The Development and Mechanism of the CAR-T Therapy

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Abstract: The immunotherapy of cancer has been a popular topic and has been developing very fast in recent years. Chimeric antigen receptor T cell therapy, as known as CAR T cell therapy is an adoptive cell therapy that modifies the T cells from the patient with specific receptors which can recognise the antigen on the surface of tumour cells, reinject the T cells back in order to kill the tumour cells efficiently. This fantastic way of modifying the patient's T cells to fight cancer has shown its value in treating B-cell malignancies and Multiple myeloma with remarkable efficiency. Further generation of CAR-T cell therapy should aim for reduced patient expenditure and side effects to encounter associated challenges. This review introduced the mechanism of the CAR-T cell therapy, the targeted cancers, challenges, and the future of the therapy.

1. Introduction

Chimeric antigen receptors (CAR-T) cell therapy is a milestone in the innovation of adoptive cell therapy (ACT) in cancer treatment and evolved from the simple blood transplant nowadays. In general, T cell is isolated via apheresis and activated by the antibody-coated beads which mimic the function of dendritic cells. Activated T cells are reprogrammed with the addition of CARs which display antigen binding fragments of the antibody to target disease. These receptors are fused to the T cell signalling domain, forming CAR-T cells and undergo expansion ex vivo. Finally, after receiving a preparative lymphodepleting regime, CAR-T cells are infused back into the patients' body for disease attacking [1]

CAR-T cell therapy has illustrated achievement on certain B cell leukaemia or lymphoma, and multiple myeloma. However, there are also many issues faced by CAR-T cell therapy, including failure in the application for solid tumours, immunosuppressive microenvironment challenges, normal tissue toxicity and antigen escape. The biggest obstacle of CAR-T technique is the not sufficient data-based analysis which can figure out the relationship between the side-effects and the properties of TCRs. This area can be a direction to eliminate the negative effect in patients and to improve the prognosis. To apply the technology into a wider range can be another direction of research which can push the method to conquer the solid tumours.

CAR-T cell therapy is an immunology therapy with a bright future and large potential to treat diseases like cancers. Although CAR-T cell therapy still has side-effects and defects, scientists already find potential methods to solve these problems. As more and more research are done, I believe CAR-T cell therapy will play an more and more important role in overcoming diseases.

2. The development of CAR-T therapy

2.1 Initiation—Blood-transplantation

CAR-T cells are so attractive that numerous scientists dedicate in immunotherapy. The relevant attempt can be tracked to 1968 when Dr Steven A. Rosenberg was during residency. He transplanted patient A remitted by himself magically to patient B suffering from cancer with the same blood-type as he thought there may have been some rare anti-tumour substance in the blood of A [2]. After getting the permission, he made a pioneering experiment—blood-transplantation. Unfortunately, just like most pioneering attempts, this experiment failed in the end [3]. Patient B was remitted in the early period after he accepted the transplantation, but the good condition seems unsustainable for the patient accepted transplantation relapsed again after the short period of good condition. Even the experiment failed this year, but it did become the fundamental of CAR-T immunotherapy nowadays [2].

2.2 Development–TIL and IL-2

The application of blood transplant for cancer therapy is very creative but not completed enough [4]. The most significant barrier is the method to assist these anti-tumour substances keep proliferating efficiently and avoiding the immune system effectively. The Tumours inundated lymphocytes, TIL discovered around some solid tumours, which can specifically identify and inactivate these cancer cells caught Rosenberg's attention [5]. In 1976, the function of IL-2 in stimulating the proliferation of T-cells became the fuel pushing the scientists to think revolutionarily to combine the two discoveries together [6]. Rosenberg and other staff members extracted and separated the TIL from patience to culture these cells in vitro at first. Before infusion back into the patient, they use the IL-2 to stimulate these T-cells to catalyse the in vivo proliferation of tumour-targeting T cells [7]. This method is a successful attempt which increases the success rate to around 40% although it's heavily costly during the cell culture process. Since then, the new method called Adoptive Cell Transfer Therapy, ACT, became a new hotspot in immunotherapy and cancer-related therapy [8].

