Chapter

# Innovations in Human Stem Cell Research: A Holy Grail for Regenerative Medicine

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## Abstract

Stem cells are unspecialized cells capable of renewing themselves and giving rise to differentiated and specialized cell subtypes. There are two general categories of stem cells, i.e., pluripotent stem cells capable of differentiation into any cell type in the human body and multipotent adult stem cells maintaining tissue homeostasis in postnatal life. Investigations in both these categories of stem cells have expanded our knowledge on human organogenesis and tissue regeneration and have suggested potential therapeutic functions of stem cells in regenerative medicine. The advent of induced pluripotent stem cell (iPSC) technology a decade ago further revolutionized stem cell biology and has given rise to the translation of stem cell-based therapies. This chapter will summarize some of the exciting progress and challenges in the applications of iPSC-derived stem cells and adult stem cells and the potential of translational and clinical research of these stem cells in regenerative medicine.

**Keywords:** stem cells, regenerative therapy, clinical studies, iPSCs, cord blood, adult stem cells

## 1. Introduction

Stem cells are a unique subset of cells that possess the biological properties of self-renewal and differentiation. The term "Stammzelle" (German for stem cell) was first used by a German biologist Ernst Haeckel in 1868 regarding unicellular organisms as the phylogenetic ancestors of multicellular organisms [1]. In 1877, Haeckel further applied the notion of stem cells to describe the fertilized egg cell as the cell of origin for all the cells in an organism [2].

Indeed, there are stem cells of different potency that not only form the foundation of any organisms but also throughout the life of the organism maintain tissue homeostasis. Following egg fertilization with a sperm, a zygote is generated (**Figure 1A**). This single-cell zygote and the cells that immediately arise in the first few divisions are totipotent stem cells, as they are capable of becoming a whole embryo including the extra-embryonic tissue, i.e., placenta. After about 5 days of human embryonic development, the zygote develops into a blastocyst. The preimplantation blastocyst consists of a mostly hollow ball of cells, with the outer cell layer as trophoblast that develops into the placenta and the inner cell mass (ICM) that is pluripotent and gives rise to all cell types in the body. These pluripotent stem

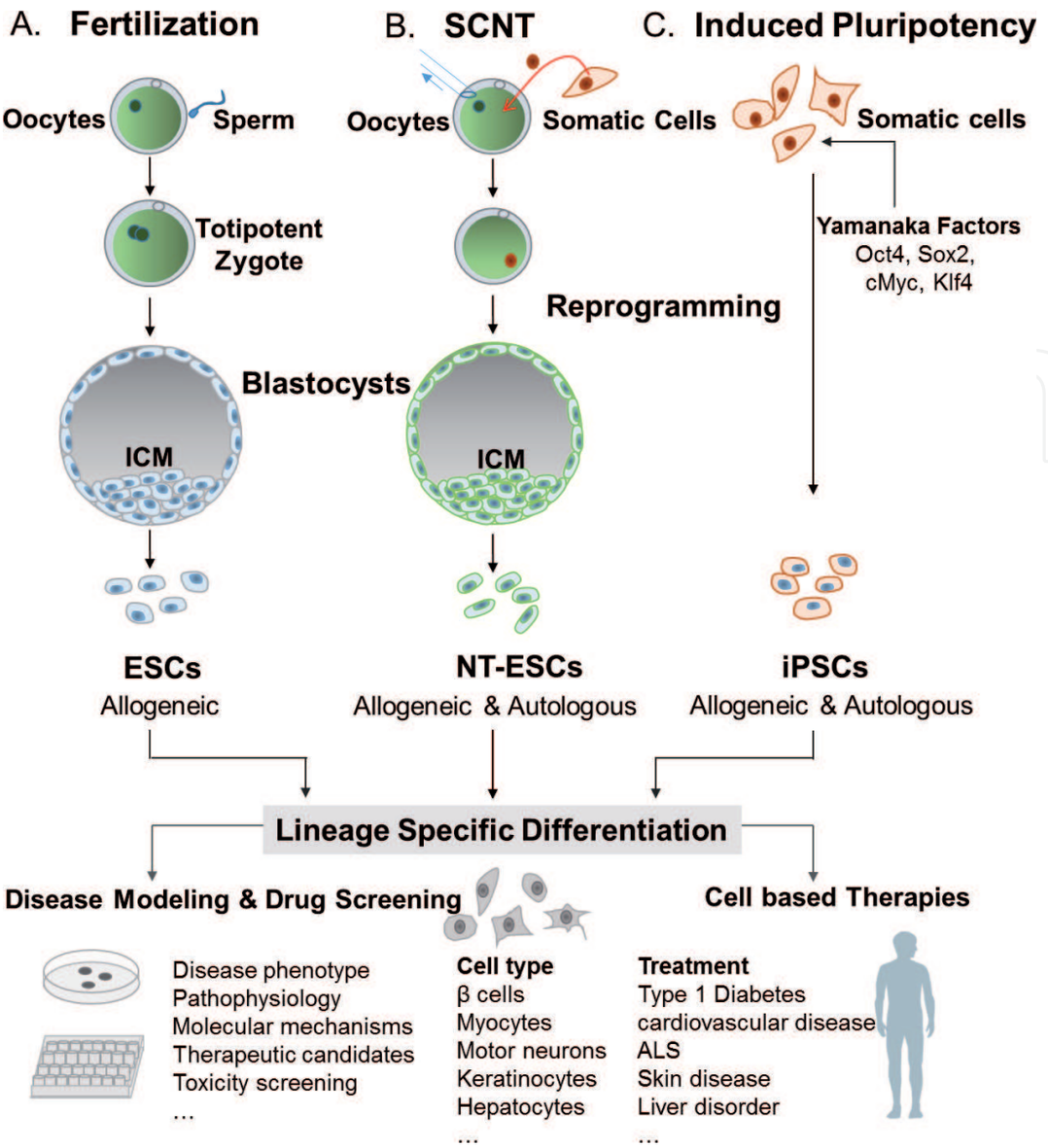


**Figure 1.**
*Derivation of pluripotent stem cells (PSCs) including embryonic stem cells (ESCs) from fertilization of oocytes and sperm (A), nuclear transfer ESCs (NT-ESCs) from somatic cell nuclear transfer (SCNT) of somatic cell nuclei into denucleated oocytes (B), and induced pluripotent stem cells from forced expression of Yamanaka transcription factors in somatic cells (C). All the three types of PSCs can be propagated extensively in vitro and undergo directed differentiation into any cell type of the body, which can be utilized in disease modeling and drug screening and developed as cell-based therapies. As generation of ESCs involve disruption of the embryos, they can only be used as an allogenic source, while NT-ESCs and iPSCs can be developed in both allogenic and autologous settings.*

cells (PSCs) in the ICM are the source for embryonic stem (ES) cells [3]. Apart from PSCs, there are tissue-specific stem cells residing in several organs such as the skin, gut, blood, and brain, also referred to as "adult" or "somatic" stem cells with self-renewal and multipotent differentiation capacity that are responsible for most regenerative activities throughout the life of the organism.

## 2. Pluripotent stem cells (PSCs)

### 2.1 ES cells

The culture of ES cells from mouse blastocysts was first reported by Evans et al. in 1981 and herein opened a new era of research in gene targeting to model human

diseases [3]. Based on this technology, Martin Evans, Mario Capecchi, and Oliver Smithies shared the 2007 Nobel Prize for their discoveries of principles for introducing specific gene modifications in mice by the use of ES cells. In 1998, Thomson and collaborators reported the derivation of ES cell lines from human blastocysts produced by in vitro fertilization (IVF) [4]. As these human ES cells are immortal and can give rise to all cell types in the body, they are invaluable tools to dissect the molecular and genetic events in human development and also allow drug testing in a wide range of diseases (**Figure 1**). Moreover, human ES cells provide an unlimited source to derive cells and tissues that could be used as cell-based therapies to treat degenerative diseases such as heart disease and type 1 diabetes (**Figure 1**). Indeed, detailed differentiation protocols have been developed to derive specific mature cells from human ES cells, for example, dopaminergic neurons, cardiomyocytes, β cells, and keratinocytes as therapies for Parkinson's disease, myocardial infarction, type I diabetes, and skin disease, respectively. However, as the derivation of ES cells involves destruction of human embryos, even though the embryos were produced by IVF and would otherwise be discarded, it nevertheless has raised ethical concerns and elicited controversial debates on the use of human ES cells [5]. Another hurdle for the therapeutic use of ES-derived cells is that they can only be used as allogeneic cells; thus they are subject to immune-based rejection in their recipients [6].

