

# mRNA Vaccines in Glioma: Antigen Selection, Immune Profiling, Comprehensive Strategies and Challenges

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**Abstract.** Glioma is one of the most invasive primary brain tumors, and the exploration of its treatment strategies has always been a focus in the field of neurooncology. In recent years, mRNA vaccine technology has shown great potential in the COVID-19 epidemic, and its application in the treatment of glioma has also attracted increasing attention. This article reviews the research progress of mRNA vaccines in the treatment of glioma, including the advantages and challenges of mRNA vaccines, the comparison of different RNA therapy methods, as well as the screening of tumor specific antigens, differentiation of immune subtypes, and development of comprehensive treatment strategies. Although mRNA vaccines have shown promising prospects in the treatment of gliomas, their clinical translation still faces challenges such as safety, production scale, and cost-effectiveness. The author believes that a deep understanding of the molecular mechanisms of glioma and the heterogeneity of the immune microenvironment is crucial for developing effective mRNA vaccines. Future research needs to make breakthroughs in improving the stability of mRNA vaccines, optimizing immunogenicity, developing personalized vaccines, and comprehensive treatment strategies.

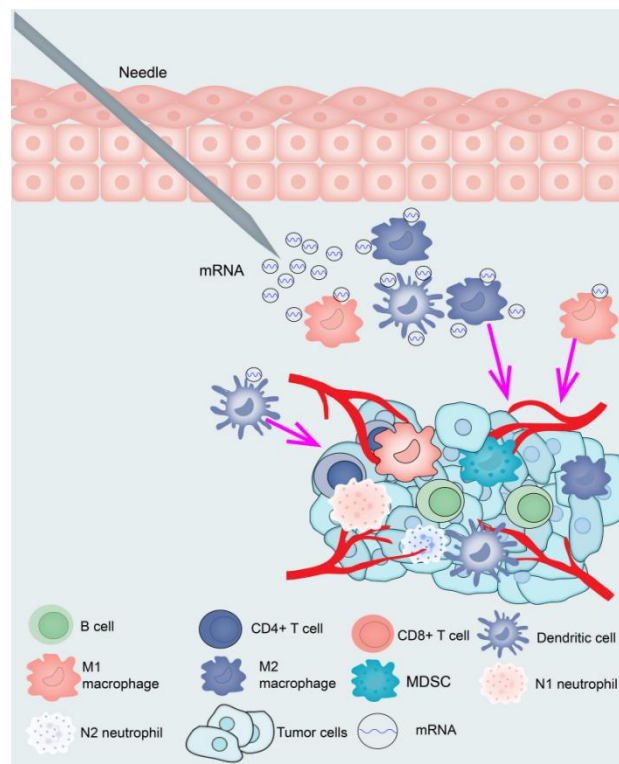
**Keywords:** mRNA vaccine, glioma, tumor microenvironment, tumor antigens, immune subtypes.

## 1. Introduction

Diffuse glioma is a highly invasive and lethal primary brain tumor, and its treatment strategy faces significant challenges. Although immunotherapy and mRNA vaccines have achieved remarkable results in various solid tumors, their application in the field of brain tumors is relatively lagging behind. This is mainly because the tumor microenvironment (TME) has noticeable immunosuppressive properties, which limit the effectiveness of immunotherapy. The TME of glioma is considered a "cold" microenvironment characterized by low levels of immune cell infiltration and weak immune response, which restricts the effectiveness of immunotherapy [1,2]. mRNA vaccines, as an emerging therapeutic approach, have demonstrated their potential in the development and clinical application of COVID-19 vaccines. However, the development and application of mRNA vaccines in glioma treatment are still in the exploratory stage. Research has shown that mRNA vaccines can activate or enhance the body's immune response to tumors by encoding tumor specific antigens or neoantigens. However, due to the unique nature of TME in gliomas, the research and application of mRNA vaccines in gliomas face a series of challenges, including how to effectively deliver vaccines to TME and how to overcome immunosuppressive factors in TME [2]. Currently, research on glioma mRNA vaccines mainly focuses on identifying tumor specific antigens and neoantigens, as well as developing effective delivery systems. Some studies have identified potential tumor antigens, such as TP53, IDH1, C3, and TCF12, which play important roles in the occurrence and development of gliomas and are closely related to the prognosis of patients (Figure 1) [3].

