Review

Stem Cells: A Primer

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Abstract

Having great therapeutic and biotechnological potential, stem cells are extending the frontier in medicine. Not only replace dysfunctional or damaged cells, the so–called regenerative medicine, stem cells may also offer us new perspectives regarding the nature of aging and cancer. This review will cover some basics of stem cells, their current development, and possible applications in medicine. Meanwhile, important remaining challenges of stem cell research are discussed as well.

Key Words: stem cells, embryonic stem cells, regenerative medicine

Introduction

One of the major goals of medicine has always been overcoming the disabling consequences of tissue and organ loss. Parkinson's Disease, chronic heart failure, spinal injuries, liver failures and diabetes mellitus are just a few examples of many disabling diseases for which no effective treatments have been developed to replace what the diseases have destroyed. Today, science has advanced to a point where immunal responses can be controlled. Organs or tissues can be transferred from one to another to replace a diseased organ. Synthetic material implants are also employed for similar purposes. Despite the achievement of transplantation medicine, organ transplantation and artificial implants alone cannot meet the increasing need for tissue replacement. Donor organ shortage, the limited lifespan of artificial implants, and the difficulties to develop a feasible transplant method (for example, the human brain) address the importance to develop new approaches (25). However, the stem cell is universally recognized as one of the promises for these devastating situations upon its discovery.

What Is a Stem Cell?

Stem cells are a group of immature cells that are capable of prolonged self-renewal. The cells may divide to produce nearly identical copies of themselves for long periods without differentiating (100). Different from most other mature cells, such as brain, cardiac, and renal cells that are specialized in performing specific functions and have limited capability of dividing, stem cells remain uncommitted and can develop into specialized cell types or all cells of the body under proper conditions (42, 76), and thus they have great potential for tissue regeneration.

Differentiation Potential of Stem Cells

Many terms were used to describe stem cells based on their proliferation and differentiation potential. Totipotent stem cells originate from a fertilized egg. The cells have the potential of dividing and differentiating into a placenta and an embryo. Pluripotent stem cells can differentiate into any cells derived from three embryonic layers–mesoderm, endoderm, and ectoderm. These three layers can give rises to all kinds of specialized cells in body tissues and organs (Fig. 1). Multipotent stem cells are progenitors of a closely related lineage of cells. The cells continuously renew the cell populations in tissues such as liver, intestine tract and skin.

Origins of Stem Cells

Stem cells can also be classified according to their origins.

Embryonic stem cells (ES cells) are usually pluripotent stem cells derived from blastocytes. Blastocytes are the cells in the inner cell mass of an

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Table 1. Adult human stem cells and their primary direction of differentiation.

Cell Type	Tissue-Specific Location	Cells or Tissues Produced
Hematopoietic stem cells	Bone marrow, peripheral blood	Bone marrow and blood lympho-hematopoietic cells
Mesenchymal stem cells	Bone marrow, peripheral blood, umbilical cord blood, amniotic fluid	Bone, cartilage, tendon, adipose tissue, muscle, marrow stroma
Neural stem cells	Ependymal cells, astrocytes (subventricular zone) of the central nervous system	Neurons, astrocytes, oligodendrocytes
Hepatic stem cells	In or near the terminal bile ductules (canals of Hering)	Oval cells that subsequently generate hepatocytes
Skeletal-muscle stem cells or satellite cells	Muscle fibers	Skeletal muscle fibers
Stem cells of the skin	Basal layer of the epidermis, bulge zone of the hair follicles	Epidermis, hair follicles
Epithelial stem cells of the lung	Tracheal basal and mucus-secreting cells, bronchiolar Clara cells, alveolar type II pneumocyte	Mucous and ciliated cells, type I and II pneumocytes
Stem cells of the intestinal epithelium enteroendocrine cells	Epithelial cells located around the base of each crypt	Paneth's cells, brush-border enterocytes, mucus-secreting goblet cells,