### 2.2 PSCs derived from reprogramming

The 2012 Nobel Prize in Physiology or Medicine was awarded to two scientists, Sir John B. Gurdon and Shinya Yamanaka, PhD, whose stem cell research was separated by over four decades but both demonstrated that the developmental clock of a specialized cell can be turned back to an immature (pluripotent) state. In John B. Gurdon's classic experiment in 1962, he replaced the nucleus in an egg cell of a frog with the nucleus from a mature intestinal cell [7]. The modified egg cell subsequently developed into a normal tadpole. Using the same principle of Gurdon's work which is known as somatic cell nuclear transfer (SCNT), the first mammalian clone "Dolly the Sheep" was born in 1996, following transfer of the nucleus from a mammary gland into an enucleated sheep egg [8]. Therefore, factors presented in mature, metaphase II-arrested oocyte cytoplasm are capable of reprogramming somatic cell nuclei to an undifferentiated state. This unique ability of oocytes has led to an important application of utilizing SCNT in therapeutic cloning to generate ES cells (ESCs) (NT-ESCs) for regenerative medicine (**Figure 1B**).

Forty-four years after Gurdon's discovery, Shinya Yamanaka took a different approach and transduced the differentiated mammalian cells, i.e., fibroblasts with a cocktail of reprogramming factors (Oct3/4, Sox2, Klf4, and c-Myc) using retroviruses (**Figure 1C**) [9]. About 30 days after transduction, induced pluripotent stem cells (iPSCs) with properties similar to ES cells were generated at an efficiency of ~0.02% [9]. After the initial discovery of Yamanaka's reprogramming techniques, several other reprogramming methods, such as transduction with nonintegrating viruses (adenovirus and Sendai virus) or transfection with protein, mRNA, Piggy Bac element, minicircle vectors, and episomal plasmids, have been developed to produce iPSCs without integration of exogenous genes to the genome of recipient cells (reviewed in [10]). iPSCs can also be generated from more easily accessible cell sources such as blood [11].

Therefore, the PSCs produced by SCNT (NT-ESCs) and forced expression of reprogramming transcription factors (iPSCs) are both alternatives to ESCs in regenerative medicine. Epigenetic and transcriptomic comparisons between

isogenic iPSCs and NT-ESCs have demonstrated that NT-ESCs more closely resemble bona fide ESCs derived from fertilized embryos [12, 13]. Moreover, a most important difference between iPSCs and NT-ESCs is the source of mitochondrial DNA (mtDNA). The mtDNA in NT-ESCs is of an oocyte germline origin, while in iPSCs is of a parental somatic origin. Due to the random nature of somatic mtDNA mutations, the frequency of mtDNA defects in iPSCs has been demonstrated to increase with the age of somatic cells [14]. Thus, NT-ESCs, carrying mutation-free mtDNA and closely resembling ESCs, represent an invaluable stem cell source for regenerative medicine. However, as the derivation of NT-ESCs requires donor oocytes, which are more technically challenging than iPSCs and are also subject to ethical and/or legal restrictions, the majority of current PSC research has been carried out using iPSCs.

### 2.3 iPSC disease modeling

These groundbreaking discoveries have revolutionized our understanding of stem cell development and created novel opportunities for human disease modeling and drug screening in "disease-in-a-dish" models (**Figure 1**) [15]. To date, significant progress has been made utilizing human iPSCs to model various neurological disorders, inherited heart diseases, and other genetic diseases such as Duchene muscular dystrophy and recessive dystrophic epidermolysis bullosa (RDEB) [16, 17]. Utilizing amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's disease as an example, ALS is a neurodegenerative disease that primarily affects corticospinal "upper" motor neurons (UMNs) and spinal cord "lower" motor neurons (LMNs), resulting in progressive muscle weakness [18]. In about 10% of patients with ALS, the disease runs in the family (familiar ALS) with mutations in around 20 genes including SOD1, TARDBP, FIS, and C9orf72 identified as common causes [18]. The remaining 90% of the patients are classified as sporadic ALS, with the causative mutations largely unidentified. As the iPSCs generated from ALS patients and differentiated into motor neurons carry the same genetic background as the patients, it represents a novel tool for studying disease pathology of ALS, particularly the sporadic form, which is not possible in the other model systems. A proof-of-principle study on derivation of iPSCs from an ALS patient and differentiation into LMNs was reported in 2008 [19]. Subsequently, Kiskinis et al. and Chen et al., respectively, established in vitro models of ALS by generating iPSC-derived LMNs from patients carrying different SOD1 mutations. Both studies recapitulated the spontaneous and progressive decrease in cell viability and ALS-related morphological changes including reduction in soma size and altered dendrites, which was linked to neurofilament aggregation [20, 21]. Chen et al. further demonstrated the pathological features of mutated SOD1 in patient-derived MNs, but not in non-MNs. Only in MNs, mutated SOD1 bound to the 3′UTR region of neurofilament (NF)-L mRNA resulted in neurofilament aggregation, restoring the expression of NF-L mitigated neurite degeneration of the ALS-iPSC-derived MNs. Meanwhile, Wainger et al. generated iPSC-derived LMNs from patients carrying SOD1, C9orf72, or FUS mutations [22]. All these ALS-iPSC-derived LMNs with distinct genetic mutations have recapitulated essential disease features and discovered common molecular pathways driving ALS pathogenesis, opening the possibility of new and effective drug screening [23]. However, challenges still remain for in vitro modeling for ALS using iPSCs [18]. Different protocols have been reported in deriving LMNs from iPSCs; thus, criteria need to be established to compare the MNs generated using different methods. Moreover, generation of UMNs from PSCs involves a series of steps and

is more challenging than that of LMNs [18]. The current protocols for deriving UMNs mostly resulted in heterogeneous, neocortical-like neurons that are immature and "stalled" at a stage resembling mid-embryonic differentiation in vivo [24]. Therefore, promoting subtype-specific differentiation and maturation will be crucial to an accurate ALS modeling. Indeed, the abilities of iPSC-derived cells to exhibit maturation and aging are crucial for accurate in vitro modeling of all the adult-onset diseases.

### 2.4 iPSC-based therapies

The most significant advantage of iPSCs lies in its application in cell-based therapies. iPSCs can be developed without destroying human embryos, therefore circumventing the ethical obstacles of utilizing and generating human ES cells. Being able to differentiate into all cell types in the body similar to ES cells, iPSCs theoretically provide an unlimited source of cells for autologous transplantation, eliminating the need for immunosuppression. Moreover, scientists have established robust directed differentiation protocols with sequential activation and inhibition of molecular differentiation pathways to generate a wide range of somatic cells from iPSCs, such as β cells and cardiomyocytes (**Figure 1**).

A challenge for the PSC or iPSC differentiation, as also mentioned above in the iPSC-derived MNs, is that the PSC-derived cells tend to be immature. This is indeed the major limitation for translating iPSC-derived red blood cells into the clinic [25]. In 2008, Lu et al. reported differentiation of human ES cells into functional oxygen-carrying erythrocytes on a large scale with up to 60% enucleation rate [26]. In comparison, differentiation of iPSCs along the erythroid lineage generated orthochromatic (nucleated) erythroblasts and reticulocytes. In most reports, the differentiated red blood cells express mainly fetal and embryonic globins, but very little adult-type (β-) globin [27]. This is likely due to the low level of erythroid Kruppel-like factor 1 (EKLF1) and absence of BCL11A in these iPSC-derived red blood cells. These two factors have been demonstrated to be essential for the developmental switch from fetal to adult globin expression [28]. Inducible expression of KLF1 during later stages of the differentiation process has been recently demonstrated to enhance differentiation and maturation of red blood cells from both human ES cells and iPSCs [29].

