deficient mice, adequately demonstrated the translational potential of hPSC-derived oligodendrocyte progenitors (40).

7.2 Pluripotent Stem Cells for Cardiac Regeneration

The applications of pluripotent stem cells for regenerative medicine become especially appreciated when juxtaposed to cardiac pathology. In a leading cause of mortality universally, pluripotent stem cells have played an integral role in the attempt to salvage deficient cardiomyocytes(44). Although patients afflicted with cardiac pathologies are well-equipped with the necessary medications, damaged cardiomyocytes are never able to return to a repaired state, limited in their regenerative potential(45). Consequently, millions of individuals annually suffer the morbidity and inevitable mortality of heart disease. However, given the ability of pluripotent stem cells to differentiate into most somatic cells, there is increasing hope that the grave consequences of cardiovascular illnesses will soon be circumvented (45).

The embryonic origin of the functioning adult cardiomyocyte is mesodermal. The signaling pathways directing normal cardiovascular development during the fetal stage are the Wnt, Nodal/Actin, and BMP-4 signaling pathways (45). First induced from mouse iPS cells in 2008, followed by the induction of human iPS cells in 2009, regenerated cardiomyocytes have been studied longitudinally (46). Several of the ethical impediments associated with cardiomyocytes derived from ES cells are avoided in the light that both iPS cells and ES cells induce similar quantities of cardiomyocytes(46). Although cardiomyocytes could be derived from iPS cells, employing iPS cells for therapeutic purposes is still inefficient, lacking meticulous methods of induction(47).

The therapeutic advantages of integrating pluripotent stem cells into cardiovascular treatment was observed in a preliminary clinical trial conducted by Menasche et al, whereby heart failure outcome was improved by the advent of embryonic stem cell- derived cardiac progenitors (45). Several hurdles must be overcome before PS cells can become established methods of therapy, one of which includes the variety of lineage cells that comprise cardiovascular cells, including cardiomyocytes, pacemaker, vascular, and mesenchymal cells, and which of these is most appropriate for regenerative purposes(47). An inextricable problem associated with stem cell manipulation, new methods must be devised to diminish tumor formation, resulting from the failure of PS cells to attain a differentiated state. Teratoma formation is evident as a specific feature of the "differentiation-resistant" phenotype characteristic of some iPS cells(47). Elimination of such impediments to PS cell usage is crucial to advancements in stem cell therapy.

Although the use of stem cells in cardiac regeneration demands critical developments, it is important to recognize that pluripotent stem cells have improved cardiac regeneration alternatives when used in conjunction with different therapeutic approaches. Such approaches include injecting cytokines and growth factors into stem cells to ameliorate cardiac function, employing biomaterials like matrix-enriched hydrogen capsules as vectors to deliver stem cells to cardiac tissue, and using Pharmacologically Active Microcarriers (PAMs) that use stem cell therapy to direct drug behavior(48).

7.3 Pluripotent Stem Cells for Hepatic Regeneration

Although the development of a functional hepatic organoid is still under development, the use of PS cells directed to hepatocyte regeneration has a promising future. Mature hepatic organoids have been produced and exhibit identical characteristics to their adult liver-derived counterparts, including similar histological characteristics and the ability to self-renew(49). Hepatic regeneration via PS cells highlights the in vitro benefits of PS cells, including the generation of identical cells that can function as experimental models to study adverse side-effects of hepatotoxic drugs (49).

Hepatocyte transplantation by means of cryoprotected hepatocytes endows physicians with the opportunity to salvage dysfunctional liver tissue. However, doing so implicates various side-effects, including instant blood-mediated inflammatory reactions (IBMIRs), whereby transplanted tissue is rejected by an interplay of activated complement and coagulation pathways(50). For hepatocyte transplantation to become a reliable source of improving patient outcome, it must become necessary to overcome the donor shortages that allow transplantation of these cells(49).

