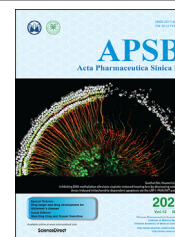




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REVIEW

Gather wisdom to overcome barriers: Well-designed nano-drug delivery systems for treating gliomas



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Abstract Due to the special physiological and pathological characteristics of gliomas, most therapeutic drugs are prevented from entering the brain. To improve the poor prognosis of existing therapies, researchers have been continuously developing non-invasive methods to overcome barriers to gliomas therapy. Although these strategies can be used clinically to overcome the blood–brain barrier (BBB), the accurate delivery of drugs to the glioma lesions cannot be ensured. Nano-drug delivery systems (NDDS) have been widely used for precise drug delivery. In recent years, researchers have gathered their wisdom to overcome barriers, so many well-designed NDDS have performed prominently in preclinical studies. These meticulous designs mainly include cascade passing through BBB and targeting to glioma lesions, drug release in response to the glioma microenvironment, biomimetic delivery systems based on endogenous cells/extracellular vesicles/protein, and carriers created according to the active ingredients of traditional Chinese medicines. We reviewed these well-designed NDDS in detail. Furthermore, we discussed the current ongoing and completed clinical trials of NDDS for gliomas therapy, and analyzed the challenges and trends faced by clinical translation of these well-designed NDDS.

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1. Introduction

Gliomas account for about 51.4% of all primary brain tumors, of which malignant gliomas that have high mortality rates account for 80%^{1,2}. Because of the characteristics of fast infiltration and growth of gliomas, clinical surgery cannot achieve the effect of eradication³. The existence of blood–brain barrier (BBB) and blood–brain tumor barrier (BBTB) prevents most drug candidates from reaching glioma cells^{1,4}. Importantly, there are not only physical barriers but also metabolic barriers in BBB and BBTB. The physical barriers is mainly based on the tight junction (TJ) between brain microvascular endothelial cells (BMVECs)⁵, and the metabolic barriers are mainly based on the drug efflux transporters and the endosomal sorting process in BMVECs^{6,7}. Some invasive drug delivery strategies, such as direct intra-brain drug delivery, can increase the concentration of the drug in the glioma to a certain extent. But it can cause irreversible damage to the patient, it is not a long-term implementation method⁸.

Therefore, from a long-term perspective, what glioma patients urgently need are non-invasive strategies to overcome BBB/BBTB. Some direct non-invasive strategies include: 1) Using some reagents to regulate the expression of the protein that controls the TJ on the BBB^{9,10}; 2) Using focused ultrasound (FUS) and microbubbles (MBs) to temporarily open the BBB¹¹; and 3) Using non-invasive nasal administration to allow the drug to be delivered to the brain through the nerves on the nasal mucosa^{12,13}. The barriers of drug entry into brain can be well overcome by these three strategies. However, there are still many normal cells in the brain of gliomas, so it is more important to accurately deliver drugs to glioma cells.

The significance of nano-drug delivery systems (NDDS) is to improve the stability of the drug in the body while promoting their distribution. In particular, NDDS with targeting function can accurately deliver drugs to the tumor. There are many overexpressed receptors/transporters on BBB/BBTB and glioma cells, which also determines that nanoparticles (NPs) modified with targeting ligands can penetrate BBB/BBTB through these receptors/transporters mediated transcytosis¹⁴. For more precise treatment, the cascade targeting NDDS that can target both BBB/BBTB and glioma cells has been developed¹⁵. With meticulous designs, the optimization and combination of different ligands making this strategy show outstanding results. Furthermore, the microenvironment of glioma also has unique properties, such as low pH, hypoxic environment and so on. These characteristics were applied to further designing NDDS that can respond to the glioma microenvironment¹⁶. These NDDS can change their properties according to specific endogenous stimuli to promote the uptake of glioma cells and the precise release of drugs in glioma cells. In order to further overcome the immunogenicity of NDDS, the biomimetic drug delivery systems (BDDS) based on endogenous cells/extracellular vesicles/proteins have been designed. But their advantages are not limited to reducing immunogenicity, other more attractive advantages have been found one by one^{17,18}. In recent years, people have been constantly screening specific active ingredients in traditional Chinese medicines (TCM) to develop drugs for the treatment of gliomas. In addition to the discovery that certain active ingredients can inhibit glioma, it has also been found that certain ingredients can be used as carriers for NDDS¹⁹. The research results of well-designed NDDS in the treatment of gliomas in recent years are summarized in detail in this article. We also discussed the current status and future development trends of these NDDS clinical translations.

2. Major barriers to overcome for drug delivery to gliomas

2.1. BBB and BBTB

The BBB is not only an important protective mechanism for the brain, but also the biggest obstacle to the delivery of drugs to the brain. It is mainly composed of BMVECs, surrounded by pericytes, astrocytes, microglia, oligodendrocytes, neurons, and basement membrane^{5,20}. And the difference between glioma and other brain diseases is the presence of BBTB²¹. These two barriers have become the biggest obstacle to the delivery of glioma drugs (Fig. 1).

2.1.1. Physical barriers

BMVECs are tightly connected non-fenestrated endothelial cells that can prevent more than 98% of small molecules and almost 100% of large molecules from entering the brain tissue²². It has been reported that only small lipophilic drugs, with a molecular weight of less than 400 Da and the form of less than 8 hydrogen bonds can cross the BBB²³. The TJ between BMVECs mainly depends on the TJ proteins between them, such as claudin (CLDN), occludin (OCLN) and zonula occludens (ZO)^{24,25}. Pericytes and the end of astrocytes cover almost all of the basal layer of brain capillaries, which is essential for maintaining the normal function of the BBB (Fig. 1C)²⁶. Pericytes can regulate BBB permeability by regulating gene expression in BMVECs, leading to up-regulation of TJ proteins. Astrocytes send signals to BMVECs through Src-suppressed C-kinase substrate, increasing TJ proteins expression²⁷. The complex extracellular matrix and matrix receptors in the brain also maintains and regulates the function of BBB, so BBB is a dynamic structure²⁸.

Malignant gliomas, such as glioblastoma multiforme (GBM), can cause the TJ of the BBB to become larger by secreting soluble factors that destroy TJ²⁹. To meet high metabolic demands, malignant gliomas will form hypoxic areas, which leads to overexpression of vascular endothelial growth factor (VEGF) and angiogenesis on the BBB³⁰. The BBTB is formed between the leaky BBB, new blood vessels and the malignant glioma tissue, and is another barrier for glioma therapy (Fig. 1B)³¹. Some aggressive tumor cells are far away from the tumor core due to the damaged BBB, reaching other healthy brain tissues³². Therefore, the tumor tissue of GBM patients cannot be completely removed, and recurrence is unavoidable. In low-grade gliomas, the structure and function of BBTB are almost the same as normal BBB, but still maintain the barrier function to a large extent, restricting the penetration of non-lipid-soluble macromolecule drugs³³. Although the formation of BBTB in GBM leads to the destruction of BBB, many GBM patients still have intact BBB in the tumor tissue. Because BBTB is mainly located in the core of the tumor, and the peripheral BBB remains normal^{34,35}. In addition, there are a large number of growth factors, cell chemokines, and various proteolytic enzymes in the BBTB microenvironment. These immuno–inflammatory reactions are very conducive to tumor proliferation, invasion, adhesion, angiogenesis, and anti-radiochemotherapy³⁶. In low-grade and high-grade gliomas, both BBB and BBTB are the main obstacles to the treatment of gliomas by preventing drug delivery. When developing an effective systemic therapy, the structural and functional heterogeneity of the BBB/BBTB in the glioma microenvironment must be considered³⁷.

2.1.2. Metabolic barriers

Theoretically, the more lipid-soluble the drugs, the easier they are to penetrate the BBB. However, it is worth noting that

there are many drug efflux transporters expressed on the BBB, and most drugs are substrates of these drug efflux transporters. These transporters can specifically and effectively remove drugs from the central nervous system, or prevent their entry from the beginning, thereby limiting the accumulation of such drugs in the brain (Fig. 1C)^{6,38}. Most of the drug efflux transporters belong to the ATP binding cassette (ABC) protein superfamily³⁹. So far, 49 ABC transporters have been discovered and divided into 7 families. They mainly include P-glycoprotein (P-gp, the encoded product of the *ABCB1* gene), multidrug resistance protein (MRP, the encoded product of the *ABCC1* gene), and breast cancer resistance protein (the encoded product of the *ABCG2* gene)⁴⁰. P-gp is the most typical drug efflux transporter, which can transport out nearly 60% of chemotherapeutic drugs. It has been proven that P-gp can be blocked by P-gp inhibitors to improve the uptake of anticancer drugs in the brain⁴¹. The astrocytes around the BBB can also up-regulate the expression of P-gp, increasing the rate of drug efflux⁴². In addition, glioma cells themselves also express a variety of drug efflux transporters. Therefore, gliomas are endowed with multidrug resistance⁶. To overcome the drug reduction mediated by efflux transporters in the brain and the drug resistance of gliomas, current preclinical research focuses on the development of transporter inhibitors⁴³. Three generations of inhibitors have been developed, such as elacridar and tariquidar, which can simultaneously inhibit *ABCB1* and *ABCG2*. Using non-human primates as test subjects, it has been found that when the drug is co-administered with transporter inhibitors, the brain penetration rate of drugs can increase by 3–7 times^{44,45}. Unfortunately, there were still no satisfactory results in clinical trials. It was considered that

these inhibitors may also inhibit *ABCB1* in normal tissues and increase the therapeutic toxicity of drugs³⁸.

Additionally, many different types of receptors and transporters are also expressed on BMVECs, which enable certain nutrients (such as glucose, amino acids and proteins) to reach sufficient concentrations in the brain to maintain the homeostasis⁴⁶. Although NPs can be designed to have ligands that can recognize specific receptors, there are still obstacles that need to be overcome during the transportation of BMVECs⁴⁷. Receptor-mediated transcytosis (RMT) depends on the vesicle transport system. When specific ligands on NPs bind to specific receptors on apical site of BMVECs membrane, receptor–ligand complexes are formed. Subsequently, the cell membrane invades to form transport vesicles encapsulating NPs. Finally, NPs are exocytosis or released in the basolateral site of BMVECs membrane, and the receptor is transported to the apical membrane or lysosomes⁴⁸. But this is a very ideal process. In fact, during this process, due to the formation of endosomes, the fate of NPs is determined by the endosomal sorting process, and NPs are very likely to be delivered to late endosomes and degraded in the lysosomes (Fig. 1D)^{7,49}. This situation mainly exists in clathrin-mediated transcytosis, such as transferrin (Tf) receptor (TfR). But this is often also related to the affinity of the ligand to the receptor. The stronger connection between ligands and receptors, the greater possibility that NPs will eventually flow to lysosomes⁴⁷. The binding of receptors and ligands can also cause caveolae-mediated transcytosis, such as low-density lipoprotein (LDL) receptor (LDLR). Compared with clathrin-mediated transcytosis, caveolae cannot deliver their contents to lysosomes, so NPs transported through this mechanism are expected to escape lysosome degradation^{50,51}.

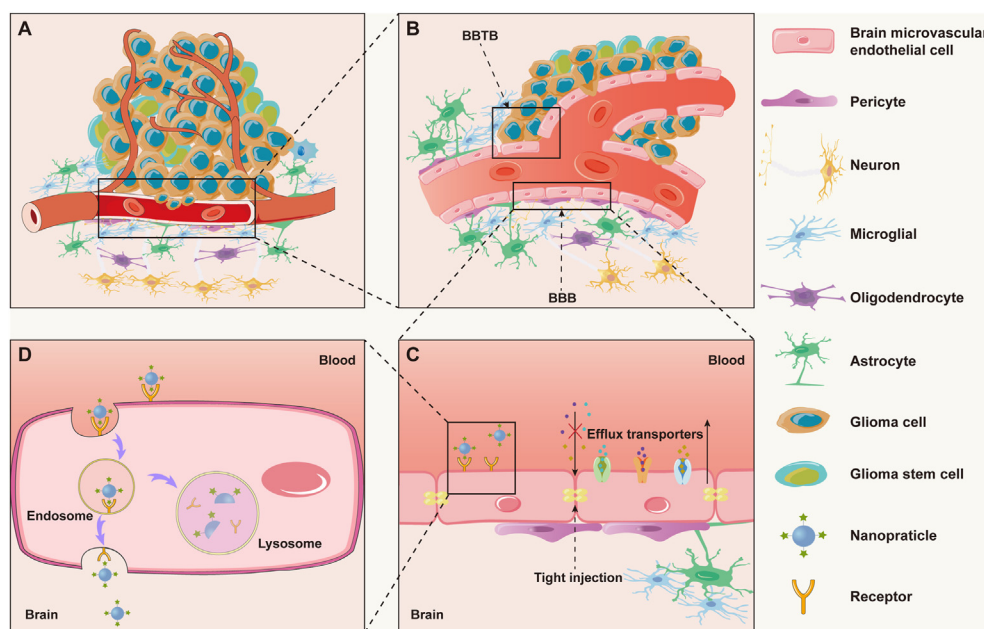


Figure 1 (A) The microenvironment of gliomas. (B) The blood–brain tumor barrier (BBTB) consists of many existing and new blood vessels. The newly formed blood vessels are highly permeable, leading to the destruction of the original TJ. Invasive tumor cells can damage other healthy brain tissues through the blood vessels, but there is still the normal BBB. (C) Tight junction (TJ) prevents the transportation of therapeutic drugs through paracellular routes. The efflux transporters promote the efflux of the drug. (D) When NPs pass through the BBB *via* receptor-mediated transcytosis (RMT), they may be hydrolyzed by enzymes in endosomes/lysosomes. Created using images from “Servier Medical Art (<https://smart.servier.com>)” under CC BY 3.0 license.