Recent advances in the development of programmable site-specific nucleases, including zinc-finger nuclease (ZFN), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein (Cas)9 system, have enabled target-specific introduction of transgene or correction of disease-specific mutations by homologous recombination, creating novel opportunities not only for disease modeling and drug testing but also generation of genetically corrected cells for autologous transplantation (**Figure 2**).

The development of iPSC technology has also revolutionized the future treatment for end-stage organ failure. Takebe et al. recently reported vascularized and functional mini-livers or liver buds created in vitro based on human iPSCs [30]. In this proof-of-concept demonstration, the authors first prepared hepatic endoderm cells from human iPSCs by directed differentiation. About 80% of the differentiated cells express liver-specific marker HNF4A. To recapitulate early organogenesis, the investigators next cultured the iPSC-derived hepatic endoderm cells with two stromal cell populations, i.e., human umbilical vein endothelial cells and human mesenchymal stem cells (MSCs), in a traditional two-dimensional culture condition. Intriguingly, the iPSC-derived hepatic cells

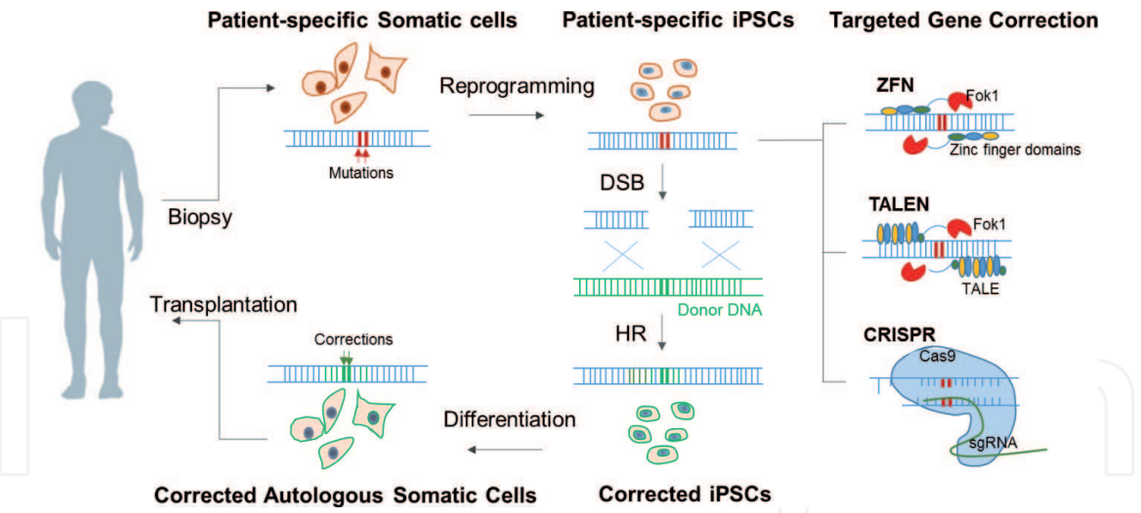


**Figure 2.**
*Development of iPSC gene correction and autologous transplantation therapy. Biopsies such as the skin or blood can be obtained from a patient with genetic mutations and reprogrammed into patient-specific iPSCs. Through targeted nuclease technologies, such as zinc-finger nuclease which contains sequence-specific DNA-binding domain fused to a non-specific* Fok1 *endonuclease enzyme, transcription activator-like effector proteins consisted of tandem DNA-binding repeats linked with the* Fok1 *enzyme, and the most recent method of CRISPR/Cas system that utilizes a single-guide RNA (sgRNA) and a protospacer adjacent motif for efficient genome targeting and binding followed by activity of the Cas enzyme, a double-stranded break (DSB) occurs at the target site of genome, inducing activation of internal DNA repair mechanism. Homologous recombination (HR) can then be achieved to incorporate exogenously transduced donor DNA to repair the mutations in the genome. After validation of the target-specific gene correction, the corrected iPSCs can be further differentiated into a cell type of preference for autologous transplantation.*

self-organized into three-dimensional cell clusters that resemble in vivo liver buds during embryonic development. Moreover, within 48 hours of transplantation into the nonobese diabetic/severe combined immunodeficiency disease (NOD/SCID) mice, the vasculatures in the iPSC-derived liver buds became functional by connecting to the host vessels, which further stimulated the maturation of iPSC-derived liver buds into the tissue resembling the adult liver. Considering critical shortage of donor organs, development of iPSC-derived organoids suggests an alternative and innovative regenerative approach for patients with end-stage organ failure.

New advances have also been made in cardiac tissue engineering for cardiovascular diseases. The protocols for differentiating ES cells or iPSCs into cardiomyocytes, smooth muscle cells, and endothelial cells that are the main functional cell types in the heart have been reported [31, 32]. However, although readily obtainable, the morphology, calcium handling, electric coupling, contraction stress, and electrophysiology of the PSC-derived cardiomyocytes have been demonstrated to be immature compared to adult cardiomyocytes (reviewed in [33]). With that, significant bioengineering efforts have been made to recapitulate environmental cues to enable maturation of newly differentiated cardiomyocytes and to promote vascular network formation (reviewed in [34]). To address the need for tissue and/or organ transplantation, there are also exciting advances in incorporating biocompatible materials, cells, and supporting components into complex 3D functional living tissues [35]. Excitingly, Noor et al. recently reported a 3D printing of thick, vascularized, and perfusable cardiac patches that fully match the immunological, biochemical, and anatomical properties of the patient [36]. This was the first report on the use of fully personalized, non-supplemented materials as bioink for 3D printing. In this study, fatty tissue was extracted from a patient. The cells from the tissue were reprogrammed to iPSCs, followed by directed differentiation into cardiomyocytes and endothelial cells. The remaining fatty tissue was decellularized and processed to generate a thermo-responsive hydrogel. The iPSC-derived cells

were then encapsulated with the hydrogel and served as the bioink for 3D printing of vascularized patches and complex cellularized structures. The investigators of this study also pointed out the obstacles that need to be overcome for a more applicable 3D printing, including efficient generation of a sufficient number of cells for the organ printing, identifying biochemical and physical cues for cell maturation and conditions for long-term cultivation and a higher-resolution imaging of the entire blood vessels for the blueprint of 3D printing, etc. Nevertheless, the results from these studies have shed light on developing autologous engineered tissue or organs for transplantation.

### 2.5 Xenogeneic generation of human organs using PSCs

With the growing knowledge of organogenesis and the aid of gene editing technologies, scientists are also pushing the boundary and creating interspecies chimeras to grow human organs in animals, which ideally could subsequently be transplanted into people. In 2017, Yamaguchi et al. demonstrated that injection of mouse PSCs in the blastocysts of apancreatic *Pdx1*$^{mu/mu}$ rats (TALEN-mediated disruption in *Pdx1* gene, a master regulator for pancreas development) which resulted in generation of a mouse pancreas in the rat [37]. Moreover, when the mouse pancreas grown in the rat was transplanted into diabetic mice, they were able to cure diabetes in the recipients without the administration of immunosuppression [37]. Wu et al. subsequently reported the creation of the first human-pig chimeras by injecting human PSCs into pig blastocysts [38]. The success rate of generating human-pig chimeras was indeed very low, and the chimeras only carried very few human cells, less than one human cell per 100,000 pig cells. To date, no one has reported using gene editing techniques performed in rat embryos to disable the pigs forming a particular organ and enable the human cells to develop more humanlike organs. Ross et al. demonstrated the generation of sheep-human hybrids, and as a step further than the reported human-pig chimeras, the contribution of human cells in sheep embryos was increased to one in 10,000 sheep cells [39]. Although these studies represent only a preliminary step toward the long-term goal, the results from these studies suggest that such creation may be eventually used to grow human organs. As the pigs and sheep are similar in size to humans, the human organs grown in these animals, the heart, liver, kidney, pancreas, lungs, and brain, could be harvested and transplanted into people, meeting the high demand for organ transplantation in the end-stage diseases. In addition, these human-animal chimeras could also be used to investigate the mechanisms of prenatal development and to investigate experimental drugs in different diseases.

