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Promise and challenges of human iPSC-based hematologic disease modeling and treatment

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Abstract Postnatal hematopoietic stem cells (HSCs) from umbilical cord blood and adult marrow/blood have been successfully used for treating various human diseases in the past several decades. However, the availability of optimal numbers of HSCs from autologous patients or allogeneic donors with adequate match remains a great barrier to improve and extend HSC and marrow transplantation to more needing patients. In addition, the inability to expand functional human HSCs to sufficient quantity in the laboratory has hindered our research and understanding of human HSCs and hematopoiesis. Recent development in reprogramming technology has provided patient-specific pluripotent stem cells (iPSCs) as a powerful enabling tool for modeling disease and developing therapeutics. Studies have demonstrated the potential of human iPSCs, which can be expanded exponentially and amenable for genome engineering, for using in modeling both inherited and acquired blood diseases. Proof-of-principle studies have also shown the feasibility of iPSCs in gene and cell therapy. Here, we review the recent development in iPSC-based blood disease modeling, and discuss the unsolved issues and challenges in this new and promising field.

Keywords Induced pluripotent stem cells · Hematopoietic disease · Disease modeling · Gene therapy · Cell therapy

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Introduction

Stem cells are responsible for tissue regeneration during normal homeostasis or injury repair. The distinct capabilities of stem cells to undergo self-renewal and multi-lineage differentiation make them ideal cell types as sources for cell therapy. The most successful and widely used cell therapy is bone marrow transplantation, which relies on the ability of hematopoietic stem cells (HSCs) to engraft in the bone marrow of patient recipients and to regenerate the whole hematopoietic and lymphatic system. HSCs give rise to all circulating blood lineages; many hematopoietic diseases can be traced back to certain defects in the HSCs or less immature but still multipotent progenitor cell compartment, collectively called HSPCs thereafter. Therefore, HSPCs are considered the ideal target cell type for gene therapy and source for modeling diseases, either in tissue culture dish or in xenograft animal models. The rarity of HSCs and the lack of efficient methods to amplify these cells ex vivo in the past decades have significantly limited the application of these versatile cells in both clinics and in research. Identifying and expanding HSCs have been actively pursued in the past two decades and significant progresses have been made. In the meantime, alternative approaches have also been developed. Development in the induced pluripotent stem cell (iPSC) technology has provided one such exciting alternative.

Pluripotent stem cells differ from tissue-specific stem cells such as HSCs in their potential to differentiate into every adult cell type in the body. The development of mouse embryonic stem cells (ESCs) has revolutionized the way we study molecular genetics and establish disease models. Derivation of human ESC lines in 1998 has provided unprecedented opportunities for studying human embryonic development in vitro [1]. Since human ESCs can be maintained and expanded in tissue culture for a prolonged period of time while maintaining normal karyotypes and pluripotency, it has been anticipated these cells can one day be used as sources for producing functional cell types for cell replacement therapy. Traditionally ESCs were derived from the blastocyst stage of early embryos; therefore, they cannot be patient-specific. In addition, there have always been controversies regarding the usage of human ESCs for purposes of research or even treatment of a human being. To overcome these limitations, various technologies have been developed to generate pluripotent stem cells from non-embryonic sources. Among them, iPSCs derived from reprogramming somatic cells by defined genetic factors have gained the most attention [2, 3]. Studies have convincingly demonstrated the pluripotency of fully reprogrammed iPSCs, although they may not be identical to ESCs derived from an early embryo. The most attractive feature of iPSCs is that they can be patient-specific or disease-specific, hence the enthusiasm of iPSCs in cell replacement therapy and disease modeling [4]. Here, we review the recent progress towards modeling and treating hematopoietic diseases using human iPSCs.