Fortunately, however, with the advent of somatic stem cell transplantation, low hepatocyte engraftment rates and shortages may become obsolete in providing patients with a functional liver(50). The ES cell demonstrates potential use in hepatocyte transplantation, possessing characteristics of mature hepatocytes after differentiation. In attempting to regenerate hepatic tissue, a complication is manufacturing the three-dimensional organoid, which consists of several types, including sinusoidal, stellate, Kupffer cells, and hepatocytes (50). Although hepatocytes have been able to be derived from ES and iPS cells, an impediment to synthesizing an entire liver from pluripotent stem cells is that they are only capable of producing two-dimensional hepatocytes and do so in a limited quantity(50). A recent advancement in the development of complex organs, however, was the creation of the vascularized human liver bud (LB). Engineered by Takebe et al., this LB was derived from human iPS cells, which, upon interaction with their endothelial and mesenchymal stem cell counterparts (a similar phenomenon occurring during organogenesis), organize into a three-dimensional LB(50). More specifically, the micro-niche required for iPSCs to differentiate into a 3D hepatic organoid necessitates human umbilical vein endothelial cells (HUVECs) and human bone marrow derived mesenchymal stem cells(51).

7.4 Pluripotent Stem Cells for Pancreatic Regeneration

Diabetes mellitus affects 450 million people globally and is a premier cause of mortality. Pancreatic β-cells, which are found in the Langerhans islets, play an important part in the deterioration of diabetic patients and have thus become a central focus when discussing diabetic therapeutic measures (52). Both transplantation treatment and diabetic disease modeling require an effective technique for producing functional pancreatic β-cells.

Human pluripotent stem cells offer an endless supply of differentiated cells for regenerative research(52). hESC or iPSC-derived cells enriched with certain transcription factors may generate glucose-responsive insulin-secreting cells in vitro, and, according to recent studies, transplantation of these cells diminished the hyperglycemic state in diabetic mice(53). In vitro and in vivo, the β-like cells resemble those from pancreatic islets in terms of gene expression, ultrastructural properties, and glucose responsiveness. The final cell population derived by multistage methods of induction contains roughly 30–60% β-like cells(53). The remainder of the cell population are relatively uncharacterized and include undifferentiated progenitors or other types of obsolete cells. The ability to create islet organoids with fully functioning mature cells has yet to be established. Creating such organoids may prove beneficial when studying the pathogenesis of diabetes and designing better therapeutic measures to combat diabetes-related side effects(54).

7.5 Pluripotent Stem Cells for Ocular Regeneration

7.5.1 Age- Related Macular Degeneration

In developed countries, age-related macular degeneration (AMD) is the major cause of blindness among the elderly. There are two types of AMD: neovascular (NV-AMD) and non-neovascular (NNV-AMD)(55). Here, retinal pigmented epithelial cells become dysfunctional and die as a result of cumulative damage to the retinal pigment epithelium, Bruch's membrane, and choriocapillaris, all fundamental components of the eye that are responsible for sight(55).

There is currently no treatment for advanced NNV-AMD. However, replacing dead or dysfunctional RPE with healthy RPE has been shown to rescue dying photoreceptors and improve vision in animal models of retinal degeneration and possibly in AMD patients(55). Differentiation of RPE from human embryonic stem cells (hESC-RPE) and from induced pluripotent stem cells (iPSC-RPE) has created a potentially unlimited source for replacing dead or dying RPE. Such cells have been shown to incorporate into the degenerating retina and result in anatomic and functional improvement(55).

7.5.2 Retinitis Pigmentosa

Like AMD, retinitis pigmentosa (RP) is a retinal disease that remains untreated, the affected patients suffering a progressive worsening of vision due to loss of visual structures required for site, specifically the photoreceptor and outer nuclear layer cells(56). Retinitis pigmentosa is a possible target illness for stem cell treatment. RP is the most prevalent inheritable eye disease that causes photoreceptor cell loss over time, resulting in gradual vision loss(57). While RP can start in childhood, the first symptoms commonly appear in early adulthood, starting with nyctalopia, loss of peripheral vision and, finally, loss of fine center vision when the core photoreceptors in the macula are damaged(57).