Despite all the promises of using human/nonhuman chimeras for regenerative medicine, they also have raised serious ethical dilemmas about the morality of these chimeras. One of the biggest concerns is whether the human cells migrate to the brain and the chimeras end up with a humanlike mind. Such issues could potentially be prevented through genetic editing to avoid the human cell differentiating into the human brain or human gonads. So far, investigations in this field are moving forward with caution, and the reported human/nonhuman chimeras have not been allowed to develop past the fetal stage.

### 2.6 iPSC banking and allogeneic cell therapies

Although the iPSC cell therapy theoretically enables autologous transplantation, which would eliminate the need for immunosuppression, the inefficiency of iPSC derivation, the time and cost for developing each personalized cell product, and the safety of the products have made such autologous therapies unpractical,

particularly for diseases that require an immediate treatment. For the clinical use of iPSC-based cell therapies, it is essential to produce high-quality and safe (no induced mutations in the genome) iPSCs. As will be mentioned below, the pioneering iPSC clinical study in Japan using patients' own iPSC-derived retinal epithelial cells for the treatment of macular degeneration was put on hold due to genomic mutations in the iPSCs. Therefore, the most feasible application of iPSC-based cell therapy would rely on the banked and human leukocyte antigen (HLA)-typed iPSCs, in which the quality and safety have been validated in advance, in the setting of an allogeneic transplantation. This use of allogenic iPSCs however means that immunosuppression would have to be applied to prevent immune rejection. Kawamura et al. recently demonstrated that even though the immunogenicity of allogenic iPSC-derived cardiomyocytes was reduced by major histocompatibility complex (MHC) class I- and class II-matched transplantation in the macaque (monkey), the recipients still required substantial and highly toxic immunosuppression for sustained allogeneic cell engraftment [40]. It has been suggested that the MHC-matched iPSC-derived cardiomyocytes were still susceptible to natural killer (NK) cell destruction, leading to their rejection in the recipients in the absence of immunosuppression [40]. Forced expression of HLA alpha chain E (HLA-E) in PSCs and their differentiated derivatives has been demonstrated to prevent allogeneic response and lysis by NK cells [41]. Recently, Deuse et al. looked into the expression of genes in syncytiotrophoblast, an interface between fetus and mother, and identified low MHC class I and II expression and a high CD47 expression as the features that are responsible for the immune tolerance of syncytiotrophoblast toward allogenic fetal antigens [42]. CD47 is a membrane protein that interacts with several cell surface receptors to inhibit phagocytosis [43]. Indeed, CD47 is a "don't eat me" signal highly expressed on the surface of cancer cells to escape the innate immune responses [44]. The authors then inactivated MHC class I and II genes through CRISPR-Cas9 targeting and overexpressed CD47 via lentiviral transduction in both human and mouse iPSCs [43]. Importantly, the engineered iPSCs and derivatives (endothelial cells, smooth muscle cells, and cardiomyocytes) lost their immunogenicity and persisted long term in fully MHC-mismatched recipients without the use of immunosuppression [43]. This suggests that hypoimmunogenic cell grafts can be engineered from iPSCs for universal transplantation without immunosuppression. These approaches are associated with potential risks of uncontrollable malignant transformation or impaired immune reactions using hypoimmunogenic cell grafts, and consideration of designing an inducible killing switch in the engineered cells to ensure overall safety should be taken into account.

### 2.7 Current clinical trials with iPSCs

In 2014, Mandai et al. reported the results of the world's first clinical study of iPSC-based therapy in patients with advanced neovascular age-related macular degeneration (AMD) [45]. In this trial, two patients were recruited, and iPSCs were generated from the skin fibroblasts and were further differentiated into retinal pigment epithelial (RPE) cells [45]. One patient received the autologous iPSC-derived RPE cell sheet under the retina. A one-year follow-up on this patient revealed no apparent improvement nor worsening in her vision, and the transplanted sheet remained intact [45]. For the other patient, however, aberrations in DNA copy number were identified in the derived iPSCs and RPE cells, but not the starting fibroblasts, implying that genome mutations occurred during the reprogramming process [45]. This patient did not receive the treatment. Indeed, this clinical trial was suspended due to the

discovery of genetic mutations in the iPSCs. In 2017, five patients were recruited and treated for the same eye condition with iPSC-derived retinal cells. However, in this trial, the iPSCs were of an allogeneic source and created at Kyoto University Center for iPS Cell Research and Application (CiRA). One of the patients developed a serious reaction to the transplant. After removal of the engineered membrane graft, the symptoms were improved [46]. The efficacy of the treatment in other patients has not been reported.

In addition to the iPSC-based therapies in AMD, in May 2018, Japan's health ministry approved cardiac surgeon Yoshiki Sawa at Osaka University to assess the safety of allogeneic iPSC-derived cardiomyocytes in patients with heart disease [47]. This research team has previously reported the efficacy of grafting of human iPSC-derived cardiomyocytes cell sheet in combination with an omental flap technique in a porcine model of ischemic cardiomyopathy [48, 49]. In the projected human trial, the treatment will initially be given to three people; then the team will seek approval to conduct a clinical trial in approximately 10 patients [47]. If these initial clinical studies prove successful, the treatment will be made commercially available soon after under a new fast-track system in Japan designed to speed up the development of regenerative therapies [47].

In October 2018, neurosurgeons at Kyoto University Hospital also performed the first iPSC cell-based therapy in patients with Parkinson's disease (https://www.kyoto-u.ac.jp/en/research/events_news/department/hospital/news/2018/181109_1.html). In this first human study, 2.4 million allogeneic iPSC-derived dopamine precursor cells were deposited into 12 sites of the patient's brain with known dopamine activity. At the time of the press conference on this procedure, November of 2018, the investigators described that the patient was "doing well." The human iPSC-derived progenitor cells have shown to improve the symptoms in a primate model of Parkinson's disease [50].

As compared to Japan's fast-forwarding pace of initiating human trials with iPSC-based therapies, the scientists and physicians in the United States are approaching this direction with more caution, even though several human ES cell-based therapies have been initiated in clinical studies [51]. For the treatment of Parkinson's disease, Lorenz Studer at Memorial Sloan Kettering Cancer Center has focused on generating dopamine neurons from human ES cells at a sufficient scale and purity and demonstrated their efficient engraftment and function in mouse, rat, and monkey models of Parkinson's disease [52]. Based on those results, the group is currently pursuing an investigational new drug (IND) application from the US Food and Drug Administration (FDA), to initiate the first human clinical use of ES cell-derived dopamine neurons [52]. In November 2018, Fate Therapeutics, Inc., a biopharmaceutical company, announced that the FDA approved their IND application for FT500, the company's universal NK cells derived from a clonal master iPSC line. Using an in vitro three-dimensional tumor spheroid model, the company demonstrated that FT500, in combination with activated T cells and an anti-programmed death (PD)-1 antibody, led to near-complete elimination of target cells (>99% reduction) [53]. The company plans to initiate first-in-human clinical testing of FT500 in combination with checkpoint inhibitor therapy for the treatment of advanced solid tumors. This is expected to be the first-ever clinical investigation in the United States of an iPSC-derived cell product.

In 2016, Cynata Therapeutics also launched a phase I clinical trial in both the United Kingdom (UK) and Australia using allogeneic iPSC-derived MSCs (differentiated from iPSCs through intermediate-stage mesenchymoangioblasts) (CYP-001) for the treatment of steroid-resistant acute graft-versus-host disease (aGvHD) in patients undergoing an allogenic stem cell transplantation. In 2018, the company reported that CYP-001 met all clinical endpoints and demonstrated positive safety and efficacy

data for the treatment of steroid-resistant aGvHD in a phase 1 trial. Cynata plans to advance the cell product into phase 2 trials for GvHD and critical limb ischemia.

