

CRISPR/Cas9 Applications in T Cell Modifications for Tumor Immunotherapy

Zhengyang Guo^{1,a,*}

¹*Department of Science, Dalhousie University, Halifax, Nova Scotia, B3H 4R2, Canada
a. zh249112@dal.ca*

**corresponding author*

Abstract: Cancer is a serious global health issue that has a high fatality rate. Therefore, immunotherapy as a promising tumor therapy has long-term, specific targets, et.al, benefits. The powerful gene editing tool CRISPR/Cas9 is commonly used for tumor immunotherapy in modifying immune cells such as T cells. The T cell modification research primarily focused on antigen editing, checkpoint knockout, and cytokine regulation to enhance cell targeting and immune functions. However, current studies justified the T cell immunotherapy feasibility for treating cancer, but the underlying mechanism in CAR and TCR and T cell function regulation is still not clear. This article concentrated on analyzing the CRISPR/Cas9 applications for editing T cell mechanisms for tumor cell targeting. For example, design new CARs or TCRs for either directly or indirectly bound to the tumor cell surface to target tumor cells and activate T cells. Also, analyzed tumor escape-related immune checkpoints (ICs) and the T cell function improvement method of cytokine regulations. Furthermore, this article also summarizes the drawbacks and possible improvement methods for T cell modification in tumor immunotherapy. This article provided the basic understanding and concepts of research and development for T cell immunotherapy for tumor treatment by CRISPR/Cas9 editing. However, the underlying mechanisms and more editing target genes for T cell enhancing need to be studied and discovered in further research to achieve a complete and thorough cure for cancers.

Keywords: CRISPR/Cas9, tumor immunotherapy, CAR-T cell, TCR-T cell.

1. Introduction

Cancer is a serious and urgent health issue, which was considered as the second major cause (17% of all cause of deaths in 2021) of death in United States is cancer, also the major cause of death in agedness (22% of all causes of death in 2021 among 60 to 79 years old) [1]. The conventional treatment of tumors includes surgery, radiotherapy, chemotherapy, and hormone therapy. However, these treatments have several drawbacks. For surgery, it proves ineffective for advanced tumors; also, it is limited by the size, location, and metastasis of the tumor [2]. In the treatment of chemotherapy and hormone therapy, the effectiveness would be suppressed by the gene mutation, transformation of cell signal pathways, or tumor heterogeneity [2]. Furthermore, damage to both tumors and healthy cells would be caused by radiotherapy, chemotherapy, and hormone therapy [2]. Therefore, the new generation of therapy is necessary for cancer patients.

On the other hand, cancer immunotherapy is the new generation of therapy. It has many branches, such as checkpoint inhibitors, cancer vaccines, oncolytic virus therapy, chimeric antigen receptor T cells (CAR T-cells), and adoptive T-cell therapy (ACT) [3]. Furthermore, the tumor immunotherapy has advantages in long-term survival rate and effectiveness, specifically targeting, wide adaptability, comprehensive and thorough elimination, and lower side effects [4]. These benefits led immunotherapy to become an indispensable research field in cancer treatment.

However, CRISPR-Cas9 technology is adapted as the cancer immunotherapy powerful tool. CRISPR/Cas9 system is a bacterial immune system, of combination system of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) for against foreign DNA. When foreign DNA invades the CRISPR gene site, CRISPR pre-RNA (crRNA) would be transcribed from CRISPR, then, combined with Cas protein and constitutively-formed trans-activating RNA to modify the gene [5]. CRISPR-Cas9 was first introduced by Emmanuelle Charpentier and Jennifer A. Doudna. They found that DNA can be modified in knock-in or knock-out nucleotides by substituting the single synthetic guide RNA (sgRNA), also named the single chimeric RNA with crRNA and tracrRNA [6]. Nowadays, CRISPR/CAS9 application in cancer immunotherapy can be used for detecting or knocking out specific tumor genes, therapies based on enhancing T cells' function, PD-1/PD-L1 targeted therapy, and resisting carcinogenic viruses [7, 8].

CRISPR/Cas9, as an emerging technology brought more possibilities in cancer immunotherapy. Therefore, this article will focus on analyzing CRISPR/Cas9 applications in modifying T cells for the identification of tumors and T cell enhancements. Moreover, organizing current challenges and discovering future development direction.