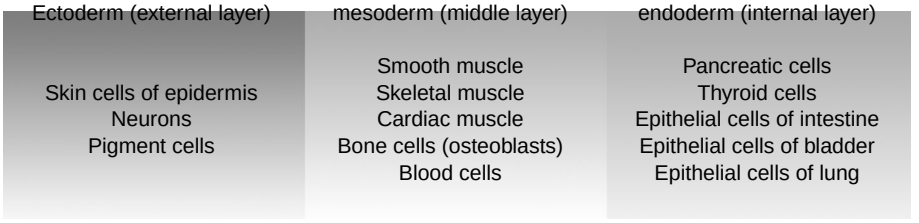

Fig. 1. The three embryonic layers–mesoderm, endoderm, and ectoderm–and their associated tissue types.

embryo before embryo implantation into a womb.

Adult stem cells are found in specialized tissues. Table 1 includes some examples. They are mostly multipotent and capable of self-renewal for the lifetime of an organism.

Cancer stem cells emerging from malignant transformation of adult stem cells or abnormal acquisition of unconstrained self-renewal by mature cells are now proposed to be the sources of tumors and tumor metastasis (18). They are also referred as tumor/cancer initiating cells and are isolated from leukemia, breast cancer, brain tumor, and colon cancer (1, 16, 32, 63, 79).

Induced pluripotent stem cells (iPS cells) are artificially created from a non-pluripotent cell, typically an adult somatic cell, by overexpressing certain combinations of proteins. iPS cells exhibit the essential properties of ES cells with respect to their morphology, cell-surface markers, gene-expression profiles and telomerase activity. Moreover, iPS cell clones can be maintained in culture at least for several months and can differentiate into derivatives of three embryonic germ layers both *in vitro* and *in vivo* (60, 85, 101).

Stem Cell Research on Medicine

Adult Stem Cells

The capacity of certain tissues in adults, such as bone, liver, and hematopoietic system to renew or to repair implies the existence of progenitor cells. The use of progenitor cells taken from adult patients may provide an easier route of cell replacement therapies. Many patients suffering from hematological disorders and undergoing extensive chemotherapy or radiotherapy have received bone marrow or cord blood stem cell transplantation for better recovery (17). Adult stem

cell derivatives transplantation is the only stem cell therapy in clinical practice so far. Recent studies have discovered the presence of intrinsic stem cells in more adult tissues that were once assumed that they did not have endogenous stem cells for self-regeneration when damaged (4, 20, 69). However, accessibility of adult stem cells poses several limitations. Particular adult stem cells are hard to retrieve from living donors. Moreover, adult stem cells cannot divide indefinitely *in vitro* (91). Thus, isolating and purifying enough amounts for clinical use is a major technical obstacle. Other concerns include the numbers and the potency of adult stem cells, which can decline with aging or be afflicted by undesirable *in vivo* environments (56, 83).

The inability of adult mammalian stem cells to differentiate across the lineage boundaries has been challenged by series of papers proposing that bone marrow cells containing hematopoietic stem cells (HSCs), which normally function to replenish mature blood cells, seem to contribute to non-hematopoietic tissues (reviewed in reference no. 29). After bone marrow cells are transferred into usually lethally irradiated recipients, expressions of donor genetic markers can be found within the skin, intestinal epithelium, liver parenchyma, pancreas, skeletal muscle, endothelium, myocardium and CNS neurons (10, 14, 24, 35, 36, 42, 46, 86, 90). Such data are expanded by some to a hypothesis that mammalian adult stem cells under certain conditions, can switch from their default pathway of differentiation and cross the lineage restriction.

Confusingly, failures to reproduce bone marrow cells or HSCs contribution to non-hematopoietic tissues in comparable experiment settings are also described (15, 68, 89, 92). Possible explanations for apparent stem cell lineage conversion, including fusions of transplanted cells and recipient cells, the de-differentiation of multipotent stem cells, and the existence of pluripotent stem cell in bone marrow, are even proposed (reviewed in reference no. 29). The issue about the authenticity of stem cell trans-differentiation has been constantly controversial and remains to be elucidated.