2.3 Improvement–CAR-T

The unavoidable limitation of the TIL based therapy is the narrow range of the target cells such as melanoma and renal carcinoma, while plenty of tumour cells can escape from TIL [9]. The solution of broadening the applicable range of ACT is the most significant progress for immunotherapy. In 1993, Hwu and Eshhar published a paper that has become a hallmark of CAR-T [9]. Based on their published data, the CAR-T can be used in breast cancer, colon cancer, and ovarian cancer. The structure of the T cell receptor, TCR, combined with alpha and beta chains which are composed of Variable (V) and Constant (C) region, two extracellular domains of TCR. What is interesting is the several similarities of antibody and TCR not only in structure but the function and even belong to the same DNA family [10]. Both TCR and antibody have two extracellular domains in structure and can identify the antigen in function, the most important point for achieving success of CAR-T technology is its similar DNA properties [8]. Based on their sophisticated technique in gene engineering and innovative thinking, the first generation of CAR-T was born. Emily Whitehead's successful treatment validates the feasibility of this new technique and encourage further development in this field [11].

3. Primary Mechanism of CAR T cells

CAR is composed of an extracellular, a transmembrane, and an intracellular domain which displays different functions in T cell modification and activation (Fig 1). The extracellular region is further differentiated into an antigen-binding region with a V and C domain and a spacer/hinge region [12]. As indicated in Figure 1, the targeted cell surface antigen from targeted cancer is recognised by the antigen-binding region and binds to CAR via a single chain variable fragment (scFV). scFV is generated by linking the heavy and light chains at variable domain through a flexible peptide linker and fused into the transmembrane through a spacer derived from IgG antibodies [13-14]. The length of the spacer determines the distance between the tumour cell and T cell, influencing the CAR function

of tumour recognition and T cell cytokine proliferation. The following structure, transmembrane domain connects the extracellular domain to the intracellular domain and stabilizes the receptor and affects CAR expression [13]. After binding to the antigen, the intracellular domain which derived from lymphocyte-specific protein tyrosine kinase (LcK) undergoes conformational changes and initiate signals from the receptor through immune-tyrosine activation motifs (ITAM)s [15].

Some studies compare the ligation of CAR and TCR in the same T cell and discovered that the immune synapse (IS) and signalling of CAR differs from the conventional T cell receptor (TCR). The IS structure of CAR consists of a small actin ring fused with disorganized LcK micro-cluster but without the conventional LFA-1 adhesion ring as same as TCR. This variance results in a shorter time to detach CAR from apoptotic target cells, leading to a more rapid signalling period and faster tumour-cell killing rates compared to TCR. Consequently, the IS in CAR is more stable and greatly contributes to the control tumour cell proliferation by stimulating different pathways [13].

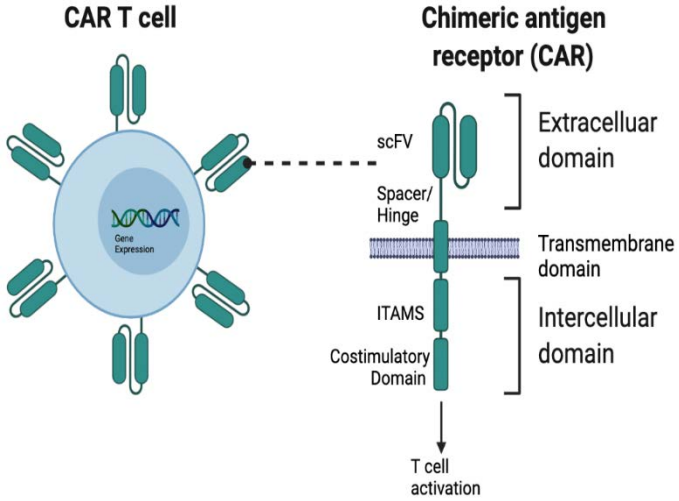

Fig. 1 The structure of chimeric antigen receptor [15]. (CAR is comprised of an extracellular domain, a transmembrane domain and intercellular domain. The extraceulluar domain is divied into a scFV antigen binding region and a spacer region, passing the signalling to ITAMs in the interculluar domain through transmembrane domain)

4. Secondary mechanism of CAR T Cells

Perforin and granzyme axis and Fas and FasL axis are utilised to eradicate antigen-positive and antigen-negative tumor cells respectively. Strome cells are sensitized by cytokine secretion (Fig 2) [15].