2.2. Direct non-invasive strategies for overcoming BBB and BBTB

At first, local delivery of drugs was considered the best way to bypass the BBB, which led to the emergence of invasive drug delivery strategies such as intrathecal delivery, intraventricular delivery, and convective enhanced delivery (CED). Intracranial implantation of Gliadel®, carmustine (BCNU) implants, has been proven to be an effective strategy in clinical trials (NCT00004892, NCT02300506, NCT00076986), but patients are still at risk of cerebral edema and infection⁵². Although CED is promising for gliomas drug delivery, the success of clinical trials is not surprising. Because the technology still has many technical flaws that need to be resolved⁵³. Besides, both of these methods require some dangerous operations and are accompanied by some serious side effects^{54,55}. Recently, many direct non-invasive strategies have been proposed to overcome BBB/BBTB to improve the drug delivery of gliomas. Including the use of biochemical reagents to modulate the BBB, FUS to open the BBB, intranasal administration to bypass the BBB, and various well-designed NDDS. In this part we will focus on the first three non-invasive methods.

2.2.1. Biochemical modulation

Using hypertonic solution to open the TJ of the BBB was the first way to be discovered. The mechanism is that BMVECs are dehydrated and contracted by injecting a hypertonic solution into the artery, and the TJ is opened in a disguised form⁵⁶. Then the therapeutic drugs are injected into the body, and the drugs can quickly enter the brain through the paracellular pathway. Mannitol is the main hypertonic agent used⁹. Although this method has been used clinically⁵⁷. There is also a certain degree of danger. Because mannitol is not selective in opening the BBB, except for the glioma area, the BBB in the normal area is treated the same. It is inevitable that some high-molecular substances will enter the brain, which will increase the water content in the brain and cause some complications⁵⁸. This method is not a sustainable development method. It is worth noting that TJ depends on the expression of TJ proteins. Therefore, the use of certain reagents to directly or indirectly regulate the expression of TJ proteins is a more promising way. Bradykinin (BK) is an active substance that can dilate blood vessels and has been shown to selectively increase the permeability of the BBB at the glioma site¹⁰. The main mechanism is

that the BK type 2 (B2) receptor is activated, which leads to the decrease of ZO-1, CLDN-5 and OCLN expression, and accelerates the formation of ATP-sensitive potassium (K_{ATP}) channels^{59,60}. Besides, the retro-inverso BK and the BK analogue RMP-7 have also been used^{61,62}. Because the expression of B2 receptor increases with the grade of glioma, this method can selectively open the BBB at the glioma site⁶³. K_{ATP} channels are highly expressed in the endothelial blood vessels of gliomas, and their main function is to increase the permeability of BBTB after being activated⁶⁴. Minoxidil sulfate is a representative K_{ATP} channel activator, which has been proven to down-regulate OCLN and CLDN-5, and this process is time-dependent⁶⁵. In addition, calcium-activated potassium channel activators, phosphodiesterase 5 inhibitors, adenosine 2A receptor activators and papaverine have also been used to turn on TJ on the BBB, which has been discussed in detail²⁵.

It has been found that certain TCM also have the effect of reducing the permeability of the BBB. Such drugs are called brain penetration enhancers (BPEs), including borneol (BO), menthol, muscone and so on⁶⁶. Among them, BO has been extensively studied to enhance the permeability of BBB⁶⁷. The accumulation of cisplatin (CDDP) in gliomas can be increased by BO, because the expression of ZO-1 and F-actin is down-regulated by BO⁶⁸. But Wu et al.⁶⁹ believe that the mechanism of BO-mediated BBB opening is mainly the increased expression of intercellular cell adhesion molecule-1 (ICAM-1). The increase of ICAM-1 expression can aggravate the damage of endothelial cells and promote the opening of the BBB. It has been found that the expression of ABC transporters such as P-gp and MRPI will also be down-regulated⁷⁰. In addition, BO can trigger mitochondrial dysfunction and enhance reactive oxygen species (ROS) levels, and cooperate with temozolomide (TMZ) to fight gliomas⁷¹.

2.2.2. FUS

FUS refers to focusing ultrasound (frequency greater than 20 kHz) to a certain part of the human body in a non-invasive way, producing thermal and mechanical effects. It is currently widely used in the research of opening the BBB^{11,72}. FUS is usually used in combination with gas-containing MBs. MBs are activated by the ultrasonic field to produce cavitation, which converts sound energy into mechanical energy, causing the tight injection of the BBB to be temporarily destroyed (Fig. 2)^{22,73}.

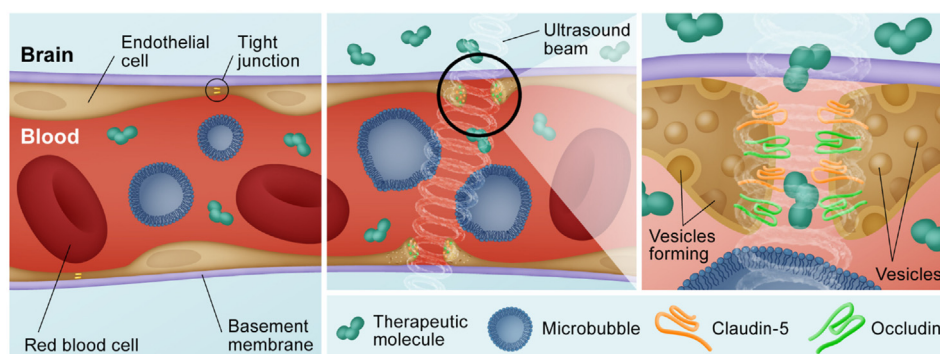


Figure 2 Mechanism of action of FUS on the cerebral vasculature-exogenous microbubbles in the circulation oscillate in the ultrasonic field, producing mechanical forces on the surrounding vasculature. This transiently opens the blood-brain barrier (BBB) by eliciting specific bioeffects such as disruption of TJs and upregulation of transcytosis, permitting transport across the BBB. Reprinted with the permission from Ref. 22. Copyright © 2019 Elsevier.

FUS and MBs can not only increase the delivery of drugs to the brain, but also extend the retention time of drugs in the brain. Park et al.⁷⁴ used 690 kHz ultrasound and MBs to destroy BBTB in a rat glioma model. The doxorubicin (DOX) concentration level measured by the fluorescence method was significantly higher than that of the control group, and there was no significant difference in the concentration between 1 and 24 h. In addition to being used to open BBB, MBs can also be used as drug carriers. Ting et al.⁷⁵ used MBs to load BCNU getting BCNU-MBs. The combination of injection of BCNU-MBs and FUS not only promotes the destruction of BBB and release of BCNU, but also prolongs the half-life of BCNU by 5 times. Boron neutron capture therapy (BNCT) is a kind of radiation therapy, and the targeted delivery of boron is the key to the successful treatment of GBM by BNCT⁷⁶. Fan et al.⁷⁷ prepared boron-containing and cationic MBs (B-MBs) containing boron. After FUS ultrasound treatment, the B-MBs can simultaneously achieve BBTB opening and delivery of boron to tumor tissues, and the effect is significantly higher than the mixture of free boron and MBs (Fig. 3A–D).

The safety of FUS still needs to be discussed in detail. Excessive ultrasound irradiation pressure can cause brain damage. Fan et al.⁷⁷ found that FUS irradiation at 0.5 MPa can observe the best BBTB opening effect (Fig. 3F and G). But severe cerebral hemorrhage occurred 8 min after FUS treatment. The strength and time of FUS are the keys to determining the security of this

strategy. To prove the safety of using magnetic resonance-guided FUS to open the BBB in clinical settings. Mainprize et al.⁷⁸ conducted a validation study on 5 patients with malignant glioma (NCT02343991). Using the optimal power of BBB opening (calculated as 50% of the power at which cavitation signals were first detected using acoustic feedback from an incremental sonication power protocol), each ultrasound is performed at a duty cycle of 0.74% for 50 s (This method referred to the research results of Huang et al.⁷⁹). It was found that there was no obvious cerebral hemorrhage or edema in the patient. Although there are some limitations, it is expected to help establish safer ultrasound parameters⁷⁸.

2.2.3. Intranasal delivery

Through the intranasal administration route, not only does it not need to cause physical damage to the body, but also can overcome the BBB/BBTB, and at the same time, the adverse reactions that occur during systemic absorption of drugs can be greatly reduced^{80,81}. So far, it is believed that there are two main routes of the drugs from nose to brain: indirect route and direct route. The indirect route means that the respiratory epithelial cells in the nose mediate the delivery of drugs to the brain through blood circulation. The direct route is composed of neurons of olfactory neurons and trigeminal neurons on the olfactory epithelium. The neurons absorb drugs and transport them directly to the brain along the axon^{13,80,82} (Fig. 4).

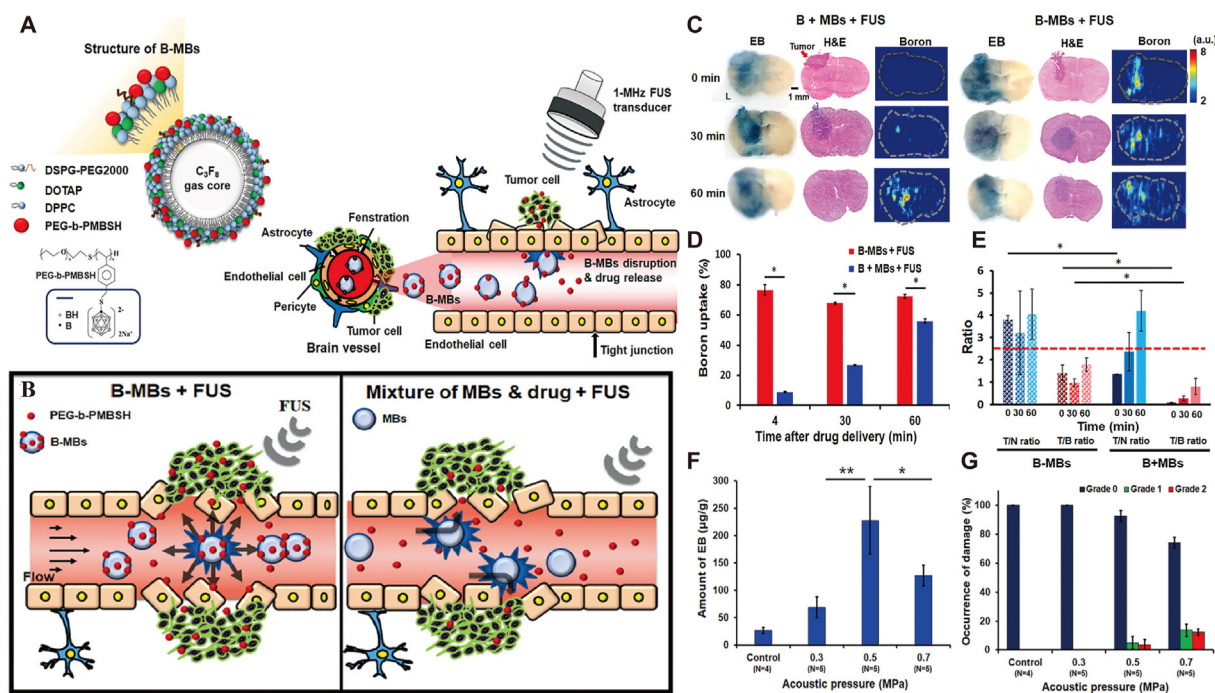


Figure 3 (A) Illustration of the B-MBs with FUS-enhanced boron drug delivery in a brain tumor. (B) Drugs (left) were loaded on the lipid shell of MBs by electrostatic interaction or (right) simply mixed with unloaded MBs. (C) Boron distribution in the tumor with different drug delivery protocols. (D) Boron cover rate in the tumor with different drug delivery protocols. (B + MBs + FUS: FUS sonication following administration of the cationic MBs with the PEG-*b*-PMBSH mixture; and B-MBs + FUS: FUS sonication following administration of the B-MBs). (E) Tumor-to-normal brain (T/N) and tumor-to-blood (T/B) ratios with FUS sonication. (Red dot line: 2.5, requirement T/N ratio for use of BNCT in clinical). (F) Quantification of Evans blue within the tumor under different FUS parameters. (G) Damage under different FUS parameters; blue bar: grade 0 (no occurrence of erythrocyte extravasation); green bar: grade 1 (occurrence of few extravasated erythrocytes without neuronal loss); red bar: grade 2 (occurrence of severe erythrocyte extravasations). Reprinted with the permission from Ref. 77. Copyright © 2018 American Chemical Society.

