Novel CAR design for tumor CAR-T cell therapy

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Abstract

Harnessing patient's own immune system to recognize and fight against cancer cells is a permanent goal of immunologists. Adoptive T cell therapy (ACT) is an important treatment approach within cancer immunotherapy, which mainly includes tumor infiltration lymphocyte (TIL) therapy, chimeric antigen receptor (CAR) T cell therapy and T-cell receptor (TCR) T therapy. Among them, CAR-T cells are emerging as a promising treatment modality in treating patients with leukemia and solid tumors. In 2017, two CAR-T therapies were approved by the US Food and Drug Administration for patients with lymphomas. However, continuous efforts are required to increase the specificity of CAR T-cells against tumor cells, and are essential to improve their anti-tumor activity in solid tumors. This review summarizes the novel designs of CAR molecule to enhance the anti-tumor activity, overcome immunosuppressive tumor microenvironment, and reduce toxicities.

Keywords

Chimeric antigen receptor, CAR-T, immunotherapy, CD19, tumor.

1. Introduction

The chimeric antigen receptor (CAR) T cell immunotherapy, is a form of genetically modified autologous T cell therapy aimed to treat leukemia and solid tumors [1]. The CAR is an antigen-binding receptor containing an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain is also called single-chain variable fragment (scFv), which is derived from variable region of light and heavy chains of antibody molecules, and connected by a glycine and serine-rich flexible linker. The scFv, similar to an antibody, can recognize and bind to a specific antigen. The hinge and transmembrane domain is a hydrophobic region in CAR that connects the scFv and the intracellular stimulatory domains, anchors them to the cell membrane, and supports their functions. The intracellular domain is composed of T-cell activation domain and co-stimulatory domain [2-4].

To generate personalized CAR-T cells, patients' autologous T lymphocytes are isolated and cultured for transduction and expansion in vitro. The CAR encoding transgenes were stably integrated into the genome of T lymphocytes mainly through gamma-retrovirus or lentivirus based transduction. The CAR T cells after transduction will be expanded for weeks to sufficient numbers, then re-infused into the patient's body. The infused CAR T cells will bind to the tumor-specific antigen and act as "living drugs" that may exert both immediate and long-term effects [5]. Such binding of the CAR T cells with tumor antigen would cause the secretion of large quantities of cytokine, inducing activation and proliferation of CAR-T cells and lysis of the tumor cells. Clinical trials have demonstrated the potent activity of anti‐ CD19 CAR T cells against multiple subtypes of B-cell lymphoma [6-8]. In August 2017, the Food and Drug Administration (FDA) approved Novartis' Kymriah, the first anti-CD19 CAR-T therapy for children and young adults with acute lymphoblastic leukemia. Just one month later, the FDA approved a second CAR-T therapy, Kite's Yescarta (axicabtagene ciloleucel) for patients with large-B-cell lymphomas whose cancer has progressed after receiving at least two prior treatment regimens.

2. Novel Design in Extracellular scFv Domain

2.1 Bispecific Chimeric Antigen Receptor

In clinical trials of CAR-T cell immunotherapies, relapse caused by antigen-escape variants of the tumor cells is common after CAR-T immunotherapy, these tumor cell variants would be left untouched by the original CAR T cells. In response to antigen-escape tumor variants, bispecific CAR-T cells, or tandem-CARs are engineered to simultaneously target two different tumor associated antigens to mitigate antigen escape and improve the anti-tumor activity of T cells [9, 10]. These dual-scFv construct consists of two scFv ligand-binding domains connected by a linker, encoded by one viral vector.

In 2013, the proof-of-concept attempt in creating a tandem CAR was explored by Grada and colleagues, they created an artificial chimeric antigen receptor that simultaneously target CD19 and HER2. This bispecific CAR design showed enhanced functionality upon simultaneous encounter of both target antigens and preserved tandem CAR T cell-induced cytolysis in a model of antigen loss [11]. Several research groups reported the anti-CD20/CD19 bispecific CAR exhibits superior anti-leukemic activity and may reduce the risk of relapse through antigen-loss in the long term [10, 12, 13]. For example, Schneider and colleagues engineered primary human T cells with a tandem-CAR encoding lentiviral transduction, allowing the CAR-T cell to recognize tumors with either CD19, CD20 or both. While being equally effective with similar cytokine production to CD19 CARs, the resulting tandem-CARs are showed to be more effective in killing tumor cells and less toxic [10].