2. Theory of CRISPR/Cas9

The CRISPR/Cas9 system is an adaptive immune defense system of prokaryotes against foreign DNA invasion (e.g., virus, plasmid) [5]. The defense mechanism involves three stages: foreign DNA acquisition, transcription, and interference [9]. First, prokaryotes integrate foreign DNA into the proximal end of the CRISPR array to respond to viral or plasmid DNA invading [9]. Then, the infected CRISPR would be transcribed into precursor CRISPR RNA (pre-crRNA) and further cleaved by the enzyme into the short crRNA. The crRNA would be able to match with trans-activating RNA (tracrRNA), forming guide RNA (gRNA), which has the ability to combine and activate CRISPR-associated (Cas) proteins and recognize and form a heteroduplex with its complementary single-stranded DNA [9, 5].

Furthermore, the protospacer adjacent motif (PAM) is another essential recognition sequence for the Cas9 protein [9]. After the Cas9 protein recognizes the PAM sequence, a site-specific cleavage would be operated after forming a DNA-sgRNA hybrid in the base-pairing complementarity between crRNA with its target DNA sequence [9]. The following two mechanisms of double-strand break DNA repair are non-homologous end-joining (NHEJ) and homologous directed repair (HDR) [5]. NHEJ leads to gene knock-out in the absence of donor DNA, rather than HDR is able to insert or correct genes in the presence of donor DNA [5].

On the other hand, Charpentier's and Doudna's discovery indicated that the complex of crRNA with tracrRNA could be rationally designed [8]. The rationally designed chimeric guide RNA (gRNA) has the same ability as tracrRNA: crRNA duplexes, which can bind and activate Cas9 protein, and are able to recognize the target sequence for editing genes [8]. In summary, CRISPR/Cas9 system achieves prokaryotes' defense mechanism by foreign DNA acquisition, transcription, and target cleavage. Nevertheless, Charpentier's and Doudna's gRNA assumption led the prokaryotes' defense system substituted into a new possibility of gene editing method.

3. Applications of CRISPR/Cas9 Editing T Cells

3.1. T Cell Receptor (TCR)-T Cells

TCR-T cells, indirectly bound target via major histocompatibility complex (MHC) [10]. The two polypeptides TCR α - and TCR β -chain combined a heterodimer TCR chain, which content variable region, constant region, and transmembrane region [10]. The variable regions of TCR chains able to recognize peptide-MHCs, and initiate TCR signaling [10]. (Lack constant region, and transmembrane region functions) However, recruit CD3 and assemble TCR-CD3 complex are necessary for intracellular signaling components to activate T cells, which TCR-CD3 complex contents three CD3 dimers totally consist ten immunoreceptor tyrosine-based activation motif (ITAM) [10]. The intracellular signaling leading to the LCK recruit causing phosphorylation of ITAMs in intracellular domains (ICDs) of CD3 chains [10]. Furthermore, the completely activation of T cells is required the signal 1 (e.g. MHC class I or MHC class II) and signal 2 (e.g. co-stimulation of receptor-ligand pair) interact with ITAMs for [10]. Thus, the TCR-T cell therapy is performed by introducing engineered TCR α β -chains forms a new recognizing target in complete TCR-CD3 complex, and removing endogenous TCR α β -chains for avoiding mispairing among the endogenous chains with TCR α β -chains [10]. According to the theory, CRISPR/Cas9 have been used for knockout endogenous TCR chains to promote the expression of engineered TCR chain transfected into the T cells [5]. For example, the New York esophageal squamous cell carcinoma 1 (NY-ESO-1) tumor-associated antigen was inserted into TRAC locus of TCR chains expressed recognition effect on the tumor cells effectively after using CRISPR/Cas 9 knockout endogenous TCR $\alpha\beta$ [5].