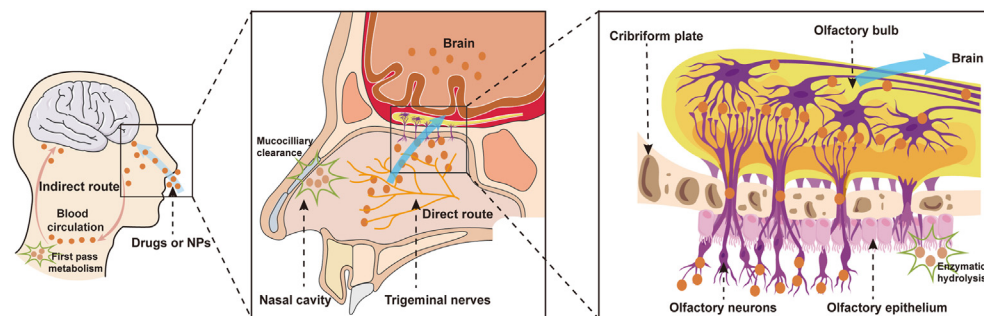


Figure 4 The direct route of olfactory nerves and trigeminal nerves play an important role in the delivery process from the nose to the brain. Drugs or nanoparticles (NPs) are transported along the intracellular axons of neurons to the olfactory bulb and then distributed throughout the central nervous system. In this process, drugs and NPs may be hydrolyzed by various enzymes. The efficiency of blood circulation is low, mainly because of the mucociliary clearance and first-pass metabolism. Created using images from “Servier Medical Art (<https://smart.servier.com>)” under CC BY 3.0 license.

Intranasal delivery of perillyl alcohol (POH) for the treatment of gliomas has now successfully entered the clinical trial stage⁸¹. da Fonseca et al.⁸³ have completed some clinical trials. After 89 patients with recurrent GBM received intranasal administration of 440 mg POH daily, the median survival time was 11.2 months. And in patients treated for more than 4 years, the side effects of POH can almost be ignored⁸⁴. There are also ongoing clinical trials, and the results are highly anticipated (NCT02704858). However, free drugs are often easily cleared by mucociliary or degraded by a variety of hydrolytic enzymes. Delivery of NDDS through the nose is expected to improve these problems and brain-targeting capability⁸⁵. Sekerdag et al.⁸⁶ reported that lipid-poly(ethylene glycol) (PEG)-poly lactic-glycolide acid (PLGA) hybrid NPs (HNP) loaded with farnesylthiosalicylic acid (FTA) were used for intranasal administration to treat glioma. It has been found that FTA-loaded HNP can effectively and quickly pass through the nasal mucosa to make FTA accumulate in the brain. Intravenous injection will cause FTA-loaded HNP to accumulate in the liver and spleen (about 10 times higher than the intranasal administration). It is worth noting that due to the existence of nasal mucosal cilia, the surface of nanocarriers also needs some mucosal adhesive to improve transport efficiency. As a good adhesion polymer, chitosan can bind well to the nasal mucosa. Colombo et al.⁸⁷ prepared nanoemulsion (KPF-MNE and KPF-NE) of kaempferol (KPF) with and without chitosan by high-pressure homogenization technology. The amount of drug entering the rat brain by intranasal administration of KPF-MNE is significantly increased (4.5 times higher than that of KPF-NE). Besides, alginate, cellulose, and PEG 400 are also widely used in assembling nanocarriers⁸⁸. de Oliveira et al.⁸⁹ compared different ratios of PEG coating and found that 5% PEGylated polymer seems to be the optimal concentration for delivery from the nose to the brain. Sukumar et al.⁹⁰ developed a therapeutic multifunctional gold-iron oxide NPs (GIONs) loaded with miRNAs against GBM. The NPs successfully improved the treatment results accompanying TMZ chemotherapy. Besides, surface-functionalized GIONs not only effectively limit the size of NPs below 50 nm, meeting the prerequisite size standards for effective intranasal delivery, but also achieve simultaneous multimodal imaging of intranasal delivery to GBM.

3. Well-designed NDDS to overcoming the major barriers

If only the above-mentioned strategies to overcome BBB are used, accurate and complete glioma treatment may not be achieved.

Because the bioavailability of therapeutic drugs in the body and the ability to target gliomas also need to be considered. Well-designed NDDS have been widely used to overcome various barriers in the treatment of gliomas⁹¹. Their development in recent years has brought new dawn to the precision treatment of gliomas. They are endowed with a variety of powerful capabilities by researchers, some rely on the carrier itself, and some rely on engineering modification. Some of them are assembled by non-toxic polymer materials, and some are derived from the living body itself. In this section, we classify the well-designed NDDS used for targeted therapy of glioma in detail, and discuss their salient features and research value.

3.1. NDDS based on cascade targeting

The prerequisite for NDDS to accurately treat gliomas is to target and pass through BBB/BBTB. The mechanism of drug crossing BBB/BBTB mainly including RMT, carrier-mediated transcytosis (CMT), and adsorption-mediated transcytosis (AMT)¹⁴. Among them, RMT and CMT depend on receptors and transporters on BBB/BBTB, respectively, and AMT depends on the abundant negative charges on BBB/BBTB (Fig. 5A). Based on these three mechanisms, NDDS is engineered to have unique ligands or properties, so that it can target BBB/BBTB actively. After that, what needs to be considered is the ability of NDDS to target the glioma lesions. Because the normal brain tissue of patients with glioma still accounts for the vast majority, if the drug continues to be dispersed in the normal brain tissue, side effects will inevitably occur. Therefore, in order to ensure the efficiency of drug entry into tumor tissues, NDDS also needs to have the ability to target glioma lesions. This concept is called cascade targeting delivery (or dual targeting delivery, Fig. 5B)¹⁵.

3.1.1. Cascade targeting based on single ligand

Fortunately, certain receptors and transporters are overexpressed on both BMVECs and glioma cells, such as TfR, LDLR, LDLR-related protein (LRP), glucose transporter (GLUT), and so on^{92–95}. Therefore, cascade targeting NDDS based on a single ligand has been widely studied for the treatment of glioma (Fig. 5B,a). The single ligand-based cascade targeted NDDS for the treatment of gliomas are summarized in Table 1^{92–94,96–118}.

Lam et al.⁹² developed Tf-modified liposome NPs (Tf-NPs) loaded with TMZ and bromodomain inhibitor JQ1. *In vivo*

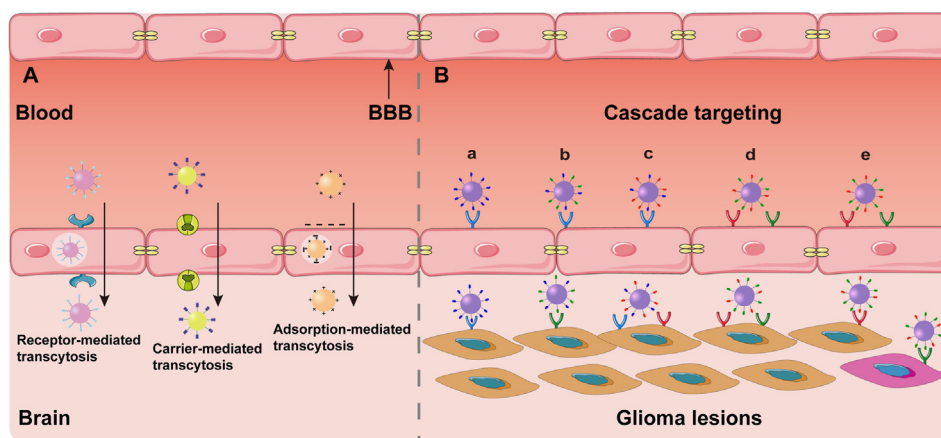


Figure 5 (A) Three mechanisms of NDDS crossing BBB/BBTB. (B) NDDS based on cascade targeting can target either BBB/BBTB or glioma lesions. (a) One ligand targets BBB and glioma cells. (b) One ligand targets BBB/BBTB and another ligand targets glioma cells. (c) One ligand targets BBB/BBTB and glioma cells, the other ligand targets glioma cells. (d) Both ligands target BBB/BBTB and glioma cells. (e) One or two ligands target the BBB/BBTB, one ligand targets glioma cells, and the other ligand targets other tumor-related cells. Created using images from “Servier Medical Art (<https://smart.servier.com>)” under CC BY 3.0 license.

imaging shows that Tf-NPs can cross the BBB and directly target tumor cells in the U87 MG and GL261 intracranial orthotopic models. It is worth noting that within a certain range, as the density of Tf on the surface of liposomes increases, liposomes are more likely to bind to tumor cells. Jhaveri et al.⁹⁷ found that the maximum cellular association for Tf-liposome was seen at 1.0%–1.5% (*mollmol*) of Tf. However, if the binding is excessive, the effect will be weakened due to steric hindrance or the formation of receptor clusters by TfR¹¹⁹. Some exogenous peptide ligands can also specifically recognize TfR. In particular, their binding site is different from that of Tf, so endogenous Tf in the blood will not competitively inhibit it⁹⁹. Bi et al.⁹⁸ constructed T7 peptide (HAIYPRH)-coupled micelles loaded with BCNU. The *in vivo* detecting results of the targeting effect using BODIPY probe show that, compared with unconjugated probe, T7-modified micelles accumulate more effectively in gliomas. However, L-Type peptides are easily hydrolyzed to affect the targeting ability. The D-Type(D-T7) peptide with reverse sequence can overcome the shortcomings of poor stability and has better targeting ability. Yu et al.¹⁰⁰ used D-T7 peptide to modify bilirubin NPs loaded with cediranib and PTX, and its penetration effect on glioma was 7.89 times higher than that of unmodified bilirubin. In addition, some TfR monoclonal antibodies have also been developed, such as OX26 and RI7217, which recognize unique epitopes on TfR, so that endogenous Tf does not inhibit its absorption^{102,103,120}. However, the preparation and quality control of these antibodies are still a major problem that needs to be overcome at present¹²¹.

Angiopep-2 is a new type of peptide composed of 19 amino acid molecules. It is a specific ligand for LRP1. Compared with Tf, it has higher brain penetration and accumulation ability⁹⁴. Jiang et al.¹⁰⁵ developed angiopep-2-modified virus-mimicking polymersome (ANG-PS) loaded with SAP, a natural protein toxin with high efficiency and selectivity for GBM tissue. ANG-PS effectively penetrates the BBB and delivers SAP to U87 MG cells *in situ*. Quantitative analysis of DiR fluorescence showed that when the ANG density was 20% (*mollmol*), the accumulation of ANG-PS in GBM was the highest. At the same time, Jiang et al.⁹³ also developed chimeric polymersome (ApoE-CP) modified by peptides derived from apolipoprotein E (ApoE). ApoE-CP loaded

with SAP can completely inhibit the growth of mouse with U87 MG GBM *in situ* (Fig. 6A). It was also found that the surface density of 20% ApoE can maximize the efficiency of cell uptake (Fig. 6B and C). In addition, it is worth noting that the delivery effect of ApoE-CP is better than that of angiopep-2-modified chimeric polymersome (ANG-CP), because ApoE can bind to the three receptors of LRP1, LRP2, and LDLR (Fig. 6D and E). Therefore, the multi-receptor targeting of ApoE peptide seems to be more efficient than single-receptor targeting. Although pretreatment of U87 MG cells with free ApoE will result in a significant reduction in ApoE-CP uptake⁹³. But interestingly, Huang et al.¹²² found that excessive but relatively low concentrations of ApoE3 (3.125–100 $\mu\text{g}/\text{mL}$) can stimulate the uptake of ApoE3-reconstituted high-density lipoprotein (HDL) by C6 cells (Fig. 6F).

ApoE peptide-modified NPs achieve better targeting effect due to the binding of three receptors, which has to trigger our thinking about whether we can develop a ligand that can bind to multiple targets on BBB or glioma cells. Liu et al.^{123,124} used the standard solid phase peptide synthesis method to couple the cyclic peptide c(RGDfK) and dGR (RGD reverse sequence) peptide with the octa-arginine (R8), respectively. The R8-RGD peptide and R8-dGR peptide were developed. R8 is a cell penetrating peptide (CPP) that carries a large amount of positive charge and can mediate the entry of therapeutic drugs into cells through AMT. But compared with RMT and CMT, it lacks specificity for BBB and glioma cells¹²⁵. RGD peptide is a specific ligand for integrin $\alpha\text{v}\beta3$, which were overexpressed on both BBB and glioma cells¹²⁶. In addition to recognizing integrin $\alpha\text{v}\beta3$, dGR can also recognize NRP-1. R8-dGR-modified liposomes loaded with PTX can induce effective glioma tissue penetration through the synergy of three ways, including electrostatic interaction, and integrin $\alpha\text{v}\beta3$ specific binding, and NRP-1 dependent penetration¹²⁴. This well-designed peptide also provides an excellent idea for solving the weak targeting of CPP.