In another report, Ruella and colleagues found that in human leukemia relapses post-anti-CD19 CAR-T treatment, CD123 was highly expressed. So they engineered a tandem-CAR targeting both CD19 and CD123, NSG mice tumor model results showed that this tandem-CAR combining CD19 and CD123 targeting has higher activity than single antigen targeting CAR against B-ALL in vivo, thus making it a promising solution to prevent relapse [9]. Positive results of tandem-CARs targeting HER2 and IL13α2 have also been shown in a murine glioblastoma models, which demonstrated enhanced antitumor activity in antigen escape tumor variants, and improved general survival in mice tumor model [14].

In general, bispecific CAR T therapy has shown promising results in antigen recognition specificity, high level of cytotoxicity, and an efficient way to prevent relapse caused by antigen-escape tumor variants.

2.2 Universal Chimeric Antigen Receptor Design

Traditional CAR is composed of a fixed antigen-specific scFv and intracellular signaling domains. This fixed design restricts the antigen specificity and affinity, limits the controllability of CAR T cell activation level, leads to the management of CAR T cell-related toxicities to be challenging [15]. Recently, Cho and colleagues designed a split and programmable CAR (SUPRA CAR), in which the scFv and signaling domains were divided into two separate parts: a transmembrane zipCAR and a soluble zipFv. The zipCAR consists of an intracellular signaling domain fused to a leucine zipper as the extracellular portion, while the zipFv portion is made of a scFv fused to a leucine zipper. When zipFv binds to the zipCAR leucine zipper, it can connect the antigen-binding domain and T cell signaling domain of the chimeric receptor to activate the anti-tumor activity of the SUPRA CAR T cells. In a cellular experiment, the addition of zipFv targeting either Her2, Axl, or both led to efficient killing of a Her2 and Axl positive K562 cell line, illustrating the potential of the SUPRA CAR system to combat antigen escape. Moreover, the SUPRA CAR system also demonstrated robust cytolytic activity in several xenograft tumor models [16]. With the ability to easily change its scFv domain, the split design of SUPRA CAR T cells overcomes major setbacks such as the need of reengineering after antigen escape and severe cytokine release storms in certain patients in the case of single-antigen or tandem-CAR T therapies, thus such therapy could be a more effective and versatile alternative to the conventional CAR T designs.

2.3 Nanobody Based Chimeric Antigen Receptors

Munter and colleagues constructed a bispecific CAR targeting CD20 and Her2, with its two scFvs derived from the VH domain of nanobody. This dual specific nanoCAR can be roughly the same size as a conventional single-antigen CAR, as the scFv domain of conventional CARs consists of both VH and VL. This more compact design of nanoCAR could improve the function and persistence of the CAR T cells by reducing potential self-aggregation. These nanoCAR T cells were then tested the antitumor effect to Jurkat cells expressing CD20 and Her2, results demonstrated that bispecific nanoCAR T cells exhibit robust killing ability [17]. So, nanoCAR can be a functional alternative to the conventional CAR design.

3. Novel Design in Intracellular Signaling Domain

The recognition of expressed tumor antigens on malignant cells by the extracellular scFv in the chimeric antigen receptor causes conformational change in the intracellular signaling domains, further initiating downstream signal transduction pathways that activates the T cell to proliferate and kill tumor cells [18, 19]. Among different generation of CAR molecules, the intracellular signaling domain changed greatly compared with the extracellular domain. In the first generation, the signaling transduction domain only contained the CD3ζ signaling module, although the CD3ζ signaling can enable some cytolytic activity, the activation strength of the signal was comparably weak, and the first generation CAR-T did not show enough anti-tumor activity in clinical trials. In the second-generation CAR, one co-stimulatory signaling domain of CD28, OX40 (CD134) or 4-1BB (CD137) was fused to the CD3 signaling domain to enhance T cell activity and cytokine production. And to further enhance the anti-tumor activity of CAR-T cells, third generation CARs incorporate two or more co-stimulatory signaling domains, which are usually chosen from CD28 and 4-1BB or OX40 [15, 20]. These optimizations made by several groups demonstrated that the addition of co-stimulatory domain of 4-1BB or OX40 can effectively enhance downstream signaling and T cell activation, further lead to higher cytotoxicity and kill efficiency, as well as higher rate of CAR-T cell proliferation [21].