3.2. Chimeric Antigen Receptor (CAR) T Cells

In chimeric antigen receptor (CAR) T cells, the CAR is designed for direct targeting of target surface antigens (non-MHC restriction) to activate T cells [10]. The CAR contains an antigen-binding domain (Fab) from an antibody, a hinge domain, a transmembrane (TM) domain, and one or more intracellular signaling domains [10, 11]. The Fab is the target antigen-specific portion of CAR, which is a single-chain variable fragment (scFv) connected to variable heavy (VH) and variable light (VL) chains [11]. The VH and VL are the complementarity-determining regions, whose lengths affect the CAR target's target epitope's affinity and specificity [11]. While scFvs exist in CARs target extracellular surface cancer antigens, which are able to activate MHC-independent T cells [11]. The hinge region is the extracellular structure region binding with the TM domain and scFv [11]. The hinge region responsible for supporting flexibility to overcome steric hindrance and providing the length allows the antigen-binding domain to associate with the target epitope (i.e., a shorter hinge length leads to a higher affinity for binding membrane-distal epitopes) [11]. The TM domain is fundamentally adapted to anchor the CAR into the T cell membrane [11]. The CAR TM domain is related to the stability, expression level of CAR, also involved in the activation of signaling, synapse formation, and downregulation of endogenous signaling molecules [11]. For the co-stimulatory domain, there are three generations of CARs. The first-generation included CD3 ζ or Fc γ signaling domain, which CD3 ζ drives immunoreceptor tyrosine-based activation motifs [10, 11]. However, the method has been identified that the CD3 ζ or Fc γ signaling domain cannot generate enough response in T cells [11]. The second-generation CARs are the compounds of a series of a co-stimulatory domain with the intracellular signaling domain of CD3 ζ [11]. Where the CAR with CD28 differentiates into effector memory T cells for aerobic glycolysis [11]; whereas the CAR with 4-1BB domain differentiates into the mitochondrial biogenesis and oxidative metabolism functions, improved expressed central memory T cells [11]. The third-generation CARs are the series of two co-stimulatory domains with the CD3 ζ intracellular signaling domain [11]. However, the third-

generation has exhibited improved function of higher synthesis of cytokine in lymphoma and pulmonary metastasis than the second-generation, but the third-generation did not present higher clinical effects in leukemia and pancreatic cancer models than the second-generation [11].

4. Human T Cell ICs in Tumor Escape

The ICs are the normal physiological immune system regulators located on the surface of immune cells [12]. They have the ability of a “checking and balancing” mechanism to avoid inappropriate immune response in healthy cells [12]. Below are the common T cell ICs involved in tumor escape.

4.1. Programmed Cell Death Protein 1/L1 (PD-1/PD-L1) Signaling Pathway

The type I transmembrane glycoprotein PD-1 is a member of the CD28 superfamily, which has a cytoplasmic domain (CD), TM domain, and a single extracellular immunoglobulin variable domain (sIgV) that contains immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) [13]. Activated T cells are the primary source of PD-1, a coinhibitory receptor for PD-L1, which is also expressed by B cells, natural killer (NK) cells, myeloid cells, monocytes, neutrophils, and dendritic cells (DCs) [13, 14]. PD-1 targeted ligand PD-L1 expressed on the surface of antigen-presenting cells, tumor cells, and normal tissue cells [13, 14] engages in trans interactions with PD-L1 to release inhibitory signaling on the surface of T cells to suppress T cell activities [14]. In addition, the cytoplasmic domain presented ITIM and ITSM would be phosphorylated after the binding between PD-1 and PD-L1, causing enhanced inhibition of TCR signaling transduction by recruiting Src homology 2 domain-containing tyrosine phosphatase 2 (SHP2) [13, 14]. Thus, the PD-L1 on the tumor cell surface benefits tumor immune escape by binding with the PD-1 receptor in the immune cells. Su et al. knocked out the PD-1 gene in primary T cells (PBMC-derived T) and analyzed the effect in M14 melanoma cells and AGS-EBV gastric cancer cells found that the T cells have a constant activation status and memory under the condition of a lack of PD-1 expression. They used interferon- γ Elispot assays to analyze the effect of PD-1 knockout, indicating that the promoted T cells have improved antigen immune response by increased IFN- γ synthesis [15]. Also, the promoted T cells have developed T cell cytotoxicity in higher tumor lysis ability after PD-1 knockout [15].