A distinctive feature of tumor cells is that they require more glucose than normal cells, which is the Warburg effect. Therefore, GLUT is highly expressed on BBB and tumor tissues of glioma patients, which can specifically recognize glucose or

Table 1 The single ligand-based cascade targeted NDDS for the treatment of gliomas.

| Target spot | Ligand | Nanocarrier | Therapeutic cargo | Therapy | Ref. | |
|---------------------------------|--|--|-------------------------------|-----------------|-----------------|-----|
| TfR | Tf | Liposomes | TMZ/bromodomain inhibitor JQ1 | Chemotherapy | 92 | |
| | | Porous silicon NPs | DOX | Chemotherapy | 96 | |
| | | Liposomes | Resveratrol | Chemotherapy | 97 | |
| | L-Type T7 peptide | PLGA-PEG micelles | BCNU | Chemotherapy | 98 | |
| | | Liposome-protamine-chondroitin sulfate NPs | siEGFR | Gene therapy | 99 | |
| | | D-T7 peptide | PEGylated Bilirubin NPs | PTX/Cediranib | Chemotherapy | 100 |
| | | T12 peptide | PEG-PLA micelles | PTX | Chemotherapy | 101 |
| | LDLR/LRP | OX26 mAb | PLGA NPs | TMZ | Chemotherapy | 102 |
| | | RI7217 mAb | Liposomes | DTX | Chemotherapy | 103 |
| | | TfRscFv | Cationic liposomes | TMZ | Chemotherapy | 104 |
| | | Angiopep-2 | Polymersomes | SAP | Proteinotherapy | 105 |
| | | | Nanocapsule | siRNA | Gene therapy | 106 |
| | | | Polymersomes | DOX | Chemotherapy | 94 |
| ApoE peptide | | Polymersomes | RGS | Proteinotherapy | 107 | |
| Chimeric polymersomes | SAP | Proteinotherapy | 93 | | | |
| Pep22 peptide | Oxidized nanocrystalline mesoporous carbon particles | DOX | Chemotherapy | 108 | | |
| RAP12 peptide | PEG-PLA micelles | PTX | Chemotherapy | 109 | | |
| Chloride ion channel | Chlorotoxin | PEI NPs | DOX | Chemotherapy | 110 | |
| EGFR/EGFRvIII | D-AE peptide | PEG-PLA micelles | PTX | Chemotherapy | 111 | |
| Interleukin-6 receptor | I ₆ P ₇ peptide | Polymer NPs | DNA | Gene therapy | 112 | |
| Interleukin-13 alpha 2 receptor | Interleukin-13 | PEG-PLGA NPs | Chelator Dp44mT | Chemotherapy | 113 | |
| nAChR | RVG29 peptide | PEG-PLGA NPs | DTX | Chemotherapy | 114 | |
| NRP-1 | ^D A7R peptide | Liposomes | DOX | Chemotherapy | 115 | |
| VAV3 protein | GICP peptide | PEG-PLA micelles | PTX | Chemotherapy | 116 | |
| Integrin receptor | TR peptide | Liposomes | PTX | Chemotherapy | 117 | |
| | sRGD peptide | PEG-PLA micelles | PTX | Chemotherapy | 118 | |

D-T7, D-type T7; DTX, docetaxel; EGFR/EGFRvIII, epidermal growth factor receptor and mutation variant III; nAChR, $\alpha 7$ nicotine acetylcholine receptor; NRP-1, neuropilin-1; PEI, polyetherimide; PTX, paclitaxel; RGS, rigosertib; SAP, saporin; Tf, transferrin; TfRscFv, anti-TfR single-chain antibody fragment.

glycoconjugates¹²⁷. Jiang et al.¹²⁸ designed 2-deoxy-D-glucose-modified polymer NPs ($_D$ Glu-NP). $_D$ Glu-NP which showed higher cellular uptake than ordinary NPs can pass through the BBB and enter the RG-2 cells through caveolae-mediated and clathrin-mediated endocytosis. Mannose tocopherol derivatives and glucose-tocopherol derivatives have been used to modify drug-loaded liposomes and micelles to achieve cascade delivery across the BBB^{129,130}. In addition, Large amino acid transporter 1, Na⁺-coupled carnitine transporter 2 and proton-coupled folate transporter are also overexpressed on BBB and glioma cells. Dual targeting NPs for these transporters have also been developed^{131–133}.

In addition, nAChRs are overexpressed in glioma vascular endothelium, glioma cells and tumor-associated macrophages (TAM)¹³⁴. Zheng et al.¹³⁵ used nAChRs-binding peptide $_D$ CDX-modified liposomes to develop CDX-LIPO that can achieve a "three-birds-one-stone" delivery strategy. CDX-LIPO can not only trigger the autophagocytosis of tumor cells, but also promote the polarization of M1 macrophages in the glioma microenvironment,

and promote mTOR-mediated reprogramming of glucose metabolism to treat GBM.

3.1.2. Cascade targeting based on dual ligands

Due to the diversity of receptors on BBB and glioma, it is clear that it is not limited to the same ligand. Using two different ligands to target BBB and glioma lesions (including glioma cells and other tumor-related cells) can often achieve better results¹⁵. The dual ligands-based cascade targeted NDDS for the treatment of gliomas are summarized in Table 2^{136–150}.

One idea is to use one ligand to target the BBB and another to target the glioma cells (Fig. 5B,b). Gao et al.¹³⁶ used TGN peptide and AS1411 aptamers to modify DOX-loaded PEG-PCL NPs to construct a dual-stage targeted delivery system AsTNP. The phage-displayed TGN peptide can specifically bind to BBB. The nucleic acid aptamer AS1411 is a G-rich aptamer derived from single-stranded DNA, which can bind to a highly expressed nucleolin on the tumor cell membrane. Compared with the single-stage targeting systems (AsNP and TNP), AsTNP showed the best anti-glioma effect.

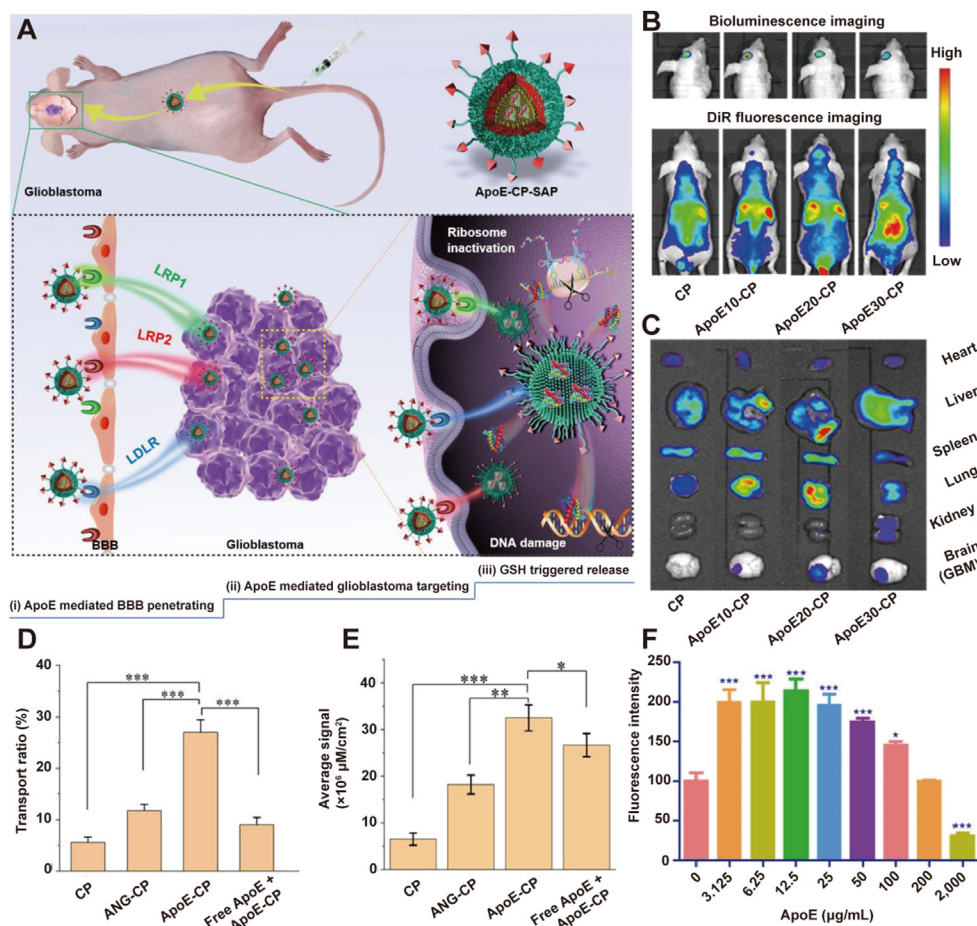


Figure 6 (A) Schematic presentation of ApoE-CP-SAP for targeted therapy for GBM. (B) Bioluminescence images of the intracranial U87 MG-Luc GBM mouse model and real-time whole-body DiR fluorescence imaging of GBM-bearing animals at 24 h after the intravenous injection of CP-DiR and ApoE-CP-DiR with varying ApoE surface densities of 10%, 20%, and 30% (*mollmol*). (C) *Ex vivo* DiR fluorescence images of excised organs from mice sacrificed at 24 h post-injection. (D) The *in vitro* BBB model transport ratios (%) of Cy5-labeled CP, ANG-CP, and ApoE-CP following 24 h of incubation. (E) Semi-quantitative analysis of DiR fluorescence intensity in GBM sections. (F) Cellular association of DiI-CaP-rHDL in the presence of ApoE at the concentrations ranged from 0 to 2000 $\mu\text{g}/\text{mL}$ in C6 cells. (A–E) Reprinted with the permission from Ref. 93. Copyright © 2018 American Chemical Society. (F) Reprinted with the permission from Ref. 122. Copyright © 2017 Nature publishing group.

Table 2 The dual ligands-based cascade targeted NDDS for the treatment of gliomas.

| Nanocarrier | Therapeutic cargo | Targeting ligand | Target spot | Targeting ligand | Target spot | Therapy | Ref. |
|--|---|--------------------------|-------------------------------|--------------------------|--|----------------------------|------|
| PEG–PCL NPs | DOX | TGN peptide | Amino acid transporter (BBB) | AS1411 | Nucleolin (glioma) | Chemotherapy | 136 |
| Fe ₃ O ₄ /Gd ₂ O ₃ | CDDP | LF | LF receptor (BBB) | RGD peptide dimer | Integrin $\alpha v \beta 3$ (glioma) | Ferroptosis therapy | 137 |
| Erythrocyte membrane-enveloped PLGA NPs | Euphorbia factor L1 | DWSW peptide | Quorum sensing receptor (BBB) | NGR peptide | CD13 receptor (glioma) | Chemotherapy | 138 |
| Hyaluronic acid nanogels | DOX | LF | LRP-1 (BBB) | Phenylboronic acid | SA (glioma) | Chemotherapy | 139 |
| DGL | DOX | Tf | TfR (BBB) | MAN | GLUT (glioma) | Chemotherapy | 140 |
| PEG–Cholic acid nanomicelles | VCR | MA | GLUT1 (BBB) | CBA | SA (glioma) | Chemotherapy | 141 |
| PEG–PLA NPs | PTX | CGKRK peptide | NRP-1 (BBTB) | ATWLPPR peptide | Heparan sulfate proteoglycan (glioma) | Chemotherapy | 142 |
| Liposomes | DOX | Tf | TfR (BBB/glioma) | Folate | Folate receptor (glioma) | Chemotherapy | 143 |
| Liposomes | DOX/VCR | T7 peptide | TfR (BBB/glioma) | ^D A7R peptide | VEGFR-2 (glioma) | Chemotherapy | 144 |
| Liposomes | DOX | Pep22 | LPR1(BBB/glioma) | c(RGDfK) peptide | Integrin $\alpha v \beta 3$ or $\alpha v \beta 5$ (glioma) | Chemotherapy | 145 |
| Liposomes | TMZ | Angiopep-2 | LPR1 (BBB/glioma) | CD133 mAb | CD133 (glioma) | Chemotherapy | 146 |
| Liposomes | DOX | ^D CDX peptide | nAChRs (BBB) | c(RGDfK) peptide | Integrin $\alpha v \beta 3$ (BBTB/glioma) | Chemotherapy | 147 |
| Liposomes | Daunorubicin | Mannose | GLUT (BBB/glioma) | Tf | TfR (BBB/glioma) | Chemotherapy | 148 |
| PEI NPs | Angiogenesis-inhibiting secretory endostatin gene | AT7 peptide | NRP-1/VEGFR-2 (BBTB/glioma) | TAT peptide | NRP-1 (BBTB/glioma) | Gene therapy | 149 |
| BSA NPs | DSF/Cu | T12 peptide | TfR (BBB/glioma) | Mannose | MR (glioma) | Chemotherapy/immunotherapy | 150 |

BSA, bovine serum albumin; CBA, 4-carboxyphenylboronic acid; DGL, dendrigraft poly-L-lysine; DSF/Cu, disulfiram/copper complex; LF, lactoferrin; MA, maltobionic acid; MAN, p-aminophenol- α -D-mannopyranoside; MR, mannose receptor; PCL, poly(caprolactone); SA, sialic acid; VCR, vincristine; VEGFR-2, VEGF receptor-2.