In the Perforin and Granzyme axis, these two cytotoxic granules are initially located at the microtubules of the target cells. After receiving synapse from the antigen-binding domain, these granules migrate to the plasma membrane via exocytosis and fuses into a vesicle. Perforin introduces pore formation on the cell membrane, allowing granzymes to cleave the key substrates of target cells and eventually induce cancer cell apoptosis. This pathway is rapid and specific [16].

The Fas/FasL axis is a calcium dependent pathway which complements with the calcium dependent granule exocytosis to perform cell-killing functions. This pathway activates caspase 8 by the trimerization of Fas receptor via Fas ligand. Caspase 8 is essential in forming death-inducing signalling complex (DISC) and mature caspase 3 which cleaves substrates of targeted cells to induce apoptosis [17]. When T cells attacks target cells during tumour regression, approximately 10% of malignant cells can secrete specific enzymes to convert non-toxic prodrug to toxic metabolites. This significant phenomenon is termed by bystander effect and affects the effectiveness of tumour regression [18]. Hong et al. illustrated in the study that the ectopic Fas expression. Hong et al. illustrated in the study that the Fas/FasL pathway enhanced the activation of CAR T-cells via the against for the bystander

tumour cells in antigen-lost diseases [19]. Consequently, this pathway can compensate for the cytotoxicity involved with the perforin and granzyme pathway [16].

Specific CAR T cells can produce cytokines which triggers interferon gamma (IFN-ɣ) receptor expression by the tumour stroma and induces the polarisation of macrophages [20], meaning that the macrophages induce anti-tumoral phenotypes as a response to the stimulation of cytokines [21]. In contrast to conventional T- cells functioning as universal killers, CAR T cells function as vehicles to secrete cytokines within the target tissue specifically [22].

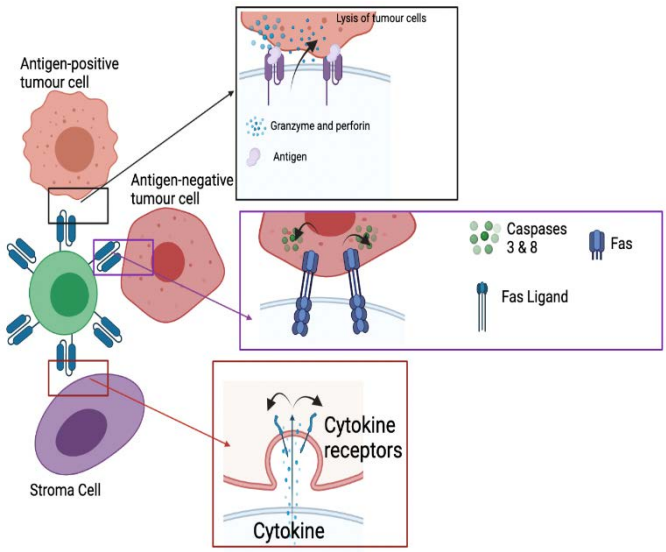

Fig. 2 CAR T cells mediate tumour killing via three axes [15]. (The mediation of antigen-positive tumour cell is achieved via the release of granzyme and perforin axis, whereas the mediation of antigen-negative tumour cell is achived via the stinulation of Fas/Fas ligands. CAR T cells cam also trigger cytokine secretion to mediate stroma cell cancers.)

