

CAR T-cell Therapy for GBM

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Abstract. Glioblastoma multiforme (GBM) is the most lethal and common primary malignant brain tumour. It has a 100% mortality rate and poor prognosis, and is currently incurable. Conventional treatments, such as surgery, can only extend patients' survival to a very limited extent and are often accompanied by severe side effects. In recent decades, chimeric antigen receptor (CAR) T-cells therapy has emerged as a novel immunotherapy with great potential in treating various tumours, raising high expectations for its application in GBM. This paper reviews the structure and function of CAR design and discusses three antigens on GBM that have completed clinical trials: EGFRvIII, HER2 and IL-13Ra2. Although each antigen-specific CAR T-cell therapy face its own challenges, the results from clinical trials have been overall inspiring. However, CAR T-cell therapy for GBM is still in very early stages and requires further research and exploration.

Keywords: Glioblastoma multiforme, chimeric antigen receptor, CAR T-cell, immunotherapy, EGFRvIII, HER2, IL-13Ra2.

1. Introduction

As modern medicine advances and global awareness of healthcare increases, more and more previously elusive diseases are being brought to light. Brain tumours are one of the illnesses that used to be poorly understood but are now receiving greater attention. The human brain itself is a highly evolved and sophisticated organ that controls every organ in the body and enables an individual to adapt to and withstand a wide range of environmental conditions [1]. A brain tumour develops as a result of a buildup of genetic alterations over time. It occurs when the brain cells succeed in evading the body's regulatory mechanisms for cell division and grow and proliferate uncontrollably. Each year, 0.005% of global population are diagnosed with primary malignant brain tumours, with malignant gliomas (MGs) accounting for 80% of these cases [2]. Glioblastoma multiforme (GBM) constitutes more than half of all MG instances and is the most common and aggressive one among all primary malignant brain tumour types [2]. It is so far an incurable disease with a median survival of only 15 months [3]. Only few patients make it to 2.5 years and less than 5% survive over 5 years [4]. According to Jigisha P Et al, the incidence rate (IR) of GBM is 3.19 per 100,000 people on average [3]. The IR is greater in men and white individuals, and the median diagnostic age is 64 years, which may indicate potential identifiable biological risk factors [3]. Since malignant brain tumours are uncommon and very secretive, the majority of risk factors remains theoretical and inconclusive, and the only association between these factors and GBM is normally just correlations. One of the very few recognised risk factors that unquestionably show a higher chance of glioma formation is ionising radiation [5]. Therefore, more study is required for

further understanding and confirmation. Early symptoms of GBM are highly varied as the size and location of the tumour and the anatomic structures of the affected brain can all have a significant impact on how the disease presents on a newly diagnosed patient, and GBM can also rarely appear in the brain stem, cerebellum, and spinal cord, in addition to the brain itself [5]. Furthermore, the human skull is a hard, volume-restricted structure, thus any unexpected development might potentially affect how humans function depending on the part of the brain that is involved [1]. However, despite GBM's lethal and unpredictable nature, there are still treatments that are expected to be able to cure the disease or at the very least extend survival. CAR T-cell therapy is one of those. It is a type of immunotherapy based on T-cells, which are a type of white blood cell also known as T-lymphocytes. It is now globally regarded as a major breakthrough in oncology as numerous types of cancer treated with it have shown impressive clinical responses [6]. Basically, It uses genetically engineered T-cells to selectively target and kill cancer cells. Original T-cells express T receptors on their surface. These receptors allow them to identify infected cells by attaching to a specific antigen on those cells and neutralise them in the process [7]. The term "CARs" refers to chimeric antigen receptors, which are artificial receptors designed to reroute lymphocytes to identify and eliminate cells with a specific antigen expression [6]. With this, T-cells are able to focus on attacking particular cells and eventually destroy a tumour. CAR T-cell therapy shows potential as an effective treatment for GBM because GBM has a large number of target antigens on its surface while only 3 of them have their clinical trials completed to date according to Luke Et al, and the rest is currently under study [8]. These 3 target antigens will be discussed in this paper along with the future prospects of CAR T-cell therapy for GBM.