Another idea is to use two synergistic ligands to produce better targeting capabilities (Fig. 5B and c–e). There is a highly specific expression of CD133 on glioma stem cells (GSCs). CD133 is also a member of the transmembrane glycoprotein family and is a specific surface marker of GBM. Kim et al.¹⁴⁶ used angiopep-2 and anti-CD133 mAb to modify liposomes containing TMZ. Compared with free TMZ and non-targeted TMZ-liposomes, the tumor size was significantly reduced after the injection of dual-targeting immunoliposome encapsulating TMZ into orthotopic tumor mice, and the median survival time increased from 23 to 49 days. There are a large number of TAM in the glioma microenvironment. TAM can be polarized into two phenotypes of TAM1 and TAM2 during the development of glioma. It has been found that remodeling the tumor microenvironment by adjusting the polarization of TAM is a new immunotherapy method¹⁵¹. Zhao et al.¹⁵⁰ found that albumin binding protein (SPARC) and MR are highly expressed on TAM2. Therefore, the BSA NPs were modified with T12 peptide and mannose. Since T12 peptide promotes BBB penetration and glioma uptake, MR and SPARC promote TAM2 uptake, this functional BSA NPs can efficiently deliver DSF/Cu to glioma cells and deliver the macrophage modulator regorafenib (Rego) to TAM2. It was shown that after

treatment with T12/Man-BSA NPs, the population of TAM2 decreases and the population of TAM1 increases, which promotes the activation of cytotoxic T lymphocytes and the immune suppression is lifted. The median survival period of C57BL/6 mice transplanted with GL261 cells *in situ* was extended to 24 days.

In the above, it is mentioned that NPs modified with high-affinity ligands are easily transported to the lysosomes of BMVECs, resulting in low efficiency of NPs reaching the brain parenchyma. Ruan et al.¹⁴⁰ used acid-cleavable Tf and MAN dual-modified DGL (DD-MCT) to overcome this problem perfectly. The exterior of the DOX-loaded DGL is connected to acid-cleavable Tf through long-chain PEG, and MAN is connected through short-chain PEG. When DD-MCT enters BMVECs through TfR-mediated transcytosis, DD-MCT undergoes Tf acid-responsive cleavage in endosomes/lysosomes. The MAN-modified DOX-loaded DGL (DD-M) escapes from the endosomes/lysosomes and then enters the brain parenchyma through GLUT-mediated exocytosis (Fig. 7A). Flow cytometry analysis showed that after DD-MCT was incubated in the Transwell model for 4 h, the amount of uptake by C6 cells was significantly higher than that of other groups (Fig. 7B). The median survival time of DD-MCT-treated mice was 223% longer than that of the saline group (Fig. 7C).

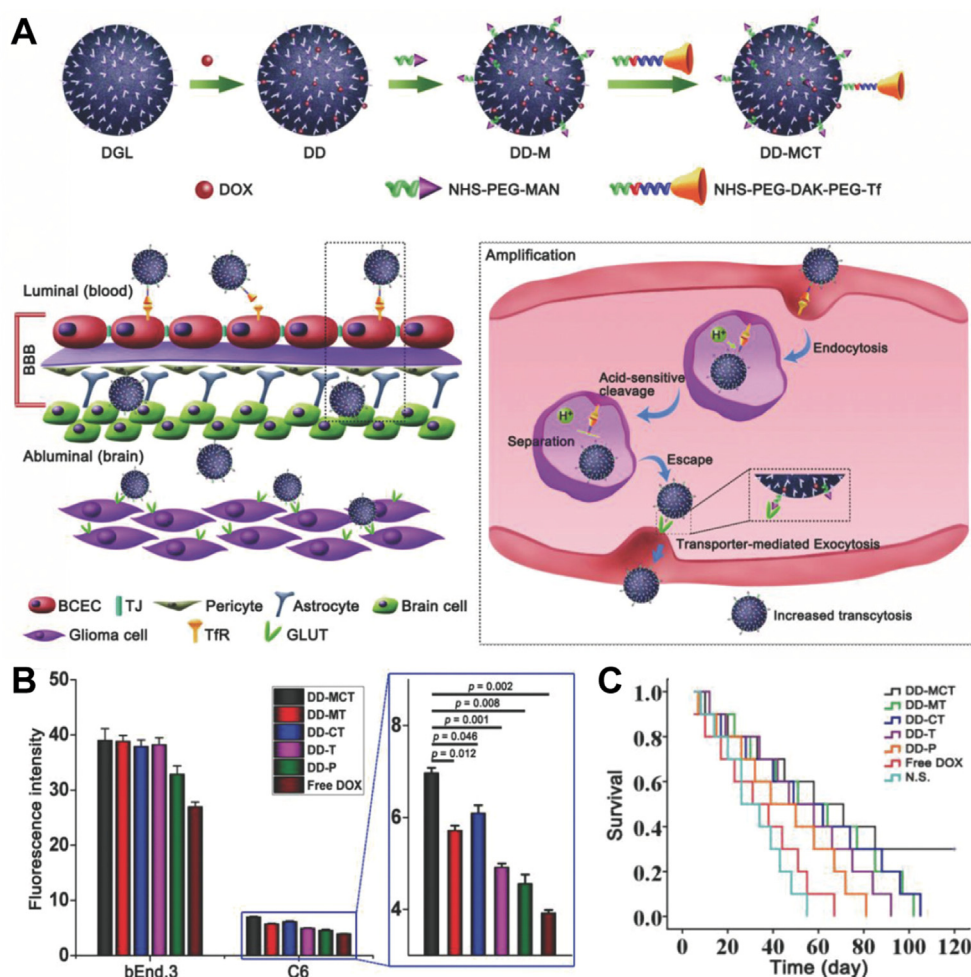


Figure 7 (A) Schematic diagram of DD-MCT preparation and cascade targeted delivery mechanism. (B) Cellular uptake of different formulations by bEnd.3 monolayers and C6 cells after introduction into the Transwell model for 4 h using flow cytometry analysis. (C) Survival curve of C6 glioma-bearing mice after intravenous administration of different formulations using Kaplan–Meier analysis. Reprinted with the permission from Ref. 140. Copyright © 2018 Wiley-VCH.

3.2. NDDS based on endogenous-stimuli-response

Cascade NDDS designed according to cell receptors or transporters shows good active targeting ability for gliomas. On this basis, some special properties of the glioma microenvironment can also be used to further enhance the absorption efficiency of NPs by glioma cells. To achieve the ideal precision treatment, it is also necessary to ensure that drugs will not leak from the carriers before they effectively reach the glioma tissue. Stimuli-responsive NDDS can effectively release the drug at the target cells after specific stimulation, thereby further optimizing the therapeutic effect¹⁵². According to the source and characteristics of the stimuli signals, stimuli-responsive NDDS can be classified into endogenous and exogenous-stimuli-responsive NDDS¹⁵³. Endogenous signals refer to the inherent characteristics of the glioma environment, such as pH, enzymes, and redox agents, while exogenous signals refer to external stimuli such as magnetic fields and ultrasound. In this section, we mainly introduce the NDDS developed based on endogenous signals.

3.2.1. Endogenous-stimuli-responsive delivery

The Warburg effect states that tumor cells mainly rely on glycolysis for energy, and a large amount of lactic acid secreted will accumulate outside the cells, forming an acidic microenvironment (pH is about 6.5). A low pH environment can induce tumor deterioration and even multidrug resistance^{154,155}. Poly- γ -glutamic acid (γ -PGA) is widely used in the development of nanocarriers because of its biodegradable, nontoxic, and anionic characteristics. But the outer anionic layer is also easily dissipated in the blood¹⁵⁶. Cationic NPs can penetrate BBB and glioma cells through electrostatic action, but they are also easily eliminated in the blood¹⁵⁷. To simultaneously deliver DOX and overcome the shortcomings of cationic NPs, a new type of dopamine-grafted- γ -PGA (γ -PGA-Dopa) was developed as a coating material. γ -PGA-Dopa can not only form a complex with DOX, but also be deposited on the surface of cationic NPs through a cross-linking reaction mediated by Michael addition reaction or Schiff reaction. γ -PGA-Dopa can shield the cationic core at physiological pH and will fall off and expose the cationic core in the acidic pH environment of gliomas. Curcumin (Cur) and DOX can be synchronously released in glioma tissue for a long time¹⁵⁶. It has been introduced before that dual targeted NDDS co-modified with other ligands is the main measure to overcome the non-specific binding of CPP. Some researchers started with CPP itself and designed a pH-responsive CPP. Tian et al.¹⁵⁸ chose arginine-rich peptide (RG)₅ as the model CPP, combined with pH-responsive polycation masking peptide (HE)₅ for charge shielding. The cumulative release of PTX in the co-modified micelles in the first 2 h was 23.1% at pH 5.0, which was ten times higher than that at pH 7.4.

Malignant gliomas show extensive areas of hypoxia, because abnormal blood vessels cannot deliver enough oxygen to all rapidly proliferating glioma cells. The glioma cells in the hypoxic area are gradually less sensitive to some antiproliferative agents due to the slower division speed. Moreover, the hypoxic environment will also induce some cells to acquire the characteristics of GSCs, and promote tumor development and drug resistance^{159,160}. In hypoxic conditions, hydrophobic nitroimidazoles will be converted into hydrophilic aminoimidazoles through a series of selective biological reduction reactions. Liu et al.¹⁶¹ using metronidazole (MI) developed ionizable liposomes, which can obtain more positive charges under hypoxic conditions, thereby enhancing the uptake of liposomes by glioma cells.

Because the liposome has tertiary amines, it can be affected by environmental pH and maintain a low cationic charge density in the blood, making it an excellent carrier for delivering siRNA for the treatment of gliomas. Radiotherapy (RT) is also a commonly used clinical method to treat tumors, but the hypoxic environment makes gliomas have a strong resistance to radiation¹⁶². MI has also been proposed as a radiosensitizer for hypoxic cells. Liu et al.¹⁶² also developed a hypoxic radiosensitizer-prodrug liposome to deliver DOX, realizing the combined chemotherapy/RT of malignant gliomas. On this basis, a novel angiopep-2-lipid-poly(metronidazoles)_n [ALP-(MIs)_n] was also designed to increase the loading of nitroimidazoles. The glioma inhibition rate of ALP-(MIs)₂₅/DOX + RT and ALP-(MIs)₄₈/DOX + RT groups were 19.6 and 13.7 (PBS + RT group was 184.5)¹⁶³.

Matrix metalloproteinases (MMP) are over-expressed in tumor tissues and are considered to be a specific trigger of NDDS¹⁶⁴. MMP promotes the infiltration of glioma cells into the surrounding tissues by degrading the extracellular matrix, allowing them to penetrate the BBB to promote the angiogenesis, invasion and metastasis of gliomas¹⁶⁵. Bruun et al.¹⁶⁶ modified siRNA-loaded cationic lipid NPs (LNP) with PEGylated cleavable lipopeptide containing glutamic acid residues. Glutamic acid residues can effectively shield the positive charge in the systemic circulation. When in a high concentration of MMP environment, the PEGylated cleavable lipopeptide coating can be cleaved to expose the positive charge, which is conducive to the endocytosis of glioma cells and the release of siRNA. The absorption rate of LNP that can be activated by the MMP is 10 times higher than that of LNP without targeting PEGylation. Chen et al.¹⁶⁷ developed a rocket-like multi-stage booster delivery system by combining a recombinant trichosanthin (TCS) fused with MMP-2 substrate peptide (MSP) and CPP with LF. First, LF helps BBB penetration by targeting overexpressed LRP-1. After reaching the glioma site, MSP is recognized and lysed by MMP-2, releasing TCS with cell penetrating ability, thereby infiltrating into the glioma cells. Using an immunologically functional C57BL/6 mouse model with *in situ* glioma, it was found that the median survival time of the recombinant TCS group was about 2 times longer than that of the saline group, which greatly improved the efficacy of glioma treatment.

3.2.2. Endogenous-stimuli-responsive release

In the endosomes/lysosomes of tumor cells, the pH is only 4.5–5.5. Since NPs internalized by endocytosis are easily trapped in endosomes/lysosomes, pH-responsive release of NDDS is very valuable¹⁶⁸. Liu et al.¹⁶⁹ developed a charge conversional biomimetic nano-platform Ang-RBCm-CA/siRNA. The core is cationic PEI complexes loaded with siRNA, the middle layer is poly-L-lysine grafted with negatively charged citraconic anhydride, and the outer shell is angiopep-2-decorated red blood cell membrane (RBCm). When Ang-RBCm-CA/siRNA reaches the endosomes/lysosomes, the negative charge of the core is converted to positive charges, causing RBCm to rupture and accelerate the release of siRNA. Using siPLK1 as a therapeutic siRNA, treatment with Ang-RBCm-CA/siPLK1 can significantly increase the survival rate to a median of 43 days, which is significantly longer than the use of Ang-RBCm-SA/siPLK1 containing pH-stable succinic anhydride.