Generation of patient-specific iPSCs

The early generations of iPSC lines were derived from fibroblasts by using integrating viruses that express several reprogramming factors [2, 3, 5, 6]. Although this approach remains to be one of the most reliable and widely used reprogramming protocols, the recent several years have seen significant progress in both the reprogramming technology and the selections of somatic cell types to make iPSCs safer and of higher quality. Several virus-free methods have been developed to reprogram somatic cells without viral genome integration. These methods still rely on the introduction of the core reprogramming factors but differ in their ways of delivering the factors into somatic cells. It has been reported that mRNA and protein forms of the reprogramming factors can be introduced into cells and successfully mediate cell fate change to pluripotency. However, further improvement of the methods is needed to enhance the reprogramming efficiencies and to make them reproducible widely in other laboratories [7, 8]. Plasmid-mediated reprogramming is currently the most reliable method for integration-free reprogramming [9–11]. The procedure is also less complicated than viral protocols which require the separated steps of virus-making and viral infection of target cells; therefore, more suitable as a routine laboratory procedure. The most reproducible plasmid system includes EBNA1 gene and the OriP DNA sequence from Epstein–Barr virus (EBV) as an autonomous DNA replication origin in primate cells. These features allow the plasmid to replicate inside cells as freestanding circular DNA (episomes). Compared to regular plasmids, this feature enables more sustained expression of reprogramming factors without integration into the cellular genome. Therefore, a one-time transfection of episomal vector(s) can achieve efficient reprogramming. The presence of the episomal vectors, though longer than regular plasmids, is usually transient due to cellular machinery-mediated epigenetic silencing of foreign vectors expressing reprogramming and EBNA1 genes. In most cases, the episomal DNA became undetectable by PCR in iPSCs 10–12 passages after initiation of reprogramming by DNA transfection [9, 10]. Whole-genome sequencing of episome-reprogrammed iPSCs has provided convincing evidence for the absence of reprogramming vectors in iPSCs, demonstrating the safety of this method [12].

Among the diverse types of cells that have been successfully reprogrammed to pluripotency, blood cells are the most accessible and the easiest to harvest. Blood sample collection and storage are among the routine procedures in hospitals and clinics. Therefore, significant efforts have been made to develop efficient and safe procedures for reprogramming various types of blood cells (Table 1) [10, 11, 13–21]. Umbilical cord blood (UCB) cell banks provide another rich source for cells with diverse HLA types. UCB cells are developmentally more immature and enriched of CD34+ HSPCs than adult blood cells. It is therefore of no surprise that the reprogramming efficiencies using UCB cells are higher than other cell types. Pioneering studies on human blood cell reprogramming all relied on CD34+ or CD133+ HSPCs as these cells offer the advantages to be greatly proliferating in culture and to be transduced at higher efficiency with retroviruses or lentiviruses by existing methods, two features that are important for reprogramming [13–15, 22, 23]. The rarity of CD34+ HSPCs in blood cell ($\sim$1 % in UCB and 0.01 % or less in adult peripheral blood) samples prompted the development of methods to reprogram other, and more abundant, blood cell types. T cells, which are abundant in peripheral blood and amenable to ex vivo expansion after stimulation of T cell receptor (TCR) activation, were the first subtypes of lineage-committed human blood cells being reprogrammed [16–18]. Analyses of the TCR genomic loci had shown the evidence of V(D)J recombination in resultant iPSCs, demonstrating their T cell origin [16–18, 24]. These studies

also provided the most convincing evidence that terminally differentiated human cells can be reprogrammed into a pluripotent state. The search for methods of reprogramming other non-T cell lineages continued, as iPSCs with V(D)J rearranged genomes may have limited potential in certain applications of cell replacement therapy especially in blood cell transplantation-based therapies. Currently, the most successful method for reprogramming unfractionated blood mono-nucleated cells (MNCs) utilizes the EBNA/OriP-containing plasmids after a brief culture of the MNCs in a condition favoring erythroblast expansion [10]. This protocol worked extremely well for UCB MNCs, as 180–450 candidate iPS colonies can be obtained from one million expanded blood cells (Table 1). These high efficiencies are equivalent to that observed in reprogramming UCB CD34+ cells. The efficiencies for reprogramming adult blood MNCs are generally 20–50 folds lower; however, multiple iPSC lines can be established from a few milliliters of blood samples [10]. In another independent study, bone marrow MNCs were also efficiently reprogrammed to iPSCs using similar episomal vectors although a different expansion condition was employed before plasmid transfection [11]. In both studies, the established iPSCs have been shown to be free of V(D)J rearrangement and vector DNA in the genomes. These successes allowed efficient generation of patient-specific iPSCs with intact genomes from convenient cell sources. More recently, it has been shown that EBV-immortalized lymphoblast cells (established originally from blood MNCs) can also be reprogrammed to EBV genome-free iPSCs [20, 21]. EBV-immortalized lymphoblasts may have additional somatic mutations (see below for discussion on somatic mutations found in iPSCs) and therefore are not preferred choice as a source of iPSCs to be used in cell therapy. However, they provide additional opportunities for disease modeling since EBV-immortalized lymphoblasts have been historically generated from many patients including those that have passed away and those with rare diseases, and maintained in many cell repositories [25]. Overall, our ability to generate iPSCs, especially the safer

