Am J Cancer Res 2025;15(3):835-854
www.ajcr.us /ISSN:2156-6976/ajcr0162064

Review Article

Multifunctional nanoplatforms based on RNA interference for glioma treatment

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Received November 16, 2024; Accepted February 19, 2025; Epub March 15, 2025; Published March 30, 2025

Abstract: Glioma is the most common malignant tumor in the central nervous system. Currently, common clinical treatments for glioma include surgery, radiation therapy, chemotherapy and immunotherapy, among which the combination of chemotherapy and immunotherapy has attracted wide attention. However, the ability of chemotherapeutic agents and immune checkpoint blockers to reach gliomas is limited due to the existence of blood brain/tumor barrier (BBB/BTB). RNA interference (RNAi) technology enables specific silencing of target genes associated with cancer therapy, so it has been used as an emerging potential cancer treatment strategy. However, Small interference RNA (siRNA) is easily degraded by serum endonuclease, which can be quickly filtered and cleared by the glomerulus. Therefore, design and construction of safe and effective delivery systems is conducive to improving the stability of siRNA and the efficiency of gene silencing. This review focuses on the research progress of nano delivery system based on RNA interference for glioma treatment.

Keywords: Nanoplatform, RNA interference, glioma, blood brain barrier, siRNA

Introduction

Gliomas are the most common and deadly malignant tumors of the central nervous system (CNS), of which glioblastoma (GBM) is the most common malignant type of glioma and is classified as Grade IV by the WHO. Glioblastoma is the most aggressive primary brain tumor in adults. It is characterized by poor prognosis and extremely low survival rate of GBM patients, with a median survival of only 15 months [1]. At present, the standard treatment for glioblastoma is mainly surgical treatment, supplemented by postoperative concurrent chemoradiotherapy and adjuvant chemotherapy. However, the prognosis for GBM patients remains poor because the effect of this treatment is often hampered by resistance to GBM chemoradiotherapy. The BBB and the complex tumor immunosuppressive microenvironment pose serious challenges in the fight against glioblastoma and other brain tumors [2]. Therefore, effective treatments need to overcome the daunting challenge of crossing the BBB.

The regulation of microRNA (miRNA) is one of the main reasons for the occurrence, development and infiltration of GBMs. Some proteins that play a key role in tumorigenesis are overexpressed in brain tumor cells, so these proteins may be considered therapeutic targets. RNAi is a promising gene regulation technique that can be used alone or in combination with other means to achieve therapeutic effects [3]. siRNA is an effector molecule of RNAi technology. RNAi technology with high specificity and low toxicity has become a new method for the treatment of glioblastoma. siRNA can silence genes responsible for increasing drug resistance and make glioma cells sensitive to the drug. RNA interference (RNAi) technology provides a new strategy for glioma treatment by specifically silencing the expression of oncogenes or genes associated with drug resistance. The current research mainly focuses on two directions: (1) Targeting oncogenes using RNA interference technology. Gliomas are clinically classified into low-grade gliomas (grades I and II) and high-grade gliomas (grades III and IV). EGFR amplifi-

cation or mutation is present in approximately 50% of glioblastomas (GBM, WHO Grade 4). Studies have shown that the nanocarrier transports temozolomide (TMZ) and EGFR-siRNA efficiently into U87 cells, causing a vigorous apoptotic response by silencing the proliferative EGFR gene and increasing the drug concentration of TMZ simultaneously [4]. Chemotherapy resistance mediated by MGMT gene is an important cause of glioma treatment failure. RNAi silencing MGMT enhances the sensitivity of TMZ to tumor cells [5]. (2) Targeting the tumor microenvironment with RNAi technology. Inhibiting the expression of angiogenic genes (such as VEGF) can block tumor angiogenesis and reduce the invasion of glioma cells [6]. Gliomas of different grades may respond differently to RNAi therapy. High-grade gliomas may be more challenging due to the presence of BBB and tumor heterogeneity.

Although researchers have achieved encouraging results in the laboratory, RNA oligonucleotides have clinical limitations, and non-characteristic distribution of siRNA in vivo after systemic administration leads to low transfection efficiency, immune response, and toxicity; siRNA is easily degraded by nuclease and cleared by reticuloendothelial system [7]; The physical barrier of vascular endothelial wall and tissue prevents the introduction of siRNA drugs into tumor cells, resulting in low uptake and endocytosis effect of siRNA by cells [8]; siRNA is unable to achieve efficient endosome escape and its off-target effect on mRNA [9]. Clinical use of RNAi therapies for GBM is hampered by a lack of safe and brain-targeted transfections. Therefore, it is very important and necessary to develop new and effective siRNA vectors to make siRNA immune to nucleases.

At present, with the application and development of nanomedicine systems in cancer therapy, multi-drug and gene delivery to improve anti-cancer efficacy and reduce side effects is a huge advantage. Nucleic acid delivery by nanoparticles (NPs) is expected to present an important impact on the treatment of GBM. However, the clinical success of GBM therapy remains a huge challenge, mainly due to the unsatisfactory delivery of therapeutic agents in glioma cells in vivo. In recent years, with the continuous development of nanotechnology, embedding drugs or genes in nanocarriers can prolong the half-life of anti-tumor drugs, improve the drug loading capacity, and have strong targeting and good biocompatibility. Compared with normal tissues, some specific macromolecular substances are more likely to penetrate into tumor tissues and stay in tumor sites for a long time under the conditions of abundant blood vessels in solid tumors, larger vascular wall space and absence of tumor lymphatic circulation, which is known as the enhanced permeability and retention effect (EPR effect) of solid tumor [10]. The nano delivery system promotes the infiltration and retention of drugs in tumor tissues through EPR effect, thus achieving passive targeting. In addition, modification on the surface of the nanocarrier can make the nanocarrier specifically recognize tumor cells, so as to realize the active targeting of tumor cells [11]. In order to improve the delivery stability of siRNA, more and more researchers focus on the research and development of siRNA nanodelivery systems. At present, the application of siRNA nanoparticle delivery carriers in tumor therapy has become a hot topic in nanobiomedicine research. Patisiran (Alnylam), a siRNA lipid nanoparticle, was approved by the FDA in 2018 for the treatment of hereditary transthyroxin protein amyloidosis and is the first FDA-approved siRNA therapeutic agent [12]. This landmark success confirms the feasibility of siRNA technology for clinical applications. siRNA-encapsulated nano delivery system is a promising way to treat GBM, which can solve the problems in the diagnosis and treatment of GBM. This article mainly reviews the recent progress in the diagnosis and treatment of GBM by nanocarrier-based siRNA delivery system in order to provide new strategies and therapies for the treatment of GBM.