Embryonic Stem Cells

Many applications have been proposed for human ES cells. The most known is their potential in transplant therapy, that is, to repair or replace damaged tissues with human ES cells. Human ES derived cells are favorable for the transplantation purpose for their two characters: superior proliferation capacity to adult stem cells *in vitro* and differentiation into any somatic cell types (2, 76). Therefore, human ES cells conceptually can be an important source for cell replacement therapy. Studies have shown the differentiation of human ES cells to neural cell types (neurons, oligodendrocytes, and glia), cardiomyocytes, cells with pancreatic beta cell-like phenotype, hematopoietic progenitors, and hepatic cells. This has revealed the great possibility of this series of work (21, 27, 40, 41, 102).

Still, it is well-known that mammalian ES cells form teratomas, typically contain a mixture of various differentiated or partly differentiated cell types, when engrafted into an immune-deficient host. Preventing formation of teratoma and other unwanted cell types is also a significant task in replacement therapy. Although the risk of formation undesired cell type can be lessened by introducing fewer undifferentiated pluripotent cells (7).

Even so, the possibility of triggering immune rejection stays to be a concern. The possibility of triggering the immune rejection is inversely related to the similarities of the major histocompatability complex (MHC) antigen between the host and the graft. Two strategies have been adopted to bypass immunal reactions. First, somatic cell nuclear transfer (SCNT), also known as therapeutic cloning, can create autologous (derived from the same individual) ES cells for a specific patient. In SCNT, a nucleus of a donor somatic cell is fused with an enucleated oocyte. The somatic cell nucleus is reprogrammed and the fusion may probably develop into an embryo (11, 12, 97). Donor-specific ES cells, with identical MHC antigen expression to the original, can be then retrieved from the embryo, and therefore avoid immunal reactions from the donor.

However, this technique is not unflawed. SCNT requires reprogramming of genetic expression of the somatic cell nucleus to a state consistent with embryonic development, but the reprogramming is usually incomplete. The embryos produced using SCNT show widespread errors in epigenetic modifications (34, 53, 78) which are vital to regulation of gene expression. Consequently, it is not surprising that some genes express abnormally in cells derived from “clone” embryos. Interestingly, cloned embryos and naturally fertilized control share similar global gene expressions (82), and ES cells created from SCNT can yield normal embryos with high efficiency (31, 38, 59, 93). Assuming it proves feasible to derive human ES cells through SCNT, there are grounds for the belief that they will develop normally and have great therapeutic potential. Regrettably, therapeutic cloning in human is only theoretical. Validated successes are still lacking so far.

Nonetheless, Shinya Yamanaka has enlightened a reproducible route to create patient-specific pluripotent stem cells. Generated iPS cells, whose properties are similar to those of ES cells (60, 85, 101), from somatic cells of a patient with correcting defected genes if necessary, and these cells, in theory, can differentiate into patient-specific cell types, avoiding the risk of immunal rejection. In addition to creating patient-