2. Glioblastoma Multiforme

GBM is one of the most aggressive and unpredictable tumours, with a nearly 100% recurrence rate and a lethal prognosis, with a typical survival of about one year, even after decades of thorough study [9]. Only a few treatments, such as radiotherapy, chemotherapy with temozolomide (TMZ), and surgical resection, have been allowed up to this point for the treatment of GBM [10]. However, conventional therapies have very limited clinical effects on prolonging the survival of patients, and are often accompanied by serious and unanticipated adverse effects. For surgical resection, surgical difficulty and operability are a huge problem - GBM cannot be completely resected due to its growth in the brain and infiltration into adjacent brain tissues [2]. Although modern medical methods can remove most of the glioma, even a small amount can cause the tumour to recur. Thus, GBM is a disease that is physically incurable today, and most therapies only marginally prolong patients' survival.

Tumour heterogeneity is one of the features of GBM and it is a major factor why the disease is extremely difficult to treat [10]. Tumour heterogeneity can be divided into two types: intra-tumour heterogeneity and inter-tumour heterogeneity [9]. In the former, cancer cells within the same tumour but at different regions display different genes, functions, and behaviours; in the latter, the same type of tumour in different individuals show different gene, functions, and behaviours. In GBM, there are distinct cell subgroups, and each one responds to therapy differently [10]. While some cells are extremely resistant to radiation treatment, others are highly resistant to chemotherapy [10]. For instance, stem cells' capability to regenerate and differentiate will constrain the effectiveness of certain chemotherapeutic medications [10].

Chemotherapy's effectiveness is also restricted by the blood-brain barrier (BBB) in the brain, and one reason why GBM patients have such a poor prognosis is because certain medications are unable to pass through this barrier [11]. A healthy and functional BBB is responsible for maintaining homeostasis of the microenvironment in the brain [12]. It shields the brain from neurotoxic substances and helps supply it with oxygen and other necessary chemicals [11]. Overall, the BBB is essential to the brain's healthy operation. However, under some pathological circumstances, the structure and function of the BBB can alter, which is called blood-brain tumour barrier (BBTB) [11]. The BBTB prevents delivery of drugs in GBM, and the large number of blood vessels it has promotes supply of oxygen and nutrients to the gliomas, enabling them to proliferate and metastasise to new areas [11].

3. CAR T-Cell Therapy

In recent decades, advances in cellular engineering have unlocked new possibilities for cancer treatment - by genetically modifying lymphocytes to express receptors that can recognise specific target antigens, more precise attacks on cancer cells become possible [8]. CAR T-cell therapy is considered a groundbreaking innovation in the field of oncology. It has shown significant therapeutic responses in treating B-cell leukaemia and other haematological malignant tumours, and therefore, its potential in treating solid malignant tumours like GBM has gathered a lot of interest from researchers [6].

CAR T-cell therapy, as the name suggests, is entirely based on T-cells from the immune system. T-cell receptors (TCRs) located on the surface of T-cells are responsible for recognising and binding to antigens presented by major histocompatibility complex (MHC) molecules. These antigens are usually small peptide fragments derived from tumour-specific proteins within the cell, which are processed by intracellular enzymes and then presented on the surface of the cell by MHC molecules. TCRs recognise and kill the target cells by binding to these MHC-peptide complexes.

CARs are artificial receptors. An extracellular target antigen-binding domain, a hinge domain, a transmembrane domain, and at least one intracellular domain which transfers activation signals make up a CAR [8].

4. CAR Structure and Composition

4.1. Antigen Binding Domain

The target antigen is recognised and bound to by the antigen binding domain, which usually derives from the variable regions of antibodies and is joined to create a single-chain variable fragment (scFv) by a flexible linker [6]. These scFvs are primarily used to recognise extracellular antigens while bypassing T-cells' dependence on MHC, although some CAR designs still rely on MHC to recognise intracellular antigens [6]. The antigen-binding domain plays a crucial role in targeting specific antigens. The function of CARs is influenced by multiple factors related to this domain, including the affinity of the scFv for the target antigen, the interaction between variable heavy (VH) and variable light (VL) chains, and other variables such as epitope location and antigen density [6].

4.2. Hinge Domain

The hinge domain is also known as the spacer domain [14]. It is located between the antigen binding domain and the transmembrane domain, playing a crucial role in connecting them [15]. It is responsible for extending the scFv to provide sufficient flexibility for the antigen binding domain to overcome steric hindrance and reach the target antigen epitope [6][15]. The length and composition of the hinge can significantly influence the CAR function; for example, spacer length influences the formation of the immunological synapse [6]. Short spacers are often better suited for binding distal epitopes on the membrane, while longer spaces are more effective for binding membrane proximal epitopes as they provide more flexibility [6]. Therefore, optimising hinge length is vital for improving performance of CAR T-cell therapy. Common sources of hinge regions include Ig segments from immunoglobulins or natural non-Ig segments from T-cells [15].