Mechanism of siRNA therapy for tumor

The occurrence of cancer is due to the mutation of tumor suppressor gene or oncogene, which leads to the uncontrolled growth of tumor cells and the inhibition of apoptosis. By comparing the efficacy of different chemotherapy drugs, it can be found that drugs that inhibit the expression of oncogenes are more effective than therapies that target cancer proteins, such as monoclonal antibodies. This is because in the tumor progression stage, the inhibited oncoproteins are easily replaced by newly

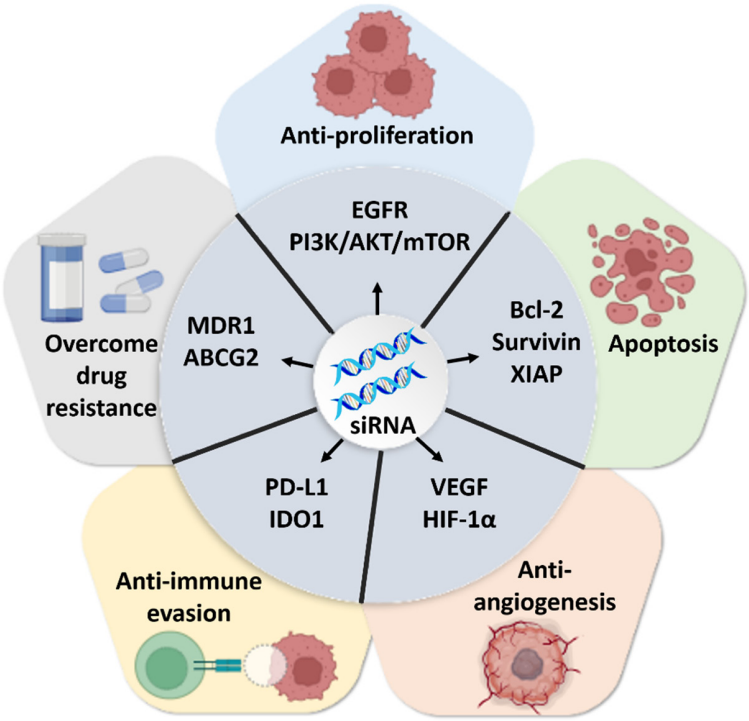

Figure 1. The mechanism of siRNA therapy for glioma. Five critical therapeutic target categories relevant to RNAi-based glioma treatment, including anti-proliferation, cell apoptosis, anti-angiogenesis, anti-immune evasion and overcome drug resistance.

expressed oncoproteins [13]. RNAi technology can play a specific role in gene regulation in anticancer therapy. siRNA is a subclass of small RNA molecules with a length of 21-23 nucleotides [14]. As one of the four subtypes of non-coding RNA, siRNA can effectively destroy the activity of messenger RNA (mRNA) in a sequence-specific way through the RNAi process, thereby mediating the silencing of target genes [15]. Gene silencing mainly occurs in two stages, namely transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS) [16]. In order to fully exert gene silencing function, siRNA needs to be fully paired with its target mRNA and inhibit the expression of the target gene by degrading the mRNA at the post-transcriptional level. Therefore, the use of RNAi technology often requires siRNA screening to obtain potential targets and the most effective siRNA sequence. The silenced genes are usually those involved in proliferation (EGFR, PI3K/AKT/mTOR signaling, etc.) [17, 18], apoptosis (Bcl-2, Survivin, XIAP, etc.) [19-21], angiogenesis (VEGF, HIF-1α, etc.) [22, 23], immune evasion (PD-L1, IDO1, etc.) [24, 25] and drug resistance (MDR1, ABCG2, etc.) [26, 27] of GBM cells as shown in **Figure 1**.

The mechanism of RNAi is triggered by the enzyme Dicer as shown in **Figure 2**, which cuts double-stranded RNA (dsRNA) into short double-stranded siRNA of 21-25 nt. Subsequently, siRNA and Argonaute 2 (Ago2) protein form RNA-induced silencing complex (RISC). At this time, the righteous strand of siRNA is dissociated from RISC, and the antisense strand sequence is paired with the mRNA complementary sequence to induce Ago2 to cut the target mRNA and finally achieve target gene silencing [28]. Due to the high selectivity and specific targeting ability of siRNA, researchers have used siRNA in the study of various cancer treatments, such as breast cancer [29], lung cancer [30], brain tumor [31], thyroid cancer [32] and bladder cancer [33], etc.

By encapsulating siRNA in nanocarriers for delivery in vivo, immunogenicity and nuclease sensitivity of siRNA can be significantly reduced [34]. Nanodrug delivery systems larger than 20 nm serve as carriers can avoid glomerular filtration of most siRNAs. Circulating nanoparticles can accumulate in large quantities at tumor sites based on the EPR effect [35]. The nanocarriers can be delivered to cells more efficiently by proper ligand modification on the surface of the nanocarriers.

Strategy for glioma treatment based on siRNA nanocarriers

The occurrence and development of tumors are usually caused by genetic abnormalities. In recent years, in order to improve the therapeutic effect of siRNA and reduce the multidrug resistance of chemotherapy drugs, researchers have designed a variety of nanocarriers to deliver siRNA to tumor sites, such as liposomes [36], polymer micelles [37], inorganic nanopar-

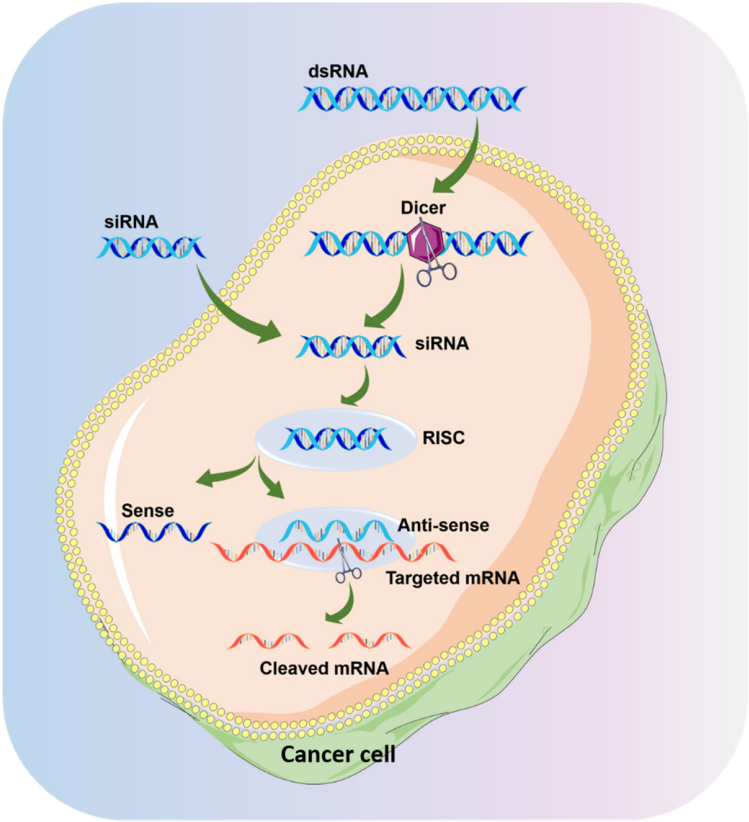

Figure 2. The mechanism of RNA interference triggered by siRNA. After the dsRNA is cut by Dicer, siRNA is produced, which binds to the RNA-induced silencing complex (RISC). The complementary pairing of siRNA with mRNA leads to the cutting or degradation of mRNA, thereby inhibiting the expression of target genes.

ticles [38], etc. These vectors are able to deliver siRNA to the tumor site, enabling drugs with different mechanisms to play a synergistic therapeutic role, inhibit the proliferation of GBM cells and control their malignancy. Sometimes targeting a single gene does not achieve an ideal curative effect, researchers design and develop chemotherapy drugs and genes co-delivery nanocarriers to reverse multi-drug resistance and improve chemotherapy drug sensitivity, so as to synergistically improve the therapeutic effect. This review summarizes the application of nano-delivery system loaded siRNA in GBM therapy. Nanocarriers for siRNA delivery in glioma therapy are summarized in **Table 1** and the nanocarriers for co-delivery of siRNA and anti-cancer drugs are summarized in **Table 2**. Strategies for glioma treatment based on siRNA nanocarriers are showed in **Figure 3**.

Nanoparticles

NPs have enhanced penetration and retention effects, enabling more precise and effective delivery of therapeutic drugs to target sites and reducing the risk of adverse reactions. The size and surface characteristics of nanoparticles play an important role in regulating the delivery efficiency of nanocarriers and the biological distribution of chemotherapy drugs. NPs have the characteristics of small size and large specific surface area, which can make them efficiently bind, absorb and release drug molecules, DNA, RNA, proteins and so on. NPs also have the characteristics of high stability, high carrying capacity, and can combine hydrophilic and hydrophobic substances, which has great clinical application potential in tumor therapy [39].