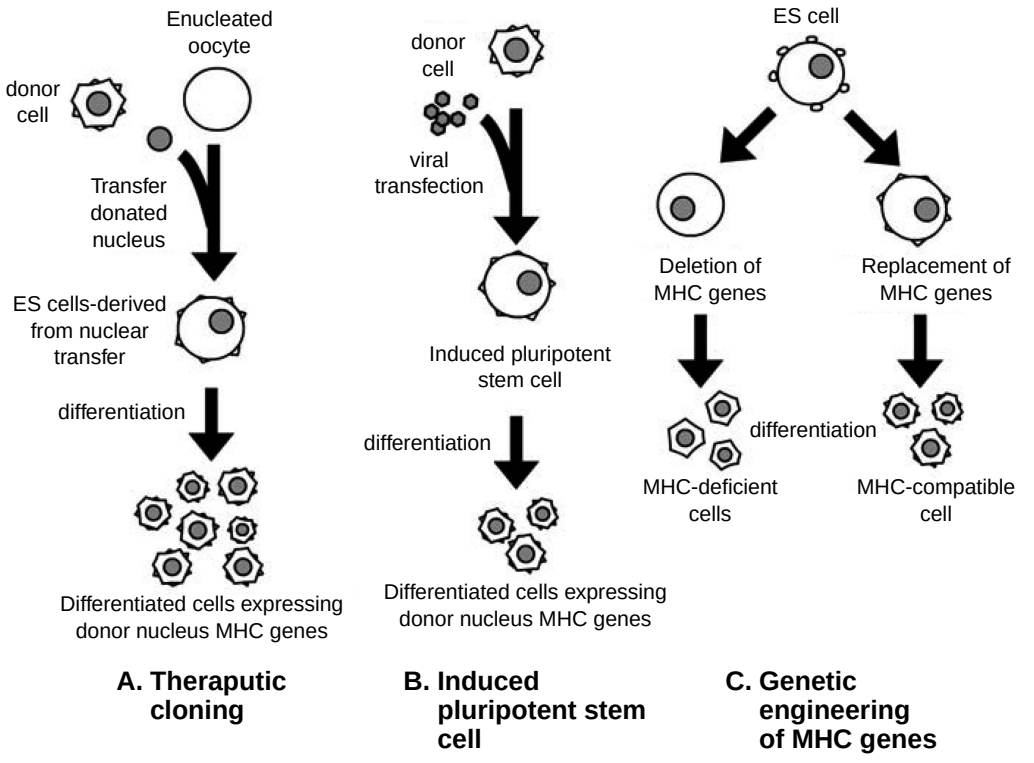

Fig. 2. Methods to reduce the immunogencity of stem cells.

specific stem cells, evasion from immune rejections can be achieved through substituting or deleting the MHC antigens. (See Fig. 2 in reference no. 64 for summary)

Fields of Stem Cell Research

Transplantation of stem cells or their derivatives, and recruiting of endogenous stem cells within a specific organ have been suggested as major blueprints for the regenerative medicine. The first design in the adult stem cell framework undergoes selection of stem cells or progenitor cells, with or without expansion, and then transplantation back into patients. Alternatively, in the ES cell model, *in vitro* expansion, directed differentiation into suitable cell types, and cell transplantation back into patients are subsequently performed. Recruiting preexisting stem cells or progenitor cells employs various cytokines to boost the proliferation and differentiation of specific types of stem cell. This strategy has been widely adopted in treating certain kinds of hematological disorders like anemia and neutropenia (17).

Type I Diabetes Mellitus

The pathogenesis of diabetes mellitus type I involves the destruction of the pancreatic beta cells and a causative absolute insulin deficiency. The destruction usually results from an autoimmunal reaction against the pancreatic beta cells. The immunological character casts a problem on transplantation of stem cell-derived beta cells to cure type I diabetes. Since autologous or MHC-matched stem cell-derived pancreatic graft displays similar surface molecules–MHC antigens–to the host beta cells, the autoimmunity may destroy the newly grown insulin-secreting cells from the graft. Meanwhile, host immunity may also wipe out the allogeneic graft expressing non-self markers. Without considering immunological factors, the ideal regime cannot be achieved.

The approach of current trials incorporates immunity attenuation with beta cell regeneration. After the transplantation of exogenous mesenchymal cell to induce chimerism (the state of an individual containing a mixture of genetically different cells), the donor immunoregulatory cells may prevent the auto-reactivity of host cells against transplants or newly generated pancreatic beta cells (30, 103). Under controlled conditions, the mesenchymal stem cells can differentiate into insulin expressing cells *in vitro* (37). Thus, the transplantation of mesenchymal cell combined with appropriate chemical signals to direct differentiation not only prevents immunal destruction of islets but also can correct the insulin insufficient state.

Parkinson's Disease

The principal pathological change of Parkinson's Disease is a loss of dopamine-secreting neurons in the substantia nigra. The lack of dopamine is responsible for most disabilities of Parkinson's Disease. Today, the dopamine precursor–levodopa–or dopamine receptor agonists are orally administered to substitute neuron-released dopamine. Dopamine-replacement, though, is effective to relieve symptoms in the early stage, but the long-term outcome is usually associated with poor responses and complications, such as motor fluctuations and dyskinesias (aberrant involuntary movements and postures). Among other attempts to avoid the disability, obviously, to restore the dopaminergic neurons and their nerve fibers from the substantia nigra to the striatum is one of the possible curative treatments.