5. Problem applying to solid tumours

Sterner from Blood Cancer Journal describes several limitations and problems faced by CAR T cells. One of them is that CAR-T cell therapy is hard to apply to the solid tumour. First, the antigens that expressed on the solid tumour for the most cases are also expressed on the normal tissues. It becomes very crucial to select the target antigen that can efficiently target the tumor cell instead of the normal cells, preventing the toxicity caused by “on-target-off-tumour” effect. There is a potential way to solve this problem, is “the targeting of tumour-restricted post-translational modifications” however, more research is needed [23]. Another problem that needs to be solved is the limited ability of the CAR-T cells to traffic and infiltrate the physical tumour barriers of the solid tumours. Strategies to overcome this problem is to use specific delivery routes instead of systemic delivery. This strategy can significantly help the CAR-T cells traffic to the tumour and at the same time reduce the toxicity caused by “on-target off-tumour” effect because the CAR-T cells directly interact with the solid tumour. This approach brings another issue that needs to be solved is that using the specific delivery routes only work with single tumour or premetastatic tumour [23]. It is also important for the CAR-T cells to be modified in order to penetrate the physical barrier of the solid tumour.

In order for the tumour to grow, these cancer cells usually develop the ability to escape from the scanning of the immune system. At the same time, the tumour also develops an immunosuppressive microenvironment to suppress the surrounding immune system, which will allow the tumour to grow better. It has been a challenge for all immunotherapies. Cells like “myeloid-derived suppressor cells (MDSCs), tumour-associated macrophages (TAMs), and regulatory T cells (Tregs)” contribute to the immunosuppressive environment and infiltration of solid tumours. These factors, again, contribute to the production of cytokines that help tumour, chemokines, and even some growth factors. At the same

time, immune checkpoint pathways play a role in decreasing antitumor immune activity. Both the immunosuppressive microenvironment and the triggered coinhibitory pathway suppressed the CAR-T cell therapy by limiting T cell expansion and shortening the T cell persistence. [19] One potential solution will be modifying the CAR-T cell, give the cell the stimulatory cytokines so that the CAR-T cells can stimulate immunostimulatory signals in order to increase the T cells' survival, replication and antitumor activity rates.

It is not possible for the CAR-T cell to eliminate large amounts of tumour burdens without toxicity to normal tissue. Cytokine release syndrome (CRS) is a common side-effect that is caused by CAR-T cell therapy. CRS is initiated when a huge number of cytokines are released because of the replication of CAR-T cells. This will over activate the immune system including symptoms like high fever, headache, trouble breathing, severe nausea, vomiting, diarrhoea and muscle pain. The seriousness of the CRS is related to the levels of burden the tumour puts on the patients' body [20].

CAR-T cell therapy also puts burden on the nervous system, the neurotoxicity. This usually happens after the infusion of CAR-T cells, especially the CD19 CAR T-cell therapy; patients will have symptoms like unconsciousness, tremor, cerebral oedema, and even seizures. The reason why there is neurotoxicity is still unclear, what we know is that the neurotoxicity is highly associated with CRS. One possible explanation is that the CRS-released cytokines (IL-6, IL-8) activate the endothelial cells in the brain cause endothelial dysfunction which cause the blood brain barrier fails to protect the cerebrospinal fluid (CSF) from the influence of serum cytokines. The leakiness of the blood brain barrier, again, fails to protect the brain mural cells from the attack of CD19 CAR T-cells. All these factors contribute to neurotoxicity.

One of the challenges of the Car-T cell therapy is that the tumour will develop resistance to the CAR construct which targets a single antigen [20]. This means that although the body showed a high response rate when the CAR-T cells which target a single antigen were first introduced in the body, as time goes by, the malignant cancer cells will show a partial or even complete loss of the expression of the targeted antigen. For example, when the patients with multiple myeloma are treated with the CAR-T cells which target the BCM, clinical researchers observed the loss of the BCMA expression in many patients. One of the solutions to prevent the relapse caused by losing of the antigen expression is targeting multiple antigens. This theory relies on the usage of dual CAR constructs or dual-scFvs containing CARs called tandem CARs which can target multiple tumour antigens. In the clinical research, the dual-targeted CAR-T cells targeting both CD19 and CD22 have shown the efficiency and most importantly the antigen escape rates are lowered [20].

