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Induced pluripotent stem cells in clinical hematology: potentials, progress, and remaining obstacles

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Abstract

Purpose of review—With the advent of reprogramming came the possibility of generating patient-specific clinical therapies. The purpose of this review is to discuss the recent key developments and remaining limitations in the stem cell and hematopoietic fields toward the goal of translating induced pluripotent stem cell (iPSC) technologies into the hematology clinic.

Recent findings—Recent progress in the hematopoietic and reprogramming fields has included identification of hematopoietic stem cells (HSCs) capable of long-term engraftment at the single-cell level, improvements in ex-vivo expansion of HSCs, transdifferentiation of somatic cells into hematopoietic progenitors, and the ‘correction’ of several disease-specific iPSCs using various gene-targeting strategies.

Summary—In light of recent advances, it is the hope that the hurdle of obtaining fully functional HSCs in a laboratory setting will be overcome through either in-vitro differentiation of pluripotent stem cells, ex-vivo expansion of HSCs obtained *in vivo*, or transdifferentiation from other somatic sources. Equally important will be for the reprogramming field to better understand the causes and consequences of the recently reported genetic/epigenetic variations present in iPSCs, especially within the context of gene-targeted strategies for correcting disease. The progress in the reprogramming and hematopoietic fields provides a strong foundation for future work toward the possible treatment of numerous hematological disorders using iPSC technologies.

Keywords

clinical hematology; hematopoietic differentiation; hematopoietic stem cell; induced pluripotency; reprogramming

INTRODUCTION

Hematopoietic stem cell (HSC) transplantation is a well established cellular replacement procedure that has been successfully used in the clinic for decades. HSC transplantation using allogeneic human leukocyte antigen (HLA)-matched bone marrow, cord blood, or

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Conflicts of interest

There are no conflicts of interest.

mobilized CD34⁺ cells is the standard of care for individuals inflicted with a variety of inherited blood deficiencies and malignancies. However, this type of procedure relies on finding an appropriate HLA-matched source as well as a sufficient quantity of donor cells. Allogeneic HSC transplantation can lead to severe graft-versus-host disease (GVHD), and therefore is associated with high morbidity and mortality. Autologous HSC transplantation carries no complications of GVHD; however, treatment of patients with genetic diseases would require the genetic defect to be corrected by gene therapy in the individual's HSCs. This approach has limitations due to difficulties maintaining and expanding HSCs in culture. With the development of induced pluripotent stem cell (iPSC) technologies, it conceptually became feasible to consider generating iPSCs from an individual patient, correcting the genetic defect using gene-specific targeting, and then differentiating the disease-free iPSCs into transplantable HSCs. Here we discuss the recent progress in the reprogramming field toward this goal, and the important obstacles that will need to be overcome before any translation of iPSCs into clinical hematology.

WHY INDUCED PLURIPOTENT STEM CELLS ARE AN IDEAL FIT FOR HEMATOPOIETIC DISORDERS

Over 30 000 cases of HSC transplantation are performed each year, but unfortunately over a third of patients who are eligible for this therapy do not find a suitable HLA-matched donor [1[•],2]. The ability to generate an endless supply of banked HLA-matched stocks for allogeneic transplantations, or to generate tailor-made cells matching an individual for autologous transplantation, would be a revolution for clinical hematology. Additional to this, however, is the possibility of curing monogenic disorders through gene correction. Indeed, the hematopoietic system serves as a perfect opportunity to alleviate and/or cure disease using these types of approaches. The decades of HSC transplantations performed in the clinic provide a roadmap for clinical use, and the fact that the hematopoietic system constantly regenerates itself provides an opportunity to replenish a patient's blood system with new or 'corrected' cells. In contrast to other complex systemic diseases, it is already known that if one can generate the right cell, that is a true HSC, it should be able to replace the patient's hematopoietic system.

The proof-of-principle of using iPSC technologies to cure hematopoietic disorders has been performed in a mouse model. The Jaenisch group first demonstrated this by showing that iPSCs generated from a humanized sickle-cell anemia mouse model could be corrected by gene-specific targeting, differentiated into hematopoietic progenitors, and then used in transplantation assays to rescue the sickle-cell anemia phenotype [3]. In our laboratory, we demonstrated this proof-of-concept in human cells by generating iPSCs from Fanconi anemia patients, and correcting them to generate disease-free hematopoietic cells [4]. Yet despite these significant advances toward clinical applications, the problem remains that human HSCs derived *in vitro* from pluripotent cells, unlike their *in-vivo* counterparts, are not able to reconstitute the hematopoietic system [1[•],5]. The challenge of obtaining a population of HSCs that can be indefinitely expanded and/or manipulated *ex vivo*, or derived from an endless supply of pluripotent stem cells, remains the holy grail in the hematopoietic field.