Laboratory studies have validated that implanted fetal dopamine neurons can survive, secrete dopamine, integrate with the striatum, and relieve Parkinsonian symptoms in rodent and primate models where the dopaminergic neurons were destroyed by toxin (67). Early human trials of dopaminergic cell transplantation also generally show good results (67, 73). However, the results of two large-scale, double-blind, placebo-controlled trials on fetal nigral transplantation only show modest improvements. The transplantation leads to long-lasting, significant improvement only in partial patients (22, 66). Some patients even develop dyskinesia, which is related to uneven distribution of graft neurons (26, 51).

The huge variation in the results of the two trials as well as the tissue availability makes it impractical and unacceptable to exploit human fetal neural tissue as a regular source for cell transplantation. Stem cells may enable the production of large numbers of dopaminergic neurons with relatively consistent properties compared to human fetal neural tissues. Figure 3 illustrates how dopaminergic neurons can be harvested from different sources of stem cell.

Until now, adult stem cells theoretically provide autologous dopaminergic neurons from trans-differentiated mesenchymal stem cells (37). Nevertheless, no convincing evidence supports that adult stem cells may efficiently trans-differentiate into dopaminergic neurons and supply sufficient cells for transplantation so far. On the other hand, in preliminary animal models, implanted dopaminergic neurons reinnervate the host striatum and reduce Parkinsonian symptoms (7). Meanwhile human ES cells have been successfully manipulated into dopaminergic neurons (70); hences human ES cells are considered as one of the most promising candidates for cell transplantation. Recently, human ES cell-derived dopaminergic neurons are reported to be partially functional and can induce behavioral improvements in Parkinsonian rats without any teratoma formation (5). In summary, long-term survival of transplanted, integrated, evenly distributed, and functional neurons are the chief goals to optimize the stem cell therapeutic effects.

Myocardial Infarction and Heart Failure

Throughout the myocardium, the newly discovered pools of mammalian cardiac progenitor cells (CPCs) can renew the myocardium by differentiating into muscle, conduction and vascular cells (4, 13). Cardiac cells expressing early transcription factors, such as isl1 (47), GATA4 (55), and Nkx 2.5 (39) may have characteristics of myogenic stem/progenitor cells. Cell marker proteins such as Kit, Sca1 and MDR1 (74, 88) have been used to extract CPCs from the heart albeit definitive markers for CPCs are lacking. Many reports have now illustrated that CPCs have a strong proliferation and differentiation potential *in vitro* (4, 13, 54, 65), but no clinical data using such cells for cardiac cell therapy are available yet. Despite the existence and the regenerative capacity of CPCs, it is generally accepted that endogenous regeneration of human myocardium may not replace the massive loss of cardiomyocytes brought by myocardial infractions or other myocardial diseases. The huge contrast between the inadequate compensation of myocardium and the robust differentiation and proliferation ability of CPCs *in vitro* raises interests to elaborate the barriers preventing regeneration, including ischemia, inflammation, and fibrosis. Adequate inflammatory signals may promote angiogenesis and recruitment of progenitor cells, while superfluous inflammatory responses may prevent the activation, recruitment, differentiation, and survival of progenitor cells. Likewise, fibrosis may partially maintain the mechanical support of myocardium, but excessive fibrosis lays a physical barrier to any cells to repair damaged sites. Initial attempts to ameliorate the hostile microenvironment, including increased vascularization and reduced fibrosis, seem to be beneficial to cardiac regenerative capacity (61).

In contrast to vastly unexplored wilderness in the field of CPC, the only myocytes or myocyte progenitors that have been studied clinically so far are isolated skeletal myoblasts. Myoblasts injected can into ischemic myocardium differentiate into myotubes improve ventricular function in animal models. However, the clinical application of skeletal myoblasts is limited, because the formed myotubes do not fully integrate electrically with remaining cardiomyocytes, and may therefore induce life-threatening arrhythmia (heart rhythm abnormalities) (48).