Inspired by the ability of natural proteins and viral particles to cross the BBB, Gregory et al. [40] designed a synthetic protein nanoparticle (SPNP) based on polymerized human serum albumin (HSA) and modified by the cell-penetrating peptide iRGD. Stat3 is a central hub related to GBM progress. The study showed that Stat3i SPNPs led to tumor growth inhibition, extended survival, and anti-GBM immune memory in 87.5% of GBM mice. The regulation of protein stability by ubiquitin proteasome system (UPS) is an important regulatory mechanism for cell growth. UPS represents a potentially valuable target for GBM treatment. Delle et al. [41] found that Praja2, a cyclic E3 ubiquitin ligase, was a key component of the signaling network that regulates the growth and metabolism of GBM cells. Praja2 was found to ubiquitinate and degrade the kinase inhibitor of Ras 2 (KSR2). Delivery of Praja2 siRNA in the brain by transferrin (Tf) targeted self-assembled NPs (SANPs) prevented KSR2 degradation, inhibited GBM growth, reduced tumor

Table 1. Nanocarriers for siRNA delivery in glioma therapy

Nanocarriers	Targeted genes	Surface modification	Effect	Ref.
Protein nanoparticle	Stat3	iRGD	Inhibit tumor growth, extend survival and activate anti-GBM immune memory	[40]
Nanoparticle gel	FAK, NOTCH-1 and SOX-2		Inhibit tumor growth	[44]
Chitosan oligolactic acid NPs	CD146	Folate	Inhibit tumor growth	[43]
Self-assembled nanoparticles	Praja2	Transferrin	Inhibit tumor growth, prolong survival time	[41]
pH-sensitive self-assembled hybrid nanoparticle	HDGF	$H_7K(R_2)_2$	Reduce the tumor volume and prolong the survival time	[42]
Cationic LNPs	CD47 and PD-L1		Activate T-cell-dependent anti-tumor immunity	[53]
LNPs	SAT1		Sensitize GB cells to radiotherapy and chemotherapy drugs	[52]
Liposome	cyclophilin A (CypA)	Aptamer-like peptide	Inhibit tumor growth	[57]
Liposome	c-Myc	Cell-penetrating peptide	Prolong survival time	[58]
Amphiphilic PAMAM dendrimer	Id1		Downregulate cell cycle-related genes and upregulate immune response-related genes	[63]
PAMAM	LSINCT5	Cell penetrating peptide	Penetrate BBB, inhibit tumor growth and activate anti-tumor immunity	[64]
Chimeric polymer	PLK1	Angiopep-2 peptide	Induce anti-glioblastoma effect and prolong survival time	[69]
Copolymer	Gli1		Induce cell apoptosis and inhibit tumor growth	[70]
Lipid polymer	Stat3		Inhibit tumor growth and prolong survival time	[71]
Amphiphilic poly (α) glutamate (APA)	PLK1	Sulfonic acid groups	Inhibit tumor cell viability	[68]
Polymer	Robo1, YAP1, NKCC1, EGFR and survivin		Reduce GBM in-migration and inhibit tumor growth	[20]
pH-responsive viral chitosan micelle	VEGF	cRGD	Inhibit tumor growth	[6]
Gold nanoparticles	Bcl-2 and VEGF	β-cyclodextrin (β-CD)	Exhibit good cytocompatibility and enhance gene silence	[80]
Porous silicon nanoparticles	multi-drug resistance associated protein 1 (MRP1)		Inhibit GBM proliferation	[82]
Magnetic nanoparticles	PLK1	Transferrin (Tf)	Increase cytotoxicity, reduce the tumor volume and prolong the survival time	[85]
Magnetic nanoparticles	tenascin-c (TN-C)		Inhibit tumor cell migration	[86]
Fe_3O_4 nanoparticles	repressor element-1-silencing transcription factor (REST)		Inhibit cell proliferation and migration	[84]

Table 2. Nanocarriers for co-delivery of siRNA and anti-cancer drugs in glioma therapy

Nanocarriers	Targeted genes	Anti-cancer compounds	Surface modification	Effect	Ref.
Nanocomplexes	MALAT1	TMZ		Improve the prognosis of GBM patients	[47]
ROS-response nanoparticle	TGF-β	TMZ		Enhance the cytotoxicity of temozolomide, improve the immunosuppressive microenvironment and prolong the survival time	[48]
Anoxic radiation-sensitive nanoparticle	MGMT	TMZ		Penetrate the BBB, inhibit GBM proliferation and prolong the survival time	[46]
pH-sensitive nanoparticles	EGFR	TMZ	Tf	Tumor cell apoptosis, inhibit tumor growth and prolong the survival time	[4]
CXCR4 targeting lipid calcium phosphate NPs	PD-L1	NO Nitric oxide	CTCE9908 peptide	Increase the infiltration, activate of cytotoxic T cells and inhibit tumor growth	[24]
Cationic lipid polylactic acid-glycolic acid (PLGA) nanoparticle	GOLPH3	Gefitinib (Ge)	Angioendothelin-2 (A2)	Penetrate the BBB and inhibit tumor growth	[54]
β-amphetamine cationic LNP	PD-L1	Paclitaxel		Cytotoxicity to GBM cell, prolong overall survival	[55]
Cationic liposomes	Survivin	Paclitaxel		Induce cell apoptosis, improve the survival rate	[59]
Liposomes	STAT3	WP1066	RGDK	Inhibit tumor growth	[60]
pH-sensitive PAMAM	c-Myc	DOX	RGD	Penetrate BBB and enhance anti-tumor activity and inhibit tumor growth	[65]
Oxidation-reducing glycolipid copolymer	VEGF	PTX	Angiopep-2 (Ap)	Inhibit glioma growth	[72]
Lactoferrin nanoparticles	Aurora Kinase B (AKB)	TMZ		Cytotoxicity and cell cycle arrest and inhibit tumor growth	[49]
Polymeric micelle	Bcl-2	TMZ	Folate	Inhibit glioma growth and prolong the survival time	[76]
Micelles	STAT3	TMZ		Penetrate BBB, inhibit glioma growth, achieve synergistic therapeutic effects	[75]
Polymer micelle	PLK1	TMZ	Angioendotheliin-2 (A2)	Cell apoptosis, inhibit glioma growth and prolong survival time	[77]
Iron oxide nanoparticles (IONPs)	GPX4	Cisplatin (Pt)		Induce iron death and improve the therapeutic effect	[88]
Iron oxide nanoparticles	MGMT	TMZ	Polypeptide	Increase sensitivity to TMZ therapy	[5]
Iron oxide nanoparticle	MGMT	TMZ		Increase apoptosis, inhibit tumor growth and prolong survival	[87]

