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Genome Editing and Chimeric Antigen Receptors T Cell Therapy

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Abstract

Recent advances in genome editing technologies have significantly enhanced making specific changes in the genomes of different types cells. Genetically engineered T cells, or the 'living drugs', is considered a new era in antitumor therapy. Current clinical trials using chimeric antigen receptors (CARs) T cells showed a promising result in patients with some intractable hematological malignancies. In this Review, some of the most recent advances in CAR T cell therapy are mentioned high lightening the use of genome editing in this field.

Keywords: CRISPR/Cas9, chimeric antigen receptor, T lymphocytes, gene therapy

Introduction

Genome editing increase our capability to explain the role of genetics to disease by enabling making more accurate models of pathological processes[1]. Gene therapy has changed intensely since the first human gene transfer experiment back in 1989. However, despite valid therapeutic hypotheses and strong efforts in drug development, there have been only a limited number of successes in using small molecules to treat diseases with strong genetic contributions[2,3].

Genetic editing technologies

Two of the most prevailing genetic editing technologies established thus far are gene therapy, which empowers rebuilding of missing gene function by viral transgene expression, and RNA interference (RNAi), which facilitates targeted repression of defective genes by knockdown of the target mRNA[4]. However, viral gene therapy may cause mutagenesis at the insertion site and result in dysregulated transgene expression. In the meantime, the use of RNAi is restricted to targets for which gene knockdown is valued[5]. A recent innovative gene editing approaches have made a progress in cancer research. For examples: zinc finger nucleases (ZFNs) are proteins that can identify a specific DNA sequence and cut the desired DNA fragment. The theory was that host cells can distinguish the cut and repair the genome segment from the foreign DNA, giving a new DNA piece into the existing genome[6]. In 2010, another gene editing technique, TALENs, transcription activator like effector nucleases, were offered. However, TALENs were tough to regulate because they required a specific protein sequence[7]. In 2012, CRISPR was proclaimed as being able to adjust host DNA sequences with only a 20-base pair RNA sequence. This gene editing method

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comprises the CRISPR-Cas9 complex, a Cas9 enzyme, acting as "molecular scissors" and gRNA (guide RNA), which escorts the Cas9 enzyme to the right place in the genome, verifying a precise cut. As the host cell identifies DNA damage, it uses the complementary RNA base pairs, through reverse transcription, to insert a new DNA sequence into the genome[8].

Therapeutic Genome Editing Strategies and CAR T Cells

Recently, the use of genome editing for therapeutic drive is superimposing with the field of cancer immunotherapy, mainly by using CAR T cells. These modified T cells prepared with tumour-targeting receptors have proved great possibility in clinical trials treating various hematological as well as solid tumors[9].

CAR T-Cell Therapy

Tumor associated antigens (TAA) expressed in the tumor microenvironment are self-antigens and endogenous T cells are tolerant due to the absence of their recognition of, or activation by, TAA. A single-chain CAR expressed on these T cells can redirect them to a TAA expressed on the cell surface independent of HLA[10,11]. After binding, the signal transduction consequences lead to activation of the T cell and directly killing the target or through relating other mechanisms of the immune system. Therefore, CARs can be used for a range of cancers by replacing their antigen-binding domains, encoded by single chain variable fragments (scFv)[12]. CD19 CARs were the first model to be used, CD19 was chosen as due to its widespread expression on B cell malignancies and its limited expression on B cells but not bone marrow stem cells. The first trials targeting CD19 tangled patients with deteriorated indolent non-Hodgkin's lymphomas (NHL) and chronic lymphocytic leukemia (CLL)[13-16].