## 3. Adult (somatic) stem cells

### 3.1 Tissue-resident adult stem cells

Tissues and organs in our body constantly regenerate throughout our lives. Human red blood cells have a defined life span of 120 days [54]. The lining in the intestine turns over completely about every 7 days [55]. Skin cells are constantly shed and then renewed. Endothelial cells in the human heart have a > 15% turnover rate per year, while cardiomyocyte exchange is highest in early childhood and decreases gradually throughout life to <1% per year in adulthood [56]. These are examples of cell turnover at a steady state. Some organs, such as the liver, display relatively slow tissue turnover at a steady state, however upon damage, undergo bursts of cell proliferation and repair [57]. Essential for such replenishment and repair in postnatal life are the activities of adult stem cells. Adult stem cells are undifferentiated tissue-resident cells capable of self-renewal and differentiation within the tissue or organ. Self-renewal allows replication of themselves to sustain their population, and differentiation enables replenishment of various mature cell types upon tissue remodeling and repair, to maintain tissue homeostasis throughout postnatal life.

Hematopoietic stem cells (HSCs) are the first identified adult stem cells [58, 59]. The idea of HSCs, however, can be traced back before the experimental proof of HSCs and following the atomic bombing of Hiroshima and Nagasaki, when the civilians exposed to irradiation were documented to have symptoms of hematopoietic failure. Subsequent experiments in mice demonstrated recovery of the hematopoietic system after radiation injury by bone marrow transplantation [60]. These studies and subsequent transplantation experiments in mice with acute leukemia [61] led to pioneering of allogeneic hematopoietic stem cell transplantation (alloHSCT) by Thomas et al. in patients treated with radiation and chemotherapy in 1957 [62]. At that time, little was known about HLAs, and there was no matching between donors and recipients, until the mid-late 1960, when methods to identify HLA were developed [63]. While many physicians had doubts on the approach of alloHSCT, Thomas et al. persevered and performed transplantation using a matched sibling donor for a patient with leukemia in 1969 [64, 65]. Eight years later, his team performed the first HLA-matched transplant from an unrelated donor, a success that led to the formation of a national registry of bone marrow donors [63]. Dr. Thomas received the 1990 Nobel Prize in Physiology or Medicine with Dr. Joseph Murray, who performed the first successful kidney transplant, for their contribution to cell and organ transplantation.

For decades, hematopoiesis has been described as a cellular hierarchy where only one type of HSC is sitting at the apex of the hierarchy and giving rise to lineage-restricted progenitors, which further differentiate into all the cell types of the blood [66]. However, accumulating data on single HSC repopulation and serial transplantation have revealed an inherent heterogeneity in self-renewal and multi-lineage differentiation of HSCs [67, 68]. These and other studies have contributed to a change of view on HSCs from a single stem cell-type hierarchy model to a consortium model where a pool of stem cells with slightly different properties regenerate all the blood types [69–71]. However, a recent study suggested that platelet-biased stem cells, primed toward the megakaryocyte lineage, reside at the apex of the HSC hierarchy [72]. To reconcile with different observations on HSCs, a new speculative

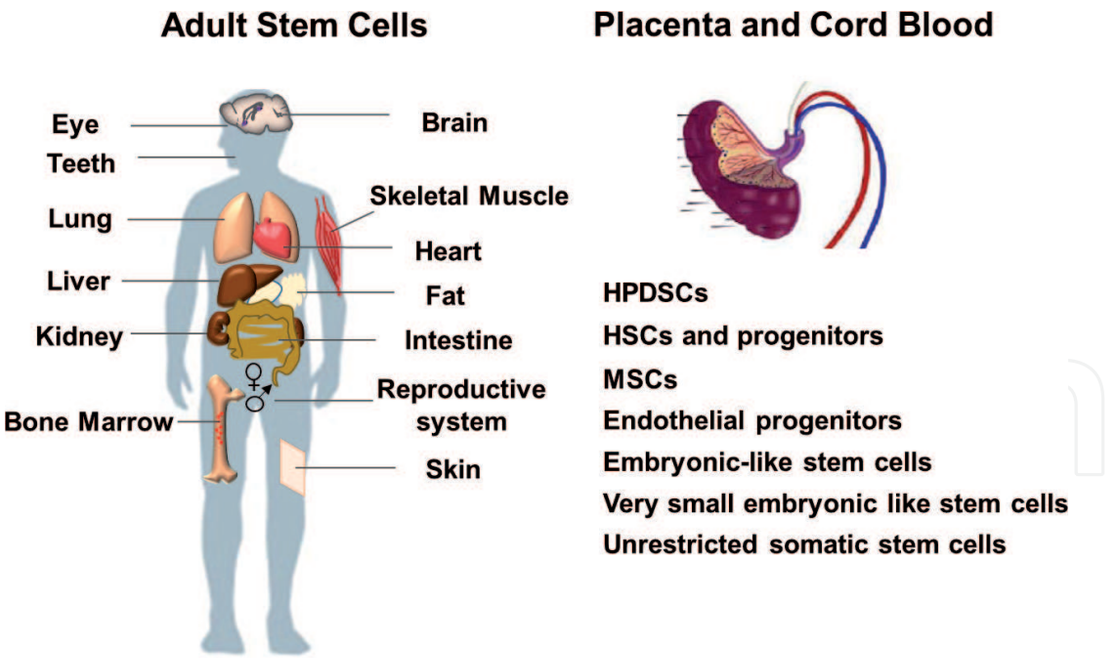


**Figure 3.**
*Identification of adult stem cells from postnatal organs and tissues such as the brain, eye, teeth, lung, heart, kidney, intestine, skeletal muscle, fat tissue, bone marrow, skin, and reproductive system. Human placenta and cord blood are also rich in different hematopoietic stem cells and progenitors, as well as non-hematopoietic stem cells.*

model was further raised suggesting that stem cells are rare reserve cells that occasionally generate lineage-restricted progenitors [73]. Some progenitors may retain a degree of developmental flexibility and, during extreme stress, can revert to a stemlike state [73].

Adult stem cells have been identified in most, if not all, tissues and organs in our body, as well as the placenta and cord blood (CB) (**Figure 3**). Extensive studies have been performed to characterize the adult stem cells in the fast-regenerating organs such as the skin and intestine [74, 75]. Moreover, the organs that were once thought to be non-regenerative, such as the brain and heart, also contain adult stem cells, i.e., neural stem cells and cardiac stem cells, respectively [76, 77]. In addition, similar to the evolving concept of hematopoiesis [73], heterogeneity of stem cell populations in distinct compartments in these organs has also been demonstrated, and the heterogeneity enables them to adapt to diverse roles and functions in response to environmental stimulation [73].

### 3.2 Adult stem cell niche and aging

It has been well accepted that the activities of adult stem cells, whether they stay quiescent or undergo activation, are largely instructed by the microenvironment they reside in, i.e., stem cell niche. Extensive studies have been performed to characterize the stem cell niche in different organ systems such as the skeletal muscle, bone marrow, skin, intestines, and brain (reviewed in [78]). There are common features of the adult stem cell niche. In general, the stem cell niche or microenvironment is composed of stem cells themselves and progeny, surrounding MSCs or stromal cells, extracellular matrix, and adhesion molecules, as well as the external cues from distant sources (long-range signaling factors) [78]. Collectively, both cellular and acellular components of the stem cell niche create a complex microenvironment maintaining stem cell fate and ensuring robust regenerative responses to external stimuli. Although other signaling pathways have been described, TGFβ

superfamily, Wnt pathway, and Notch signaling have been identified in different model systems as key regulators of stem cell quiescence, survival, maintenance, and activation [78]. For example, the TGFβ superfamily not only contributes to stem cell quiescence maintenance [79–81], it also plays an important role for stem cell activation and aging. TGFβ has been found to be upregulated in both the satellite cells (skeletal muscle stem cells) and serum of aged mice, which induces high levels of pSmad3 in satellite cells and interferes with their regenerative activities [82]. Importantly, Notch signaling antagonizes pSmad3 and controls satellite cell proliferation by blocking TGFβ-dependent upregulation of cyclin-dependent kinase inhibitors [82]. Meanwhile, increased Wnt signaling in the aged satellite cells has been demonstrated to contribute to cell fate conversion of satellite cells from a myogenic to a fibrogenic state [83].