4.3. Transmembrane domain

The transmembrane domain anchors the CAR to the T-cell membrane [6]. Although it is an very important component of the CAR, research on the transmembrane domain is relatively limited compared to other regions of the CAR [6]. Nevertheless, some studies have demonstrated that it significantly affects the expression level and stability of CARs [6]. According to research by Fujiwara et al., CARs containing both the transmembrane domain and hinge domain show higher expression levels than those with only the hinge domain [15]. The transmembrane domain is usually derived from type-I single spanning proteins, with CD28 being one of the most stable options [15].

4.4. Intracellular signalling domain

The intracellular signalling domain is located at the distal part of the CAR structure [16]. It plays a crucial role in CARs by transmitting the signal generated from the binding of CARs to antigens to the T-cells, thereby activating the immune response of the T-cells [17]. The design of CAR T-cells has undergone several upgrades based on this domain [6]. Second and third-generation CAR T-cells are equipped with co-stimulatory domains joined to the intracellular signalling domain to enhance intracellular signalling and T-cell expansion [16].

5. Targeting EGFRvIII, HER2, and IL-13Ra2 for GBM Therapy

The precision of CAR design and its highly personalised medical pattern make it promising in the treatment of various cancers. For GBM, a complex and unpredictable tumour type, CAR T-cell therapy still have a lot of potentials. The antigenic heterogeneity of GBM increases the complexity of target-specific therapy but also provides multiple potential targets for CAR T-cell therapy. To date, there are three antigens in total have their clinical trials competed: EGFRvIII, HER2, and IL-13Ra2 [8].

5.1. EGFRvIII

EGFRvIII, or the full name 'Epidermal Growth Factor Receptor Variant III', is a deletion mutation of the EGFR gene, characterised by the loss of exons 2 to 7 in its coding region [8][18]. This structural alteration leads to the constitutive activation of EGFRvIII, promoting tumour growth and invasion [18]. On the other hand, this makes it an ideal tumour-specific target, as it is commonly found in various tumours but is normally not found in healthy tissues [18]. EGFRvIII is expressed in gliomas in approximately 30% of GBM patients [8]. EGFRvIII-targeted CAR T-cells have shown favourable anti-tumour ability in preclinical settings [8]. However, in clinical application, effects of therapies based on this antigen are very limited due to various factors [8]. In a Phase I clinical trial, 10 patients with recurrent GBM received a single dose of EGFRvIII-targeted CAR T-cells [19]. Due to the mutation which leads to the formation of an unique immunogenic epitope at the junction of exons 1 to 8, no on-target off-tumour toxicity was observed during the treatment (although no significant improvement was noted either) [8][19]. This may be because, according to O'Rourke et al. (2017), patients receiving EGFRvIII-targeted CAR T-cell therapy for GBM after relapse developed adaptive resistance and antigen loss, leading to poor treatment outcomes [8]. Most patients showed a loss of EGFRvIII expression in their tumours after receiving CAR T-cell therapy, suggesting that CAR T-cells may have successfully targeted and eliminated EGFRvIII-positive tumour cells [19]. However, adverse events were also observed in clinical trials. In an early clinical trial, when the dose was increased to 3×10^{10} CAR T-cells, one patient underwent severe side effects, which are hypoxia and dyspnea, and when the dose was increased to 6×10^{10} , another patient died 4 hours after administration due to severe hypotension and acute dyspnea [8]. These adverse events are likely due to the excessive number of T-cells causing congestion in the pulmonary vasculature, reflecting the potential risks associated with high-dose administration and the route of delivery [8].

5.2. HER2

HER2, or Human Epidermal Growth Factor Receptor 2, is found in normal cells but is over expressed in various cancer cells [20]. According to Mineo et al. (2015), HER2 is over expressed in around 80% of GBM [20]. In one study, a patient with colon cancer died after receiving an intravenous injection of HER2-targeted CAR T-cells, with CAR T-cell accumulation and cytokine storm syndrome (CSS) observed in multiple organs [8]. Cytokine release syndrome (CRS) is a common side effect in patients receiving immunotherapies, such as CAR T-cell therapy, and CSS is an extreme form of CRS. Elevated inflammatory cytokines, which are immune-regulatory proteins that signal and activate immune cells such as monocytes and macrophages, can be released when CAR T-cells are massively activated [6][8]. This extreme death case indicates that not all targets are suitable for CAR T-cell therapy, and it is essential to avoid selecting antigens that are also expressed in normal cells whenever possible. However, there