Table 1 Human iPSC generation from different blood cell types

Type of tissue	Cell types	Reprogramming methods	Efficiency (per 10^6 cells)	References
Cord blood	CD34+ or CD133+ stem/progenitor cells	Retrovirus	50–500	Ye [14]
				Giorgetti [15]
		Plasmid	50–200	Chou [10]
	Unfractionated MNCs (after erythroblast culture)	Retrovirus	67	Unpublished data (Cheng lab)
		Plasmid	180–450	Chou [10]
	Unfractionated MNCs	Plasmid	2–17	Hu [11]
Bone marrow	CD34+	Retrovirus	50	Ye [14]
		Plasmid	6	Chou [10]
	Unfractionated MNCs	Retrovirus	N/D	Kunisato [19]
	Unfractionated MNCs	Plasmid	352–2,095[a]	Hu [11]
Peripheral blood	CD34+	Retrovirus	100	Loh [13]
		Plasmid	9	Chou [10]
	Myeloid	Retrovirus, lentivirus	2–5	Kunisato [19]
				Loh [17]
				Staerk [18]
	T linage	Retrovirus	100	Brown [16]
		Lentivirus	5–10	Loh [17]
				Staerk [18]
		Sendai virus	1,000[a]	Seki [24]
	Unfractionated MNCs (after erythroblast culture)	Retrovirus	10	Unpublished data (Cheng lab)
		Plasmid	7	Chou [10]
EBV-transformed lymphoblasts	Immortalized B cell lines	Plasmid	4–130	Choi [20]
				Rajesh [21]

N/D not determined

[a] Efficiency estimated based on alkaline phosphatase (AP) staining. Others: based on TRA-1-60 or TRA-1-81 staining or purely on human ESC/iPSC morphology

iPSCs without genomic alteration, opened up the opportunities for developing models and treatment for many diseases.

Advantages of iPSC-based disease modeling

The major motivations for modeling diseases with iPSCs usually came from either the lack of animal models, especially mouse models, or the difficulty to isolate and maintain relevant human tissues in a dish. Mouse models have been invaluable for understanding mechanisms of many types of disease; however, for certain diseases, the same genetic mutation(s) observed in patients did not result in similar disease phenotypes in mouse due to the fundamental differences between man and mouse. In other cases, the mouse models were unavailable because the underlying molecular defects occur in multiple pathways that are less defined or different between murine and human. In these cases, the ideal alternative would be isolating relevant human tissue/cells to conduct ex vivo experiments or to create xeno-transplantation models. The major limitation of these approaches is that many disease-relevant tissues are either difficult to access or highly heterogeneous in cell types. The best example in hematology research is the general lack of ability to isolate clonal HSCs and to maintain them in the laboratories. The inability to maintain and expand these primary cells also limited the ability to conduct genetic modifications which are essential for understanding precise roles of candidate genetic variations in diseases. The pluripotent iPSCs can theoretically solve some of these problems due to the following properties: (1) iPSCs can be patient-specific and disease-specific; retain all the genetic, and to certain degree epigenetic, identity of the parental primary cells from the patient. (2) iPSCs can be clonally derived and expanded; this is an important feature as it provides a potential solution to the heterogeneity issue when dealing with many types of diseases. (3) iPSCs have the potential to generate all cell types, not only the cell type of origin before reprogramming, but other types of cells. This is becoming more significant as it is more and more evident that, in many diseases, interactions between different cell types play important roles in disease development. Examples are the interactions between HSCs or its cancerous counterparts and their niches, or the interaction between non-immune cells and the immune system. (4) It is more feasible in iPSCs than in most adult stem cells (e.g., HSCs) to perform genetic modifications using current technologies such as those based on low-efficiency homologous recombination, giving the iPSC models advantages for understanding the molecular mechanisms of underlying diseases and for developing potential gene and cell therapy.