Recently, Tikhonova et al. mapped the transcriptional landscape of mouse bone marrow microenvironment (HSC niche) at a single-cell resolution and reported previously unappreciated levels of cellular heterogeneity within the niche and with defined distribution of pro-hematopoietic factors [84]. Furthermore, bone marrow niche underwent transcriptional remodeling under stress conditions, leading to a significant upregulation of adipogenesis-related pathways and a global reduction in osteo-lineage-related gene expression [84]. There was also a downregulation of vascular-endothelial-expressed Notch ligand DLL4, which skewed bone marrow hematopoiesis toward a myeloid transcriptional program [84]. These results indeed provided an explanation to the observation that the HSC populations in the elderly exhibit myeloid skewing and lymphoid lineage deficiency [85].

The notion of aging has been raised as the ratio of tissue attrition to tissue regeneration [86]. This process is accompanied by reduced regenerative capacity of adult stem cells at levels of both self-renewal and differentiation (reviewed in [87]). The decline in regenerative function of adult stem cells also contributes to pathophysiological alterations in age-related diseases. Meanwhile, current studies also indicate that age-imposed biochemical changes in the stem cell niche are responsible for such regenerative declines of tissue maintenance and repair. Indeed, several studies have revealed that regenerative potential of stem cells was not controlled by the age of the stem cells themselves, but by the age of the niche they stay in [86]. In a classic experiment, minced skeletal muscle tissue containing satellite cells from young rodents was transplanted into the muscle of aged hosts, and conversely muscle tissue from old rodents was placed into the muscle of young hosts [88]. Such heterochronic transplantation demonstrated that the age of the host was more important than the age of the transplanted stem cells in muscle regeneration [88]. Serial transplantation of spermatogonial stem cells to the testes of young male mice demonstrated that spermatogenesis from that stem cell could continue for more than 3 years, long past the normal life span of the animal, when the stem cell is continually maintained in a young niche [89]. The importance of stem cell niche is further revealed by the heterochronic parabiotic studies [83, 90, 91]. In such experiments, two rodents, young-to-old (heterochronic) and young-to-young and old-to-old (isochronic), are surgically connected through a large flap of the skin, allowing vascular circulation between the two connected animals. In these studies, the isochronic parabionts were not significantly different in tissue regeneration than their respective non-parabiotic age-matched controls. However, in the heterochronic parabionts, regeneration in the muscle, liver, and brain was all significantly improved for the old animal and was decreased for the young animal [83, 90, 91]. As there was no evidence of blood cell exchange between the two connected animals, the studies suggested that tissue regeneration in the stem cells of an old animal could be promoted by "young" systemic factors (long-range factors), while

the aged circulation might have "negative" regulators that suppress the regenerative activities of "young" adult stem cells [86]. Examples of identified "negative" factors include p16INK4a, C-C motif chemokine 11 (CCL11), TGFβ, and TNFα, and "young" factors are sirtuin 6 (SIRT6) and Delta/Notch [82, 91–94].

All these studies underscore the importance of understanding how the stem cell microenvironment controls stem cell dynamics and function. Identifying specific molecular and cellular mechanisms that either rejuvenate or compromise the regenerative capacity of adult stem cells will be instructive in developing therapies antagonizing aging and age-related pathological changes. In animal studies, it has been demonstrated that forced activation of Notch signaling and inhibition of TGFβ and Wnt pathways could restore the regenerative capacities of aged muscle [83, 95].

Understanding the impact of microenvironment on stem cell activities is also essential for the development of an effective stem cell therapy. Whether the cells are derived from pluripotent stem cells or isolated from adult tissue, ex vivo culture or manipulation in a microenvironment that maintains cell identity and potency is critical for the efficacy of cell transplantation. Furthermore, a substantial challenge in the regenerative therapy is acute cell death after transplantation of the cells into a degenerative/pathological microenvironment. A recent clinical islet transplantation study quantitated the level of circulating cell-free (cf) DNA as a biomarker for the dead beta cells after transplantation [96]. The authors reported that a distinctive peak of cfDNA was observed 1 hour after transplantation in 83.8% of patients. The cfDNA was also detected 24 hours posttransplant, and that signal was correlated with overall poor clinical outcome (higher insulin requirement, lower stimulated C-peptide level, and decreased 3-month engraftment) [96]. Instant blood-mediated inflammatory response has been speculated to contribute to the significant graft attrition at this early stage of transplant [97]. Therefore, modulation on microenvironment that ensures the survival, integration, and function of the therapeutic cells after transplantation would greatly improve the efficacy of cell therapies.

### 3.3 Mesenchymal stem cells

MSCs were initially identified as rare non-hematopoietic colony-forming units following plastic adherence of bone marrow cells (reviewed in [98]). Although the original notion of MSCs specifically referred to cells in the bone marrow (also called bone marrow stromal cells, BMSCs), MSCs have been derived from other sources such as cord blood, adipose tissue, and dental pulp. MSCs can be identified by the expression of cell surface markers including CD90, CD73, and CD105 and lack of expression of hematopoietic and endothelial cell markers. MSCs have the capacity to differentiate along mesoderm lineage into osteoblasts, chondrocytes, adipocytes, and fibroblasts. MSCs were also reported to give rise to other mesodermal cell types such as cardiomyocytes and endothelial cells, as well as the cells of other lineages such as neurons and hepatocytes. However, as these claims were mainly based on the expression of the markers and not functional studies, whether MSCs truly have such differentiation capacities require more extensive validation.

There are also controversial results on the age-related changes in MSCs. Some studies reported an age-dependent reduction in the number of MSCs isolated from human bone marrow, while others demonstrated no correlation between MSC numbers and age, even in patients with osteoarthritis [99–102]. Functionally, Sun et al. demonstrated using young and aged mice that although the frequency of MSCs was not significantly different between the young and old bone marrow, the

self-renewal and bone formation capacity of old MSCs were significantly compromised as compared to the young MSCs [103]. Moreover, similar to the finding in the satellite cells, exposure of the aged MSCs to a young extracellular matrix rejuvenated these functions of aged MSCs [103]. Recent studies also demonstrated that with age as well as treatment of antidiabetic drugs, MSCs favored differentiation into adipocytes resulting in an increased number of adipocytes and a decreased number of osteoblasts, which may be related to osteoporosis. Downregulation of a transcription factor c-Maf has been identified as the age-related switch in MSC differentiation [104].

### 3.4 Human umbilical cord blood and placental stem cells

The idea to use human umbilical cord blood arose in the early 1980s, when experiments using cord blood from near-term mice demonstrated hematopoietic reconstitution in lethally irradiated mice [105]. Further, cord blood-derived HSCs were found to have a higher proliferative capacity than those in bone marrow and sufficient doses of HSCs, and hematopoietic progenitor cells were contained in a single collection of cord blood [106]. In 1988 a 5-year-old boy with Fanconi anemia underwent the first cord blood transplantation from his HLA-identical newborn sister [107]. In 1993 the first unrelated cord blood transplantation was performed in a 3-year-old with refractory T-cell acute lymphoblastic leukemia. Since then, related and unrelated cord blood has been increasingly utilized as an alternative source of HSCs [108].

In addition to the HSCs and hematopoietic progenitors, many populations of non-hematopoietic stem cells have been reported in human cord blood, such as MSCs, endothelial progenitor cells [109], embryonic-like stem cells (CBE) [110], very small embryonic-like (VSEL) stem cells [111], multi-lineage progenitor cells (MLPC) [112], and unrestricted somatic stem cells (USSCs) (**Figure 3**) [113]. There have been controversial reports on the identity of VSEL. VSEL stem cells were isolated based on the sorting of $CXCR4^{+}SSEA\text{-}1^{+}Sca\text{-}1^{+}Lineage^{-}CD45^{-}$ cells originally from murine bone marrow cells and subsequently in human CB [111]. Several other groups also reported the isolation of VSEL cells from different adult tissues and implied that VSEL cells were originated during embryonic development and deposited in bone marrow and other organs as dormant precursor cells of adult stem cells (reviewed in [114]). These cells are reported to be very small (3–5 μm), possess large nuclei, express pluripotency markers Oct4 and Nanog, and undergo multi-lineage differentiation. However, the stem cell characteristics of this cell type were challenged by the other group [115].