Zhou et al. successfully identified three key antigens in diffuse glioma: KDR, COL1A2, and SAMD9 through systematic analysis, providing an important reference for the development of mRNA vaccines [4]. This study utilizes high-throughput sequencing technology and immunohistochemical analysis to accurately screen target antigens suitable for vaccine design. Trivedi et al. further explored the potential of mRNA vaccines in accurately targeting neoantigens and tumor associated antigens, and found that these vaccines can significantly activate anti-tumor immune responses in vivo [5].

They demonstrated the effectiveness of mRNA vaccines through mouse models and preclinical trials. In addition, the study also found that the immune subtypes of gliomas are related to TME and patient survival rates, which provides a possibility for developing personalized mRNA vaccines [4,6]. Despite some progress in research, mRNA vaccines still face many challenges in the treatment of glioma. For example, how to improve the delivery efficiency of mRNA vaccines, how to overcome immune suppression in TME, and how to select appropriate vaccine strategies based on the patient's immune subtype. Future research needs to delve deeper into these areas in order to develop more effective glioma mRNA vaccines [5]. In summary, the treatment of glioma requires new treatment methods, and mRNA vaccines provide a potential treatment strategy. However, to achieve effective application of mRNA vaccines in glioma treatment, further in-depth research is needed on their behavior and mechanism of action in the glioma immune microenvironment, as well as the development of more effective vaccine design and delivery strategies.



**Fig. 1** Application mode 3 of mRNA vaccine in glioma [3].

## 2. Advantages and Disadvantages of mRNA Vaccines

Based on existing research, this article summarizes the advantages and challenges of current mRNA vaccines. The main advantages of mRNA vaccines are: strong targeting:

- 1) mRNA vaccines can encode tumor specific antigens and trigger immune responses against tumors [7].
- 2) Stimulating long-term immune memory: As found in Jinjin Chen et al.'s study, mice treated with mRNA cancer vaccines exhibited long-term immune memory [7].
- 3) Higher safety: mRNA vaccines do not integrate into the host genome, reducing the risk of gene mutations [8].
- 4) Multi modal activation of immunity: mRNA vaccines can activate the immune system in various ways, including dendritic cell vaccines, nanoparticle mediated antigen presentation, etc [8]. The challenges faced by mRNA vaccines mainly include the inhibitory TME of gliomas, poor delivery efficiency and targeting, weak stability, relatively short half-life, and certain immunogenicity.

### 3. Comparison of mRNA Therapy Methods

**Table 1.** Types, effects, mechanisms, advantages, and challenges of six mainstream mRNA therapies

mRNA vaccine type	Effect	Mechanism	Advantages	Challenge
RNA interference (RNAi)	Reduce the expression of specific genes through siRNA or miRNA.	The naturally occurring mechanism of gene silencing.	Specific and non-specific gene silencing.	1. May lead to non target gene suppression, poor delivery efficiency and stability.
Antisense Oligonucleotides (ASOs)	Prevent the translation of target mRNA and reduce the production of specific proteins.	Single stranded DNA or RNA molecules that complement and pair with the target mRNA.	High specificity, capable of efficiently inhibiting the production of specific proteins.	1. It may not be able to effectively penetrate the cell membrane, resulting in low intracellular delivery efficiency. 2. Specificity may also lead to non target effects, affecting the expression of other genes.
Small Activating RNA (saRNA)	Changing chromatin structure to promote or enhance transcription of specific genes.	RNA molecules that specifically activate gene expression.	Enhance host immune response and assist in vaccine efficacy.	1. Stability and delivery efficiency need to be improved.
mRNA-based therapeutics	Translating functional proteins in the body.	mRNA molecules encoding therapeutic proteins.	Rapid development, high production efficiency, and strong scalability.	1. Insufficient stability, delivery efficiency and immunogenicity need to be optimized. 2. Long term expression and potential insertion mutation risks are also challenges faced.
Circular RNA (circRNA)	As a miRNA sponge, it regulates miRNA	Stable RNA molecules formed through	High stability, suitable for the development of new	1. Further research is needed on the function and mechanism of