THE MAIN REMAINING CLINICAL HEMATOPOIETIC HURDLE

Prominent researchers throughout the world have been working on ways to develop HSCs *in vitro* that could be used to reconstitute the entire human hematopoietic system. These efforts began a decade ago following the discovery and characterization of human embryonic stem cells (ESCs) [6], with scientists trying to recapitulate the ability of murine ESCs to generate HSCs [7]. However, transduction with HoxB4, which was necessary for murine ESCs to develop into fully functional HSCs, was not sufficient for obtaining fully functional HSCs from human ESCs [5]. Human HSCs derived *in vitro* from pluripotent cells display characteristics of HSCs obtained from in-vivo sources (e.g. express HSC markers, show multilineage hematopoietic potential in clonogenic assays), but unlike in-vivo human HSCs are not able to reconstitute the hematopoietic system of recipient mice (Fig. 1) [1^{**}]. Over the years numerous strategies, including coculturing of human ESCs on primary cells derived from essential regions of hematopoietic development, have been performed to generate HSCs *in vitro* that are capable of long-term engraftment *in vivo*, but this hurdle remains.

Part of the obstacle has been the ability to identify a pure HSC, rather than a group of markers that were representative of an enriched population containing HSCs capable of engraftment. The pioneering work of John Dick's group in this area culminated this past year with a profile of markers that distinguished a HSC that was capable of long-term engraftment at the single-cell level [8^{**}]. Whether this profile of markers can be recapitulated *in vitro* from pluripotent stem cells remains to be tested. However, the identification of a human HSC is an important step for understanding the molecular profile of a HSC. It is thus the hope that this recent discovery will enable an examination of the exact molecular differences that exist between in-vivo and in-vitro obtained HSCs, and provide a more precise understanding of the molecular profile we seek to obtain *in vitro*. For instance, are there certain developmental checkpoints the cells being generated *in vitro* have not achieved? The differences that exist between these populations (Fig. 1) are likely to be due to a combination of intrinsic and extrinsic factors, and insight into these specific molecular differences may more strategically guide the next phase of research in this area. For example, differences in transcription factor networks could be addressed by gain or loss-of-function methodologies (e.g. ectopic HoxB4 was necessary for mouse HSCs to fully engraft the hematopoietic system of recipient mice); signaling pathway variations could be addressed by modifying particular growth factors during differentiation; and differences in adhesion networks could be used to adjust stroma conditions during in-vitro cultures.

In addition to the possibility of generating HSCs by in-vitro differentiation of pluripotent cells, recent advances in other areas suggest alternative future possibilities. Mickie Bhatia's group reported the ability to generate multilineage blood progenitors by transdifferentiating fibroblasts using a single transcription factor, Oct 4 [9]. It is possible that future demonstrations of transdifferentiation from other somatic cell types may contain the proper genetic and/or epigenetic mechanisms that are required for long-term hematopoietic stem cell potential. Alternatively, and a greater focus for many researchers, is the possibility that the field will solve the problem of generating HSCs at a high number through ex-vivo expansion of HSCs obtained from the bone marrow or cord blood. One report found that by

chemically inhibiting the aryl hydrocarbon nuclear receptor protein, human HSCs could be expanded *ex vivo* approximately 17-fold, and still retain the ability to engraft immunodeficient mice [10]. Another study showed clinically relevant ex-vivo expansion of hematopoietic stem and progenitor populations by activating the Notch signaling pathway [11]. Most recently, HSCs were expanded by controlling the inhibitory feedback signaling that occurs in ex-vivo HSC cultures through an automated media dilution approach [12*]. Future studies examining these types of chemical and/or growth factor control treatments may provide improved methods of expanding HSCs *ex vivo* that could be used in a clinical setting [13]. Furthermore, many groups are exploring methods to use iPSCs to generate specific mature blood cell types that could be used clinically, as described in a recent review [14].

PROGRESS IN AND TOWARD THE CLINIC

Assuming we can someday overcome this main hurdle in the hematopoietic field, recent advances in stem cell biology have begun to put the remaining mechanisms required for clinical success in place. Numerous strategies for generating safe, non-integrative methods of reprogramming have been reported [15*]. What remains for the reprogramming field to determine are the functional consequences, if any, for the various genetic and epigenetic aberrations that have been recently reported in iPSCs [16–19,20*,21–24]. What will be equally as important is to determine whether any functional consequences that exist outweigh the potential benefits to the patient.

