Modulating the blood–brain tumor barrier for improving drug delivery efficiency and efficacy

Yujun Song | Chuan Hu | Yao Fu | Huile Gao

Abstract
The blood–brain barrier (BBB) is a highly regulated and efficient barrier that controls the mass transfer between blood and brain, severely limits brain penetration of systemically administered therapeutics. During the onset and progression of brain tumors, BBB alters, and the blood–brain tumor barrier (BBTB) forms. Though BBTB differs from BBB in certain aspects, such as neuronal connections and aberrant pericyte distribution, it retains critical aspects of BBB. Hence, one major challenge in the development of therapeutics for brain tumor is to achieve sufficient BBTB penetration thus improving the efficiency of drug delivery to the tumor site. In this review, we summarize the strategies that can overcome BBTB and improve BBTB penetration efficiency from the perspectives of regulating BBTB structure and transport processes.

KEYWORDS
active efflux transport, blood–brain tumor barrier, carrier-mediated transport, receptor-mediated transport, tight junction

1 INTRODUCTION
The incidence of brain tumors has shown an upward trend in recent years. Malignant brain tumors, including primary brain tumors, (e.g. glioblastoma multiforme) and metastases, are aggressive and lethal entities for the majority of affected patients. Following the spread of cells from a primary tumor through the blood to the brain microvasculature, brain metastases are more common than primary brain tumors. Lung cancer, breast cancer, melanoma and many other types of cancers may all lead to brain metastases. Currently, standard treatment options, including surgery, radiotherapy and chemotherapy, do not improve the survival rate of patients considerably. Faced with this
situation, scientists are actively seeking more efficient and effective strategies. However, the presence of blood–brain barrier (BBB) and blood–brain tumor barrier (BBTB) present major barriers to the therapeutic delivery to brain tumors.1–5

BBB strictly controls the substances in the blood to enter the central nervous system (CNS) through tight junction (TJ), cell uptake and so on, adjusts the steady state of the CNS, protects the stability of the microenvironment, so that it is not affected by harmful substances, such as toxins, pathogens and inflammatory cells, in the surrounding circulation, but provides obstacles for the transport of drugs to the CNS.6,7 In Alzheimer’s disease, gliomas, neuroinflammation and other pathological conditions, the structure and function of BBB alter. In brain tumors, BBB’s TJ has been destroyed by tumor cells and micropores appeared on the surface of vessels. The tumor vasculature cannot meet the growing demand of tumor cells, which forms hypoxic and acidic regions within the tumor, and the expression of vascular endothelial growth factor (VEGF) increased. The overexpression of VEGF stimulates angiogenesis, which leads to heterogeneous neovascularization with chaotic branches in the vascular system, and ultimately leads to the formation of BBTB. The BBTB is characterized by aberrant pericyte distribution and loss of astrocytic endfeet and neuronal connections.8 The presence of BBTB structure can disrupt the transport of certain drugs into the brain tumor microenvironment. Also, BBTB displays heterogeneous permeability which further limits the evenly penetration and diffusion of drugs within tumors. Therefore, it is important to improve the permeability of BBTB to improve the delivery efficiency.8–10

Nowadays, most reviews discuss how to change the permeability of BBB to increase the efficiency of drug delivery, whereas very few articles focused on how to change the permeability of BBTB to improve delivery. Herein, we aim to review how to overcome the barrier effect of BBTB to improve drug delivery efficiency and efficacy (Table 1). We will elaborate on two aspects: modulating the structure of BBTB and adjusting the transport process of BBTB.

2 | MODULATING THE STRUCTURE OF BBTB

TJ between brain cerebral microvascular endothelial cells (BMECs) is the structural and functional base of BBB. Though BBTB differs from BBB in some aspects, such as neuronal connections and aberrant pericyte distribution, it still retains critical aspects of BBB including TJ. Therefore, the permeability of BBTB can be changed by modulating TJ to increase the efficiency of drug delivery.

2.1 | Osmotic BBTB disruption

Injecting hyperosmotic solution, such as 25% mannitol solution, into the carotid or vertebral artery can cause BMECs contraction, thus, opening the TJ of BMECs and causing temporary and reversible destruction of the barrier.11,12 In addition, mannitol can not only open BBTB, but also reduce the hydrostatic pressure in the tumor, thus enhancing the transportation of chemotherapeutic drugs through passive diffusion.13 At present, permeability barrier destruction is used to increase the application of chemotherapeutic drugs, antibodies, and nanoparticles (NPs) in the treatment of human brain tumors.14

So far, osmotic destruction has shown very promising clinical results, especially as a first-line treatment of chemical-sensitive brain tumors. Osmotic disruption is mainly used in combination with doxorubicin (Dox), methotrexate, carboplatin, cyclophosphamide, etoposide and other chemotherapeutic drugs, which can be used as drugs carried on NPs targeted to brain tumors.15 Osmotic disruption delivery significantly improves the antitumorefficacy of BR96-Dox (a tumor-specific immune complex).16 Mannitol super-selective intraarterial cerebral infusion combined with cetuximab is proven safe and tolerant in the treatment of recurrent malignant glioma. Phase I/II clinical trials are currently under way to determine the efficacy of treatment in patients with high-grade gliomas.15 In principle, the combined therapy can improve the efficiency of targeted drug delivery system by increasing the permeability of BBTB. However, the neurotoxicity and safety of the combination therapy need to be further explored.