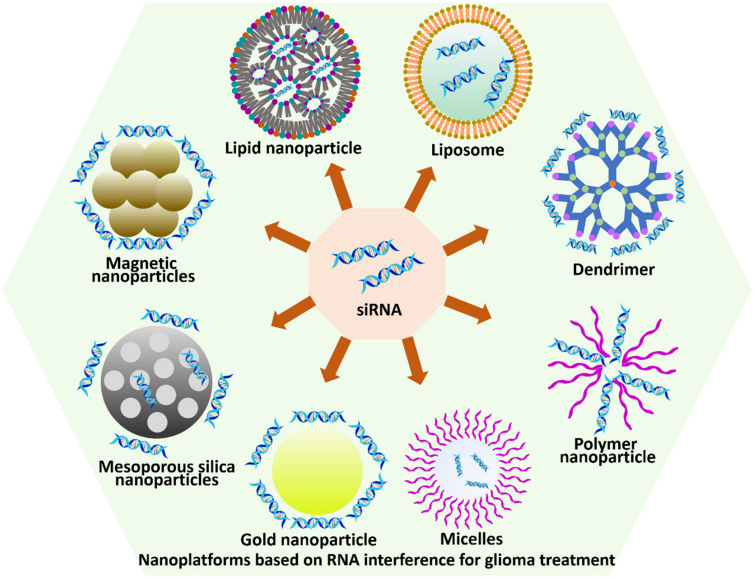

Figure 3. Simplified diagrams of representative nanocarriers applied for siRNA delivery in gliomas. Common siRNA nanodelivery systems include lipid nanoparticle, liposome, dendrimer, polymer nanoparticle, micelles, gold nanoparticle, mesoporous silica nanoparticle, magnetic nanoparticle, et al.

size, and prolonged survival in treated mice. Hepatocellular carcinoma-derived growth factor (HDGF) is considered as a potential therapeutic target for glioma, and the expression level in glioma is positively correlated with the degree of malignancy. Zhou et al. [42] developed a pH-sensitive self-assembled hybrid NP package siHDGF modified with the peptide H7K(R2)2. Studies showed that the NPs effectively delivered siHDGF to the brain and malignant glioma cells, down-regulated the expression of HDGF, inhibited the malignant phenotype of glioma cells, reduced the tumor volume of U251 human glioblastoma nude mice, and prolonged the survival time. CD146 is mainly expressed in the division of glioma stem cells (GSCs) and regulates the cell cycle process. Fukui et al. [43] developed glioma gene therapy targeting GSCs using chitosan oligolactic acid (COL) NPs coupled to folate-polyethylene glycol (FA-PEG-COL NPs) for the delivery of CD146 siRNA (siCD146). The results of glioma model study in mice showed that siCD146 NPs had obvious inhibitory effect on brain tumor growth. Delivery of siCD146 significantly reduced Ki-67 index in residual tumor tissue compared to control mice.

The combination of multiple genes targeting different targets will provide better efficacy. Manju et al. [44] designed a multi-gene (FAK, NOTCH-1 and SOX-2) targeted siRNA NP gel (NPG) prepared by self-assembly of protein-hyaluronic acid combination. The results showed that gene silencing FAK, NOTCH-1 and SOX-2 inhibited neuroglobule formation, while normal stem cells were unaffected and retained the ability to differentiate neurons. GBM PDX model studies showed that the tumorigenic potential of NPG treated tumor cells was significantly impaired. Intracerebral injection of NPG inhibited tumor growth in rat brain tumor models in situ.

Chemotherapy drug Temozolomide (TMZ) is the first-line chemotherapy regimen for glioma. However, drug resistance has limited the efficacy and clinical application of TMZ. TMZ also shows poor targeting specificity and usually leads to decreased drug concentration and increased side effects in GBM [45]. In order to increase the sensitivity of TMZ-based chemotherapy and radiotherapy, Xie et al. [46] synthesized an anoxic radiation-sensitive NP for co-delivery of TMZ and O6-methylguanine-DNA-methyltransferase siRNA (RDPP(Met)/TMZ/siMGMT). Downregulation of MGMT expression could significantly activate cell apoptosis, inhibit DNA damage repair, and enhance TMZ sensitivity. Studies shown that RDPP(Met)/TMZ/siMGMT could effectively penetrate the BBB, accurately targeted GBM cells, and inhibited GBM proliferation. And RDPP(Met)/TMZ/siMGMT significantly improved the survival time of GBM mice in situ. Relevant studies have shown that long noncoding RNA (lncRNA) and metastasation-associated lung adenocarcinoma transcript 1 (MALAT1) affects tumor cell invasion and GBM therapy resistance. Kim et al. [47] constructed tumor-targeting nanocomplexes loaded with MALAT1 siRNA. The study showed that the silencing of MALAT1 significantly increased the sensitivity of GBM cells to the chemotherapy drug Temozolomide (TMZ). The study confirmed that combining standard TMZ therapy with targeted therapy of lncRNA nanocomplexes signifi-

cantly improved the prognosis of GBM patients. Chemotherapy for gliomas is often influenced by the immunosuppressive tumor microenvironment, in particular the tumor growth factor β (TGF-β), an immunosuppressive cytokine, which is severely hindered. The researchers modified the tumor immune microenvironment through RNAi-based immune regulation to improve the effectiveness of chemotherapy. Qiao et al. [48] established a NP with double targeting and ROS response (Angiopep Lipo-PCB (TMZ +BAP/siTGF-β), ALBTA) for the treatment of intracranial glioblastoma. The results showed that the NPs enhanced the cytotoxicity of temozolomide and improved the gene silencing efficiency of siTGF-β. ALBTA significantly improved the immunosuppressive microenvironment of glioma mice and prolonged the survival time of glioma mice. Overexpression of epidermal growth factor receptor (EGFR) leads to chemotherapy resistance of GBM. To overcome these obstacles, Wang et al. [4] prepared pH-sensitive GBM-targeting NPs. Chemotherapy drugs TMZ and EGFR inhibitors (EGFR siRNA) were co-encapsulated in nanocarriers (Tf-PEG-PAE(SS)/TMZ@siEGFR). Studies showed that the nanocarriers induced strong apoptosis by silencing proliferating EGFR gene while increasing TMZ drug concentration. Studies on glioma mice showed that the accumulation of nanocarriers at tumor sites significantly inhibited tumor growth and prolonged the survival of mice by intracranial injection of Tf-PEG-PAE(SS)/TMZ@siEGFR. To investigate the efficacy of siRNA delivered by lactoferrin NPs (LfNPs) through the BBB in the treatment of glioblastoma multiforme (GBM) and enhance the efficacy of conventional TMZ chemotherapy. Kumari et al. [49] designed Aurora Kinase B (AKB) siRNA-supported NPs (AKB-LFNPS) were prepared by water-in-oil emulsion method using milk protein and lactoferrin as raw materials. AKB-LfNPs was detected in cell lines and GBM in situ mouse models with and without TMZ treatment. These LfNPs have been shown to be effective in AKB silencing, cytotoxicity and cell cycle arrest of GL261 cells. In mice treated with AKB-LfNPs alone and in combination with TMZ, tumor growth was significantly reduced and survival was improved by 2.5 times.

Nitric oxide (NO) plays a regulatory role in the BBB and tumor blood vessels, so NO can be delivered to disrupt the BBB and improve the delivery of immunotherapy drugs into GBM tumors. Hsieh et al. [24] reported an immunotherapy approach using CXCR4 targeting lipid calcium phosphate NPs to deliver NO and immune checkpoint ligand inhibitor programmed cell death 1 ligand 1 (PD-L1) siRNA. Delivery of NO leads to increased BBB permeability, which improves gene transmission across BBB. PD-L1 siRNA could significantly increase the infiltration and activation of cytotoxic T cells in GBM tumors, and inhibit the progression of GBM.

Lipid nanoparticles

Lipid nanoparticles (LNPs) are lipid vesicles with homogeneous lipid nuclei. LNPs can deliver nucleic acids to cells and is composed of ionizable cationic lipids, neutral helper phospholipids, cholesterol and PEG lipids. These vesicles are widely used for the delivery of small molecule drugs and nucleic acids, and have recently received a lot of attention for their remarkable success as delivery platforms for COVID-19 mRNA vaccines [50, 51].