Blood disease modeling using human iPSCs

Despite these potential advantages of iPSC-based modeling, there have only been very limited numbers of iPSC-based blood disease models that have shown either the disease features or the feasibility of gene therapy (Table 2) [14, 26–30]. The first comprehensive proof-of-principle study to demonstrate the potential of iPSCs in any disease modeling and treatment was conducted in the mouse model

Table 2 Published modeling of blood disease using human patient-specific iPSCs

Disease	Cell type origin for reprogramming	Known mutation(s) retained in iPSC	Disease feature(s) recapitulated	Gene correction	References
X-linked chronic granulomatous disease (X-CGD)	BM-MSCs	Point mutation in CYBB gene	Lack of ROS production in neutrophils	AAVS site targeting for *CYBB* transgene expression	Zou [28, 29]
Fanconi anemia	Dermal fibroblast (FAA and FAD2 corrected)	Variant in FA group	Disease free	Lentivirus-mediated transgene expression	Raya [26]
	Dermal fibroblast; bone marrow-derived fibroblast	Variant in FA group	N/D	Complementation before reprogramming	Müller [30]
Sickle cell disease (SCD)	Unfractionated PB-MNCs; skin fibroblasts	Point mutation in HBB gene	Lack of wild-type beta globin expression	HR mediated on-site gene correction	Zou [28, 29]; Sebastiano [27]; Li [33]
Polycythemia vera (PV)	Hematopoietic progenitor cells (CD34+CD45+)	JAK2-V617F	Enhanced erythropoiesis in iPSC-derived CD34+CD45+ cells	N/D	Ye [14]

N/D not determined

of sickle cell disease (SCD) [31]. iPSCs were generated from skin fibroblasts of mice with human sickle cell mutation knock-in. Corrected iPSCs through homologous recombination were then differentiated into HSPCs and were transplanted back into sickle mice to correct the SCD point mutation [31]. This study provided a proof-of-principle for developing iPSC-based gene and cell therapy for patients with SCD or other inherited blood disorders. Inherited diseases with well-defined genetic lesion(s) like SCD are the most straightforward to develop iPSC models. Human SCD iPSCs have been derived for developing disease models and treatment [10, 20, 27, 29, 32, 33]. Besides SCD, X-linked chronic granulomatous disease (X-CGD) is another example of such recessive genetic diseases. Similar to SCD, the genetic mutation in X-CGD is inherited and therefore disease-representing iPSCs can be generated from a broad range of cell types. X-CGD iPSCs generated from BM-MSCs carried the same disease-causing gp91^{phox} deficiency [28]. Importantly, lack of ROS production in neutrophils due to the absence of oxidase activity was observed after directed differentiation of the patient-specific iPSCs [28]. Another category of inherited diseases such as Fanconi anemia (FA) are more complex than SCD or X-CGD in that disease-causing genetic mutations can occur in any of a group of genes that are required for certain pathways [34]. Thus, every patient may possess distinct mutations from many others that suffer from the same disease. In order to model this type of disease, a panel of iPSCs from various patients may be required to comprehensively represent the genetic complexity of the disease [26, 30].

Recent progress in reprogramming blood cells, especially the episome-mediated integration-free reprogramming, has made it more feasible to develop iPSC models for studying acquired blood diseases such as myeloproliferative neoplasms (MPNs), aplastic anemia, myelodysplastic syndrome (MDS), paroxysmal nocturnal hemoglobinuria (PNH) and many forms of leukemia. Many of the disease-relevant mutations are restricted to hematopoietic lineages; therefore, the traditional iPSC generation from fibroblasts would have missed the genetic information important for disease development. The fibroblast-derived iPSC would still be important for disease modeling as they serve as germ line controls that may contain certain predisposing mutations or polymorphisms. The first acquired hematopoietic disease modeled by iPSCs was polycythemia vera (PV), one of the three major BCR-ABL negative MPNs [14]. Human iPSC generated from PV patient blood cells contained the JAK2-V617F point mutation, the most frequently observed acquired mutation in PV blood. More importantly, the CD34+CD45+ HSPCs generated from the PV-specific iPSCs showed enhanced erythropoiesis compared to healthy control iPSCs-derived HSPCs, recapitulating the major clinical feature of red blood cell over-production in PV patients [14].