4.2. Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) Signaling Pathway

The combination of CD28 and CD80/86 forms a co-stimulatory complex that stimulates T cell activation by improving T cell proliferation and differentiation, and enhancing the progression of the cell cycle [13]. However, CTLA-4 is an inhibitor for the co-stimulatory complex that has higher affinity than CD28 to complete CD80/86 sites with CD28 [13, 14]. CTLA-4 is located on the activated conventional T lymphocytes only, and expressed primarily on the surface of immunosuppressive T regulatory cells (Tregs) [13]. CTLA-4 signaling pathways include surface localization regulation, intracellular signal inhibition, enhancement of Treg function, and exogenous ligand regulation [14]. For surface localization regulation, CTLA-4 could be phosphorylated to suppress CTLA-4 removing from CD80/86 from binding between CTLA-4 and endocytic adaptor protein AP-2 leading to the continuously transmit inhibitory signals [14]. In intracellular signal inhibition, CTLA-4 able to recruit SHP2 and has possibility to recruit PP2A causing to inhibit activation of T cells [14]. The CTLA-4-induced contact-dependent suppression of Treg cells attracts PKC η to the immunological synapse, where it forms the CTLA-4/PKC η complex [14]. Additionally, it recruits the GIT2- α PIX-PAK complex [14]. CTLA-4 able to lead inhibition of extrinsic mechanisms of T cell signaling in reducing CD80/86 expression and expressing CTLA-4 causes indoleamine 2,3-dioxygenase (IDO) to tryptophan depletion and T cell suppression [14]. In summary, CTLA-4 competitive binding with

CD28/CD80/86 to suppress the co-stimulatory signal mediated by CD28 and induce inhibition signal cause T cell activities reducing. Shi et al. [16] research tested and found CTLA-4 knockout T cells have enhanced anti-tumor activity on HCT116 colon cancer cell line. Lack expression of CTLA-4 weakened the tumor cell activation in 58.26% [14]. Also, CTLA-4 knockout T cells doubled the amount of late apoptosis tumor cells by enhanced the caspase-3, caspase-8, and caspase-9 in 10 to 8 times than control [16]. Moreover, CTLA-4 knockout T cells exhibited cytokines promotion in over 2 times expression of TNF- α and IFN- γ [14]. And the promoted T cells decreased the fatality rate of the mice from 100% to 10% at 65 days after HCT116 colon cancer infection [16].

4.3. Lymphocyte Activation Gene-3 (LAG-3) Signaling Pathway

LAG-3 is an inhibitory transmembrane receptor for effector T cells (Teffs), Treg, NK cells, activated B cells, and plasmacytoid DCs [13]; also, it is a ligand of MHC-II [13]. LAG-3/MHC-II has higher affinity than CD-4/MHC-II, the binding between LAG-3 with MHC-II lead to competitively inhibits CD-4/MHC-II interaction, leading inhibition of CD4 T lymphocytes' activation in T cells' proliferation, activation, cytotoxicity and cytokine production [13, 14]. However, the binding mechanism between LAG-3 with MHC-II still not identified, LAG-3/MHC-II complex considered as directly binding, while the new studies illustrated there might has more controversial ligands between LAG-3 and MHC-II [13, 14] (e.g. LSECtin, galactin-3 (Gal-3), and fibrinin-as-protein 1 (FGL1) [13]). Furthermore, the expression of LAG-3 on Treg surface has been found to show that the interaction causes the inhibition of proliferation and immunosuppressive function in Tregs mechanism is unclearly and needs to be further clarified [14]. Thus, LAG-3 as an inhibitory transmembrane receptor for CD-4/MHC-II complex cause the inhibition of T cells and tumor cell escape, while the completely interactions of LAG-3 with Treg for T cells still need to further researches. Durham et al. detected LAG-3 knockout influence in T cells indicated the LAG-3 knockout T cells have enhanced proliferation activation of CD4+ T cell, higher resistivity for Treg, and tended development into TH1 phenotype (increased IFN- γ and Tbet expressions) [17].