Multipotent adult stem cells are thought to be another route to improve cardiac function on the basis of early studies indicating that bone-marrow originated mesenchymal stem cells can differentiate

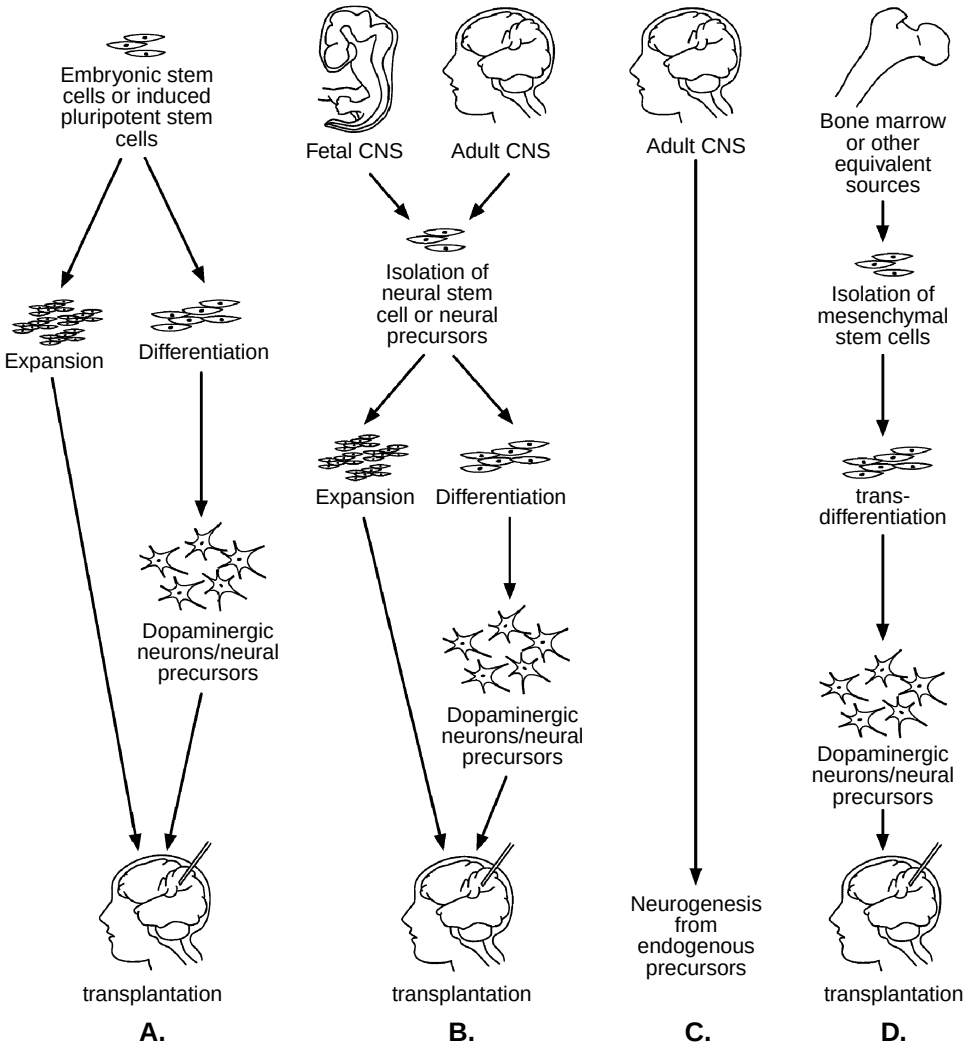

Fig. 3. Possible pathways of stem cell therapies for Parkinson's disease (A) Embryonic stem cells (or other equivalent stem cells) are inducted to differentiate before transplantation into dopaminergic neurons or neural precursors, or are directly transplanted after expansion. (B) Neural stem cells or neural precursors may be isolated from fetal brain or sub-ventricular zone of adult brain. Isolated cell can be expanded and differentiated to dopaminergic neurons or more differentiated neural precursors for transplantation. The alternative method can be direct transplantation after expansion of isolated cells. (C) Endogenous neural precursors may divide and differentiate into dopmainergic neurons under proper stimulations. (E) Stem cells form other tissues may be trans-differentiated into neural lineage. Cells expressing dopaminergic markers and with partial neuronal properties have been generated from adult mesenchymal stem cells in mouse models. CNS, central nervous system.