In 2015, a unique strategy was developed to screen for RPE-differentiation promoting factors(57). Several embryonic and iPSC lines have been shown to produce functional RPE using both spontaneous and targeted differentiation approaches. RPE differentiation that mimics normal development was recently discovered. With several therapies now in clinical trials, RPE cells produced from hESCs are the first to show a good translational capacity. However, there are still key challenges to consider, such as ethics and limited sourcing. Although this method has shown to be beneficial, the ideal cure would be to simply replace the dying photoreceptors(56). This treatment would be available everywhere and would be unaffected by the underlying pathology or injury. Recent advancements have established a solid foundation for successfully generating retinal cells from pluripotent stem cells(58).

8. Challenges Related to Utilizing Pluripotent Stem Cells for Regenerative Medicine

There are some major roadblocks to hESC development for clinical translation. The first pertaining to the ethical concerns that rise from the etiology of these cells being one that is derived from the inner cell mass of a human embryo and the second- the immunological rejection issues because these cells are obtained from an allogeneic source (59). Currently, several nations permit the derivation of hESCs from donated excess IVF embryos under certain conditions, although doing so may implicate the wellbeing of the patient and has unstudied consequences. This raises the question of where and under what conditions may legal and ethical research be conducted to study the medicinal potential of human embryonic stem cells. (34). However, the usage of ESCs and/or ESC-derived cells is limited or illegal in many other nations(34).

8.1 Pluripotent Stem Cells and Tumorigenic Potential

The relationship between pluripotency and tumorigenicity has been studied since the 1960s(60). The induction of pluripotency has been connected to tumorigenic transformation, causing chromosomal and sub-chromosomal genomic abnormalities. A single nucleotide polymorphism, or SNP, is a difference in the DNA sequence at a single point or base pair among individuals(61). Even if a single SNP does not cause a disease, it may be linked to specific pathologies. It was demonstrated that numerous deletions of tumor-suppressor genes were found in iPSCs shortly after pluripotency induction, which were absent from the somatic cells from which they pluripotent stem cells were induced(62). *De novo* mutations during pluripotency induction are most likely caused by the replication stress associated with the reprogramming process, suggesting that DNA demethylation causes structural instabilities in the genome(62).

Despite such clinical roadblock, progress has been made in lowering the risk of tumor formation following transplantation using iPSC-based therapy. For example, the removal of the oncogene *c-Myc* (from transducing vector) which promotes cancer by overstimulating cell growth, and suppressing genomic instability during reprogramming, are the mechanisms by which tumor formation can be diminished(63). Given the risk of mutagenesis and cancer formation associated with viral vectors, a move toward insertion-free procedures for generating iPSCs has been proposed(63). Finally, caution should be used while selecting the somatic origin of iPSCs. Cell lines produced from mouse tail-tip fibroblasts and hepatocytes are more likely to form teratomas than cell lines derived from other tissues(64). This is most

likely due to these cell lines' greater resistance to differentiation when compared to other somatic cell lines(64).

8.2 Deriving Human Embryonic Stem Cells

Human Embryonic Stem Cells (hESCs) are derived from the surplus of embryos from in-vitro fertilization(65). Cryopreserved embryos may be discarded, by donation transferred to other potential parents or donated for research(66). Research using excess embryos should comply, in the light of previous informed consent, with guidance by institutional review boards (IRBs) or comparable organizations. Specifically, the generation of hESC lines typically occurs utilizing existing and excess embryos initially produced for assisted reproductive technology and no longer necessary for reproduction(59). Many standards for human testing have been put out years ago, including the Nuremberg Code (1947) and the Helsinki Declaration and the Belmont Report (1978) in order to prohibit unethical research and human therapy(67). With the advancement of technology in the field of biomedicine and the emergence of new fields such as stem cell research and genome editing, these new technologies necessitate the inclusion of a set of new specific rules in the regulations to allow their application in these broad fields, particularly in the field of regenerative medicine(68).