4.4. T-cell Immunoglobulin and Mucin-Domain Containing-3 (TIM-3) Signaling Pathway

The extracellular N-terminal variable immunoglobulin (IgV) domain of TIM-3 can form a binding with its ligands, which include Gal-9, carcinoembryonic antigen associated cell adhesion molecule 1 (CEACAM1), non-protein ligand a mixture of (PtdSer), and high mobility group box 1 (HMGB1) [13, 14]. The binding between TIM-3 and Gal-9 leads to Th1 effector cells' apoptosis and limits the synthesis of IFN- γ [13]. CEACAM1/TIM-3 complex formation inhibits cell-mediated autoimmunity and anti-tumor immunity [13, 14]. The non-histone chromatin-associated protein HMGB1, which could be released into the tumor microenvironment (TME) [14]. The binding with TIM-3 would suppress the apoptotic tumor cell nucleotide acid recruitment onto the endosome of dendritic cells (DCs) [14]. The binding of PtdSer and TIM-3 induces the clearance of efferocytosis-recognized apoptotic cells, leading to a reduction in the reorganization of the immune system [13, 14]. The Tregs, CD4+ T cell subsets (Th1/Th17), and CD8+ T cell subsets (Tc1) have TIM-3 expression to suppress T cell activation and proliferation, and downregulate immune responses by decreasing cytokine synthesis, leading to downregulation of anti-tumor immunity [14].

5. CRISPR/Cas9 Enhances T Cell Functions by Gene Modifications

Heeren's research (2020) [18] gathered statistics about CIRSPR/Cas9 application in enhancing T cells' cytokine synthesis by editing T cell genes. The author collected the impact of cancer types by human T cell gene editing led to the cytokine production amounts in vitro and vivo [18]. The research exhibited some valuable gene editing in DGKA/DGKZ, IFNG 3'UTR, PTPN2, and TGFB2, also

compared the secretion changes in IFN- γ , TNF- α , and others (e.g. IL-2, IL-17, CD107a, and Gzm family) [18].

5.1. Interferon- γ (IFN- γ)

Interferon- γ (IFN- γ) is mainly produced by activated NK cells and T helper 1 (TH1) cells, also minorly produced by DCs, B cells, and macrophages (M ϕ) [19]. IFN- γ and IL-12 are able to co-induce the transcription factor T-bet expression; also, the inducing cause stimulation of IFN- γ expression by the T-bet encoded region IFNG locus chromatin remodeling and regulation of T-bet [19]. In addition, in NK cells, the type I interferons (e.g., IFN α , IFN β) can induce IFN- γ expression via STAT4-mediated signaling, while the coordinate induction of IL-12 and IL-18 results in NK and CD8 T cells producing IFN- γ [19].

IFN- γ regulates M ϕ , DCs, T cells, and T follicular helper (TFH) cells [19]. IFN- γ enhances the expression of IRF1 and IRF5 in M1 polarized M ϕ , respectively, resulting in induces pro-inflammatory gene expression and the promotion of the production of nitrogen and oxygen intermediates after IRF1 enters the nucleus, and the stimulation of pro-inflammatory cytokines expression and downregulation of synthesis of anti-inflammatory cytokine IL-10 are caused by IRF5 [19]. Furthermore, IFN- γ is also involved in DCs, non-hematopoietic cells, and other M ϕ s' functions [19]. For example, it improves the antimicrobial activity by enhancing microbicidal materials formation and phagocytic capacity; it has been identified participating in granuloma-containing lymph nodes exhibition caused granuloma formation and the suppressive effects of anti-IFN γ antibodies caused granuloma formation; IFN- γ can enhance chemokines and adhesion molecules secretion; also, it triggers CXC receptor 3 (CXCR3) ligands (e.g. CXCL9, CXCL10, and CXCL11), leading to DCs, M ϕ , and non-haematopoietic cells activations [19]. In other words, it can promote DC maturation and T cell differentiation to improve the adaptive immune response during the early phases by triggering TH1 cell and TH2, and TH17 cell responses [19]. IFN- γ can also induce T-bet expression, benefiting the B cell lymphoma-6 (BCL-6) protein in enhancing TFH cell deafferentation and improving immunoglobulin class in B cells [19].

5.2. Tumor Necrosis Factor- α (TNF- α)

One of the best-characterized pro-inflammatory signaling pathways in the context of cancer and other pathological conditions is identified as TNF- α and its receptor TNFRs [20]. It is a multipurpose pro-inflammatory cytokine that can control a wide range of processes related to inflammation, vascular functions, insulin sensitivity, coagulation, metabolism, cellular apoptosis, and tumor growth and invasion [20]. Through its receptor TNFR1, TNF- α promotes the migratory and morphological alterations of lymphatic endothelial cells (LECs), which is linked to the enhancement of lymphangiogenesis [20]. TNF- α also stimulates TAMs lead to VEGF-C expression, further promoting lymphatic vessel network formation by activating the VEGFR3 signaling pathway [20]. Additionally, TNF- α overexpression is highly related to the density promotion of lymphatic vessels within the tumor and increased lymph node metastasis [20].

