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Research paper

Precise immune tolerance for hPSC derivatives in clinical application

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ABSTRACT

Human pluripotent stem cells (hPSCs) promise a foreseeing future for regeneration medicine and cell replacement therapy with their abilities to produce almost any types of somatic cells of the body. The complicated immunogenicity of hPSC derivatives and context dependent responses in variable transplantations greatly hurdle the practical application of hPSCs in clinic. Especially for applications of hPSCs, induction of immune tolerance at the same time increases the risks of tumorigenesis. Over the past few years, thanks to the progress in immunology and practices in organ transplantation, endeavors on exploring strategies to induce long term protection of allogeneic transplants have shed light on overcoming this barrier. Novel genetic engineering techniques also allow to precisely cradle the immune response of transplantation. Here we reviewed the current understanding on immunogenicity, and efforts have been attempted on inducing immune tolerance for hPSC derivatives, with extra focus on modifying the graft cells. We also glimpse on employing cutting-edge genome editing technologies for this purpose, which will potentially endow hPSC derivatives with the nature of wide spectrum drugs for therapy.

1. Introduction

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), have the ability to differentiate into all types of cell. hESCs and hiPSCs share substantial similarities in terms of pluripotency and differentiation, but are derived from different origins. hESCs are established from the inner cell mass of human blastocyst [1], while hiPSCs from somatic cells by reacquiring pluripotency, either through ectopically expression of a set of transcription factors or by chemical based regimens [2,3]. In defined culture system, hPSCs can differentiate into all three germ layers [4–6], as well as various types of functional cells of a specific lineage. These derivatives together with the process of obtaining them, provide a valuable system in exploring human embryonic development, modeling genetic diseases *in vitro* and developing cell based therapy [7]. Upon transplantation into disease models such as Parkinson disease [8], Huntington disease [9], Amnesia [10], or heart failure [11,12], the hPSC derived functional entities exhibit remarkable effectiveness and reasonable safety. In most of the cases, transplantations were carried out in experimental settings to verify the functionality of hPSC derived cells, which did not take the possible immune incompatibility into consideration. Although immune tolerance strategies for organ transplantations to certain degree work in allogeneic cell transplantation, advantages of cell therapy are comprehensively compromised by these systematic immune suppressions.

hPSCs are once thought to have low immunogenicity because of low level expression of HLA class molecules or co-stimulation molecules [13]. Yet, this happens mostly in experimental settings of an unrealistic circumstance. Since immune response is an adaptive reaction between graft and host, specific gene expression and epigenetic abnormality of the graft in local niche could potentially lead to immunologic rejection regardless of the cell types. iPSCs in theory should be immune compatible to the individual where they are generated, but depending on types of cell graft, approaches of reprogramming or tissue origins from which iPSCs are generated, there remains variable immune response upon autogenic transplantation of iPSCs or their derivatives [14].

Abbreviations: AAV, adeno-associated virus; ATIIC, lung alveolar epithelial type II cell; B2M, b-2-microglobulin; BAC, bacterial artificial chromosome; CIITA, class-II MHC transactivator; CP hESC, hESC lines constitutively expressing CTLA4Ig and programmed death-ligand 1 (PDL1); CTLA4Ig, cytotoxic T lymphocyte antigen 4 fused with immunoglobulin; HSCs, hematopoietic stem cells; HDR, homology directed repair; hESC, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; HLA, human leukocyte antigen; KD, knockdown; KO, knockout; mAbs, monoclonal antibodies; MHC, major histocompatibility complex; MKPs, megakaryocyte progenitors; MPCs, mesenchymal progenitor cells; MSCs, mesenchymal stromal cells; NK, natural killer; PD-L1, programmed death ligand-1; PG, parthenogenetic; TALEN, transcription activator-like effector nuclease; TAP1, antigen presentation 1; TAPBP, TAP-associated glycoprotein; Tol-DC, tolerogenic dendritic cells; ZFN, zinc finger nuclease

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Further operations on the iPSCs, such as correcting defects of iPSC for autologous based therapies, alterations of the functional genes or introducing of extra DNA fragments into genome may also be immunogenic, triggering de novo immunologic responses. PSCs established through somatic nuclear transfer (SCNT) is another example which should be immune compatible to the nuclear donor, however, because of the mismatched mitochondria, they can trigger certain levels of host immune response as well [15]. Inflammatory environment and cytokines in the recipients are strong triggers of immune response and upon transplantation, HLAs are usually upregulated by these complicated stimulations, which induce immune rejection to the cell transplants [16]. Thus, it remains challenging in the stem cell field to overcome immune rejection for fruitful cell replacement therapy.

Based on current understanding on hPSCs, their immunogenicity and responses that are induced upon transplantation, it is possible to tackle this question either through repressing immune system of the host, or through modifying stem cells to produce immuno-compatible cells for transplantation. Like that in allograft organ transplantation, immunosuppressive drugs can be used to block the immune rejection to hPSC derivatives at multiple steps of the immune response. However, long term use of immunosuppressive agents could generate severe side effects, especially the possible tumor formation or serious infection [17]. With some success in animal models, safer and more effective approaches to induce non-systemic immune tolerance are on their way to clinic. Here, we reviewed updates of how native immune system recognizing and reaction to the transplanted hPSC derivatives, and how novel strategies are developed to induce precise immune tolerance for effective and safe cell transplantation therapy. Importantly, we introduce how the stem cell community employs the fast-developing genome editing technologies to engineer human embryonic stem cells escaping immune surveillance without causing systemic immune repression [18–20].

