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General review

CRISPR/Cas9 genome editing: Fueling the revolution in cancer immunotherapy

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ABSTRACT

The development of genomic editing technologies expands the landscape of T cell engineering for adoptive cell therapy. Among the multiple tools that can be used, CRISPR/Cas9 has been shown to be relatively easy to use, simple to design and cost effective with highly efficient multiplex genome engineering capabilities. Allogeneic universal chimeric antigen receptor (CAR) T cells can be produced by disrupting T cell receptor (TCR) and beta-2-microglobulin (B2M) in CAR T cells or by directly knocking in a CAR at the disrupted TRAC locus. The anti-tumor function can be further boosted by simultaneous ablation of PD-1 and CTLA-4. The anti-tumor activities and safety of TCR-transferred T cells can be improved by knocking out endogenous TCR, which avoids the use of affinity-enhanced TCRs that may lose specificity and cause severe adverse effects. Therefore, CRISPR/Cas9 technology holds enormous promise to advance the field of adoptive cell therapy.

1. Introduction

Adoptive T cell immunotherapy in cancer has entered an era of rapid growth, especially after the approval of Kymriah and Yescarta, which used genetically modified living T cells with chimeric antigen receptors (CARs) as drugs for the treatment of B cell leukemia and lymphoma [1,2]. Apart from modification with a CAR, T cells can also be redirected to target tumors by transfering with a T cell receptor (TCR) [3]. Both CAR- and TCR-modified T cells can recognize tumor antigens and kill tumors. Each strategy has unique advantages and disadvantages. CARs only recognize cell surface antigens, and CAR T cells can be activated in an HLA-independent manner without providing additional co-stimulation. TCRs recognize peptides derived from either cell surface or intracellular proteins that are presented by the MHC (pMHC) in an HLA-dependent manner. Adoptive T cell immunotherapy for cancer treatment, especially for solid tumors, is still facing enormous challenges, such as target specificity, T cell exhaustion and suppressive tumor microenvironments. With a much deeper understanding of cancer immunology and T cell biology, together with the recent clinical experiences with some successful adoptive T cell therapies, efforts and progress have been made to improve the current T cell-based therapies, including, but not limited to (a) improving T cell trafficking by the introduction of chemokine receptors into CAR T cells [4]; (b) improving tumor recognition and bystander discrimination by targeting a broader range of intracellular tumor neoantigens [5], engineering more sophisticated recognition receptor recognition AND-gate circuits [6–8] or improving the sensing of antigen densities by affinity-tuned receptors [9]; and (c) overcoming the tumor suppressive microenvironment by combination therapy with checkpoint inhibitors [10] or engineering cells to ignore suppressive signals by expressing a dominant-negative form of the TGF beta receptor [11], the PD1-CD28 switch receptor [12], cytokines [13,14] or soluble payloads to remodel the tumor microenvironment [15–17]. An effective T cell therapy requires the T cells to persist, proliferate and maintain effective function. Therefore, in addition to the strategies developed thus far, treating cancers is still a complex multifactorial problem. Thus, the toolbox needs to be expanded to equip T cells with most essential capabilities to face the challenges presented by individual cancer types. The development of genomic editing technologies will further expand the landscape of T cell engineering. There are three genomic editing technologies, including zinc-finger nucleases (ZFNs) [18], transcription activator-like effector nucleases (TALENs) [19] and CRISPR/Cas9 [20], which have already been used in the development of T cell products for use in clinical trials to treat different diseases, such as HIV [21] and cancers [22]. The CRISPR/Cas9 system is presently one of the most developed of these tools

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because it is relatively easy to use, simple to design, cost effective, and most importantly, possesses highly efficient multiplex genome engineering capabilities [23–26]. In this review, we summarize the recent work using CRISPR/Cas9 in T cell engineering applications with a focus on the pre-clinical development of off-the-shelf universal CAR T cells and improving the efficacy and safety of TCR re-directed T cells that led to a clinical trial involving the first use of CRISPR/Cas9 for cancer treatment in humans in the U.S.A.