Among the other cord blood-derived non-hematopoietic stem cells, USSCs are the best characterized stem cell population and represent a novel universal allogenic stem cell source for degenerative diseases. USSCs are generated from full-term CB based on outgrowth of plastic adherent and spindle-shaped colonies in the presence of 30% fetal bovine serum, $10^{-7}$ M dexamethasone, and 2 mM ultra-glutamine in low glucose DMEM [113]. USSCs possess the ability to differentiate in vitro into the bone, cartilage, adipocytes, hematopoietic cells, and neural cells and in vivo into myocardial cells, Purkinje fibers, and hepatic cells [113]. Although USSCs share cell surface marker with MSCs, they have distinct gene expression, epigenetic signatures, and cytokine profiling [113, 116–120]. USSCs have high proliferation and expansion properties. They can be cultured for more than 20 passages without any spontaneous differentiation or slowing down doubling time [113, 116]. This is a significant advantage of USSCs over MSCs, the doubling time of which dramatically increases after four passages, leading to cell senescence [121]. Significantly, even after 13 passages, the average telomere length of USSCs is 8.6kbp, which is significantly longer than

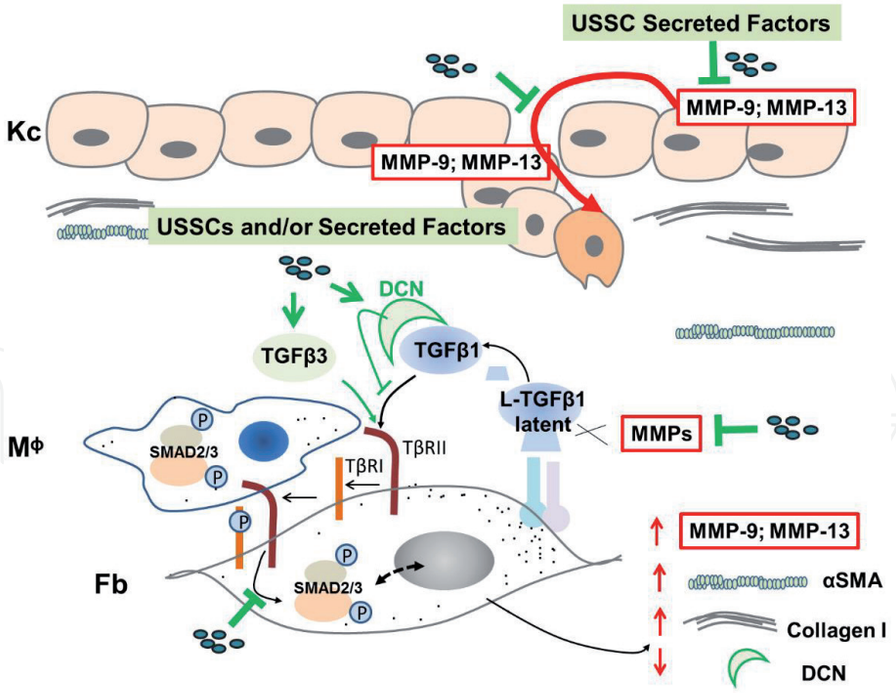


**Figure 4.**
*Dual effects of cord blood-derived USSCs on modulating RDEB skin microenvironment. USSCs exert anti-fibrotic function by suppressing phosphorylation of Smad2/3 in the fibroblasts (Fb) and macrophages (MΦ) of the skin, inhibiting matrix metalloproteinases (MMP)-9 and -13 dermal expression and upregulating anti-fibrotic TGFβ3 and DCN expression. USSCs also attenuate secretion of MMP-9 and MMP-13, which correlate with epithelial malignant transformation, from keratinocytes (Kc) and cutaneous squamous cell carcinoma (cSCCs) derived from patients with RDEB. This figure was adapted from* Stem Cells *with permission [125].*

the 7.27kbp telomere length of MSCs at passage 4 [113]. In preclinical studies, USSCs demonstrated the ability to alleviate myocardial infarction, liver injury, spinal cord injury, and intraventricular hemorrhage [122–124]. Moreover, USSCs also promote wound healing and improve manifestation of an inherited skin blistering disease, i.e., RDEB [120, 125]. Suppression of TGFβ-mediated fibrosis and modulation of extracellular matrix remodeling have been accounted as part of the mechanisms of action of USSCs in the treatment of RDEB (**Figure 4**) [125].

Once considered as a medical waste similar to CB, the human placenta has also been demonstrated to provide a novel stem cell source for cellular therapy. Hematopoietic stem and progenitor cells were identified throughout gestation from human placental blood, vessel perfusate, and cells from digested placenta tissue, and stromal cells generated from placenta possessed pericyte characteristic and may be a supportive microenvironment for hematopoiesis [126]. Recently Celgene Cellular Therapeutics, Inc. manufactured human placental-derived stem cells (HPDSCs) from full-term donor placentas following saline perfusion, red blood cell depletion, and volume reduction. The overall cell types as determined by flow cytometry analysis are similar between HPDSCs and CB [127]. However, HPDSCs contain a significantly higher level of both hematopoietic and non-hematopoietic stem and progenitor cells than CB. In addition, HPDSCs have a lower percentage of T cells than CB and are largely negative for MHC class II molecules, indicative of their potential use as both autologous and allogeneic cells. A pilot clinical study demonstrated that adding HPDSCs as universal donor cells with CB transplantation in patients with malignant and nonmalignant diseases had no adverse effects and may reduce the incidence of aGvHD [128]. Administration of HPDSCs in a mouse model of RDEB, in the absence of any conditioning regimen, also resulted in significant improvement on the survival and disease manifestation of the recipient mice [127].

As will be mentioned below, CB hematopoietic progenitor cells are among the few stem cell products that are approved by the FDA. Over 40,000 CB transplantations have been performed worldwide in both adults and children for the treatment of around 80 different disorders [129]. The advantage of using CB and cord blood CB-derived stem cells compared to other adult stem cell sources is the fast availability and ease in collection without causing any discomfort or risk to the donors. Moreover, being early in development, CB stem cells have not been exposed to immunological challenge and are less likely to carry somatic mutations than other adult cells. Any age- or stress-related transcriptional remodeling that might have impacted the stem cell function of adult stem cells, as discussed above, would not be of an issue in CB stem cells.

### 3.5 Examples of clinical studies with adult stem cells

As of today, FDA-approved cellular and gene therapy products are limited to the hematopoietic progenitor cells from CB for alloHSCT in patients with disorders affecting the hematopoietic systems, autologous chondrocytes on a porcine collagen membrane for repair of cartilage defects of the knee, allogeneic cultured keratinocytes and fibroblasts in the treatment of mucogingival conditions, and a few chimeric antigen receptor (CAR) autologous cellular immunotherapies (https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products).

In addition to the treatment of the disorders affecting hematopoietic system, CB alloHSCT has also been applied in clinical trials to treat nonmalignant diseases. More than 20 years ago, CB transplantation had been initiated in infants and children with Krabbe's disease [130, 131]. Krabbe's disease is an autosomal recessive disorder due to deficiency of the lysosomal enzyme galactocerebrosidase, leading to progressive neurologic deterioration and death in early childhood. These transplantation studies demonstrated that CB cells, both hematopoietic and non-hematopoietic in origin, could engraft in the patients' central nervous system, providing the missing enzyme and facilitating neural cell repair. Particularly, the young patients that underwent transplantation before the development of symptoms showed significant improvements in developmental skills, while the children who underwent transplantation after the onset of symptoms had minimal neurologic improvement [130, 131]. A long-term follow-up (median 9.5 years, range 4–15 years) study further demonstrated that the surviving patients who underwent early CB transplantation function at a much higher level than untreated children or children who were symptomatic at the time of alloHSCT [132]. Based on the observed efficacy of CB transplantation on improving neurological outcome, Cotten et al. conducted a pilot study on intravenous infusion of autologous CB in 184 pediatric patients who had their CB banked at birth and were subsequently diagnosed with acquired neurological disorders [133]. This investigation demonstrated the safety and feasibility of autologous CB infusion in these pediatric recipients. Subsequently, Cotten et al. also reported the safety and feasibility of autologous CB infusion in neonates diagnosed as hypoxic ischemic encephalopathy [134].