	activity.	reverse splicing.	vaccines.	action.
Self-Amplifying mRNA (SAM)	Generate a large amount of antigen proteins in a short period of time.	The mRNA form that can self replicate within host cells.	Efficient antigen expression enhances immune response.	1. Safety needs to be evaluated, stability and delivery are challenges.

Based on literature review, this article summarizes six mainstream mRNA therapy types, mechanisms of action, effects, advantages, and challenges faced by machines (Table 1). Among them, Zhang et al.'s research elucidated the effects, mechanisms, advantages, and challenges of five mRNA therapy techniques: RNA interference (RNAi), antisense oligonucleotides (ASOs), small activating RNA (saRNA) therapy, mRNA-based therapies, and circular RNA therapy (circRNA) [8]. Iavarone, C., The researchers investigated issues related to self amplifying mRNA (SAM [9]). This article summarizes their research findings (Table 1).

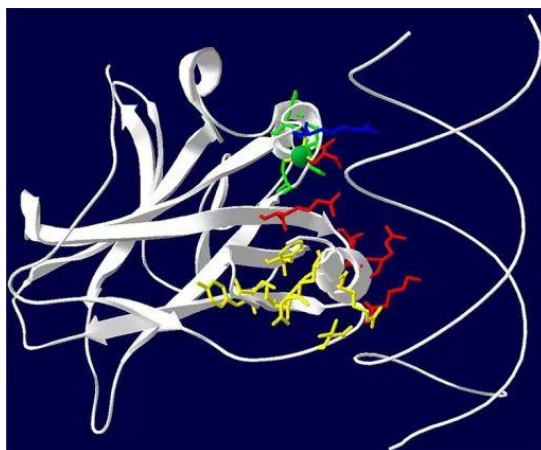
#### 4. Specific Antigen Screening and Immune Subtype Differentiation

##### 4.1. Specific Antigen Screening

The screening of specific antigens is the core of mRNA vaccine development. This review summarizes eleven specific antigens that can be used for mRNA vaccine development, namely TP53, IDH1, C3, TCF12, KDR (VEGFR-2), COL1A2, SAMD9, FCGBP (Fc Fragment of IgG Binding Protein, FLNC (Filamin C), TLR7 (Toll Like Receptor 7), CSF2RA (Colony Stimulating Factor 2 Receptor, Alpha). This article will provide a detailed introduction to TP53, IDH1, and CSF2RA.

##### 4.1.1. TP53

The TP53 gene encodes the tumor suppressor protein p53, which plays a critical role in cell cycle control, DNA damage response, and apoptosis. TP53 mutations are found in over 50% of malignant tumors and are closely associated with tumor development, progression, and prognosis. MRNA vaccines may promote the restoration of normal function of p53 protein by encoding the wild-type form of TP53, thereby inhibiting tumor cell proliferation and inducing apoptosis. It is expected to enhance immune surveillance in the TME, improve patient survival rates and quality of life (Figure 2) [3].



**Fig. 2** Three dimensional band diagram (white) of p53 DNA BD and line diagram of DNA double helix skeleton in the p53 DNA binding domain model structure group.