As these studies progress, it is important to note that there have been significant recent advances in gene-targeted correction in disease-specific pluripotent cells. These methods of performing homologous recombination have included helper-dependent adenoviral vectors [25,26], zinc-finger nucleases [27–29] and bacterial artificial chromosome [30] approaches. Importantly, these studies have shown the ability to generate ‘disease-free’ cells. Specific to any future clinical use for the hematopoietic field, correction of monogenic hematopoietic disorders (e.g. sickle-cell disease) have also been performed in integration-free patient iPSCs [26]. Although in some cases minimal increases in mutational burden were found to be present following gene correction, a better understanding of the causes and consequences of changes in mutational load that occurs within each process is required before any future use of these technologies in a clinical setting. Nevertheless, the progress of genetic correction in pluripotent cells nicely parallels the promising recent clinical success of gene therapy for the treatment of hematopoietic disorders [31**,32**]. These combined results suggest that if the field can obtain HLA-matched HSCs at unlimited or at least sufficient quantities *ex vivo*, they could be readily translated to the clinic.

In light of these many advances toward the possible use of pluripotent-derived cells in the clinic, within this past year we have also seen the use of ESC-based therapies in the media spotlight once again. Late last year Geron announced that the first clinical trial utilizing human ESCs, controversial even within the stem cell community, was going to be halted [33]. Importantly, this was a business decision on Geron’s part, rather than a decision made by any early clinical safety concerns. But in the beginning of this year came the first report of the promise of pluripotent stem cells in the clinic. A group led by Steven Schwartz and

Robert Lanza [34**] transplanted retinal pigment epithelium, derived from human ESCs, into the retina of patients suffering from Stargardt's macular dystrophy and dry age-related macular degeneration, which are leading causes of blindness in the developing world. These cells integrated into the host retina with no signs of rejection or abnormal growth after 4 months, and, significantly, some patients showed signs of visual improvement, albeit modest ones [34**]. Although these cells have been injected into an immunoprivileged site, and are therefore not necessarily indicative of the possible consequences of utilizing pluripotent-derived cells in other tissue types, these encouraging results mark the important beginning of the clinical road-map for pluripotent stem cell use. After years of controversy, these early results provide a welcome reminder of the hopeful possibilities that stem cell research may provide in the clinic.

CONCLUSION

The hematopoietic field will expand on this year's significant advances and continue to tackle the hurdle of obtaining fully functional HSCs in a laboratory setting, whether it be from in-vitro differentiation of pluripotent stem cells, ex-vivo expansion of HSCs obtained *in vivo*, or transdifferentiation from other somatic sources. The reprogramming field will aim to understand the causes and consequences of genetic/epigenetic aberrancies present in iPSCs, and expand on the recent advances in genetically correcting disease. And the roadmap of pluripotent stem cell uses in the clinic has begun. All of these steps will guide us toward the possible treatment of numerous hematological disorders using iPSC technologies.

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KEY POINTS

- The ability to generate an unlimited supply of HLA-matched hematopoietic cells for transplantation would prevent the need for waiting for donor matches, and could enable the possibility of gene corrected hematopoietic transplantation therapies for patients suffering from monogenic hematopoietic disorders.
- Recent progress in the reprogramming field has demonstrated gene-targeted corrections in several disease-specific iPSCs, including those from monogenic hematopoietic disorders.
- Key recent findings in the hematopoietic field have included identification of hematopoietic stem cells (HSCs) capable of long-term engraftment at the single-cell level, progress in ex-vivo expansion of HSCs, and transdifferentiation of somatic cells into hematopoietic progenitors.
- The functional consequences, if any, of the genetic and epigenetic aberrancies recently found to be present in iPSCs will need to be explored before any translation of iPSCs into the clinic.
- The main hurdle of obtaining fully functional HSCs from pluripotent stem cells still needs to be overcome before the full potential of iPSC-derived hematopoietic cells in transplantations can be reached.

FIGURE 1.

Summary of the advances and limitations in human pluripotent stem cell-mediated HSC differentiation. (a) Mouse ESCs or iPSCs can be cultured *in vitro* to differentiate into HSCs that when transplanted into irradiated immunocompromised recipient mice are able to fully reconstitute the entire blood system of the mouse. (b) Human ESCs or iPSCs can be differentiated *in vitro* into cells that display characteristics of in-vivo HSCs (e.g. express HSC markers, form colony-forming units) but when transplanted are unable to fully reconstitute the hematopoietic system of the recipient mice. (c) In the clinic, human HSCs are collected and used for bone marrow transplantations to treat hematopoietic disorders.

Human HSCs obtained from in-vivo sources are also able to fully reconstitute the blood systems of mice. The molecular differences that distinguish human HSCs grown *in vitro* from their fully functional in-vivo counterparts are unknown, and understanding and correcting these differences is an essential step toward unlimited potential to treating hematopoietic disease. ESC, embryonic stem cell; HSC, hematopoietic stem cell; iPSC, induced pluripotent stem cell.

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