Considering the complexity of tumor microenvironment, application of CAR-T immunotherapy to solid tumors requires high level of T cell expansion and persistence. Guedan and colleagues found that the signaling module of ICOS was a potent co-stimulatory domain to enhance CAR T cell effector function and in vivo persistence. ICOS is a member of the CD28 family, capable of activating PI3K and downstream AKT signaling to promote cytokine release and T cell activation. In the Capan-2 cell-derived pancreatic xenograft tumor model, CAR-T cells with fused domains of ICOS, 4-1BB and CD3ζ (ICOSBBz) demonstrated enhanced tumor eradication after 4 weeks, with a robust expansion of CD4+ and CD8+ along with high persistence. Experiments further suggested that lower expression level of the ICOSBBz receptors not only affect the anti-tumor activity, but also maintain high persistence and low level exhaustion of CAR-T cells [22].

Previous studies have shown that the JAK-STATs signaling pathway, which can be activated through IL-2R β-chain and IL21 receptor that engaged by IL2, IL7, IL15 and IL21 cytokines, can result in the transcription of various downstream genes involving T cell activation, proliferation, memory cell formation and effector cell differentiation. Kagoya and colleagues constructed a novel CD19 CAR which encodes a truncated intracellular signaling domain from the IL-2R β-chain (IL-2Rβ) and a STAT3-binding tyrosine-X-X-glutamine (YXXQ) motif, fused with the CD3ζ and co-stimulatory domains of CD28 (28-ΔIL2RB-z(YXXQ)). The 28-ΔIL2RB-z(YXXQ) CAR-T cells showed antigen-dependent activation of the JAK kinase and of the STAT3 and STAT5 transcription factors signaling pathways. Further studies demonstrated that this CAR design promoted CAR-T cells proliferation and prevented terminal differentiation in vitro and superior in vivo persistence and antitumor effects in different NSG mice tumor models, compared with control CAR-T cells expressing a CD28 or 4-1BB co-stimulatory domain alone [23].

4. Inducible and Controllable CAR Design

One of the major side-effects of CAR-T therapy is the cytokine release storm, which may cause fever, tachycardia, hypotension and even fatal events. The off-tumor CAR-T targeting also led to adverse effects and toxicities. Another potential risk is insertional mutagenesis in CAR-T cells, caused by the random insertion of lentivirus [24, 25]. Given the potential risk of CAR-T cell immunotherapy, several control elements have been engineered into the CAR design. One of such designs is the addition of "suicide genes", such as inducible caspase-9 (iC9). Caspase-9 was fused to FK506 protein, then through a chemical inducer of dimerization (CID) effect, the activated caspase-9 causes the activation of the mitochondrial apoptotic cascade and eventually lead to the apoptosis of CAR-T cells [26, 27].

Another approach is to use Tet-on inducible system to control the expression of CAR molecule, which can only be induced by doxycycline (Dox) [28, 29]. For example, Sakemura and colleagues tested this Tet-on inducible system in the Raji xenograft mouse tumor model and observed significant antitumor activity of the Tet-on CD19CAR-T cells, which is comparable to that of conventional anti-CD19 CAR-T cells. However, without Dox, these Tet-on CD19CAR T cells lost CAR expression and cytolysis function in vitro and in vivo, indicating this design can be a “switch” to control the activity of CAR-T cells through Dox administration [29]. So, the application of this Tet-on inducible CAR can be an effective solution to control CAR-T toxicity and lower the risk of CAR-T immunotherapy.

5. Conclusion Remarks

In recent years, CAR-T cell immunotherapy has achieved great success and progress in treating hematological malignancies. But many questions, such as the toxicity and high rate of relapse, still remain to be solved. To effectively treat different tumors with low toxicity and relapse rate, especially in the treatment of solid tumors, novel design of CAR molecules must be further developed and created. In this review, we summarized the recent-designed CAR constructs within the extracellular and intracellular domains, which can enhance the activation, proliferation and persistence of CAR-T cells, and result in robust anti-tumor activity. We expect the reliable, safe, and effective CAR-T cells and extend it toward the treatment of a broad range of tumors in the near future.

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