Spermidine/spermidine N1-acetyltransferase 1 (SAT1) is responsible for cellular polyamine catabolism and is overexpressed in GBM. It is a potential therapeutic target due to its role in tumor survival and promoting resistance to radiation therapy. Vinith Yathindranath et al. [52] prepared LNP-based siRNA delivery system (LNP-siSAT1) to selectively knockdown SAT1 enzyme in human glioblastoma cell lines. LNP-siSAT1 effectively down-regulated SAT1 expression on mRNA and protein levels in U251, LN229 and 42MGBA GB cells and other brain-associated endothelial cells (hCMEC/D3), astrocytes (HA) and macrophages (ANA-1). Enhancing the delivery of LNP-siSAT1 in BBB cell culture models by circumventing the BBB could safely and effectively reduce SAT1 expression and sensitize GB cells to radiotherapy and chemotherapy drugs.

CD47 is a transmembrane glycoprotein, also known as integrin-associated protein, is overexpressed in several tumors. Liu et al. [53] prepared a new cationic LNPs to deliver CD47 siRNA and immune checkpoint ligand inhibitor PD-L1 siRNA to the BBB and the target mouse brain for regulating the tumor microenvironment and for GBM immunotherapy. The NPs significantly enhanced the cellular uptake and

endosomal escape of siRNA, effectively transferred siRNA to mouse brain GBM through the BBB, and down-regulated the expression of target genes in tumors, thereby synergically activated T-cell-dependent anti-tumor immunity in in-situ GBM.

Gefitinib (Ge) is an EGFR tyrosine kinase inhibitor (TKI). The expression of Golgi phosphorylated protein 3 (GOLPH3) is associated with poor prognosis in glioma. Down-regulation of GOLPH3 can promote EGFR degradation. Ye et al. [54] developed an angioendothelin-2 (A2) -modified cationic lipid polylactic acid-glycolic acid (PLGA) NP (A2-N) for the delivery of Ge and GOLPH3 siRNA (A2-N/Ge/siGOLPH3). The study demonstrated that A2-N/Ge/siGOLPH3 successfully crossed BBB and targeted gliomas. The released siGOLPH3 effectively inhibited the expression of GOLPH3 mRNA and further promoted the degradation of EGFR and p-EGFR. The released Ge also significantly inhibited EGFR signaling. Psychostimulants such as amphetamines and methamphetamines can penetrate the BBB. However, it is rarely used in nanomedicine delivery due to toxicity. Saha et al. [55] designed and synthesized 3 different β-amphetamine cationic LNP for the first time. The LNP were found to be nontoxic and could cross BBB through endocytosis. The LNP could simultaneously encapsulate paclitaxel (PTX) and PD-L1 siRNA. Dual-loaded LNP showed cytotoxicity to GL261 cells and improved overall survival in mice with in-situ GBM compared to non-targeted controls.

Liposomes

Liposomes are widely studied and applied as drug carriers at present. Currently, there have been approved several liposomes in clinic, indicating that the research on liposomes has reached a relatively mature stage [56]. The structure of lipid bilayer of liposome is similar to that of human biofilm, so it has the characteristics of low immunogenicity, low toxicity and degradability after injection into human body. There is a double-layer membrane structure of hydrophilic and hydrophobic cavities in liposomes, so compared with other carriers, liposomes can contain both hydrophilic and lipophilic drugs for the treatment of the same disease, which can greatly improve the therapeutic effect. It has great advantages and development prospects in the application of drug carriers for the treatment of brain glioma.

Saw et al. [57] developed an aptamer-like peptide-modified liposome nanoplatform for siRNA delivery and GBM targeted therapy. The nanoplatform is mainly composed of the following key components: (i) Classical liposome structure and the therapeutic siRNA is loaded in watery core; (ii) Hydrophilic PEG chains on the shell, which prolong blood circulation; (iii) Surface-encoded peptide, the outer domain B of fibronectin overexpressed on glioma cells. These liposomes could target glioma cells and effectively inhibit the growth of GBM tumors by silencing the expression of cyclophilin A (CypA), which was upregulated in brain cancer and played an important role in malignant transformation of brain cancer and maintenance of stem cell character of glioma cells. The malignant degree of GBM is closely related to the up-regulation of oncogene c-Myc expression. Nasal mucosa administration is an effective treatment. Permeability of the nasal mucosa, glioma targeting, and avoidance of premature release during remote transport are necessary conditions to ensure therapeutic effectiveness. In order to solve the above problems, Hu et al. [58] constructed a penetrin-derived peptide-modified liposome for containing c-Myc siRNA (sic-Myc) pre-compressed with octoarginine, named 89WP. It was found that within 4 hours, siRNA was released into the cytoplasm through the endosomal escape, which induced the expression of c-Myc mRNA and protein in glioma cells to be significantly down-regulated. In addition, due to the enhanced permeability of the nasal mucosa, liposomes delivered more siRNA to the in-situ glioma after nasal administration, thereby prolonging the survival time of glioma mice by inducing apoptosis. This liposome improves the efficiency of gene delivery for intranasal drug delivery and is promising in selectively silencing disease-related genes in intracranial tumors.

To overcome the drug resistance of paclitaxel (PTX) in glioma, Sun et al. [59] designed a low-density lipoprotein receptor-related protein and a RNA aptamer bound CD133 were used as dual-targeting ligands to prepare dual-modified cationic liposomes (DP-CLPs) loaded with survivin siRNA and PTX (DP-CLPs-PTX-siRNA) for actively targeting imaging and treating CD133+

glioma stem cells after passing through the BBB. Studies shown that DP-CLPs exhibited strong targeting ability and delivered drugs (PTX/siRNA) to CD133+ glioma stem cells, which induced selective apoptosis of cells, promoted cell differentiation into non-stem cell lineages, and significantly inhibited tumorgenesis. The apoptosis of CD133+ glioma cells in nude mice with intracranial glioma was induced to improve the survival rate. Vangala et al. [60] constructed RGDK-lipopeptide-modified liposomes that co-deliver JAK/STAT pathway small molecule inhibitors (WP1066) and Stat3 siRNA, which can be targeted and bound to integrin α5β1 receptor on the cell surface and internalized in GL261 cells. Studies shown that the liposome significantly inhibited in situ GBM in mice compared to untreated mice (inhibition rate is 350%).

Dendrimers

Dendrimers are a new type of functional polymers developed in recent years. They are highly branched and structurally accurate molecules synthesized by repeated growth reaction [61]. Polyamide-amine dendritic macromolecules (PAMAM) are most commonly used to prepare novel nanoscale dendritic macromolecules. PAMAM is monodisperse and highly branched, with a particle size ranging from 1.5 to 14.5 nm. It has a cavity inside, which can wrap drug molecules of different sizes, and the end groups can connect bioactive substances such as antibodies through appropriate modification, thus forming a stable system with longer cycle time in the body [62]. Compared with linear macromolecules, PAMAM has the advantages of regular structure, definite molecular weight and molecular size, precise control of molecular shape and functional group, stability, non-immunogenicity, and high transport efficiency for bioactive agents. These characteristics make PAMAM one of the research features in the field of biomedicine [62].

Ellert-Miklaszewska A et al. [63] evaluated the siRNA delivery and gene knockout function of amphiphilic dendritic molecules (AD) in primary microglia. Studies showed that AD effectively delivered siRNA to primary microglia, reduced the expression of target genes and proteins, and led to transcriptome changes. Jin et al. [64] developed a novel bi-functional tree-polymer drug delivery system loaded with siLSINCT5 via PAMAM and surf-modified cell penetrating peptide tLyp-1 (tLypNP-siRNA) to overcome BBB. In addition, to overcome the immunosuppressive microenvironment within GBM tissues, the researchers applied a checkpoint inhibitor called anti-NKG2A monoclonal antibody (aNKG2A), which is able to promote antitumor immunity by releasing T and NK cells, further by pH-sensitive chain binding to the surface of siLSINCT5-loaded NPs. Studies showed that the delivery system effectively crossed the BBB and inhibited GBM by simultaneously inhibiting LSINCT5-activated signaling pathways and activating anti-tumor immunity.