into cardiomyocytes (87). Subsequent clinical trials on transplantation of multipotent adult stem cells from other solid organs and bone marrow tried to prove their potential to repair myocardium after myocardial infarction (6, 77, 87). However, subsequent investigations show inconsistent therapeutic benefits (84, 98) and the original results that multipotent adult stem cells trans-differentiate into cardiomyocytes may be artifacts (3, 58, 62). The consensus now is that the improvement in cardiac function after bone marrow cell transplantation results from salvage of ischemic cardiomyocytes predominantly through paracrine effects of transplanted cells (23).

Human ES cells remain a promising approach to repair myocardial infarction even with the debates of trans-differentiation of adult stem cells. Derived from the inner cell mass of an embryo before implant, human ES cells have better capacity for self-renewal and indubitable potential to give rise to cardiac cells and the protocols for ES cell derivation and differentiation into cardiomyocytes are well established. *In vitro* studies, ES cells not only successfully differentiate into cardiomyocytes but also create connections with pre-existing cardiomyocytes allowing synchronized pulses. Human ES cell-derived cardiomyocytes have shown structural, contractile, and electro-physiological

properties of an adult myocardium (41, 57, 99).

In addition to these similarities to their adult counterparts, the human ES cell-derived cardiomyocytes can survive, proliferate, and mature both *in vitro* and after *in vivo* transplantation into a mouse heart in contrast to the greatly limited proliferative ability of adult cardiomyocytes (44, 45, 49, 80, 99). This unique feature implies that fewer cells may be required to reach optimal therapeutic effects and gradual expansion of the graft which may allow angiogenesis to support new cardiomyocytes. In mice which received human ES cell-derived cardiomyocytes, cardiac function improved at 4th weeks after transplantation as compared with those receiving non-cardiomyocyte derivatives (44, 49), though long-term outcomes need to be confirmed on animal models with slower heart rates, which are much closer to the intrinsic beating frequency of human cardiomyocytes.

Hematopoietic Malignancy

The HSC is the common progenitor of all blood cells. Mature blood cells are replenished continuously by more-primitive precursors that are differentiated from HSCs. Normally, the HSC is the lifetime source of blood cells, but when mutations malignantly transform HSCs or progenitor blood cells into cancerous stem cells, these cancerous stem cells produce all forms of leukemic cells, *i.e.*, abnormal blood cells (16, 32).

Although chemotherapy and radiotherapy can eliminate most cancerous cells, fractional cancerous stem cells still can survive allowing the cancer to relapse. Leukemic stem cells are highly refractory not only to chemotherapy because they are dormant and excrete toxic drugs but also refractory to radiotherapy because they repair DNA efficiently, and resist apoptosis (19). Nevertheless, HSC transplantation holds the key to eradicate the cancer.

Allogeneic grafts of HSCs can trigger immunal responses if the immune profiles of the recipient and the donor are different. T cells can determine the difference and launch immunal responses by their binding to an antigen. Recipient T cells can identify the foreign donor cells and can reject the graft, while donor T cells can identify recipient antigens and cause graft-versus-cancer effects and/or graft-versus-host disease. The severity of the responses depends on the degree of the difference. Major difference (unmatched major histocompatability complex antigens) between the host and the graft may invoke lethal graft-versus-host disease. In this disease, the donor immune system attacks the recipient organs and probably causes organ failures. In contrast, minor difference (unmatched minor histocompatability complex antigens) can initiate beneficial graft-versus-cancer effects (9, 75). Donor T cells recognize recipient minor histocompatability complex antigens on leukemic cells and cancerous stem cells. Consequently, both leukemic cells and cancerous stem cells can be eliminated by donor immune system (8). Through the graft-versus-leukemia effects, appropriate allogeneic grafts of HSCs reduce the recurrence rate (33).