Over the last two decades, there have been an increasing number of clinical trials involving stem cell treatment for various degenerative diseases. For cardiovascular disease alone, there have been more than 200 clinical trials using various cell types including skeletal myoblasts, autologous bone marrow mononuclear cells (BMMNCs), $CD133^+$ bone marrow cells, endothelial progenitors, autologous $CD34^+$ cells, MSCs, cardiopoietic stem cells that were generated by

treating MSCs with a cocktail of trophic factors, allogeneic and autologous c-kit$^+$ cardiac stem cells isolated from biopsies obtained during coronary artery bypass grafting, and cardiosphere-derived cells generated from culture outgrowth of heart biopsies (reviewed in [135]). Most studies demonstrated that the tested cells were safe and the procedures were feasible. However, the efficacy of these cell therapies remains inconclusive. Early clinical trials, ranging from pilot to phase III double-blinded placebo-controlled studies, such as TOPCARE-AMI, BOOST, FNCELL, and REPAIR-AMI, demonstrated significant efficacy (evaluated-based on left ventricular ejection fraction, LVEF) of BMMNC treatment in patients with acute myocardial infarction. However, recent multicenter, double-blinded, and placebo-controlled studies, such as TIME, LateTIME, and SWISS AMI trials, found no improvements after BMMNC administration in LVEF and other parameters measured by cMRI. Even the same center, which reported significant improvement in heart function in the initial pilot trial and subsequent partly randomized and open-label study on autologous CD133$^+$ bone marrow cell infusion during coronary artery bypass graft surgery [136, 137], later revealed no significant benefits of CD133$^+$ bone marrow cell infusion in a strictly double-blind, fully randomized, and placebo-controlled trial (CARDIO133, NCT00462774) [138]. It has also to be mentioned that a report on the initial results of a phase I, open-label, and randomized trial using autologous c-kit+ cardiac stem cells in patients with ischemic cardiomyopathy was recently retracted from the journal, due to lack of reliability on the production of cells and integrity of the data [139].

### 3.6 Lessons learned from clinical stem cell therapies

Although there are a substantial number of clinical studies, it is difficult to compare results between trials. Sources of cell products, mode of cell delivery and cell dose, timing of administration, the age and complications of patients, and the choice of surrogate endpoint markers are all the variables for the efficacy of each clinical study.

When it comes to a stem cell therapy, it is tempting to think of it as the cell replacement therapy, that is, the stem cells engraft and differentiate to replace the damaged cells in vivo. However, although a few studies demonstrated that MSCs could undergo tri-lineage differentiation and human CD34$^+$ cells could fuse with cardiomyocytes and also transdifferentiate in animal models [140, 141], most studies reveal no evidence of therapeutic cells to undergo real cell replacement in vivo. Even c-kit$^+$ cardiac stem cells and cardiosphere-derived cells do not become cardiomyocytes in experimental animals with infarction [142, 143]. Rather, paracrine effects, e.g., secretion of growth factors to enhance tissue preservation and/or recruitment of endogenous repair, have been considered as the major mode of action of stem cells [137,138]. Similar lack of differentiation has also been reported when neural stem cells or progenitors were transplanted in animal models [144]. It would be interesting to see whether administration of human ES or iPSC-derived mature cells, such as cardiomyocytes and neurons as mentioned in the first part of the chapter, would result in more effective outcomes in the recruited patients, as in preclinical studies, these fully differentiated cells appeared to be able to functionally integrate in the recipient tissue [49, 50].

For cellular therapies, there is a choice of using either autologous or allogeneic cells. The autologous cells have the advantage of circumventing immunogenicity; however, the cell dose could be limited per isolation and the quality may not be reliable. Moreover, as mentioned above, the function of tissue stem cells declines

with age. It was also reported that BMMNCs isolated from patients with chronic ischemic heart disease have a significantly reduced migratory and colony-forming activity in vitro and a reduced neovascularization capacity in vivo, as compared to healthy controls [145]. Therefore, for the patients that are elderly and suffer from pathological insults, they may not benefit from treatment using their own cells. For allogeneic cells, the cell dose is less of an issue than autologous cells. As off-the-shelf products, they are presumably validated for safety and efficacy before being applied to patients. However, generation of cell lots with consistent potency and comparability has been recognized as a significant issue for clinical translation. Recently, two independent investigations reported that clinical-grade human neural stem cell product (HuCNS-SC; proprietary of StemCells, Inc.), in contrast to the research-grade NSCs provided by the same company, failed to demonstrate the efficacy in animal models of spinal cord injury and Alzheimer's disease, respectively [144, 146]. However, despite being informed of the negative impact of stem cell engraftment on functional outcome in the animal model, the company initiated a clinical trial testing this product in patients with cervical spinal cord injury (NCT02163876) in December 2014 and subsequently reported a small improvement in motor strength in 4/5 subjects in the 6-month interim report. However, the clinical trial was terminated in May 2016, due to a lack of significant improvements and the lack of a trend for improvements over time [146]. In this study, there were no details on how the clinical- and research-grade products were made differently that might contribute to the disparity on the efficacy of animal studies. HuCNS-SC was derived from donated fetal brain tissue based on fluorescence-activated cell sorting of $CD133^+$ cells and expanded as neurospheres. It is possible that the clinical- and research-grade products were from different donors with varying genetic background and/or developmental stage. It is also possible that the scale-up production of the cell products under good manufacturing practice (GMP) unfavorably changed the therapeutic function of the cells. Nevertheless, the lack of efficacy of the cell product in the animal model was consistent with the failure of the clinical trial. It has to be noted that in vivo preclinical testing of the final clinical product is not required by the FDA, because "human-derived cellular therapy products intended for clinical administration in animals may not be informative" [147]. As a result, stem cell products are increasingly entering clinical studies for various disease conditions without prior efficacy studies in animal models, outpacing our understanding on their potential mechanisms of action. Although it is true that not all the animal models recapitulate spectrum of human diseases and it is difficult to extrapolate the results from the animal study to human, the lesson learned from the failures of clinical studies including HuCNS-SC is the importance of robust and reliable potency assays to characterize the cells and to ensure the consistency between different manufacturing lots before applying to patients. Recently, the International Society of Stem Cell Research (ISSCR) released updated guidelines on stem cell research and clinical translation, recommending that the cells entering clinical trials are based on sound scientific rationales with robust manufacturing and animal efficacy data, in addition to a safety package to support clinical trials [148].

## 4. Conclusion

We are at the end of the beginning in the field of stem cell research and stem cell regenerative medicine. Particularly, the development of iPSCs and gene editing techniques have opened a new era of disease modeling and personalized

medicine. However, although it is feasible, the time and cost for production and validation of autologous iPSC-derived cellular therapeutics have made such personalized medicine unpractical, especially for diseases requiring an immediate therapeutic intervention. Therefore, a more feasible use of iPSCs and iPSC-derived cell products would rely on an HLA-matched allogeneic setting. Moreover, targeted differentiation of PSCs into distinct cell subpopulations and proper cell maturation remain to be a challenge in the development of iPSC therapies. In the end, whether the cell products are derived from iPSCs or adult stem cells, they face the same challenges as cellular therapies, that is, large number of cell death after transplantation and poor functional integration of the survived cells. The current efforts on tissue engineering and organoid system have demonstrated promises in overcoming these difficulties. Understanding tissue microenvironment is also the key to develop effective therapies that ensure exogenous cell engraftment and integration and/or augment endogenous tissue stem cell function for regeneration.

With the excitement of stem cell research, more subtypes of stem cells are entering into clinical studies, and there is a growing interest in commercializing and marketing of these stem cell products. However, caution should be maintained to ensure the quality of cell products and the scientific rationale and rigor for their clinical translation. Lastly, a randomized and controlled clinical trial with large sample size and multiple surrogate endpoints are essential to determine the safety and efficacy of stem cell therapy.

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