6. Future direction of CAR T therapy

6.1 Techniques in select appropriate T-cells

In the past, the sorting method with magnetic beads was more popular. The drawback of the magnetic beads is the unavoidable residues which can be threatening for patients. Nowadays, there are new kinds of material that can be used in the selection process which can be decomposed to small molecules without any affection to humans. In the next five to ten years, the tools for the selection process can be updated along with the development of new material which can be implied in CAR-T technique [24]. Besides that, technology combination is the major trend in the development of CAR-T, the selection process can be applied as well. The material comes from living organisms can be more acceptable to simplify the decomposed process by highly efficient catalysis–enzymes. Some companies have put their eye on this aspect which seemingly is an ignorable process but in fact, plays a significant role in the success rate of CAR-T technique.

6.2 Technique in reprogramme

With the irresistible favour of scientists to enhance the CAR-T method, most of them keep on finding the best programmed TCR which can combine all the advantages. Nowadays, the TCR has been renewed to the fourth generation but the price for that has not been loved until now [5]. The fourth generation can be much more effective than before in accuracy, sensitivity, and efficiency.

However, it still can't control the cytokine storm either, which has been shown in plenty of cases and the biggest threat to patients' lives. It is the target for TCR relevant research in the future and a great step for patients' satisfaction [24].

6.3 Technique in cell activation and culture

In most cases, the reprogrammed CAR-T cells need the CD3 and CD28 to combine with TCR so that the T-cell can be activated. In this process, the efficiency is hard to predict. The new technique to activate T-cells is by a series of decomposable gel beads combined with the CD3 and CD28 in the surface. In this way, the beads can be decomposed by a kind of specific buffer solution to avoid the side-effects caused by the impurity components. In cell culture, the low success rate can be regarded as one reason for the high price. T-cells need an appropriate environment to finish its expansion process. In the future, that can be a direction enhancing the success rate to lower cost with lower request for huge and delicate machines. What makes patients and their family members worried about is the security during this process. The methods of avoiding the pollution by bacteria and mutated substances need to be taken into consideration when improving the technique during the activation and expansion procedure. There is not so adequate data about the CAR-T therapy on it's different side-effects and the relationship of the other potentially influential factors in this process with the sorts of side-effects. In the future, there are some possibilities to improve the monitor and nursing care during the infusion process.

6.4 Prognosis of patient side effects

The side-effect of CAR-T technique influences the prognosis of almost all the patients. The most famous case, Emily, suffered from the Cytokine Storm Syndrome known as CCS as well. One of the major issues is the conflict between the treatment of CCS and the function of the inner CAR-T cells. Fortunately, Emily hasn't relapsed until now, but this risk is a problem that needs to be solved in the future [25]. There is still not enough data for analysing and selecting the most appropriate therapeutic strategy for CSS without the negative influence on the CAR T cells. Both data and clinical cases are required as support to unveil the most effective combination achieving the best prognosis.

7. Conclusion

CAR-T cell therapy is newly developed technique in treating cancer cells, pioneering in personalised medicine and providing new research areas for further cancer study. It modifies patients' own T cells with a CAR region to attack the tumour cells specifically. However, this technique is not mature enough to be widely utilised in clinical applications. First finding ways to lower the cost of this therapy will be an important aspect that will help the popularity of CAR-T cell therapy. Reduce side-effects and increase the safety will be another problem that scientists will face in developing the CAR-T cell therapy. Finally, expending the scope of treatment is essential for the CAR-T cell therapy. One direction is to increase that receptors to target, for example not only focus on CD19, but also target other receptors like CD22 and CD 123 to expand the range of diseases that the CAR-T therapy can treat. Another direction is to overcome the problem that the CAR-T cell therapy is hard to apply to solid tumours. Overall, further studies and research should be conducted in the aim of generalising into more types of cancers and reducing side effects. Combination of different technologies is a necessary way to promote these techniques. CAR T cell therapy is a prospecting field and a milestone in cancer treatment.

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