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Rise of iPSCs as a cell source for adoptive immunotherapy

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Abstract

Adoptive T-cell transfer is a potentially effective strategy for treating cancer and viral infections. However, previous studies of cancer immunotherapy have shown that T cells expanded in vitro fall into an exhausted state and, consequently, have limited therapeutic effect. One way to overcome this obstacle is to use induced pluripotent stem cells (iPSCs) as a cell source for making effector T cells. In recent years, there have been several reports on generating effector T cells suitable for adoptive immunotherapy. The reported findings suggest that by using iPSC technology, it may be possible to stably derive large numbers of juvenile memory T cells targeted to cancers or viruses. In this review, we describe a strategy for applying iPSC technology to immunotherapy and the characteristics of T cells derived from iPSCs. We also discuss how these

technologies can be applied clinically in the future.

Introduction

T cells play a central role in acquired immunity against pathogens. Since the identification of tumor-specific antigens and their epitopes in the 1990's (1), substantial progress has been made in our understanding of T cell-mediated antitumor responses. From that understanding emerged adoptive cell transfer (ACT) therapy for cancer, which makes use of tumor-infiltrating lymphocytes (TILs) and has achieved some success. For example, Rosenberg's group in National Cancer Institute reported that after lymphodepleting systemic chemotherapy, adoptive transfer of TILs induced clear and reproducible responses in nearly 50% of melanoma patients (2). Despite the therapeutic potential of TIL infusion therapy against cancer and chronic viral infections, there remain two important biological problems must be overcome (3-5). One is that cancer and viruses often hamper or escape T cell immunity by increasing regulatory T cell (Treg) counts, expressing one our more inhibitory molecules (e.g., PD-1L), or suppressing antigen presentation. This can be partially remedied by removing/remodeling the host immune system using chemoradiotherapy or newly developed monoclonal antibodies that have immunoregulatory effects (2, 6). The other

problem relates to the quality of T cells expanded ex vivo. As currently performed, in vitro expansion for ACT induces T cells to differentiate into a late effector state and increase killer activity; however these cells then fall into an exhausted state and do not survive or proliferate in vivo after infusion. Consequently, they have little therapeutic effect (2,7). Optimization of the in vitro culture protocol may improve outcomes, but for now the problem remains to establish antigen-specific T cell clones that need repeated stimulation in long-term culture periods.

To overcome that problem, the therapeutic potential of induced pluripotent cells (iPSCs) is drawing attention (8). iPSCs have the capacity for self-renewal while maintaining pluripotency and could potentially be a major cell source for induction of juvenile T cells suitable for ACT therapy. T cells recognize antigens via their T cell receptors (TCRs). Because TCR genes are irreversibly rearranged in the thymus during T cell maturation, iPSCs derived from T cells (T-iPSCs) retain the rearranged TCR genes of the original cell. It would therefore be expected that redifferentiating T-iPSCs derived from antigen-specific T cells would produce large numbers of juvenile antigen-specific T cells. In mouse, the therapeutic potential of this approach was successfully demonstrated through nuclear transplantation and subsequent in vivo maturation using toxoplasma gondii antigen-specific T cells and alpha-GalCel-specific

Valpha14 NKT cell nuclei (9,10).

Generation of iPSCs from T cells was initially difficult. But over the years several groups, including ours, have succeeded in using integrated viral vector systems to produce T-iPSCs from mouse and human polyclonal and antigen-specific T cells (11-16). This has enabled the potential of T-iPSCs as a cell source for ACT to be explored all around the world.

Generation of rejuvenated antigen-specific T cells using T-iPSCs

In 2013, a series of studies were reported on the establishment of T-iPSCs from antigen-specific cytotoxic T cells (CTLs) and redifferentiation of the T-iPSCs into functional CTLs (16,17). Two Japanese groups, including ours, each succeeded in using a Sendai viral vector to establish T-iPSCs, our group from a HIV nef protein-specific CTL clone and Kawamoto's group from a melanoma MART-1 antigen-specific CTL clone. To differentiate T-iPSCs into CTLs, we employed an in vitro differentiation protocol entailing co-culture with C3H10T1/2 and Delta-like 1-expressing OP9(OP9-DL1) stromal cells. After 35-40 days of differentiation, we obtained CD3+ CD4+ CD8+ double-positive (DP) stage T cells. It is at this stage during physiological thymocyte development that TCR-α gene rearrangement occurs (18, 19). With

T-iPSC-derived DP cells, activation of Rag-1 and Rag-2, two genes related to recombination machinery, was observed, as was a reduction in the percentage of original antigen-specific cells. To create mature CD8 single-positive (SP) CTLs from T-iPSCs without TCR gene revision, it was necessary to artificially end the TCR-α rearrangement. TCR signaling via the peptide-major histocompatibility complex (MHC) during positive selection is known to downregulate RAG expression and prevent further rearrangement of TCR (19). In addition, stimulation using a anti-CD3 antibody is known to mimic TCR signals (20). Both our group and Kawamoto's stimulated differentiated T-iPSCs on days 35-40 using anti-CD3 antibody or PHA and succeeded in producing CD8 SP cells. In our hands, redifferentiated CD8 SP cells recognized the same peptide on HIV antigen as the original CTLs, and immunological assays revealed that these cells exhibit normal cytolytic activity, INF-γ secretion and degranulation when stimulated by their target peptide. In addition, proliferation assays revealed that redifferentiated T-iPSCs had greater expansion potential and longer telomeres than the original cells, indicating CTLs can become "rejuvenated" by passing through the T-iPSC state.