5.3. Other Cytokines

IL-2 has functions of promoting CD4 $^{+}$ and CD8 $^{+}$ T cell proliferation, and regulating T cell differentiation in the upregulate effect for TH1, TH2, TH9, and downregulating effect for TH17, and TFH [21]. By preventing Teff activation and proliferation and controlling the placement of Treg cells inside organs and tissues, IL-2 can also preserve Treg cell survival and enhance its functions [21]. IL-2 is related to the determination of CD8 $^{+}$ cell fate, the differentiation of effector cells into short-lived effector cells to promote the immediate clearance caused by high expression of IL-2R α , while

effector cells differentiate into long-lived memory cells (TMem) promote long-term immune protection under low IL-2R α expression [21]. Also, IL-2 is able to induce activation-induced cell death (AICD) and eliminate self-reactive cells, and enhance NK and CD8⁺ T cell cytotoxins [21].

IL-17 functions in mucosal sites (e.g., respiratory, gastrointestinal, and cutaneous mucosa) to improve tissue re-epithelialization and barrier repair [22]. Also, it can enhance the immune defense of antimicrobials by triggering metabolic adaptation and inflammatory responses, and promote wound repair in improving epithelial cell proliferation and recovery of barrier function [22].

CD107a (also known as LAMP-1) is a lysosome-associated membrane protein that is expressed on the inner membrane of the lytic granules of NK cells and cytotoxic T cells (CTLs) [23]. When granular contents are released into the synapse, CD107a would be transported to the cell surface after NK or CTL recognized target cells [23]. This feature leads to CD107a being adapted as the functional marker of cell degranulation activity [23].

Granzymes (Gzm) are a family of serine proteases, also known as cytotoxic initiators of target-cell death [24]. GzmA and GzmB are synthesized by CTL, NK, and NKT cells, whose intracellular functions lead to cell apoptosis and pyroptosis. GzmA can trigger thrombin receptor activation, muscle fiber injury, pro-inflammatory cytokine production, osteoclastogenesis, and multiple-organ inflammation in the extracellular space [24]. Additionally, GzmB can enhance the generation of anoikis and autoantigens, the separation of the dermal–epidermal junction, cytokine processing, the malfunction of the epithelial barrier, the remodeling of the extracellular matrix, and the release of growth factors [24].

5.4. Consequences of Different Gene Site Edition in T Cells

Enzymes diacylglycerol kinases (DGKs) for diacylglycerol metabolism catalysis, which were identified as potential immunotherapy targets for suppressing CD3 signaling and T cell effector function [18]. While DGKA/DGKZ double knockout in human T cells improved INF- γ and IL-2 secretions and enhanced T cell cytotoxicity in vitro, and increased INF- γ and GzmB productions result in Glioblastoma suppression in vivo [18]. After knocking out TGFBR2, human T cells present the upregulation in IFNG, GZMA, and GZMB expressions in vitro; also, cause to the lung tumor regression by developing INF- γ and IL-2 expressions [18]. Additionally, the knockout of protein tyrosine phosphatase non-receptor 2 (PTPN2) lead to the increased expressions of INF- γ and IL-17 [18]. On another hand, editing of the AU-rich elements (AREs) site of IFNG gene's 3'UTR able to enhance INF- γ expression [18]. ARE encoded in the 3' UTR of T cell IFNG gene for suppress INF- γ production during resting state, while knock out ARE, the T cell would express INF- γ mRNA to synthesize INF- γ in both resting and activating states of T cells [18].