Potential of iPSCs for developing cell therapy and drug treatment for blood diseases

In addition to providing insight into disease mechanisms through disease modeling, advances in iPSC technology can also directly benefit disease treatment in two other ways. The patient-specific feature of iPSCs made them the ideal cell types for developing cell therapy. Autologous iPSC-derived functional progenies are less likely to result in immune rejection or GVHD. For certain acquired blood diseases, iPSCs derived from tissues other than blood may be used as sources for production of disease-free autologous donor cells. In other cases, gene therapy to repair genetic lesions may be considered before cell replacement therapy. Diseases with well-defined monogenic mutations are excellent candidates for developing gene therapy. Proof-of-principle studies have been carried out using X-CGD as a model disease [28]. Functional correction of the X-CGD defect in the patient-specific iPSC was achieved following zinc finger nuclease (ZFN)-mediated site-specific HR targeting of a gp91^{phox} minigene to the AAVS1 (safe harbor) locus. Introduction of gp91^{phox} minigene facilitated the production of mature neutrophils with restored oxidase activity [28]. These types of inherited diseases are results of loss-of-function gene mutations; therefore, the gene addition approach can be sufficient for correcting disease phenotypes. In other cases such as SCD, the presence of a mutant form of protein is a major disease-causing mechanism. Targeted gene correction would be a preferred method for gene therapy. Progress in gene targeting technology has allowed, similar to what was achieved in the mouse model, the precise homologous recombination-mediated gene correction of SCD mutation in patient-specific iPSCs [29].

In addition to cell therapy, drug screening and testing is another area through which patient-specific iPSCs can have major contributions to therapeutic development. Traditional drug development relied heavily on cell line-based compound screening and animal-based testing. Although a number of drugs have been successfully developed through these approaches, the lack of relevance to human diseases of these systems has resulted in high failure rates and added significant burden to the lengthy and costly clinical trials, contributing to the high costs and long timeline of drug development [35]. Disease-specific iPSCs can be maintained and expanded in culture and, with proper differentiation conditions, can generate a large quantity of cells displaying disease phenotypes; therefore, serving as an ideal alternative to primary patient cells which are usually

limited by their availability. Although by far the only successful report of compound screening using disease-specific iPSCs was conducted with iPSC-derived neural crest cells which had identified candidate drugs for treating familial dysautonomia (FD) [36], it is anticipated that this approach will also lead to novel drug treatment of blood diseases.

Challenges and unsolved issues

As discussed in the previous sections, there have only been limited successes in iPSC-based disease modeling and therapeutic development despite the great potential. There still are several major challenges that limit the broader applications of this new technology.

Clinical grade iPSC generation and differentiation

The reprogramming technologies are evolving. Besides the virus-free integration-free reprogramming methods, conditions for mouse feeder-free iPSC culture are being constantly improved to make iPSCs safer and more suitable for clinical applications [37–41]. However, it is still a challenge to establish systems that can sufficiently support human iPSC self-renewal, genetic modifications and differentiation while compatible with clinical production under current Good Manufacturing Practice (cGMP) standards.

Disease types may affect reprogramming process

Another issue related to iPSC generation is that some disease-causing genetic mutations may also negatively regulate the reprogramming process. Alternative approaches therefore will need to be considered. It has been reported that attempts to reprogram fibroblast cells from various Fanconi anemia patients failed, suggesting that the FA mutations associated with DNA repair also blocked reprogramming. The fibroblasts that had been genetically corrected did give rise to iPSCs and can be re-differentiated back to phenotypically normal hematopoietic progenitors, providing potential cell sources for future cell therapy [26, 30].

Somatic mutations captured and found in iPSCs

iPSCs are derived from somatic cell types by a clonal fashion and theoretically retain all the genetic information of the cell of origin. It was first reported from an exome sequencing study of human iPSCs that on average 5–6 point mutations are found in the exome of a given iPSC as compared to the parental fibroblast cell population [42]. Sensitive PCR methods found that at least 50 % of such point mutations, or more actually single-nucleotide variants (SNVs), can be detected in the original fibroblasts albeit at a low frequency. In order to assess the extent to which reprogramming may accelerate the accumulation of genetic mutations, whole-genome sequencing of three episomal vector-reprogrammed human iPSC lines derived from two cell types of one adult donor were performed [12] or mouse iPSC lines [43]. The human study provided direct evidence of integration-free reprogramming as the vector sequence was undetectable in the deeply sequenced iPSC lines. Among 1,058–1,808 heterozygous single-nucleotide variants (SNVs) identified in each iPSC line, only 6–12 were within the coding regions. The ratio of synonymous changes to nonsynonymous changes is roughly 1:1 and the mutations are not selectively enriched for known genes associated with cancers [12]. Similarly, a recent study using mouse iPSC lines concluded that background mutations in parental somatic cells account for most of the genetic heterogeneity of derived iPSCs [43]. These recent results suggest that most of the genetic variation in iPSC clones is not caused by reprogramming per se, but is rather a consequence of cloning individual cells, which "captures" their mutational history. However, we will need to pay attention to the existing residual SNVs in the starting somatic cell population, if the goal is to discover disease-related SNVs or mutations.