CAR structure

The prototypical CAR uses a mouse monolonal antibody (mAb) that harbors a chosen cell-surface TAA activating favorite T-cell activation and effector functions. The specificity of a CAR is attained by its exodomain which is designed from the antigen-binding motif from a mAb that links VH with VL sequences to build a single chain fragment variable (scFv) region[17,18]. Exodomains are finalized by the addition of a flexible (hinge), such as from CD8α and is expressed on the T-cell surface through a transmembrane domain[19]. Upon binding TAA, the CAR triggers T cells via an endodomain which comprises cytoplasmic domains from CD3 or high-affinity receptor FcεRI. This docking approach between CAR and TAA offers the genetically modified T cell with a fully-competent stimulation signal, recognized as CAR-dependent killing, proliferation, and cytokine production[20]. Many adjustments were made to the CAR design to scope some functions, for example first-, second-, and third-generation CARs designed with one, two, or three signaling motifs within an endodomain (Figure 1) that include cytoplasmic signaling motifs resultant from CD28, CD134, CD137, ICOS, and DAP10[21].

New modification for CAR T CELL

Humanized scFv regions were used to decreases CAR immunogenicity. These humanized CARs are designed to avoid immune-mediated recognition leading to abolition of the genetically altered T cells.

Approaches to present CAR constructs into T cells

Numerous approaches are used to introduce CAR constructs into T cells, *non–viral-based DNA transfection* was originally used because of cost and the low risk of insertional mutagenesis. However, it needs

long-term culture and antibiotic selection. *Transposon-based systems* can integrate transgenes more competently than plasmids that do not contain an integrating element[22,23]. *γ retroviruses* are very famous among many researchers; they are easy to produce, they can transduce T cells competently and more important permanently and they are innocent from an integration point in primary human T cells[24]. *Lentiviral vectors* can also proficiently and permanently transduce T cells but are more expensive to making; but safer than retrovirus based on integration preferences. Use of specific promoters in grouping with lentiviral transduction has empowered sustained surface expression of CARs on T cells which ultimately will extend the endurance of CAR T cells *in vivo*[25].

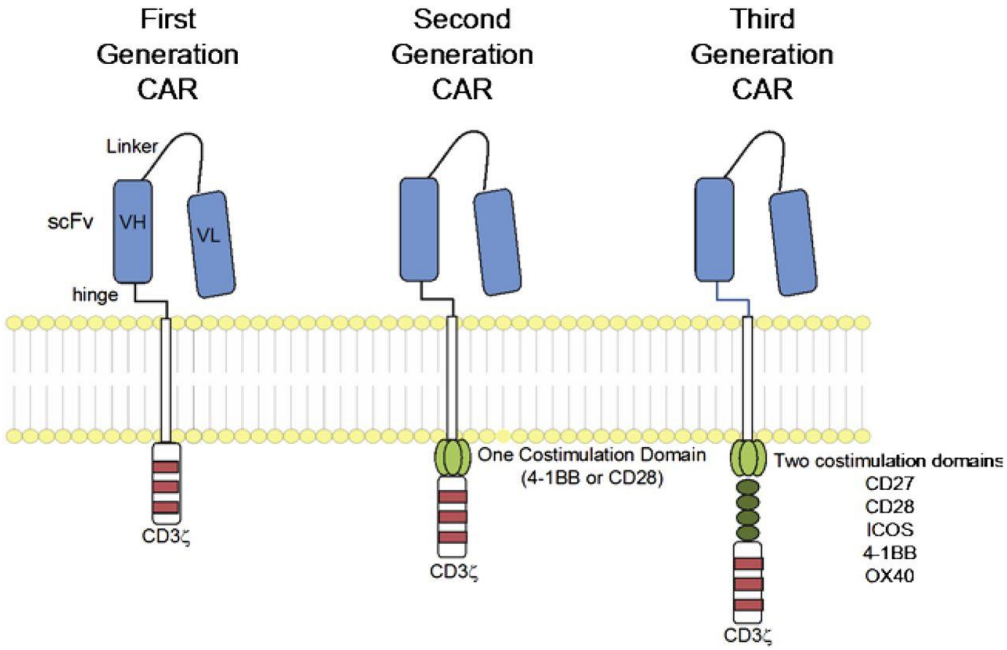

Figure 1: three generations of CAR T cells[21]