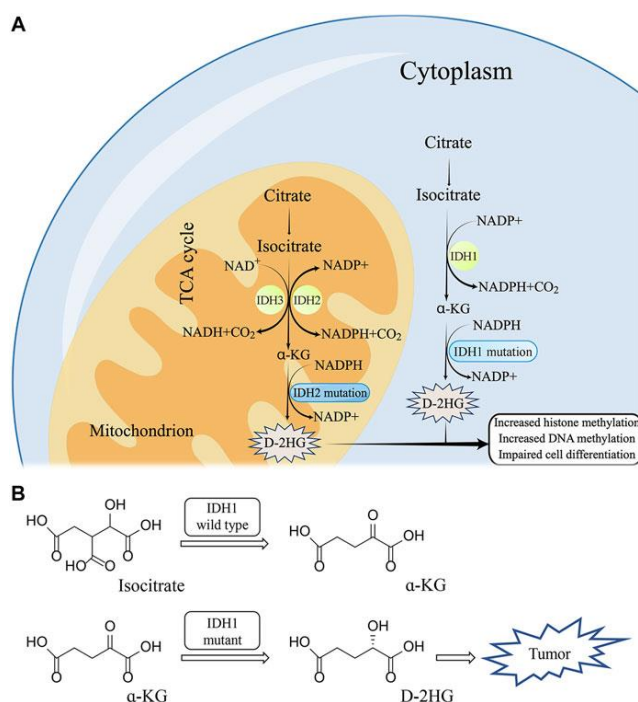
Red: Residues that directly interact with the main slot of DNA. Blue: R248 residue, directly interacts with DNA grooves.

Yellow: SLH motif stable residues support the tertiary structure opposite to the DNA main slot binding interface.

Green: Zinc ions and zinc ion coordination residues stabilize some DNA binding interfaces [10].

#### 4.1.2. IDH1

IDH1 is a key metabolic enzyme, and its mutants are commonly present in gliomas, which are related to the metabolic reprogramming and enhanced proliferation ability of tumors. Figure 3 shows the molecular mechanism of IDH1 mutations and the corresponding structures of substrates. mRNA vaccines can be designed to target IDH1 mutants, promoting the immune system to clear tumor cells expressing the mutated IDH1 by activating specific immune responses. May enhance patients' immune response to tumors, prolong progression free survival and overall survival [3].



**Fig. 3** Molecular mechanism of IDH1 mutation (A) and corresponding structure of substrate (B) [11].

#### 4.1.3. CSF2RA (Colony-Stimulating Factor 2 Receptor, Alpha)

CSF2RA is the alpha chain of granulocyte macrophage colony-stimulating factor receptor, involved in regulating the proliferation and activation of immune cells. In the TME, CSF2RA may affect the polarization state of tumor associated macrophages. mRNA vaccines may contain the coding sequence of CSF2RA. By regulating the expression of CSF2RA, it may help to alter the composition of immune cells in the TME, promote anti-tumor immune responses, facilitate the transformation of tumor associated macrophages into anti-tumor phenotypes, enhance the attack ability of immune cells on tumors, and improve the therapeutic efficacy of vaccines [12].

#### 4.2. Differentiation of Immune Subtypes

Understanding the characteristics of different immune subtypes is crucial for optimizing the efficacy of mRNA vaccines. Different studies have different insights into the differentiation of immune subtypes in glioblastoma. This article mainly summarizes the existing research subtypes of GBM (glioblastoma) and does not provide reference for future studies. Wu et al. classified GBM into four subtypes [13,14], namely IS1-IS4 (Table 2). Lin et al. classified GBM into three subtypes, namely IS1-IS3 [15] (Table 3).