Epigenetic memory

The similarity and difference between ESCs and iPSCs as well as that among iPSCs of various origins have been topics of extensive investigation since the beginning of the modern reprogramming era. Virtually all the published data have demonstrated that the epigenetic reprogramming process was generally extensive in bona fide iPSCs, as the iPSCs displayed distinct global DNA methylation patterns from somatic cells and are similar to ESCs. Studies using mouse system have first suggested that certain epigenetic marks that were distinct from ESCs had failed to be reset and were retained in the iPSCs after reprogramming [44, 45]. The phenomenon of epigenetic memory has also been documented recently in a human system [46]. A study using human iPSCs derived from pancreatic islet beta cells has shown that these iPSCs can be distinguished by their epigenetic profiles from other pluripotent stem cells and that they had increased ability to differentiate into insulin-producing cells [46]. On the other hand, studies from both mouse and human iPSCs have suggested that the epigenetic memory is likely to be observed at early passages of iPSCs and can be erased through continuous passaging [45, 47]. Functional studies have also shown that with improved differentiation conditions, iPSCs of diverse origins can be

differentiated into functional cell types such as neural cells and hepatic cells at comparable efficiencies even though they can be distinguished by epigenetic profiles [48, 49]. It is being appreciated that there are also differences within the group of ESCs and whether the level of these differences is significantly lower than what is observed between iPSCs and ESCs will require more comprehensive studies with larger numbers of pluripotent stem cell lines. Overall, the significance of epigenetic memory is still an unsolved issue of basic biology; however, it is reasonable to predict that its practical impact on the modeling of most (genetic) diseases and therapeutic development would be minimal.

Generation of functional cell types from iPSCs

The biggest challenge for successful iPSC-mediated disease modeling and therapy is to generate functionally relevant cell types from iPSCs. The most relevant cell types for blood disease are HSCs and their functional progenies such as red blood cells, neutrophils, T and B cells. Current protocols are limited in their ability to recapitulate the process of definitive hematopoiesis in a dish; therefore, no HSCs have been generated from pluripotent stem cells. This significantly hampers our efforts to create in vitro or xeno-transplantation models. Unlike in the mouse system where ectopic expression of the HoxB4 gene can facilitate the generation of transplantable HSCs [50], no pathways have been identified that have the ability to push the human iPSC blood differentiation through primitive hematopoiesis stages, even though pathways such as NOTCH have been shown to be essential for HSC formation [51, 52]. Insights from studies of early blood development in multiple animal models (e.g., mouse and zebrafish) will likely shed light on the molecular mechanisms of this process.

Genetic modifications in iPSCs

The ability to conduct efficient targeted genetic modifications is essential for the iPSCs-based disease models and cell therapy to be effective. Traditionally the efficiency of homologous recombination-mediated gene modification in pluripotent stem cells had been extremely low due to the low clonal expansion rates of human ESCs/iPSCs and the even lower HR rates [53]. However, this is a field that has seen tremendous progress in the past few years. Besides the improved culture conditions, particularly the discovery of ROCK inhibitor-mediated clonal cell growth, there have been some major breakthroughs in our efforts to enhance the recombination rates. The development of zinc finger nuclease (ZFN) technology and, more recently, the transcription activator-like effector nuclease (TALEN) technology have allowed significant enhancement of gene targeting efficiency in iPSCs [28, 29, 54–58]. These exciting new developments will no doubt have impact on the realization of iPSC potentials.

Conclusions

Compared to the traditional approach of using postnatal hematopoietic stem cells, iPSCs offer several advantages. It allows the clonal expansion of cells that contain genetic signatures of rare clones of HSCs. It would facilitate the genetic modifications that are often difficult to perform on HSCs. These advantages are the reasons that patient-specific iPSCs may serve as ideal sources for disease modeling and for gene and cell therapy. The challenges of the current technologies include the standardization of reprogramming methods for generating safe and high-quality iPSCs, developing efficient and scalable differentiation protocols for generating functional cell types. Recent development in gene targeting technologies has provided additional valuable tools for realization of the great research and therapeutic potentials of iPSCs.

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