CAR T cells and Genome editing approaches

Presently, most CAR T clinical trials apply autologous T cells which is hindered by the poor quality and quantity of T cells and by the time and expense of engineering autologous T cell products. Allogeneic universal donor T cells can be of a great benefit to CAR T cell therapy, as it can increase the number of patients who could be treated by a single CAR T cell product. Though, endogenous TCR on allogeneic T cells may recognize the alloantigens of the recipient, leading to graft-versus-host disease, furthermore, the expression of HLA on the surface of allogeneic T cells leads to quick rejection by the host immune system. That's why, ZFNs and TALENs have been used to knock out endogenous T cell receptor genes in T cells, which could prevent the previous unwanted complications[26]. Another approach to prevent the CAR T cell rejection by the recipient through genome editing strategies by eliminating the

expression of histocompatibility antigens on the donor T cells[27].

Genome editing using CRISPR/Cas 9 and CAR T cells

Complex genome editing is one of the great potentials for advancing T cell–based adoptive immunotherapy. However, the low guiding efficacy of DNA transfection limits the use of complex genome engineering in primary T cells. A new approach named "hit-and-run" delivery was established by using electroporation of Cas9 mRNA and gRNA to present CRISPRs to T cells[28]. A current tripartite gene interruption of TRBC, B2M, and PD1 generated double-negative CD3 and HLA-I. This procedure produced specific CAR T cells to CD19 targets, resilient to host rejection, and incapable of triggering GVHD, therefore opening the way to produce multi-functional universal CAR T cells by using CRISPR/Cas9 techniques[28]. Another method by using multiple sgRNAs aiming at the same genes to advance gene disruption capability, which may potentially increase the off-target effects. Ren et al 2017 established a One-shot CRISPR system, by incorporation of multiple gRNAs in a CAR lentiviral vector. In this way, effective multiple gene modification can be achieved by a single electroporation of various Cas9 mRNAs[29].

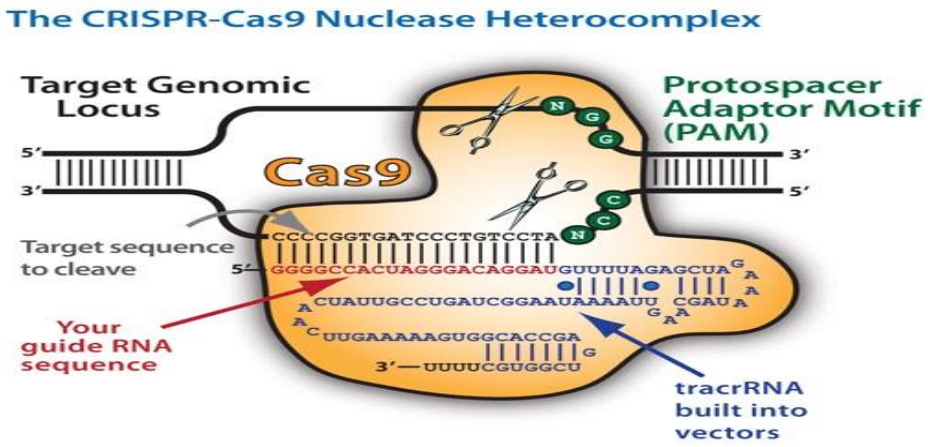

Figure 2: CRISPR-Cas 9 gene system[27]

Summary

The use of CAR T cells has led to fruitful clinical outcomes, particularly in CD19-expressing B-cell acute leukemia. Up-to-date there are three noteworthy evolutions for T-cell-based therapies. First, CAR T cells designed by using CRISPR–Cas9 gene-editing tools that made more-effective antitumor responses than CAR T cells produced using standard methods. Second, the targeted nature of the recent CAR integration might establish safer than random integration. Third, this approach might permit off-the-shelf CAR T cells, a universal cell: not coming from a patient's own T cells. This would make engineering of CAR T cells easier and inexpensive, a very helpful for the treatment of severely immunocompromised patients.

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