**Table 2.** Four immune subtypes of GBM.

Immunophenotyping	Features	The connection with mRNA vaccines
IS1	<ol style="list-style-type: none"> <li>1. Immune cold phenotype</li> <li>2. Low immune cell infiltration</li> <li>3. Low expression of immune checkpoint (ICP) related genes</li> </ol>	May have good responsiveness to mRNA vaccines
IS2	<ol style="list-style-type: none"> <li>1. Immunothermal and immunosuppressive phenotypes</li> <li>2. High expression of ICP related genes, such as PD-L1 and CTLA4</li> </ol>	May have good responsiveness to mRNA vaccines
IS3	<ol style="list-style-type: none"> <li>1. Relative immune cold phenotype</li> <li>2. There is a certain degree of immune activation</li> </ol>	Has a certain reactivity to mRNA vaccines and may require combination with other treatments
IS4	<ol style="list-style-type: none"> <li>1. Moderate tumor immune microenvironment</li> <li>2. Immune cell infiltration and expression levels of immune regulatory molecules are moderate</li> </ol>	May require a combination of mRNA vaccines and multiple treatment strategies

**Table 3.** Three immune subtypes of GBM

Immunophenotyping	Features	The connection with mRNA vaccines
IS1	<ol style="list-style-type: none"> <li>1. Lack of regulatory immune cells</li> <li>2. Lack of immunosuppressive antigen-presenting cells</li> </ol>	May lead to T cell activation and survival advantage, suitable for mRNA vaccines
IS2	<ol style="list-style-type: none"> <li>1. Immunosuppressive type</li> <li>2. Tumor microenvironment that may have immune suppression</li> </ol>	Need to combine immune checkpoint inhibitors and mRNA vaccines
IS3	<ol style="list-style-type: none"> <li>1. Immune promoting type</li> <li>2. Having an inflammatory microenvironment</li> <li>3. There are more activated B cells, cytotoxic T cells, and NK cells</li> </ol>	May be the best candidate for mRNA vaccines

In addition, there are some studies targeting low-grade gliomas, such as Ye, L. et al. classified low-grade gliomas into three subtypes, namely IS1-IS3 [2]; And, Zhou, Q. et al.'s study on diffuse glioma divided it into three subtypes, namely Ims1-Ims3 [4]. The characteristics of immune typing are closely related to the potential efficacy of mRNA vaccines. Based on different immune microenvironment states, more personalized immunotherapy plans can be designed for glioma patients.

## **5. Comprehensive Strategy and Challenges Faced**

The application of comprehensive strategies can significantly enhance the efficacy of mRNA vaccines in the treatment of glioma. The following summarizes two comprehensive strategies that are at the forefront of research.

### **5.1. Combining Liposomes and mRNA Vaccines**

Combining liposomes and mRNA vaccines can improve delivery efficiency and stability. Chen, J. and Wang, C. et al. demonstrated the advantages of liposomes as mRNA delivery carriers, including improved *in vivo* stability and immune response [7,16]. Researchers have designed a lipid nanoparticle (LNP) -113-O12B, specifically designed to encapsulate and deliver mRNA<sup>7</sup> encoding specific tumor antigens [7]. The researchers encapsulated mRNA encoding TRP-2 peptide (TRP2180-188) using 113-O12B, which is a tumor associated antigen overexpressed in various tumors. In this way, 113-O12B LNP can effectively deliver mRNA to lymph nodes, which are key sites for immune cell activation and proliferation. In lymph nodes, encapsulated mRNA is taken up by APCs and subsequently translated into TRP-2 peptide. This process activates specific CD8<sup>+</sup>T cell responses, which are the main effector cells of anti-tumor immune responses. Chen et al. reported that 113-O12B encapsulated mRNA encoding TRP-2 peptide (TRP2180-188) exhibited excellent tumor suppressive activity [7]. This indicates that the mRNA vaccine has significant effects in tumor suppression. In addition, when the 113-O12B encapsulated TRP-2 mRNA vaccine is used in combination with anti-PD-1 therapy, it achieves a complete response rate of 40% in the conventional B16F10 tumor model. This result further demonstrates the potential of mRNA vaccines combined with checkpoint inhibitors in tumor treatment, providing a scientific basis for the development of new tumor immunotherapy regimens.