Co-delivery of chemotherapeutic drugs with tumor-specific siRNA, as a new therapeutic approach, provides a promising strategy for cancer treatment. Huang et al. [65] designed and constructed a pH-sensitive nanosystem decorated with PAMAM-RGD to deliver doxorubicin (DOX) and c-Myc siRNA (RGD-SeNPs/siRNA) for the combined treatment of glioblastoma. PAMAM-RGD surface modification significantly enhanced the uptake of RGD-SeNPs/siRNA by cells and increased the selectivity of cancer cells. More importantly, in the case of pH 5.3, the duration of taking the drug is extended and adverse side effects are reduced. The BBB model established in vitro showed that the nanosystem effectively penetrated the BBB and enhanced anti-tumor activity. In addition, the nanosystem also showed excellent advantages in the penetration ability and inhibition effect of U251 tumor spheres, demonstrating its anti-cancer potential in vivo.

Polymer nanoparticles

Nanocarriers based on polymers show great advantages, including convenient design and synthesis, appropriate particle size and shape, good biocompatibility, high drug loading efficiency, biodegradability and so on. Therefore, polymer-based nanocarriers has aroused the wide attention of researchers [66]. The polymeric NPs constructed by Karlsson et al. [67] contain ester bonds for hydrolytic degradation and disulfide bonds for the release of environmentally triggered siRNA in the cytoplasm. These photocrosslinked bioreducible NPs (Xb-NPs) have a shielded surface charge that reduces the adsorption of serum proteins.

XbNPs facilitated siRNA-mediated in vivo knockout in melanoma colonized in the lungs after systemic administration. Thus, by photocrosslinking, biodegradable polymer NPs exhibit greater colloidal stability and efficient delivery of RNA therapeutics under physiological conditions.

GBM chemotherapy resistance is often caused by increased activation of genes associated with DNA repair, such as overexpression of typical genes associated with cell proliferation and tumor progression, such as polo-like kinase 1 (PLK1). Therefore, Krivitsky et al. [68] used amphiphilic poly (α) glutamate (APA) targeting siRNA polymers of PLK1 to sensitized resistant GB cells. The brain targeting was improved by modifying nanocarriers with sulfonic acid groups. Sulfonated nanocallers have superior selectivity for P-selp (SELP), a transmembrane glycoprotein that is overexpressed in GB and angiogenic brain endothelial cells. Self-assembled polymers of sulphonated APA and siPLK1 internalize into GB cells and our unique three-dimensional (3D) GB spheres, inducing specific gene silencing. In addition, RNAi nanotherapy effectively reduced the cell viability of chemotherapy-sensitive and chemotherapy-resistant GB cells. Similarly, Shi et al. [69] constructed angiopep-2 peptide-modified chimeric polymer (ANG-CP) as a non-toxic brain-targeting non-viral vector to deliver siPLK1. In vitro experiments showed that ANG-CP significantly silenced PLK1 mRNA and corresponding oncoprotein in U-87 MG cells, prolonged the cycle time of siPLK1, and promoted its accumulation in GBM. siPLK1 induced a stronger anti-glioblastoma effect and significantly improved the survival time of glioblastoma-carrying mice.

Gli1 is an ideal candidate target for tumor gene therapy and plays an important role in tumor genesis. Zhou et al. [70] constructed a novel self-assembled gene delivery system using the copolymer of 1, 2-diacyl-3-trimethylpropane and methoxy-polyethylene glycol-lacroester (DMP) as materials. Studies showed that DMP- siGli1 exhibited anti-glioma effect by inducing apoptosis and inhibiting cell growth in vitro. In addition, in subcutaneous tumor bearing mice, DMP- siGli1 complex significantly inhibited tumor growth by inhibiting Gli1 protein expression, promoting apoptosis and reducing proliferation. Signal transduction and transcriptional activator 3 (Stat3), a key signaling protein that drives the major markers of cancer, is a promising target for glioblastoma therapy. Linder et al. [71] delivered Stat3 siRNA using a nanoscale lipid polymer (LPP) based on polyethylenimine and phospholipid 1, 2-dipalmitoylsan-glycerol-3-phospholipine choline. Studies showed that LPP-mediated siRNA delivery mediates effective Stat3 knockdown in Tu2449, U87 and Mz18 glioma cells, inhibited Stat3 activity and cell growth. Intracranial application of siRNA LPP led to downregulation of Stat3 target gene expression, reduced tumor growth, and significantly longer survival in Tu2449 glioma mice compared to animals treated with negative controls.

Vascular endothelial growth factor (VEGF) is considered to be a key regulator of tumor neovascularization. siRNA inhibited VEGF expression but could not completely inhibit angiogenesis and tumor growth. In order to improve the therapeutic effect of glioma, Wen et al. [72] developed an angiopep-2 (Ap) -modified oxidation-reducing glycolipid copolymer that co-delivers siVEGF and PTX, called AP-CSSSSA/P/R complex. Studies showed that the Ap-CSssSA/P/R complex simultaneously delivered siVEGF and PTX to tumor cells, which had great advantages in inhibiting glioma growth through receptor-mediated targeted delivery and apoptosis, and exhibited inhibitory effect on VEGF gene silencing induced neovascularization.

Kozielski et al. [20] synthesized a bioreducible and biodegradable polymer that can package and deliver hundreds of siRNA molecules into a single NP, facilitating combination therapy against multiple GBM-promoting targets. siRNA targeting several anti-GBM genes (Robo1, YAP1, NKCC1, EGFR and survivin) can be delivered simultaneously within the NP. Delivery of Robo1 (circular homologous 1) siRNA via biodegradable particles was found to trigger GBM cell death, as was non-viral delivery of NKCC1, EGFR, and survivin siRNA. Incorporating several anti-GBM siRNAs into NP preparations resulted in high GBM cell mortality, reduced GBM inmigration, and reduced tumor burden after administration.

Micelles

Bader et al. [73] first applied polymer micelles to drug carriers in 1984. Since then, micelles

have gained more and more attention. Compared with other nanocarriers, micelles exhibit unique properties: (1) Micelles can penetrate solid tumors through EPR effect. The targeting molecules attached to the micellar shell can exhibit targeting effect. (2) Drugs that are insoluble in water can be wrapped in the core-shell structure of micelles to improve the solubility and bioavailability of drugs. (3) Micelles are thermodynamically stable and biocompatible in water-based media, which can reduce toxic side effects. (4) The preparation process is simple with high drug load. (5) Micelles prolong the retention time of drugs in the blood circulation [74].

siVEGF shows great potential in inhibiting tumor growth, proliferation, and migration by reducing the proliferation of blood vessels. Zhang et al. [6] prepared a new pH-responsive viral chitosan micelle as a siRNA delivery system based on the bionics principle. The cyclic (arg-gly-asp-d-ph-lys) (cRGD) modified polyethylene glycol was coupled to the HA2-modified chitosan by the hydrazone bond to form micelles. cRGD -modified chitosan micelles could accurately target glioma U87MG cells with high expression of αvβ3. siVEGF is loaded into the core of the micelle through electrostatic and hydrophobic interactions. Intracellular drug release is achieved through pH-responsive cleavage of the kersome to the hydrazone bond in an acidic environment. In addition, the micelles could effectively transmit siVEGF and silence VEGF gene expression in U87MG cells, thus significantly inhibiting tumor growth. This study showed that the micelle was able to deliver and release siVEGF in a controlled manner and tracked it through a fluorescence resonance energy transfer (FRET) system, enabling RNAi based tumor anti-angiogenesis therapy in vivo.