2. CRISPR/Cas9 genomic editing technology in the generation of allogeneic universal CAR T cells

The current sources of the T cells used in adoptive immunotherapy are primarily from the patients themselves in an autologous setting, albeit in some clinical settings, allogeneic donor T cells are used, such as in the situation of allogeneic bone marrow transplantation in which the immune tolerance between the donor and the recipient patient has been established. While the use of autologous CAR T cells has produced successful results in some clinical trials, limitations and constraints exist; for example, a large portion of cancer patients are unable to be treated or are treated with poor-quality T cells at insufficient quantities. Furthermore, the time and expense of manufacturing autologous T cell products also limits the use of the technology by a broad array of cancer patients. The use of genetically modified allogeneic T cells from healthy donors as off-the-shelf "universal" T cells can circumvent the limitations and can potentially be developed into a next-generation highly efficient therapy. Ideally, a batch of universal CAR T cells could be used to treat multiple patients without causing graft versus host disease (GVHD), and the T cells would persist for a long enough time to efficiently eradicate tumors. Therefore, the major barriers that prevent the successful use of "universal" T cells are GVHD and rejection of the infused allogeneic T cells. Recognition of recipient alloantigens by allogeneic T-cells can induce GVHD; conversely, recognition of foreign HLA molecules on donor T cells may lead to rapid rejection. Therefore, both TCR and HLA should be silenced or disrupted in allogeneic universal T cells. Early studies used siRNA silencing to knock down the expression of endogenous TCR to improve the safety and efficiency of introduced TCR by preventing the mispairing of the introduced TCR with the endogenous TCR [27]. The development of genomic editing technologies enables easy and highly efficient gene knockout. ZFNs are engineered endonucleases composed of a tandem array of zinc finger DNA-binding domains that can selectively bind and cut a DNA sequence of choice using the catalytic restriction enzyme FokI [12]. ZFNs were first used to knockout endogenous TCRs to improve the safety and function of TCR-transduced T cells [28]. Later, ZFNs were used to disrupt the endogenous TCR or HLA in T cells to generate CD3/TCR-negative CD19 CAR T cells [29] and HLA-negative CD19 CAR T cells [30]. ZFNs have also been used to ablate PD-1 in the tumor-infiltrating lymphocytes from patients with metastatic melanoma [31]. Similarly, TALENs have been used to disrupt TCR alpha and beta chains to improve the functionality of transduced virus-specific TCRs [32]. Torikai et al. generated universal CAR T cells by genetically editing CD19-specific CAR T cells to eliminate the expression of the TCR alpha and beta chains via TALEN genomic editing to prevent GVHD without compromising CAR-dependent effector functions [32]. Valton et al. recently reported the generation of CAR T cells with a TCR alpha chain and a disrupted deoxycytidine kinase [33]. This study led to a report that two infant B-ALL patients were treated with TALEN gene-edited universal CD19 CAR T cells [34]. The Servier/Pfizer/Cellectis trials using the TALEN technology (UCART19, CALM trial in adults and PALL trial in pediatric patients), which results are anticipated at the EBMT meeting this month. To generate the most high quality universal T cells, a genomic editing tool is required to have highly efficient simultaneous multiplex genomic editing capabilities to ensure that as little manipulation as possible is needed for product processing to produce final products at high quantities. The clustered regularly interspaced short palindromic repeats (CRISPR) system is a newly developed, simple, but very versatile, and precise gene-editing technique with the unique ability to easily achieve highly efficient multiplex genomic editing [23,35]. CRISPR/Cas9 genomic editing of human primary T cells has been proven to be a simple and easy method of achieving high-efficiency multiplex genomic editing, which makes it feasible to simultaneously knock out multiple gene loci in primary human T cells with very high efficiency [36–38].

Using multiplex CRISPR/Cas9 genomic editing, allogeneic universal CAR T cells that are deficient in the TCR beta chain, beta-2-microglobulin (B2M), PD-1 and CTLA-4 have been generated [38–40]. These CAR T cells maintain function both in vitro and in vivo without causing GVHD. Furthermore, with the additional disruption of PD-1, TCR and HLA class I-negative CAR T cells have exhibited significantly improved in vivo anti-tumor activities [39]. CRISPR/Cas9 can also be used to target a CAR to the TRAC locus to generate universal CAR T cells. It has been demonstrated that directing a CD19-specific CAR to the T-cell receptor α constant (TRAC) locus not only results in uniform CAR expression in human peripheral blood T cells but also enhances T-cell potency; these edited cells vastly outperformed conventionally generated CAR T cells in a mouse model of acute lymphoblastic leukemia [41].

Disrupting the expression of HLA alone may not be sufficient to enable universal T cells to survive long enough to completely control a tumor. Additional manipulations of the T cells may be required to ensure that the T cells survive and persist for long periods; such manipulations include those mentioned above regarding issues with NKs and pre-conditioning regimens. Clinical trials and further studies will identify key molecules that are also involved in the rejection of allogeneic universal T cells. Furthermore, the quality and quantity of the T cells are critically important for determining whether off-the-shelf universal T cells could represent an effective therapy. This issue may not be a problem for some hematological malignancies, such as B cell leukemia and lymphoma, for which the outcome of the treatment is not closely associated with the dose of T cells infused over a large dose range; however, the substantial obstacle of generating sufficient numbers of T cells to treat multiple patients from a single manufacturer remains. This obstacle is especially relevant for solid tumors; in this case, an effective dose for a patient could be as high as a few hundred billion [37], which is a number that is close to the maximum yield of one current standard T cell manufacturer. Ex vivo expansion of large amount of T cells is feasible, but potentially at the price of exhaustion and/or generation of short-lived effector T-cells. Efforts to improve the manufacture of T cells with both high quality and yield will eventually move the field forward [42–45].

Knocking out B2M brings down the expression of HLA class I antigen on the cell surface, which raises the issue of whether such HLA class I-negative T cells will be the target of NK cells, which would result in the rejection of the infused HLA class I-negative universal T cells. Whether the HLA class I-negative cells will be rejected sooner than their HLA class I-positive counterparts, which can be quickly rejected by the recipient's T cells, remains an open question. The answer to this question may differ in different clinical settings because the immune systems of cancer patients are often compromised, and the use of a pre-conditioning regimen also influences the outcome. There are some solutions for counteracting NK-mediated rejection, such as using an anti-NK cell depletion antibody or engineering T cells with HLA-E [30,46].