Glutathione (GSH) is an active tripeptide naturally present in human cells. Because tumor cells lack a normal GSH regulatory mechanism, the intracellular GSH concentration can even be 1000 times higher than the extracellular concentration¹⁷⁰. Most redox-

responsive NDDS depends on compounds with disulfide bonds. In a high-concentration GSH environment, the disulfide bonds will undergo a redox reaction to break into sulfhydryl, allowing the drug to be accurately released in the tumor tissue¹⁷¹. Zou et al.¹⁰⁶ developed an angiopep-2-functionalized redox-responsive siRNA nanocapsule [Ang-NC ss (siRNA)]. The unique small size (25 nm) and the targeted modification of angiopep-2 make it highly accumulate in GBM, and the encapsulated amount of siRNA can even reach 100%. *N,N'*-Bis(acryloyl) cystamine containing disulfide bonds is a crosslinking agent for nanocapsules. Under physiological conditions without GSH, less than 20% of siRNA was released within 24 h, while in the presence of 10×10^{-3} mol/L GSH, nearly 80% of siRNA was released within 24 h.

In addition to high concentrations of GSH, due to factors such as increased metabolic activity and abnormal mitochondrial function, the level of ROS in tumor cells increases, which leads to increased oxidative stress¹⁷⁰. Zheng et al.¹⁷² designed a ROS-responsive siRNA nanomedicine functionalized with angiopep-2 (Ang-3I-NM@siRNA). The hydrophobic effect of phenylboronic ester serve is one of the reasons for enhancing the stability of the delivery system. In addition, phenylboronic ester serve will lose the stability of hydrophobic interaction in the environment of high concentration of ROS, and randomly generate carboxyl groups to interfere with other stabilizing factors (electrostatic and hydrogen bond interactions). Finally, the effective release of siRNA will be realized in glioma cells. When treated with H₂O₂ at concentrations above 0.1×10^{-3} mol/L, the release amount of siRNA and the particle size of 3I-NM@siRNA increased with the increase of H₂O₂ concentration. Dong et al.¹⁷³ used *p*-(boronic ester)benzy to link PEG with PTX, and the self-assembled PEG-B-PTX micelles were very stable under normal physiological conditions. *In vitro*, phorbol-12-myristate-13-acetate (PMA) was used to incubate U251 cells to create an environment with high levels of ROS in the cells. It was found that PEG-B-PTX can reduce the IC₅₀ value of U251 cells by 11 times through PMA stimulation. It was confirmed that high concentration of ROS in glioma cells can trigger the 1,4-elimination reaction of *p*-(boronic ester)benzy and release PTX. In addition, the release of the by-product quinone can deplete the GSH in tumor cells and play a synergistic therapeutic effect.

3.3. BDDS based on endogenous substances

As materials science is in the process of rapid development, a wide range of nanocarriers are used to design NDDS that can accurately treat gliomas. The nanocarriers of NDDS introduced above mainly include liposomes, polymer NPs, nanomicelles, dendrimers and so on. At present, the commonly used method is to modify the surface of nanocarriers with PEG. PEGylated nanocarriers are not easily taken up by the reticuloendothelial system, which can prolong the circulation time of nanocarriers in the body¹⁷⁴. However, it is found that some people will also have an immune response to PEGylated nanocarriers, and the PEGylated nanocarriers given a second time will also be quickly removed¹⁷⁵. Here, we will introduce biomimetic drug carriers designed by endogenous substances, including cells, extracellular vesicles and endogenous proteins (Fig. 8). These biomimetic carriers have unique advantages over synthetic carriers (Table 3^{176–195}).

3.3.1. BDDS based on cells or cell membrane coating

Red blood cells (RBCs) are the most abundant cells in the blood and have a long life span in the circulatory system. Mature RBCs

lack nuclei and organelles, which makes the RBCm easy to extract and purify¹⁹⁶. The good biocompatibility and prolonged circulation time are the excellent characteristics of BDDS designed by RBCm^{197,198}. Zou et al.¹⁷⁶ used the angiopep-2-modified RBCm to coat pH-sensitive NPs containing DOX and lexiscan (Lex). It was found that the elimination half-life ($t_{1/2,\beta}$) of Ang-RBCm@NM-(DOX/Lex) nanomedicine is 9.3 h, while the $t_{1/2,\beta}$ of NM-(DOX/Lex) without RBCm is 2.4 h. It has been showed that the RBCm biomimetic strategy successfully minimized the non-specific clearance rate in the blood, thereby prolonging blood circulation. Due to the CD47 transmembrane protein on the surface of natural RBCm can bind to SIRP α expressed by macrophages, so that the RBCm can escape the clearance and degradation controlled by immune cells¹⁹⁹. Chai et al.²⁰⁰ successfully improved the stability and controlled release behavior of drug nanocrystals (NCs) through RBCm. It has been shown that NCs loaded with DTX will quickly aggregate within 48 h, while NCs coated with RBCm (RBC-NCs) hardly change in particle size within 48 h. Moreover, RBC-NCs loaded with DTX showed good release behavior in pH 7.4 PBS and 10% fetal calf serum, compared with NCs. In addition, ligand-modification can confer the characteristics of excellent BBB permeability, superior tumor accumulation, and extremely low adverse effects to RBCm-coated NPs¹⁷⁶.

It has been found that the cytokines and chemokines secreted by tumor cells can induce the accumulation of stem cells in tumor tissues, so that stem cells have a natural tendency to home to tumors²⁰¹. Roger et al.¹⁷⁸ proposed that the combination of mesenchymal stem cells (MSCs) and drug-loaded NPs is a promising strategy in the treatment of gliomas. Malik et al.¹⁸⁰ used non-viral drug polylysine-modified PEI copolymer to produce genetically engineered MSCs with suicide genes, herpes simplex virus thymidine kinase and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Compared with single-plasmid-transfected and untransfected MSCs, double-transfected MSCs and the prodrug ganciclovir can produce a significant synergistic therapeutic effect to glioma *in vivo* and *in vitro*. To improve the delivery efficiency of TRAIL, Suryaprakash et al.¹⁷⁹ proposed hybrid MSCs/nanomedicine spheroid system. Compared with the single MSCs method, the hybrid sphere showed higher retention of nanocomposite in tumor tissues, thereby significantly inhibiting tumor growth. Neural stem cells (NSCs) have been proved by many studies to have intrinsic tumor-tropic properties, which can migrate arbitrarily throughout the brain and target the lesion and infiltrating sites of gliomas²⁰². Portnow et al.²⁰³ used retroviruses to transfer a type of NSCs capable of expressing cytosine deaminase (CD). CD, as a prodrug converting enzyme, can convert the prodrug 5-fluorocytosine into 5-fluorouracil which has a killing effect on glioma cells.

Neutrophils (NEs) and monocytes/macrophages are very important immune cells in the body and participate in inflammation which plays an important role in all stages of tumors, including tumor occurrence, invasion, and metastasis^{204,205}. It has been found that these immune cells can penetrate the BBB and can infiltrate glioma cells^{206,207}. Different from the mechanism by which RMT and CMT make NPs target gliomas, NEs spontaneously transport NPs to infiltrating glioma cells by identifying and verifying signals. Xue et al.¹⁸¹ incubated PTX-loaded cationic liposomes (PTX-CL) with NEs to obtain NEs containing PTX-CL (PTX-CL/NEs). When glioma mice undergo surgical resection, inflammation occurs at the wound site, releasing a large amount of inflammatory factors, such as TNF- α and CXCL1/KC. These inflammatory factors can induce NEs to cross the BBB, causing

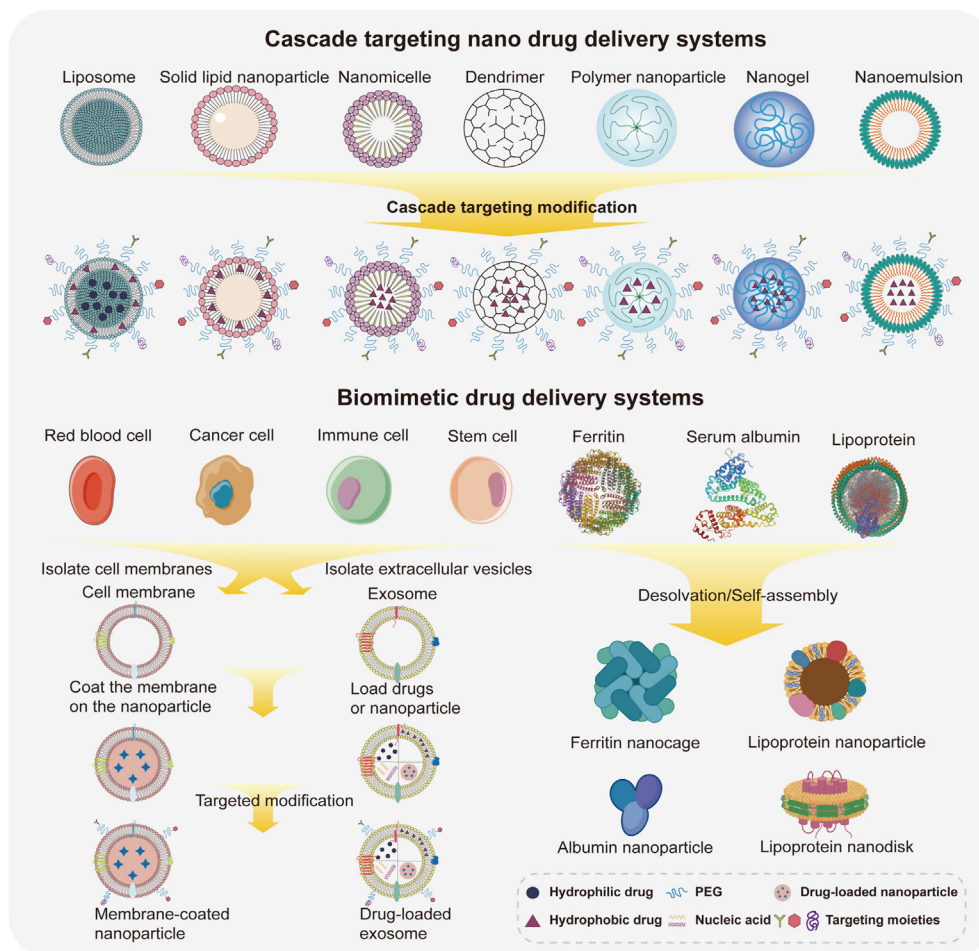


Figure 8 Synthetic nanocarriers and endogenous biomimetic carriers that have been extensively studied. Created using images from “Servier Medical Art (<https://smart.servier.com>)” and “<https://biorender.com>” under CC BY 3.0 license.

them to accumulate at the site of inflammation. Then PTX-CL/NEs are over-activated by concentrated cytokines to release PTX-CL. Mice after tumor resection were treated with PTXCL/NEs whose PTX dose was half of PTX-CL. It was found that the median survival was as high as 61 days, while PTX-CL was only 38 days. PTX-CL/NEs can prolong the survival time of mice after surgery and effectively prevent the recurrence of glioma after resection. Wu et al.¹⁸² co-incubated magnetic mesoporous silica NPs loaded with DOX with NEs of healthy mice to obtain the biomimetic drug delivery platform ND-MMSNs. After treating mice with ND-MMSNs, the number of apoptotic tumor cells was $20.92 \pm 2.28\%$, which was significantly higher than that of the control group and MMSN-treated mice. In addition, monocytes/macrophages are also expected to be used in the design of BDDS^{183,208–210}.

There are many unique proteins and receptors on the cell membrane of cancer cells, so they can evade immune surveillance and proliferate indefinitely. If the cancer cell membrane is coated on the surface of NPs, it can effectively improve the circulation time and the homogenous targeting ability of NPs²¹¹. The indocyanine green (ICG)-loaded and cancer cell membrane-coated NPs can be disguised as cells to reduce the interception of liver and kidney, and can simultaneously identify and treat tumors²¹². Since most cancers, such as lung cancer, breast cancer, and melanoma, have a greater probability of metastasis to the brain to

form secondary brain tumors, it is known that these cancer cells can cross the BBB²¹³. The attachment of these cancer cells to BMVECs and their transendothelial migration are mediated by the interaction of proteins on cancer cell membranes and adhesion molecules of BMVECs such as integrins, selectins, and chemokines²¹⁴. Wang et al.¹⁸⁴ designed polymer NPs loaded with ICG in the cell membrane of brain metastasis tumors for brain tumor imaging and photothermal therapy, and injected the biomimetic NPs into normal mice, early orthotopic brain tumor models, and advanced orthotopic brain tumors. The results showed that the NPs coated with brain metastasis tumor cell membrane can pass through the BBB more efficiently, and show a better effect of inhibiting the growth of brain tumors. Jia et al.¹⁸⁵ found that using the protein on the C6 glioma cell membrane to modify DOX-loaded liposomes can form more stable BDDS. The biomimetic liposomes of glioma cells showed excellent homology targeting capability in 3D tumor-spheres, and the ability of penetration was increased by 2.25 times. After intravenous injection of C6 glioma tumor-bearing nude mice, the tumor inhibition rate reached 93.3%.