Genetic Disorders

The fundamental concept of gene therapy is that transfer of genetic material or entire genome will lead to a phenotype modification for therapeutic purposes. Theoretically, the most straightforward strategy is to correct or compensate for the defected gene expression by altering the genotype. Alternatively, the gene therapy can be designed to generate normal cells or organs, in the case of stem cell therapy, by manipulating wild-type or genetically corrected stem cells.

Bone marrow stem cell transplantation has been used to treat various inherited diseases, including hemoglobuniopathy, immunodeficiency, leukodystrophies, and lysosomal storage diseases, although bone marrow transplantation bears the risk of the probability to develop graft-versus-host disease and the requirement for immunosuppression of the host (43, 50, 52, 71, 72, 81, 94-96). Another potential application of stem cells for hereditary disease is transplantations of Pluripotent stem cell-derived cells or tissues. Pluripotent stem cells with are differentiated *in vitro* into specialized cells or their progenitors and then transplanted *in vivo* to replace the diseased organs or cells. The threat of the immunorejection of the transplanted cells by the host can be evaded through autologous cells with transfer relevant wild-type gene to host-specific pluripotent cells, which can be ES cells created from therapeutic cloning or iPS. Recently, Jacob Hanna and his colleagues (28) provided a prefatory mouse model of iPS-cell-based treatment in combination with repair of sickle-cell anemia genetic defects.

Crucial Issues and Challenges

Accumulated genetic mutation and aging can make stem cells suboptimal to continue research. Scientists are unable to collect sufficient stem cells from slowly growing cell lines. ES cells may be subjected to epigenetic changes with prolonged culture, affecting their phenotypes. Noteworthily, genetic mutations may evolve into malignant transformation of stem cells. That is, recipients who are transplanted with mutant stem cells may develop cancer. Additionally, human embryonic stem cells lines acquired an immunogenic, non-human sialic-acid residue after long-term culture on mouse feeder cells (the previous-used stem cell culture method). The contaminated cells can trigger unwanted immune responses in human body.

Apart from the mutations, epigenetic abnormality, and contaminations of stem cells, there are many technical challenges to overcome to realize the promise of stem cells. The primary biology of stem cells has set the background for better differentiation and expansion of stem cells. Further inquiries involve signals that direct differentiation, *e.g.*, cytokine stimulations and associated environment that stem cells are exposed to. It is also important to investigate into identification of the apposite cell, whether it is a differentiated progenitor or a more primitive stem cell, from tissues or culture colonies.

Beyond basic science, before stem cell therapy is put into clinical practice, new concerns rise. First, since the quality and the quantity demand of stem cell-derived cells can be satisfied with enough knowledge to differentiate the appropriate cell types and identify appropriate cells, a transplant protocol that provides optimal graft survival without uncontrolled cell overgrowth. Which kind of cell should be used? By what route the engraftment will be more successful? The answers vary case by case; still, these issues are unexplored.

Second, adult stem cell trans-differentiation or therapeutic cloning can reverse the differentiated state of the cell and direct it into desirable tissues, whereas the derived tissues inherit the genetic defects that the cell carries. Defects can be congenital, acquired during the aging process, or mutant caused by the environment, but all of them impair the therapeutic effects of stem cell transplantation. To solve this problem requires the incorporation of gene therapy to repair the microscopic genetic defects.

Lastly, the assurance that stem cell transplantation is not associated with teratoma, undesired cell types, malignant transform of transplanted stem cells or other undesirable side effects should be obtained. The unbridled differentiation and proliferation can be circumvented by transfer of a suicide gene to stem cells. A suicide gene encodes a protein that can convert a non-toxic prodrug to a cytotoxic compound and thus confer selective and specific eradication of cells carrying a suicide gene when cooperated with prodrug. A further solution is transplantation of more purified and terminal-differentiated cells. Ultimately, the goal largely requires long-term clinical practice and follow-up to fine-tune the transplantation therapy.

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