Using TMZ as a model loaded drug, siRNA micelles achieved effective synergistic therapeutic effects by targeting Stat3, a key gene in the TMZ resistance pathway. Jiang et al. [75] prepared a novel siRNA micelle self-assembled from siRNA-disulfide-poly (n-isopropylacrylamide) diblock copolymer to contain Stat3 siRNA. siRNA micelles not only exhibit extended blood circulation time, superior cellular uptake, and efficient in situ siRNA release, but also achieve effective BBB penetration. In addition, since they are non-cationic, these siRNA micelles show no charge-related toxicity. Studies shown that the new RNAi nanomaterials inhibited the growth of in situ U87MG xenografts without adverse reactions, and significantly improved the survival efficiency. Bcl-2 is an oncogene, which exhibits obvious inhibitory effect on cell apoptosis. Peng et al. [76] synthesized a polymeric micelle for delivering TMZ and Bcl-2 siRNA (TMZ-FaPEC@siRNA) used folate-conjugated triblock copolymer (Fa-PEG-PEI-PCL, Fa-PEC) of poly (ε-caprolactone) (PCL), poly (ethylenimine) (PEI) and poly (ethylene glycol) (PEG). The results showed that the nanocoliters induced apoptosis of tumor cells by silencing anti-apoptotic gene Bcl-2 and activating pro-apoptotic gene Bax. Intracerebral injection of TMZ-FaPEC@siRNA can significantly inhibit the growth of orthotropic glioma in rats and prolong the survival time of the animals. PLK1-targeted therapy leads to G2/M block and increases glioma sensitivity to TMZ. Therefore, in order to limit TMZ resistance in gliomas, Shi et al. [77] developed a TMZ-embedded angioendotheliin-2 (A2) modified polymer micelle (A2PEC) and a PLK1-targeting siRNA (TMZ-A2PEC/siPLK). TMZ is wrapped in A2PEC by hydrophobic interaction, and siPLK1 is complex with TMZ-A2PEC by electrostatic interaction. In vitro experiments have shown that TMZ-A2PEC/siPLK can effectively enhance the cellular uptake of TMZ and siPLK1, leading to obvious apoptosis and cytotoxicity of glioma cells. In vivo experiments showed that glioma growth was inhibited and survival time was significantly prolonged after injection of TMZ-A2PEC/siPLK1 through tail vein.

Inorganic nanoparticles

Inorganic nanomaterials play an important role in the field of the application of nanocarriers in the diagnosis and treatment of tumors. Inorganic nanocarriers show many advantages in the field of tumor therapy, including a wide variety of materials, easy synthesis and modification. Suitable ligands are critical to the colloidal stability and function of NPs. Inorganic NPs, including gold, silver, iron oxide, carbon, quantum dots and other materials, have been used in preclinical and clinical studies for the detection, diagnosis and treatment of a variety of tumors [78].

Gold nanoparticles: Gold NPs are nanoscale metal materials. Among many nanomaterials, gold NPs have the advantages of low IV toxicity,

easy control of particle size and shape, and local surface plasmonic resonance, etc. Therefore, the application of tumor sensors, drug release agents and enhancers in plasmonic photothermal therapy has been widely studied [79].

Qiu et al. [80] described a safe and efficient non-viral vector system based on β-cyclodextrin (β-CD)-modified dendritically wrapped gold NPs (Au DENPs) for improving siRNA delivery to glioblastoma cells. Au NPs were captured by grafting β-CD onto the 5th generation polyamines (amines) dendrimers as nanoreactors. The obtained β-CD-modified Au DENPs (Au DENPs-β-CD) is complexed with two different types of therapeutic siRNA (Bcl-2 siRNA and VEGF siRNA). The results showed that the formed Au DENPs-β-CD vector effectively delivered siRNA to glioma cells, exhibited good cellular compatibility, and enhanced gene silencing to inhibit the expression of Bcl-2 and VEGF proteins.

Mesoporous silica nanoparticles: Mesoporous silicon is a biocompatible material with stable skeleton, high specific surface area, uniform pore volume and other characteristics, which is very suitable for drug carrier. The surface of mesoporous silicon NPs (MSN) has unique physical properties, which can affinity and adsorb liposomes in the phospholipid layer of cell membrane, and improve the non-specific uptake efficiency of MSN through the intracellular protein mediated endocytosis pathway and pinocytosis. The surface modification of MSN by different chemical functional groups can regulate their form of uptake by cells (phagocytosis or receptor-mediated endocytosis) and efficiency (electrostatic adsorption and specific receptor recognition). MSN is a good drug carrier with low toxicity and good biocompatibility [81].

Overexpression of multi-drug resistance associated protein 1 (MRP1) plays an important role in chemotherapy resistance of GBM. Porous silicon NPs (pSiNPs) can be used for high-capacity loading and delivery of siRNA in vitro and in vivo. Tong et al. [82] established pSiNPs of polyethylenimine (PEI) cap that could load siRNA in large volumes and optimize the release profile (70% is released within 24 to 48 hours). The pSiNPs are biocompatible, which can be absorbed by cells and effectively inhibit the expression of MRP1 in GBM. In addition, siRNA delivery was found to significantly reduce GBM proliferation as a related effect. In mouse experiments, the study confirmed the silencing effect of MRP1 in GBM tumors (an 82% reduction in protein levels within 48 hours after injection) and an anti-proliferative effect in GBM by reducing the number of proliferating cells.

Magnetic nanoparticles: Magnetic targeted drug delivery system is a new type of targeted drug delivery system that has been studied more in recent years. Usually, the carrier is directed to the target area under the action of sufficiently strong external magnetic field, so that the drug contained in the carrier can be released and play a role in the lesion site with the characteristics of high efficiency, rapid effect and low toxicity. BBB blocking and low drug concentration in the tumor area are two important factors that restrict the therapeutic effect of glioma. Magnetic targeted drug delivery system brings the light to solve this problem. It can successfully carry some diagnostic and therapeutic drugs through BBB, directly into the brain tumor tissue of the intercellular fluid, reduce the drug to normal brain cells and bone marrow and other sensitive organs of the toxic side effects [83].

Repressor element-1-silencing transcription factor (REST) plays an important role in different types of tumors, and can be used as a carcinogenic factor in neurogenic tumors such as glioma Wang et al. [84] developed polyethylenimine (PEI) coated Fe_3O_4 NPs (NPs) for siRNA delivery into GBM cells to silence REST. Studies showed that the transcription and translation levels of REST were significantly reduced. It was demonstrated that the PEI coated Fe_3O_4 NPs effectively inhibited cell proliferation and migration. In order to enhance the penetration of siPLK1 across the BBB and treat GBM, Liu et al. [85] prepared Tf modified magnetic NPs (Tf-PEG-PLL/MNP@siPLK1). We demonstrated that Tf-PEG-PLL/MNP@siPLK1 enhanced cellular uptake of siPLK1, thereby increasing gene silencing and cytotoxicity in U87 cells. In addition, Tf-PEG-PLL/MNP@siPLK1 significantly inhibited the growth of U87 glioblastoma spheres in an in vitro BBB model and significantly improved the penetration efficiency of siPLK1 through BBB under magnetic field in vitro. After injection of Tf-PEG-PLL/MNP@siPLK1 through tail vein, siPLK1 selectively accumulated in the