6. Challenges and Improvement Methods of CAR and TCR T Cell Therapies

TCR and CAR T cell therapies commonly have conquer the challenges of antigen escape, toxicity risks, and immune microenvironment inhibition, also some unique disadvantages [11, 25]. Some types of tumor cells able to escape the engineered T cells' target by losing of target antigen during long-term treatment. For examples, the target antigen losing occur in MAGE-A3 targeted therapy of TCR lead to recurrence [25], while CAR targeted IL13Ra2 in glioblastoma exhibited IL13Ra2 expression downregulating causes tumor recurrences [11]. The off-target toxicity feature in both TCR and CAR occurs in engineered T cells targeting and function on the normal cells, such as excessive TCR affinity induced cross-reactivity in the target of MAGE-A3, and CAR induced neurotoxicity (ICANS) and cytokine release syndrome (CRS) [11, 25]. Furthermore, the inhibitory factors in TME result in T cell exhaustion to decrease TCR T cell functions [25], and fibrotic matrix of solid tumors and myeloid suppressor cells (MDSCs) suppress CAR T infiltration inhibit its activity [11]. On

another hand, TCR and CAR therapies have their unique challenges. For examples, in TCRs, the downregulation of MHC in some tumor cells cause TCR lose efficacy, and the heterodimer formations of exogenous TCR and endogenous TCR result in new auto-reactivity generation and graft-versus-host disease (GVHD) [25]. In CARs, some health cells possibly have target antigen that leads to the target-induced tumor regression toxicity, the scFv in the CAR structure may lead to non-specific activation due to the excessive affinity or deviation in epitope recognition, and the mismatch between the physical barrier and chemokines cause weak in migration and infiltration abilities [11].

The improvements of TCR and CAR involve in multiple targets, cell function development, and cytokines regulation [11, 25]. Multiple targets designed T cells able to against antigen escape, such as simultaneously targeting NY-ESO-1 and MAGE-A3 in TCR, and dual-targeting CD19 and CD22 in CAR [11, 25]. Cell gene editing strategy able to improve T cell availabilities. For examples, knock out endogenous TCR in TRAC and TRBC able to downregulate heterodimer formation and simultaneously knock out PD-1 to develop anti-exhaustion capacity in TCR, and knock out PD-1 or CTLA-4, or introduce dominant negative TGF- β receptor resistance to enhance TME inhibition in CAR [11, 25]. Cytokine regulation for improving T cell survival and function [11, 25]. Regeneration of thymus can be improved to achieve the the T cell pool restoring in TCR [25]. While design T cell for upregulating IL-12 or IL-15 synthesis able to increase proliferation and anti-TME ability [11].

7. Conclusion

CRISPR/Cas9 is a powerful gene editing tool, which able to perform gene knockout by the process named NHEJ, and gene designing by the process named HDR. The two of common editing T cells target mechanisms are designing chimeric antigen receptor (CAR) and TCR for tumor immunotherapy. CAR able to directly target cell surface antigens to induce T cell immune responses. While TCR target cell leads to T cell activation by indirectly bound through MHC. However, tumors able to escape T cell immune responses in the physiological immune system regulators ICs. Thus, CRISPR/Cas9 knock out T cell ICs enhances T cell capacities and prevent tumor escape from occurring. For example, the bounding between tumor receptor PD-L1 and TCR PD-1 inactivate T cell achieving tumor immune escape, and T cell PD-1 knockout help to prevent tumor escape as well as enhance T cell cytotoxicity in higher tumor lysis capacity. Furthermore, T cell can be improved by gene modifications. The double knockout genes in DGKA and DGKZ justified cause the cytokines T-bet expression and T cell differentiation related IFN- γ and cell apoptosis and pyroptosis related GzmB secretion improvements. However, CAR and TCR immunotherapies have some drawbacks. The antigen escape, off-target toxicity, and TME inhibition are common challenges; while the TCR unique disadvantages tumor downregulation of MHC and heterodimer formation between exogenous TCR and endogenous TCR, and CAR has unique drawbacks in target healthy cells, non-specific activation, and weaker migration and infiltration abilities. Thus, the T cell developments are necessary, which basically prevent in multiple targets designing against immune escape, and T cell enhances ICs knockout and cytokine regulations. Overall, this article primarily focused on the tumor immunotherapy in T cell modification methods in targeting and function developments. However, this article only gave one of the concepts in tumor immunotherapy, and more potential methods can be adapted. Therefore, the other potential T cell identification mechanisms, IC and gene sites in enhancing T cell functions, and immune cells could be focused on in further research of CRISPR/Cas9 applications in tumor immunotherapy.

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