3.3.2. BDDS based on extracellular vesicles

Extracellular vesicles (EVs) are intramembrane vesicles produced by almost all cells. According to their source and size, they are mainly divided into exosomes (Exos) (30–150 nm), microvesicles

Table 3 Advantages of BDDS from different sources.

| Source | Advantage | Ref. |
|----------------|---|---------|
| Red blood cell | The most abundant blood cells; The most accessible cells; Extending circulation time; Evading immune clearance | 176,177 |
| Stem cell | Tumor homing ability; Evading immune clearance | 178–180 |
| Immune cell | Cross BBB transportation; Tumor targeting ability | 181–183 |
| Cancer cell | Extending circulation time; Tumor homing ability; Immune modulation | 184,185 |
| Exosome | Wide variety of sources; Natural vesicles; Cross BBB transportation; Tumor homing ability; Easy to modify | 186–188 |
| Ferritin | Evading immune clearance; Receptor-mediated active targeting capacity; pH-Triggered disassembly/assembly facilitates encapsulation of drugs | 189,190 |
| Serum albumin | Evading immune clearance; Redox reactivity; High drug load; Cross BBB transportation; Easy to modify | 191–193 |
| Lipoprotein | Evading immune clearance; Receptor-mediated active targeting capacity; High drug load; Easy to synthesize | 194,195 |

(50–1000 nm) and apoptotic bodies (1–10 μ m). They have a lipid bilayer structure like cells, which can encapsulate and transport lipids, nucleic acids and proteins²¹⁵. Among them, the research on Exos as glioma drug delivery systems is the most prominent. Various adhesion proteins and ligands on the Exos membrane can cross the BBB through three mechanisms (RMT, lipid raft-mediated and micropinocytosis)²¹⁶.

Bai et al.¹⁸⁸ compared macrophage-derived Exos (R-Exos) with blood serum-derived Exos (B-Exos). When used in combination with FUS, R-Exos and B-Exos showed no significant differences in physical properties, drug release, tumor targeting and cytotoxicity. However, due to its high yield and easy availability, B-Exos has a higher clinical potential. Above, it has been mentioned that the NEs can be used as a drug delivery system to cross the BBB and target the glioma lesion site. Similarly, Exos derived from NEs (NEs-Exos) also have BBB penetration and glioma targeting. Wang et al.²¹⁷ obtained NEs-Exos/DOX by loading DOX into NEs-Exos by sonication. The zebrafish model verifies that NEs-Exos can pass through the BBB in large quantities and enter the brain. Moreover, the inflammatory response increases the recruitment of NEs-Exos. Quantitative fluorescence analysis showed that the fluorescence intensity of brain tumors on Day 15 of NEs-Exos/DOX was 3.52 and 2.74 times lower than that of saline and free DOX groups, respectively.

The Exos produced by human embryonic stem cells (ESCs) have been confirmed to be able to inhibit the carcinogenic phenotype of cancer cells²¹⁸. Zhu et al.²¹⁹ prepared c(RGDyK)-

modified and PTX-loaded ESCs-derived Exos (cRGD-Exo-PTX). Compared with unmodified Exos, cRGD-Exo-PTX significantly improved the efficacy of PTX in the treatment of GBM in mice by enhancing targeting capability. Lang et al.¹⁸⁶ used genetically engineered bone marrow MSCs to produce miRNA-124a-loaded Exos (Exo-miR124a) which can target glioma. After carotid injection of glioma mice, the Exo-miR124a acts by down-regulating FOXA2 (a known target of miR-124a), significantly inhibiting the survival of GSCs.

At present, there are also some obstacles in the development of drug-loaded Exos. For example, methods of drug loading and targeted modification. Salarpour et al.²²⁰ used U87 MG cells-derived Exos to encapsulate the chemotherapy drug PTX. Comparing the two loading methods of incubation and sonication, it was found that sonication can load more PTX on the Exos, but it will cause the Exos to become larger after loading. If the Exos are to be loaded with large nucleic acids such as mRNA, the effects of electroporation, induced transfer and other methods are still not ideal. Yang et al.²²¹ developed a cell nanoporation method that can encapsulate functional mRNA into Exos on a large scale. Thakur et al.²²² to enhance the loading efficiency of DOX in Exos, developed Exo-Load, a microfluidic device. The loading efficiency of DOX into U251 cells-derived Exos was significantly improved to 31.98%, which demonstrated that Exo-Load could be a potential drug-loading device into Exos with further optimization. Jia et al.¹⁸⁷ and Zhu et al.²¹⁹ respectively modified the drug-loaded Exos to make the engineered Exos better target the glioma site. Better modification effects can be achieved by combining with click chemistry or transfecting cells with plasmids. Gradually improving drug loading efficiency and targeting capability is the key to studying Exos for the treatment of glioma.

3.3.3. BDDS based on endogenous proteins

As an indispensable substance for living organisms, protein also plays an irreplaceable role in the treatment of glioma. As mentioned above, Tf and LF can be used as targeting ligands^{92,137}, and SAP can be used as a therapeutic agent⁹³. More importantly, certain endogenous proteins can also serve as good drug delivery carriers due to their non-toxic and non-immunogenic properties.

It is known that TfR is overexpressed on BBB and glioma cells, so many TfR-based cascade targeting NDDS have been developed. Ferritin is the endogenous binding ligand of TfR1. Importantly, HFn itself can carry therapeutic drugs. Ferritin is composed of 24 polypeptide subunits and arranged symmetrically into an octahedral cage-like nanostructure, so ferritin has good thermal and chemical stability²²³. The subunits of mammalian ferritin include the heavy (H) chain and light (L) chain. Ferritin can reversibly assemble/disassemble at different pH. When $\text{pH} < 3$ or $\text{pH} > 10$, it decomposes into H and L chain. However, when the pH has restored physiological conditions, they can reversibly recombine into ferritin²²⁴. It is on this basis that ferritin nanocages loaded with drugs have been developed. Fan et al.¹⁸⁹ found that only H-ferritin (HFn) can pass through the BBB, while L-ferritin cannot. More importantly, the structure of HFn remains intact after passing through the BBB. The results of immunofluorescence experiments show that HFn is hardly internalized into the lysosomes of BMVECs, but after being endocytosed by U87 MG cells, HFn accumulates in lysosomes. Therefore, DOX-loaded HFn (HFn-DOX) can significantly inhibit the growth of glioma *in situ*. On this basis, Wang et al.¹⁹⁰ replaced the fifth helix of HFn with a functional motif composed of hydrophobic-hydrophilic-RGD peptides, and constructed a

simultaneous loading of camptothecin (CPT) and epirubicin (EPI) protein-carrying nanocage (EPI@Am-PNCage/CPT). 132 hydrophilic EPI molecules and 50 hydrophobic CPT molecules are respectively loaded in the inner cavity and outer shell of each EPI@Am-PNCage/CPT. In addition, EPI@Am-PNCage/CPT can cascade targeting Tfr1 and integrin $\alpha\beta3$, prolonging the median survival of U87-luc tumor-bearing mice to 38 days.

Serum albumin is the most abundant extracellular protein in serum. It is non-immunogenic and has a long circulating half-life. The albumin-based PTX preparation (called Abraxane®) was launched in 2005 and has made huge profits²²⁵. It proves that the development of serum albumin as a drug carrier appeared bright prospect. At present, the commonly used serum albumin as carriers are BSA and human serum albumin (HSA). Due to the active metabolism of gliomas, serum albumin can pass through the BBB by over-expressed SPARC and glycoprotein 60 and be transferred to glioma cells^{191,226}. Lin et al.¹⁹¹ designed BSA NDDS (L-BSA-NP) co-encapsulated with PTX and fenretinide, and used the LMWP (low molecular weight protamine, a CPP) to enhance its penetration. The synthesis of L-BSA-NP relies on a green method, first using urea and NaHB₄ to break the disulfide bond in albumin and denature it. The denatured albumin has a linear structure, which can self-assemble into NPs with two hydrophobic drugs through hydrophobic bonds, and then the disulfide bonds are restored to maintain the stability of NPs. Because the abundant amino acids on the albumin molecule contain 17 pairs of disulfide bonds. Therefore, albumin-based NDDS has inherent redox response release characteristics. Ruan et al.¹⁹² prepared substance P (SP) peptide-modified and PTX-loaded HSA NPs (SP-HSA-PTX NPs). When SP-HSA-PTX NPs were exposed to the concentration of GSH in tumor cells, about 75% of PTX was released within 48 h, which was nearly twice as high as in PBS. Due to the stabilizing effect of intermolecular disulfide bonds, SP-HSA-PTX NPs can maintain integrity and stability in the extracellular environment. Since the efficient delivery of siRNA *in vivo* has been challenging. Gregory et al.¹⁹³ used polymerized HAS, oligoethylene glycol, and iRGD peptide to engineer synthetic NPs (STAT3i SPNPs) that can efficiently deliver STAT3i (siRNA against STAT). Under the combined treatment of STAT3i SPNPs and focused RT, the level of STAT3 in 87.5% of the mice was significantly reduced, there was no obvious residual tumor, and the long-term survival time point was reached.

Lipoproteins are NPs that are naturally assembled from lipids and proteins. They have a core-shell structure, the hydrophobic core is mainly composed of triglycerides and cholesterol esters, and the hydrophilic outer shell is composed of phospholipids and apoproteins¹⁸. Such properties allow lipoproteins to be loaded with drugs. In addition, lipoproteins have better structural stability than liposomes²²⁷. Both LDL and HDL have been studied for the delivery of anti-tumor drugs. LDL itself is the endogenous ligand of LDLR, so it naturally has the ability to target BBB and glioma cells. Because it is a vehicle for delivering cholesterol to tissues. If the developed LDL-based NDDS has low drug loading efficiency, it indirectly means that a high concentration of LDL is required for one administration, which may affect the body's cholesterol metabolism²²⁸. It seems that this problem can be solved by reconstructing LDL (rLDL)²²⁹, but it has not yet been applied to the treatment of glioma. Therefore, the current research is mainly based on HDL-based NDDS. The structure of HDL is similar to LDL, and its function is opposite to LDL. Its main function is to transport excess cholesterol in the body to the liver²³⁰. The size of HDL is relatively small (<12 nm), and it has two forms: discoidal

or spherical morphology¹⁸. In order to facilitate large-scale production, HDL-based NDDS customized according to requirements is an excellent choice. Huang et al.¹²² constructed ApoE3-reconstituted HDL (ApoE-rHDL) which has been used in the treatment of Alzheimer's disease and GBM. It was found that macropinocytosis may be the unique mechanism of GBM-specific accumulation of ApoE-rHDL. Apolipoprotein-I (ApoA-I) peptide is an essential component to stabilize HDL. Kadiyala et al.¹⁹⁴ develop ApoA-I mimetic peptide-based synthetic HDL mimicking nanodiscs (sHDL) loaded with DTX and Toll-like receptor 9 agonist CpG (DTX-sHDL-CpG). DTX-sHDL-CpG has the functions of chemotherapy and immunotherapy at the same time. DTX-sHDL-CpG can mediate a powerful immune response through the activation and expansion of tumor-specific CD8⁺ T cells. In addition, after combined with RT, 80% of mice had tumor regression and long-term survival. On this basis, Scheetz et al.¹⁹⁵ screened out three neo-antigen peptides from the GL261 mouse model, and synthesized each neoantigen peptide-conjugated sHDL nanodiscs vaccine. The incorporation efficiency of antigen peptides can be as high as 90%. The vaccine can induce the neoantigen-specific CD8 α^+ T cells to fully infiltrate the GL261 tumor microenvironment, achieving a powerful immunotherapy effect.

3.4. NDDS based on active ingredients of TCM

As we all know, the chemical components contained in TCM are complex, and one component or multiple components may play a therapeutic role. The above-mentioned resveratrol, shikonin, and Cur are effective ingredients of certain TCM^{97,187,231–234}. Loading them with suitable targeting carriers achieves a better therapeutic effect on gliomas. In addition to the therapeutic effect of inhibiting gliomas, these effective ingredients of certain TCM have more attractive features, which make a unique contribution to the optimization of NDDS (Fig. 9).

Meng et al.²³⁵ coupled BO with 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] to prepare DOX-loaded nanomicelles. The permeability of BO-modified micelles to BBB was significantly higher than that of other groups. And it has been discovered that BO can regulate HIF-1 α expression through the mTORC1/eIF4E pathway to promote human glioma cell apoptosis²³⁶. Besides, aromatic resuscitation drugs derived from TCM, such as menthol and muscone, have the same effect as BO in promoting brain penetration⁶⁶. Liang et al.⁶⁶ confirmed the targeted drug delivery effect of BSA NPs modified by three BPEs of BO, muscone, and menthol on gliomas, and compared them with T7-modified BSA. It has been found that menthol-modified BSA (MeB) is the best brain targeting carrier. MeB can be captured from the bloodstream and penetrate the fenestrae in the pineal gland. Compared with Tfr-mediated endocytosis, this novel approach allows drugs to pass through the BBB more effectively. In summary, the use of modern technology to combine these BPEs selected from TCM with nanocarriers has shown great potential and is expected to become a safe and effective strategy for the treatment of gliomas.