### **5.2. RNA Vaccine Combined with Immune Modulators and ICIs**

In addition, combining mRNA vaccines with immunomodulators and ICIs can further enhance the effectiveness of the vaccine. Segmenta Collar et al. studied the strategy of combining tumor neoantigen vaccines with immune modulators, and the results showed that this method can promote tumor attack through synergistic effects, reduce the risk of tumor recurrence through adaptive immune response, and significantly enhance anti-tumor immune response [17].

## **6. Challenges**

Although mRNA vaccines have shown potential in the treatment of gliomas, their clinical application still requires overcoming multiple challenges such as antigen selection, immunosuppressive microenvironment, and personalized treatment. Firstly, the stability and delivery efficiency of mRNA vaccines are key issues. Due to their large molecular size and negative charge, mRNA molecules have poor stability *in vivo* and are difficult to penetrate cell membranes [18]. To address this issue, researchers have developed various delivery systems, such as lipid nanoparticles (LNPs), to protect mRNA molecules and improve their delivery efficiency and expression levels *in vivo* [19,20]. Secondly, the immunogenicity and safety of mRNA vaccines are also the focus of research. Although mRNA vaccines have shown good immunogenicity and safety in COVID-19 vaccines, further research is still needed in the field of glioma treatment to ensure their safety and efficacy [21]. In addition, the immune reactions that mRNA vaccines may trigger, such as allergic reactions and cytokine release syndromes, also need to be strictly monitored in clinical applications [22]. Thirdly, the production process and cost-effectiveness of mRNA vaccines are also challenges. The large-scale production of mRNA vaccines requires highly specialized equipment and technology, while maintaining cost-effectiveness to ensure the popularization and accessibility of vaccines [23]. Finally, personalized and precise treatment with mRNA vaccines is also a direction for future development. With a deeper understanding of the molecular mechanisms of diseases, the development of personalized mRNA vaccines will become possible, which will provide patients with more precise and personalized treatment plans [24]. In summary, although mRNA vaccines have enormous

potential and advantages, they still face challenges in terms of stability, immunogenicity, production processes, and personalized treatment, and require further research and optimization.

## 7. Conclusion

mRNA vaccines have shown unprecedented potential in the field of glioma treatment, particularly in activating immune responses against tumor specific antigens and promoting the implementation of personalized treatment strategies. In view of this, the author believes that future research must focus on several key areas to ensure the effectiveness and practicality of mRNA vaccines. Firstly, improving the stability and delivery efficiency of mRNA vaccines is a prerequisite for their clinical application. Due to the instability of mRNA molecules and their rapid degradation in vivo, the development of advanced delivery systems, such as lipid nanoparticles (LNPs), is crucial for protecting mRNA molecules, enhancing their delivery efficiency and expression levels in vivo. Secondly, optimizing the immunogenicity of mRNA vaccines is key to activating effective immune responses. By precisely designing antigen sequences and selecting appropriate delivery carriers, the immune response triggered by vaccines can be enhanced, thereby improving the recognition and clearance ability of tumor cells. More research is needed in this area. In addition, developing personalized mRNA vaccines is a crucial step towards achieving precision medicine. Considering the molecular mechanisms of glioma and the heterogeneity of TME, future research requires a deeper understanding of the molecular characteristics of tumors and the immune status of each patient, in order to design vaccines that can target individual patient tumor features. At the same time, developing comprehensive treatment strategies that combine mRNA vaccines with other treatment methods such as chemotherapy, radiotherapy, targeted therapy, and ICIs may not only improve treatment efficacy, but also achieve synergistic effects, thus playing a greater role in glioma treatment. Finally, issues such as safety assessment, production scale, and cost-effectiveness must be taken seriously in the vaccine development process. Qualified safety assessment is a necessary condition to ensure that vaccines do not cause adverse reactions, while production scale and cost-effectiveness are related to the popularization and accessibility of vaccines. In summary, future research needs to make breakthroughs in improving stability, optimizing immunogenicity, developing personalized vaccines, and comprehensive treatment strategies to achieve the widespread application of mRNA vaccines in glioma treatment and ultimately overcome challenges in clinical translation.

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