are studies that have shown that peritumoral injection of HER2-specific CAR T-cells is more effective than intravenous injection of those for treating GBM, as better tumour infiltration of HER2-targeted CAR T-cells is observed in the peritumoral injection group [20]. The poor efficacy of traditional intravenous injection may be due to the blood-brain barrier obstructing CAR T-cells from entering the brain [20], leading to their accumulation in healthy organs and potentially causing attacks on these organs, as HER2 is also expressed in normal tissue cells. Third-generation HER2-targeted CAR T-cells have been proved to have an outstanding in vitro GBM cell killing ability, according to another study [8]. Moreover, with the introduction of the CD28 intracellular domain, these CAR T-cells no longer exhibited adverse reactions such as CRS after administration [8]. Among 17 patients receiving this therapy, 8 showed clinical improvement [8]. Additionally, the median survival of patients receiving these CAR T-cells was up to 25.5 months, significantly exceeding the regular median survival [8].

In summary, while the expression of HER2 in normal tissues increases the risk of on-target off-tumour effects and CRS, its high expression frequency in GBM and its favourable clinical performance in treating this tumour still make it a promising target.

5.3. *IL-13Ra2*

Interleukin-13 (IL-13), which is secreted by T-cells, functions as an immune-regulatory cytokine [8]. In normal cells, it binds to the interleukin-13 receptor alpha 1 (IL-13Ra1) which has a low affinity for it, and activates the STAT-6 signalling pathway, regulating N6 growth arrest-specific genes, which promotes apoptosis [21]. However, in GBM, there is a highly expressed decoy receptor known as interleukin-13 receptor alpha 2 (IL-13Ra2), which has a high affinity for IL-13 [21]. This receptor is more likely to bind IL-13, and since it lacks the intracellular signalling domain, it cannot activate STAT-6, thereby inhibiting apoptosis and giving the cell cancerous characteristics [21]. Studies have shown that the expression level of IL-13Ra2 is positively correlated with the malignancy of GBM [21]. Furthermore, IL-13Ra2 is expressed in 75% of gliomas, while it is almost absent in normal brain tissues and most other normal tissues [8][21]. For these reasons, IL-13Ra2 has been considered a promising target antigen. In clinical trials, according to Keu et al. (2017), patients receiving IL-13Ra2-targeted CAR T-cells had a median survival of 19.7 months, and according to Brown et al. (2015), patients had a median survival of 11 months after relapse, showing positive outcomes [8]. These data reflect the initial success of IL-13Ra2-targeted CAR T-cell therapy.

6. Discussion

GBM is one of the most aggressive and lethal malignant brain tumours, and it is currently incurable. Although conventional treatments are well-developed, these methods only extend patient survival to a very limited extent and often come with severe side effects. In recent years, advancement in immunotherapy, particularly CAR T-cell therapy, have provided a new direction for GBM treatment. CAR T-cells have high specificity and efficacy by recognising target-specific tumour antigens, showing broad potential in the treatment of various cancers. However, GBM's tumour heterogeneity, the blood-brain barrier, and the diversity of target antigens still present significant challenges.

This paper explores the basic structure of CARs and focuses on the three target antigens that have completed clinical trials: EGFRvIII, HER2, and IL-13Ra2. Although these antigens have shown positive clinical responses, each has its own disadvantages and limitations. EGFRvIII-targeted CAR T-cell therapy faces issues such as antigen loss and resistance, and there have been cases of treatment-related deaths in clinical trials. This reduced the enthusiasm for this target; HER2's expression in normal tissues increases risks and is more likely to cause cytokine release syndrome (CRS) compared to the other two antigens, but its high expression in GBM and significant extension of patient survival make it still an interesting subject; In comparison, IL-13Ra2, with its high expression close to HER2 and its rare expression in normal tissue cells, has become a relatively safe and effective target. The clinical trial results of it also show promising efficacy.

Overall, CAR T-cell therapy shows great potential in the treatment of GBM, but widespread application still requires further research and optimisation to address existing technical and safety issues.

Current research on antigens has provided a solid foundation for further studies. These studies could focus on optimising the therapy to minimise CRS, on-target off-tumour toxicity, and other side effects, improving the delivery methods, and exploring more highly specific target antigens with rare expression in healthy tissues. Through continued exploration and clinical trials, it is hoped that more precise and personalised treatment options can be developed, ultimately improving patient survival and quality of life.

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