Clonal expansion of acquired immune cells is very useful for clinical application, as has been seen with B cell monoclonal antibodies. This strategy to generate T cells by

passing them through T-iPSCs enables us to expand clinically applicable T cell clones stably and without limit, which could drive innovation in ACT therapy.

Decades of research into cancer immunology have established that strong lymphocyte infiltration is associated with a good outcome in many types of tumors, including melanoma and head and neck, breast, renal, bladder, urotherial, ovarian, colorectal and pancreatic cancers, among others. This provides hope that ACT could become an effective therapy for numerous tumor types (21). At present, however, clinical trials of ACT are performed only in a few tumors, like melanoma. This is in part because of the difficulty of achieving proliferation of tumor-specific CTLs, which must differ radically depending on the immunogenicity of the tumor (22). A stable and unlimited supply of tumor-specific CTLs derived from T-iPSCs will facilitate research into ACT therapy for many types of cancer not treatable with immunotherapy at present.

iPSC based expansion of Mucosal-associated invariant T

In T cells there are some subsets of innate T cells as represented by natural killer T (NKT) cells other than acquired immune T cells. Mucosal associated invariant T (MAIT) cells belong to the innate T cells and are abundant in humans, representing up to 50% of the resident T cells in the Liver, 10% of the peripheral blood mononuclear

cells (PBMCs) were known to play a pivotal role in host defense against a wide range of bacterial and fungal infection including mycobacterium. Recently, Wakao et al reported generating T-iPSCs from human cord blood MAIT cells by using Sev vector and redifferentiated them into MAIT cells. Redifferentiated MAIT (re-MAIT) cells could produce various cytokines such as IFN-γ , TNF-α in the presence of bacteria-fed monocyte as original MAIT cells do. Infusion of re-MAIT cells into immunocompromised nice showed their antimycobacterial activity in vivo (23).

This result not only demonstrate the clinical potential of T-iPSCs derived innate T cells, but also show the possibility of T-iPSC technology as a device to explore the rare or difficult to culture T cell populations

Combination of T-iPSC and CAR technology

Genetic engineering of T cells to express chimeric antigen receptors (CARs) has recently emerged as a promising approach to rapidly generating tumor-targeted T cells endowed with enhanced antitumor properties (24). Most CARs utilize an antigen-derived antigen-binding motif to recognize their target (25). This enables them to recognize cell surface antigens with a higher affinity than TCRs and to function in an HLA-independent fashion, thereby eliminating the need to consider HLA restriction and

overcoming some tumor escape mechanisms. Recently clinical trials of CD19 CAR-modified T cells demonstrated their efficient targeting of acute and chronic lymphoblastic leukemias (26-30). Moreover, promising results on the use T-iPSCs as a cell source for CAR-modified T cells were recently reported (31). By itself, use of CAR technology is a new and potentially problematic strategy, and severe adverse effects have been reported in several clinical trials. But although many aspects of this approach require improvement, the combination of iPSC and CAR technologies offers a promising avenue to the treatment of cancers.

Discussion

T-iPSCs possess great potential as a cell source for ACT therapy. Establishing T-iPSCs from a patient's own antigen specific T lymphocytes and redifferentiating them for ACT therapy is an ideal way to tailor immunotherapy. But this strategy still has significant problems, including the time required and cost. For example, it takes at least 3 months to establish T-iPSCs and redifferentiate them into T cells, and establishing iPSCs for each patient may be too costly for routine use. One possible solution is to generate a T-iPSC bank for common combination of cancer antigen and HLA haplotypes (i.e HLA-A2 restricted cancer antigen WT1 derived specific peptide) and repress

allo-antigenic HLA expression through genomic modification (32,33). If such an antigen-HLA-identified T-iPSC bank could be established, then the dream of coordinating antigen-specific CTLs for timely ACT therapy based on T-iPSCs with matched HLA-type might come true. This banking concept would match well with CAR or exogenous TCR transduction technology to T-iPSC.

Increasing the efficiency of T-iPSC generation and redifferentiation, establishment of feeder-free culture systems for T cells, cost reduction, and many biological and technical challenges lie ahead. But if it were possible to achieve T-iPSCs as a cell source for ACT therapy, then the treatment of malignancies and chronic viral infections would be completely changed, and the impact on medicine would be enormous.

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