2. Immune rejection to graft of hPSC derivatives

Mammalian immune system recognizes “self” and “non-self” through a built-in machinery derived during development. Both pathogens and transplanted cells/tissues, or alteration of native cells can activate innate immunity or/and acquired immunity. MHC molecules present fragments of a protein (epitope) to the cells surface, which could be identified as “self” or “hostile”, to T cell. In the context of cell transplantation, both cytotoxic T lymphocytes ($CD8^+$ T cell) and natural killer cell (NK cell) take responsibility to mediate attacks of donor cells. $CD8^+$ T cells of recipient recognize specific antigens presented by class I major histocompatibility complex (MHC-I) molecules. Class II MHC molecules which normally expressed by Antigen Presenting Cells (APCs) can trigger $CD4^+$ T cell to secrete proinflammatory cytokines such as Interferon-γ (IFN-γ), Tumor Necrosis Factor α(TNF-α), Interleukin 12(IL-12) and Interleukin 17(IL-17), and in turn enhance the $CD8^+$/NK cell performance.

Discoveries of the major histocompatibility complex (MHC), known in humans the human leukocyte antigen [21] significantly valued the practice of transplantations [21]. HLAs are mapped to multiple genetic locus of chromosome 6 and are main molecular targets of allograft rejection by host immune system. The class I HLAs, including HLA-A, HLA-B, HLA-C, are expressed on almost all nucleated somatic cells and contain β2 subunits that can only be recognized by $CD8^+$ T cells, a process called cellular immunity. The class II HLAs are HLA-DR, HLA-DQ, and HLA-DP, which are mostly expressed on APCs (macrophage or dendritic cells) [22]. By interacting with $CD4^+$ molecules on surfaces of helper T cell, HLAs class II participate to the establishment and augmentation of the adaptive immunity (Fig. 1). HLAs seem to play a critical role in graft rejection of stem cell transplant, especially when MHCs are upregulated [23], either because of the stages of differentiation, inflammatory cytokines stimulation [22], or particular situation such as teratoma formation [24].

Undifferentiated hPSCs, proved firstly in hESCs express very low level of MHCs, which can protect themselves from being recognized by native T cells in recipient. Directly targeting MHC expression in PSCs might be able to produce HLA-class-I-knockdown hESC lines, which was once thought inducing less immune response upon transplantation of the differentiated derivatives [25]. However, this was proved not the case and graft cells are instead susceptible to be recognized and eradicated by NK cells. Low level of MHC-I on cell surface would lead to NK cell recognition and NK cell-mediated killing, in which Inhibitory killer immunoglobulin-like receptors (KIRs) are actively involved. In normal cells, interaction of KIRs and the appreciated MHC-I molecules protect these cells from cytotoxicity. Without MHC-I expression identifying “self”, the hPSCs will not be recognized by KIRs and become vulnerable to NK cells [26]. Indeed, in the NK-deficient SCID beige mice, teratoma grow much faster than that in normal mice [27], indicating the effective NK-mediated rejection. Similarly, transfusion of iPSC-derived hematopoietic progenitors also causes NK-mediated immune rejection [28]. Thus, destroying the interaction between MHCs and T cell might from one aspect reduce the rejection, it cannot guarantee long-term survival of PSC derivatives [29,30].

In adaptive immune response, beside the primary antigen-specific recognition between T-cell receptor (TCR) and MHCs, a secondary confirmation is needed for full activation of T cells, the so called “co-stimulatory signal”. Varies co-stimulatory molecules coordinately and strictly control the intensity and range of immune response before its “over-heating”. Undifferentiated human PSCs express low levels of co-stimulation molecules but unlike class I MHCs, neither incubation with proinflammatory cytokines nor differentiation increases their expressions [31]. Short-term blockage of co-stimulatory signals extends survival time of the hESC derived pancreatic endoderm grafts [32]. Programmed cell death protein 1 (PD1) and B7 homolog 1 (B7H1; also, known as PDL1) are proved to play an important role in T cell activation, and antibodies targeting these proteins have shown satisfactory therapeutic potentials. In summary, interplay between graft and recipient generates complicated outcomes in stem cell therapies in which the immune recognition and immune responses play critical roles. Graft cells are harshly interrogated by host immune system, and in the presence of co-stimulation signals, mismatched MHCs expressed on PSCs or their derivatives will be directly presented to T cells. NK cells, as well, participate in the immune rejection of graft cells in a MHC independent manner.

3. Immunogenicity of iPSCs

iPSCs hold great promise in “personalized medicine” because of their potential as “customized” cell sources for individual therapy. iPSCs are directly derived from somatic cells, meaning they maintain both the nuclear and mitochondrial genomes and bear MHCs compatible to individuals from whom the iPSCs are generated, thus their derivatives theoretically are recognized as “self” upon autologous transplantation. A patient who suffering from age-related macular degeneration became the first recipient who received autologous iPSC-derived retinal pigmented epithelium in her 70 s [33]. Although this trial greatly stirred the iPSC as well as the stem cell field, it also raised concerns on risks of the reprogramming-related mutations and a reconsideration of the overall advantages of autologous versus allograft transplantations using iPSC derived cells. In this regard, a public bank of HLA-typed iPSCs would be a more practical approach for HLA-matched cell and tissue transplantation.

Because multiple factors affect the immunogenicity of iPSCs which could potentially trigger rejection [34], the concept of iPS cell-based personalized medicine remains controversial [34,35]. Infiltrating T cells were found in graft of undifferentiated iPSCs but not in that of iPSC derived differentiated cells upon transplantation into syngeneic mice [36,37]. Such immunogenicity might be negligible and without clinical significance, at least in the corresponded tissues that receive