Table 3. Mechanism of siRNA nanocarriers in treatment of glioma

Mechanism	Targeted genes	Ref.
Promote tumor cell apoptosis	Gli1; Survivin; EGFR; Bcl-2	[4, 59, 70, 76, 80]
Inhibit tumor proliferation	Stat3; FAK, NOTCH-1 and SOX-2; Praja2; CypA; c-Myc; PLK1	[40, 41, 43, 44, 57, 58, 60, 65, 68, 71, 75, 77, 85]
Inhibit tumor invasion and migration	Robo1, YAP1, NKCC1, EGFR and Survivin; TN-C; REST	[20, 84, 86]
Enhance sensitivity to chemoradiotherapy	SAT1;MGMT; GOLPH3; TGF-β; MALAT1	[5, 46-48, 52, 54, 87]
Overcome drug resistance	MRP1	[82]
Enhance immune response	CD146; CD47; PD-L1; Id1; LSINCT5	[24, 43, 53, 55, 63, 64, 69]
Inhibit angiogenesis	VEGF	[72, 88]

brain tissue of tumor-bearing mice, significantly reduced the tumor volume and prolonged the survival time of tumor-bearing mice. The extracellular matrix glycoprotein tenascin-c (TN-C) can be used as a target for GBM, and downregulating TN-C via RNAi is a very promising cancer treatment strategy. Grabowska et al. [86] proposed a scheme using MNP@PEI as ATN-RNA carrier in GBM cells. The obtained polyethylenimide (PEI) coated with magnetic NPs bound to dsRNA has a high ATN-RNA delivery efficiency, which not only leads to a significant downregulation of TN-C expression level, but also inhibits the migration of tumor cells.

MGMT can repair DNA damage induced by TMZ, leading to TMZ resistance. Therefore, the combination of MGMT inhibitor and TMZ may reduce chemotherapy resistance and improve efficacy. Chung et al. [5] designed polypeptide-functionalized iron oxide NPs (NP-CTX-R10) to deliver siRNA to silence MGMT to sensitize tumor cells to TMZ. NP-CTX-R10 forms a complex with siRNA through electrostatic interaction and is able to deliver siRNA to different glioma cells. NP-siRNA can silence up to 90% of genes. Glioma cells transfected with MGMT-targeting NP-siRNA showed significantly increased sensitivity to TMZ therapy. Similarly, Wang et al. [87] constructed an iron oxide NP for targeted delivery of siMGMT. Studies showed that NP overcame biological barriers and downregulated MGMT expression in mouse tumors with in-situ GBM. Compared with TMZ alone, sequential treatment with NP and TMZ resulted in increased apoptosis of GBM cells, inhibition of tumor growth, and significantly longer survival.

Cisplatin (Pt) is a platinum-containing anticancer drug that has shown efficacy in a variety of solid tumors. Therefore, researchers apply Pt to degrade nuclear DNA and mitochondrial DNA, leading to apoptosis and the production of H_2O_2. GPX4 is related to the metastasis and invasion of glioma. Zhang et al. [88] constructed iron oxide NPs (IONPs) based on gene therapy to treat GBM through iron death and apoptosis. siRNA targeting glutathione peroxidase 4 (si-GPX4) and Pt can be co-delivered by modifying the porous structure with ion chelate protein attached to carboxyl group, with high drug delivery efficiency. During intracellular degradation, IONPs significantly increases iron (Fe^{2+} and Fe^{3+}) levels, while Pt damages nuclear and mitochondrial DNA, leading to apoptosis. In addition, IONPs increases H_2O_2 levels by activating reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX). The Fenton reaction between Fe^{2+}, Fe^{3+} and intracellular H_2O_2 produces potent reactive oxygen species (ROS) to induce iron death, and co-releases si-GPX4 to inhibit GPX4 expression, thus improving the therapeutic effect through synergistic mechanism with iron death.

Mechanism of RNAi technology in treatment of glioma

RNAi technology inhibits the occurrence and development of glioma through different mechanisms, including promoting tumor cell apoptosis, inhibiting tumor proliferation, invasion and migration, enhancing sensitivity to chemoradiotherapy, overcoming drug resistance, enhancing immune response and inhibit angiogenesis, et al. (**Table 3**). RNAi technology offers a powerful and versatile approach to inhibit the occurrence and development of glioma through multiple mechanisms. By targeting key genes involved in apoptosis, proliferation, invasion, angiogenesis, drug resistance, and immune

evasion, RNAi holds great promise for improving the treatment of this devastating disease.

Discussion and conclusions

Although RNAi therapy shows great potential, there are many challenges in clinical transformation, such as stability and immune response. siRNA is easily degraded during blood circulation. Chemical modifications such as 2'-o-methylation were used to improve stability. Nano delivery vectors can also greatly improve the stability of siRNA. RNAi therapy may trigger an immunogenic response. Optimizing siRNA sequences, such as avoiding immune-rich activation sequences (such as GU-rich sequences), may be able to overcome the immunogenicity challenge. Combined with other treatments, such as chemotherapy, radiation, or immunotherapy, can enhance the efficacy of treatment while reducing immunogenic-related side effects. The complexity of the blood-brain barrier (BBB) and tumor microenvironment also limits the application of RNAi therapies. Recent studies have designed novel delivery systems (e.g. exosomes, lipid nanoparticles) that will overcome these challenges. Clinical trials of RNAi therapy in glioma are still in the early stages, but have shown benefits. By targeting key genes (EGFR, MGMT, STAT3, etc.), RNAi therapies are expected to inhibit tumor growth and enhance the effectiveness of existing therapies. In the future, as delivery systems are optimized and clinical trials advance, RNAi therapy may become an important tool for glioma treatment.

With the rapid development of nanotechnology and modern medical technology, the targeted treatment of brain glioma has made amazing achievements. Researchers have also made in-depth exploration of the occurrence and development mechanism of brain gliomas, and according to its characteristics and properties, a series of nanotargeted delivery systems have been developed to treat brain gliomas. Although these new dosage forms have different targets and different mechanisms of action, they can all show therapeutic effects on brain glioma. The emergence of nanomedicine marks an unparalleled opportunity to improve drug efficacy by enhancing the ability to cross biological barriers. The size and morphology of NPs have a critical impact on their efficiency through BBB and subsequent diffusion in the brain.

However, there are still many difficulties to overcome in the application of nanocarriers in clinical glioma therapy. First of all, although nano drug delivery carriers can act on brain glioma, they also have toxic side effects on other normal tissues. Despite improved targeting through target-modified nanocarriers, BBB and BBTB still act as a strong barrier to the nanodelivery system, greatly limiting the enrichment of anticancer drugs in the brain. Therefore, it has become the main direction of current research to develop a nano drug delivery system that targets only the treatment site and reduces the accumulation of drugs at non-target sites.

Secondly, although the application of nanocarriers to glioma has played a remarkable therapeutic effect. However, compared with other diseases, the targeted treatment of glioma by nanocarriers started late. In spite of this, nanocarriers still surpass the traditional drug delivery forms with their unique advantages and show broad application prospects.

Thirdly, the current studies have only been limited to in vitro cell investigations, subcutaneous or orthotopic tumor transplantation in mice and dogs. However, rats, dogs are much different with clinical patients. Researchers will explore and apply human transplant models that are closer to patients for anti-tumor experiments. Therefore, suitable nanodelivery vectors can be effectively screened for clinical transformation and effective treatment of glioma.

To explore the intracellular mechanism of nanomedicine and elucidate the release and therapeutic mechanism of nanomedicine in vivo is helpful to better design and development of nanomedicine. We believe that with the continuous development of nanotechnology and biomedicine, researchers will develop more stable and safer nanomedicine for clinical application. The treatment of glioma will reach new heights and benefit more patients.

Acknowledgements

We thank for the support. This study was funded by Basic research project of Science and Technology Department of Yunnan Province (202401AU070041, 202401AU070047); and Doctoral Research Foundation of the First

Affiliated Hospital of Kunming Medical University (2022BS001, 2022BS002).

Disclosure of conflict of interest

None.

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