Immunogenicity and tumorigenicity of human pluripotent stem cells

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Abstract

Introduction
Pluripotent stem cells (PSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), are capable of unlimited self-renewal and differentiating into all types of cells. Therefore, human PSCs hold great promise for regenerative medicine. Despite rapid progress in translating hESCs into human therapy, many challenges must be overcome for the clinical application of hESCs, such as the allogeneic immune rejection of hESC-derived cells. These problems could be mitigated by the development of iPSCs, reprogrammed from somatic cells by the introduction of defined reprogramming factors Oct3/4, Sox2, Klf4, and cMyc. While great progress has been made in improving the safety and efficiency of reprogramming, iPSCs show flaws in epigenetic and genetic abnormalities associated with tumorigenicity, raising safety concerns that must be addressed before the clinical application of hiPSCs. In this review, we will discuss recent progress in our understanding of the immunogenicity and tumorigenicity of pluripotent stem cells.

Conclusion
In summary, iPSC-derived cells could be immunogenic in syngeneic recipients but immune tolerated due to immune suppressive microenvironment. Significant progress has been made to establish novel strategies to induce immune tolerance of allogeneic hESC-derived allografts.

Introduction

Human embryonic stem cells (hESCs), derived from the inner cell mass of blastocysts, are able to undergo unlimited self-renewal and to differentiate into any somatic cell types belonging to the three embryonic germ layers. hESC-derived cells have been shown to possess regenerative capabilities in animal models of spinal cord injury, Parkinson’s disease, Type 1 Diabetes, cardiomyopathy, and macular degeneration. But there are many challenges such as the ethic burdens and allogeneic immune rejection by the recipients that hinder the clinical application of hESCs.

The iPSC technology developed by Yamanaka group in 2006, could avoid these problems. iPSCs reprogrammed from patient-specific somatic cells, are similar to hESCs in morphology, self-renewal, gene expression of ESC-specific markers. The cell types successfully reprogrammed include neural progenitor cells, mesenchymal cells, pancreatic β cells, blood progenitor cells. Human iPSCs provide enormous opportunities in the biomedical sciences in both cell therapies for regenerative medicine and human diseases modeling. In spite of these encouraging advances, there remain major obstacles blocking the clinical development of human iPSCs-based therapies in patients. Two such major obstacles are the immunogenicity and tumorigenicity of human PSC derived cells.

Discussion

Immunogenicity of hESCs and their derivatives

Allogeneic immune rejection is mediated primarily by T cells activated by foreign major histocompatibility complex (MHC) antigens.

The major histocompatibility complex (MHC), termed Human Leukocyte Antigen (HLA) in humans, consists of glycoproteins encoded by highly polymorphic genes on chromosome 6. The encoding molecules for MHC class I, which are co-dominantly expressed on the surface of almost all vertebrate cells, and MHC class II, which are expressed on the surface of antigen presenting cells, are responsible for antigen presentation. MHC molecules are the main targets of allograft rejection. hESCs express low levels of MHC molecules, leading to low allogenicity. Alvarez and colleagues reported that the expression levels of MHC molecules are decreased during reprogramming of somatic cells into iPSCs, while are increased when iPSCs re-differentiated.

They also demonstrate that low expression levels of MHC class I molecules are associated with the absent or reduced expression of TAP-1 and tapasin components in hESCs and iPSCs. The other analysis of the reprogramming somatic cells and iPSCs by microarray reached similar results. Therefore, the elucidation of the mechanism governing the expression of MHC molecules in pluripotent stem cells is of great significance to decide how to control the immunogenicity of PSCs.

To prevent the allogeneic immune rejection, powerful immune suppressants are available to achieve persistent immune suppression, however, immunosuppressants are highly toxic to the patients with disability diseases, and persistent immune suppression will lead to an increased risk of cancer and infection. Therefore, in designing hESC-based cell therapy of patients with chronic diseases such as various neurodegenerative diseases, new immune tolerance strategies must be developed to resolve the bottleneck of immune...
Immunogenicity of Autologous iPSCs
To avoid the immune bottleneck of hESC-based therapy, it has been assumed that the autologous cells derived from patient-specific iPSCs should be immune tolerated when transplanted into the same patient. In contrast to the popular assumption, Zhao et al. has reported that the inbred C57BL/6 (B6) iPSCs and their derived teratomas induce T cell-dependent immune responses after transplantation into the syngeneic B6 mice. Using the same inbred mouse strain, another study showed consistent result showing that the cardiomyocytes differentiated from B6 iPSCs in vitro are highly immunogenic when transplanted into B6 mice, while B6 iPSCs-derived skin tissue in the chimeric mice is completely immune tolerated by B6 recipients, suggesting that cells derived from iPSCs in vivo will have low levels of immunogenicity.