For a long time, the main design concept of nanocarriers has been to ensure that the carriers will not cause adverse effects on the body while improving the efficacy of the drug. The new carriers that are screened out must pass many tests and spend a lot of manpower and material resources. Therefore, researchers envisioned whether drug active molecules could be used as carriers or optimized carriers. Due to the special chemical properties of the

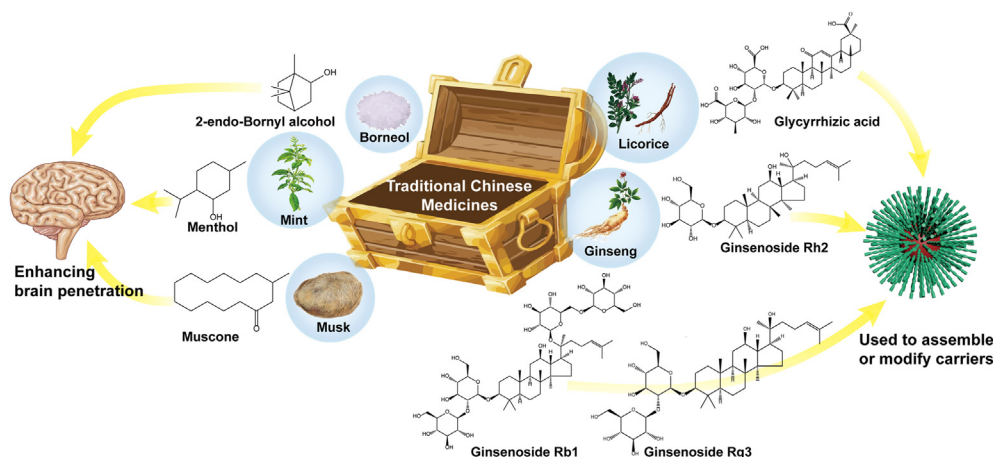


Figure 9 The unique contribution of some active ingredients of TCM to brain drug delivery. Created using images from “Servier Medical Art (https://smart.servier.com)” under CC BY 3.0 license.

active ingredients of TCM, molecular recognition and self-assembly technologies can make these active ingredients become carriers for drug delivery²³⁷. Saponins are a large group of ingredients widely derived from TCM. They have a special chemical structure, among which saponins have varying degrees of lipophilicity, and sugar chains have strong hydrophilicity¹⁹. This unique property makes them used in the development of drug carriers. Glycyrrhizic acid (GA) is one of the main saponins of the TCM licorice, which has anti-inflammatory, anti-cancer, and antibacterial effects²³⁸. Importantly, GA can not only assemble with some lipophilic drugs into complex micelles to increase the solubility of the drug, but also interact with cholesterol and phospholipids on the cell membrane to enhance the permeability of the drug to cells²³⁹. Zhang et al.²⁴⁰ prepared a solid dispersion (SD) of Cur and disodium glycyrrhizinate (Na_2GA) by a mechanochemical method. When the SD is dissolved in water, Na_2GA automatically forms Cur-loaded micelles. The cytotoxicity of Cur-loaded micelles against U87 MG cells is higher than that of free Cur. Compared with free Cur, the oral bioavailability of rats increased by about 19 times. Moreover, it was also found that GA has *in vitro* inhibitory effects on the growth of U251 GBM cells²⁴¹. Therefore, GA not only plays a role as an excipient but may also play a synergistic effect on the treatment of gliomas. Ginsenoside, the main active ingredient in ginseng genus medicinal materials, also functions as drug carriers²⁴². Dai et al.²⁴³ found that ginsenoside Rb1 can self-assemble with certain lipophilic anticancer drugs, such as betulinic acid, dihydroartemisinin, and hydroxycamptothecin, to form stable NPs. In addition to its self-assembly properties, since ginsenoside is a steroid compound, it may be a reliable substitute for cholesterol as a liposome component²⁴⁴. Lipid accumulation order of phospholipid bilayer, size, and surface status of liposomes could be greatly changed with the insertion of ginsenoside. Hong et al.²⁴⁵ found that ginsenoside Rh2-functionalized liposomes have longer blood circulation. And the glucose group of ginsenoside Rh2 can also be used as a ligand for GLUT. On this basis, Zhu et al.²⁴⁶ developed a multifunctional liposome system (Rg3-PTX-LPs) based on ginsenoside Rg3 and loaded with PTX. Rg3-PTX-LPs enhanced the chemotherapy efficacy of PTX by synergistically killing tumors, regulating the tumor microenvironment, and activating the immune microenvironment in gliomas, thereby significantly improving the anti-glioma effects. Rh2 and Rg3 not only act as

membrane materials, but also play a synergistic therapeutic effect²⁴⁵.

4. Clinical translation and future prospects

It is undeniable that NDDS is the excellent strategy for overcoming multiple obstacles to deliver drugs to gliomas. But it cannot be denied that it is not easy for NDDS to enter in clinical stage. At present, most of the NDDS used to treat gliomas are still in the preclinical stage. NDDS, which has entered clinical trials for the treatment of glioma, mainly focuses on liposomes. The NDDS currently used in glioma clinical trials are summarized in Table 4.

Among them, the most studied liposomes are loaded with DOX or irinotecan, which are the combination products of the most commonly used anti-tumor drugs and the simplest nanocarrier. However, the combination of Caelyx®, TMZ, and RT did not significantly improve the prognosis of GBM patients (NCT00944801)²⁴⁷. The clinical research results of Onivyde® have not yet been released, but they are worth looking forward to (NCT03119064, NCT02022644, NCT03086616). Targeting NDDS has also been represented clinically, such as 2B3-101 with GSH as the targeting ligand and C225-ILs-dox with Cetuximab as the targeting ligand (NCT01386580, NCT03603379). Unfortunately, due to the insufficient number of subjects, SGT-53 with Anti-TfR antibody as a ligand failed to complete the phase II clinical trial (NCT02340156).

Therefore, greater efforts are needed to develop products that can be used in the clinical treatment of glioma. It is not difficult to see that the well-designed NDDS has shown many impressive results in preclinical trials. However, the difficulty in scale-up of the manufacturing process and the complexity of quality control have become the factors that limit its entry into clinical development. Secondly, there is the challenge of the body's immune recognition. This is also the reason for the rapid development of BDDS in recent years. The marketing of Abraxane® proves that endogenous protein can be used as a drug carrier for clinical use²²⁵. Exos have also been used in clinical trials to diagnose pancreatic ductal adenocarcinoma (NCT03032913). Tumor antigen-loaded dendritic cell-derived exosomal vaccines have proven safety and feasibility, and are expected to be used to treat patients with non-resectable non-small cell lung cancer

Table 4 Overview of glioma clinical trials based on NDDS.

| Name | Carrier | Cargo | Targeting moiety (if any) | Combination therapy (if any) | Current ClinicalTrials.gov identifiers (phase) |
|-----------------------------|------------------------------|---|---------------------------|---|---|
| Myocet® | Liposome | DOX | — | — | NCT02861222 (phase I: completed) |
| Caelyx® | Liposome | DOX | — | — | NCT00944801 (phase I, II: completed) |
| Onivyde® | Liposome | Irinotecan | — | TMZ | NCT03119064 (phase I, II: completed) |
| Lipodox | Liposome | DOX | — | — | NCT00019630 (phase I: completed) |
| Nanoliposomal CPT-11 | Liposome | Irinotecan | — | — | NCT00734682 (phase I: completed) |
| NU-0129 | Gold NPs | Spherical nmembrane protein mRNA Nucleic acid | — | — | NCT03020017 (Early phase I: completed) |
| 2B3-101 | Liposome | DOX | GSH | Trastuzumab | NCT01386580 (phase I, II: completed) |
| C225-ILs-dox | Liposome | DOX | Cetuximab | — | NCT03603379 (phase I: completed) |
| Onivyde® | Liposome | Irinotecan | — | — | NCT02022644 (phase I: active, not recruiting) |
| Onivyde® | Liposome | Irinotecan | — | — | NCT03086616 (phase I: recruiting) |
| 186Rhenium Nanoliposomes | Liposome | Rhenium | — | — | NCT01906385 (phase I, II: recruiting) |
| EGFR (V)-EDV-Dox | Bacterially-derived minicell | DOX | Bispecific antibodies | — | NCT02766699 (phase I: recruiting) |
| ABI-009 | Albumin | Rapamycin | — | Bevacizumab/TMZ/Lomustine/ Marizomib/Radiation | NCT03463265 (phase II: recruiting) |
| RNA-LPs | DOTAP liposome | Autologous total tumor mRNA and pp65 full length lysosomal-associated membrane protein mRNA | — | — | NCT04573140 (phase I: not yet recruiting) |
| DepoCyt | Liposome | Cytarabine | — | TMZ | NCT01044966 (phase I, II: terminated) |
| SGT-53 | Cationic liposome | P53 cDNA | Anti-TfR antibody | TMZ | NCT02340156 (phase II: terminated) |

—, not applicable.

(NCT01159288). However, the study of Exos as drug carriers has not yet entered the clinical stage, possibly because the methods of separation and purification are currently not suitable for large-scale clinical production²⁴⁸. Cell-loaded or cell membrane-coated NPs can be endowed with long circulating half-life and targeting properties. Their large-scale production is achievable, but the batch-to-batch difference of the products produced is the main challenge that needs to be overcome¹⁹⁷. In the future, researchers should turn the design ideas of NDDS to clinical actual needs as much as possible, and combine multiple strategies to overcome the obstacles in the treatment of gliomas one by one.

5. Summary and outlook

Although the strategies for the treatment of glioma have made great progress, the current treatment strategies for glioma based on NDDS are still in its preliminary clinical stage. We believe that researchers in the field of nanomedicine should focus on the following aspects, so as to further promote the clinical translation of NDDS for the treatment of glioma.

Currently, the clinically marketed NDDS are mainly liposomes and polymer NPs with relatively simple processes. Although the cascade targeted NDDS has unlimited development prospects in preclinical research, there are more factors to be considered in the preparation process. The density of the targeting ligands on the NDDS must be appropriate, too low or too high density will affect the targeting effect. If multiple ligands are used in combination, appropriate ratios need to be considered. Since NDDS targets glioma cells, transcytosis of BMVECs is required. In this process, the hydrolysis of NDDS by endosomes/lysosomes needs to be considered. This process also depends on the extent to which the ligand binds to the receptor. The acid-responsive cleavage ligand developed by Ruan et al.¹⁴⁰ is expected to be a perfect strategy to solve this problem. In addition, the modification of the ligand also determines the translation and rotation kinetics of NDDS on the cell membrane. Understanding the complex relationship between these will facilitate the selection of more suitable ligands²⁴⁹. NDDS, which responds to the glioma microenvironment, has acquired the ability to accurately release drugs on the basis of precise targeting. Among them, NDDS with charge reversal properties not only ensures the stability in the circulatory system, but also ensures that NDDS can avoid endosomes/lysosomes hydrolysis. In the future, researchers can combine multiple stimuli to ensure a sensitive response²⁵⁰. However, the complexity of the preparation process of these responsive NDDS is also the main reason that affects clinical translation. Compared with traditional synthetic NDDS, BDDS based on endogenous substances also shows a very prominent advantage in the treatment of glioma. But the source of these biological materials determines the body's immune rejection response. And the large-scale preparation cost must also be considered, especially the purification and characterization of Exos has not yet established a standardized method.

In addition, the currently used *in vitro/in vivo* BBB model still has many limitations. This is also the reason that hinders the clinical translation of NDDS. Although the most commonly used Transwell model *in vitro* is easy to operate and low in cost, it cannot accurately represent the physiological/pathological complexity of BBB²⁵¹. Zebrafish and mice are currently the most current and longest used animal models, which can provide real-time brain imaging. However, the physiological properties of

their BBB (such as the type and number of markers, the inner diameter of the blood vessel, etc.) are different from those of the human body, and they require relatively high operational skills for researchers²⁵². Therefore, a suitable BBB model is also one of the prerequisites for clinical translation of NDDS. Recently, Peng et al.²⁵³ developed a microfluidic-based human BBB (μ BBB) platform, embedding U87 MG cells in it for 3D culture. μ BBB-GBM can accurately predict BBB penetration of NPs and U87 MG cells uptake in real time.

We suggest that on the basis of the development of well-designed NDDS/BDDS, combined with other non-invasive strategies to overcome BBB (such as BPEs, FUS, nasal administration, etc.), so as to achieve the greatest possible clinical translation.

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Author contributions

Jiwei Cui and Ruoning Wang conceived and designed this review. Jiwei Cui, Yuanxin Xu and Haiyan Tu analyzed the literatures and summarized the results. Ruoning Wang, Huacong Zhao, Honglan Wang and Liuqing Di reviewed and edited this review. Jiwei Cui and Ruoning Wang revised this review. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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