8.3 Genetic Material and Confidential Personal Information

iPSCs generated from any individual carry a tremendous quantity of private information (DNA) that, if misused, may violate law, morals, and individual privacy. Even if the initial cell donor is no longer living, the iPSCs contain information even about his or her near relatives, thus posing ethical and legal concerns about individual privacy(67). Furthermore,

total anonymization of the donor's data is rarely desirable, because future iPSC research may require continuing access to information regarding the donor's health state, which necessitates knowledge of the donor's name and address(69). In analyzing data, researchers may unintentionally learn that the donor has a genetic condition that they are unaware of. Many countries' ethical codes forbid researchers from disclosing such information to employment agencies, employers, third parties, or even patients without their consent(69,70).

8.4 Pluripotent Stem Cells and Cloning Potential

PSCs, SCNT technology, and any other approach for human reproductive cloning are all banned, unlawful, and punitive around the world. However, human therapeutic cloning for people who require a transplant to treat their disease and obtain genetically similar tissue from a blastocyst may be possible especially with the advent of iPSCs(67). Using iPSCs for purposes like these could theoretically be possible thanks to using several technologies, such as tetraploid complementation(67).

8.5 Cost-Related Issues of Pluripotent Stem Cell Utilization

However, one important ethical hurdle in the development of patient-specific medicines and customized medicine is the expense(70). Concerns about the distribution of novel, expensive, but potentially lifesaving, patient-specific medicines center on a lack of equal access to treatment depending on socioeconomic level and health-care quality(71). Because iPSCs are easier to obtain than ESCs due to fewer research limitations and greater simplicity of manufacture, they may one day provide viable and economical choices for mass production and routine clinical usage of patient-specific therapies (72). If this is the case, iPSCs may be able to address some of the ethical issues about unequal access to medical interventions based on wealth. In general, producing a research-grade iPSC line costs between $10,000 and $25,000. From patient recruiting to final characterization, the entire procedure takes 6 to 9 months, with another 3 to 6 months required to develop large scale iPSC derivatives(70). According to previously published data, creating a clinical grade iPSC line cost around $800,000(32).

8.6 Challenges Pertaining to Pluripotent Stem Cells and Intellectual Property

Even though the first attempts to manufacture ESCs were patented, it has been questioned whether patents that entail the killing of human embryos for ESC creation should be allowed. Patent obstacles may be imposed on iPSC technology in the future. Some patent groups are opposed to the assumption that ESCs and iPSCs are the same entity and so have the same patent process(67). The EU Biopatent Directive (Directive on the Legal Protection of Biotechnological Inventions) was designed to provide standardized patent protection for biotechnological inventions throughout the European Union (73). However, patent filings for induced pluripotent stem cell (iPSC) methods have surpassed those for other stem cell technologies, raising fears that overlapping intellectual property rights would stymie research and innovation (74). Intellectual property (IP) rights are at the heart of the commercialization process, acting as a powerful motivator to unlock the therapeutic potential of technology(73).

8.7 Standardizing Regulatory Pathways for Stem Cell Lines

Investigators must submit their new stem cell–derived therapy portfolio to regulatory agencies for independent review and approval once all preclinical and commercialization

challenges have been addressed. The purpose of these agencies is to examine and oversee stem cell–based clinical trials to assure their safety and efficacy, and research submitted to the Food and Drug Administration (FDA) must exhibit scientific value and credibility in order to do so (70). Despite a vast interest and financial expenditures, only a handful regenerative medicines have been authorized by the FDA. Most of them have been stem cell therapies produced from umbilical cords that have been utilized to treat blood malignancies and other immune-related disorders (75).

9. Conclusion

Pluripotent stem cells are of particular interest due to their ability to differentiate into a wide spectrum of cell types in situations where functional adult stem cells are unavailable. Induced pluripotent stem cells are a promising alternative to pluripotent embryonic cells, eliminating

the ethical concerns that come with their usage while also providing a better model for researching human diseases and maybe developing more effective therapeutics. Despite the advances made thus far, more intense research on the features of human iPSCs is required to both understand the basic biology of pluripotency and cellular differentiation and to address all the challenges related to therapeutic applications. The use of iPS techniques has ushered in a new age in stem cell research, with exciting prospects for patient-specific pluripotent cell-based regenerative medicine.

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12. Biography

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