In addition, iPSCs and their differentiated cardiomyocytes are immune rejected when transplanted into the heart of the syngeneic mice. In contrast, when B6 iPSCs and their derived cells are transplanted under the kidney capsule of the B6 mice, all graft survived without any evidence of immune rejection, indicating that transplantation site might determine the levels of immunogenicity of iPSCs. Furthermore, cells isolated from these grafts did not provoke a secondary T cell response either in vitro or in vivo.

Another study has directly compared the immunogenicity of neural progenitor cells derived from autologous and allogeneic iPSCs in the brain of nonhuman primates, showing that neural progenitor cells derived from iPSCs induce a minimal immune response in the brain, while the allografts cause an acquired immune response. In summary, these studies suggest that certain cells derived from iPSCs might be immunogenic in response to autologous or syngeneic immune system.

Therefore, it is critical to establish a physiologically relevant system to evaluate the immunogenicity of the cells derived from hiPSCs.

Epigenetic and Genetic abnormalities of iPSCs
Epigenetic and genetic abnormalities may occur during the generation, differentiation, long-term culture of PSCs, reprogramming of iPSCs, and early embryogenesis in vivo.

The genetic abnormalities include chromosome aneuploidy, subchromosomal aberrations (gene duplications, deletions and point mutations), and X chromosome inactivation. It has been reported that genomic abnormalities in iPSCs are derived from the genomic varieties of the precursor cells. In addition, recent studies have shown that programming process is associated with a higher coding sequence mutation rate and chromosomal abnormalities in newly generated iPSCs.

At least some of the coding sequence mutations are induced during the reprogramming process. The cause of the somatic mutations remains unclear, but oxidative stresses induced by reprogramming process could play an important role. Recent studies also indicate that pluripotent stem cells generated by somatic cell nuclear transfer harbored significantly fewer exome mutations than iPSCs, indicating that reprogramming conditions can be improved to minimize the genetic instability.

Gene profiling studies have demonstrated that hESCs and iPSCs share similar global gene expression patterns, but are distinguishable by certain transcriptional signature.

Researchers have reported aberrant epigenetic patterns and residual epigenetic memories inherited from their precursor cells. The epigenetic memory might contribute to the immunogenicity of iPSC-derived cells. Some evidence also suggests that some epigenetic and genomic abnormalities can lead to cancer risk. Thus, the genetic and epigenetic abnormalities of iPSCs could contribute to the immunogenicity and tumorigenicity of iPSCs.

The Relationship between the Pluripotency and Tumorigenicity of Human iPSCs
Teratomas are defined as benign germ cell tumors (GCTs), which consist of mature and well-differentiated tissues (mature teratomas), or embryonic and less-differentiated tissues (immature teratomas) with a normal karyotype. Pluripotent stem cells can form teratomas when transplanted in vivo. In addition, many types of human cancer have been shown to express the genes that are used in reprogramming somatic cells into iPSCs, including c-Myc, Klf4, and the pluripotency genes Oct4, Sox2, Nanog, indicating that these pluripotency genes are oncogenic.

In support of this notion, Nanog has been shown to promote epithelial-mesenchymal transition and metastasis of cancers. In addition, recent study has reported that Oct4 suppresses the differentiation of ESCs and promotes reprogramming into iPSCs by inactivating p53 through inducing sirt1-mediated deacetylation of p53. Considering the important roles of p53 in suppressing pluripotency, the transient or partial inactivation of p53 might be required for cells to be reprogrammed into pluripotency. Recent studies have compared the production of iPSCs and oncogenic foci (OF), a form of in vitro generated tumor cells, suggesting that induced pluripotency and tumorigenesis are related processes. Therefore, there is close relationship between pluripotency and cancer.

Conclusion
While human PSCs offer unprecedented opportunities to develop into human...
disease models and provide the renewable sources of lineage-specific cells for cell therapy, many challenges remain to be overcome in order to achieve the full potential of human PSCs. In this context, much needs to be learned about the genomic stability, cancer risk and immunogenicity of human PSCs and their derivatives.

Recent technical advances, such as whole genome genetic and epigenetic analysis as well as the development of humanized mouse models with functional human immune system, have allowed significant progress in addressing the issues of genetic and epigenetic regulation and immune tolerance of PSC-derived cells. Further understanding of the cross-talk between the pluripotency pathways and tumorigenic pathways will help to develop new strategies to minimize the cancer risk associated with PSCs.

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