There are also problems with the use of hyperosmotic solutions to improve the permeability of brain barriers. For example, therapeutics with large sizes, such as immune-coupling (about 150 kDa in mass), NPs (30 nm in diameter), or virus particles (200 nm in diameter), can only be effective in a very short period of time (15 min).15 Moreover, the opening of BBTB is non-selective. When BBTB is turned on, normal BBB will also be impacted. Thus, the selective opening of BBTB is particularly important for future studies.17,18

2.2 | Focused ultrasound

Focused ultrasound (FUS) uses ultrasound as the energy source, focuses on the target tissue, and utilizes acoustic wave and thermal energy transformation to perform hyperthermia therapy on the tumor. FUS with circulating microbubbles (MBs) can temporarily penetrate BBTB, which is considered an effective noninvasive
| Methods                          | Types                        | Mechanisms                                                                 | Noteworthy                                                                 |
|---------------------------------|------------------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Osmotic disruption              | Modulating the structure of BBTB | Hyperosmotic solutions lead to BMECs contraction and TJ opening.            | Osmotic disruption is nonselective and the opening time is uncertain.      |
| Regulating by FUS               | Modulating the structure of BBTB | FUS modulate the BBTB ion channel and receptor activity, by the same time control a microdamage of the brain vasculature. | FUS is not specific to BBTB, it also affects BBB. The effective load ability of MBs and the safe level of FUS intensity need to be carefully considered as well. |
| Regulating by vasoactive substances | Modulating the structure of BBTB | Vasoactive substances cause the conversion of intracellular calcium and phosphatidylinositol increased, then isolate the TJ. | Temporarily cannot effectively control and use the time dependence and selectively open BBTB. |
| Regulating by EMAP-II           | Modulating the structure of BBTB | EMAP-II activates the corresponding pathway, then downregulates the expression of TJ proteins. | Often used in combination with other anti-tumor drugs.                     |
| Regulating by Non-coding RNA    | Modulating the structure of BBTB | Some non-coding RNAs regulate the expression of TJ proteins through the corresponding pathways. | As a potential strategy for the treatment of brain tumors, more researches are needed. |
| Regulating by RNA-binding proteins | Modulating the structure of BBTB | The up-regulation or silencing of RNA binding proteins regulate the expression of TJ proteins. | More researches are needed.                                                |
| Regulating by K\textsubscript{Ca} and K\textsubscript{ATP} channels | Modulating the structure of BBTB | K\textsubscript{Ca} channel accelerates the formation of pinocytotic vesicles, which can transport drugs across BBTB. K\textsubscript{ATP} channel modulates the relaxation mechanism of cerebral vessels by coupling intracellular metabolic changes with plasma membrane electrical activity. | K\textsubscript{Ca} and K\textsubscript{ATP} channels agonists can only increase the permeability of BBTB, but have no effect on BBB. |
| Regulating RMT                  | Regulate the transport process of BBTB | The binding of targeting ligands to receptors leads to endocytosis and the transport of complexes passing through the BBTB. | The widespread expression in other tissues, small dissociation rate, off-target effect, and potential toxicity in practical use should be considered. |
| Regulating CMT                  | Regulate the transport process of BBTB | Inhibit glucose uptake by tumor cells.                                       | Should be used in combination with other drugs or treatments.             |
| Regulating AET                  | Regulate the transport process of BBTB | The missing or suppressing of efflux transporters can increase the permeability of BBTB. | Because these ABC transporters are expressed on the BBB, as well, the specificity and safety need to be concerned. |
| Regulating by Mfsd2a             | Regulate the transport process of BBTB | The absence of Mfsd2a resulted in a leaky barrier due to an increased transcytosis across the endothelial cytoplasm. | More researches are needed.                                                |
Vasoactive substances

Bradykinin is an inflammatory mediator that increases the vascular permeability, and the bradykinin-type 2 (B2) receptor is expressed on BMECs. Studies show that bradykinin can significantly increase the ion permeability of BBTB. Cereport\textsuperscript{(R)}, an analog of bradykinin, has a longer plasma half-life and a greater receptor selectivity than bradykinin. It selectively binds to B2 receptor and activates a typical bradykinin-like secondary messenger system. The conversion of intracellular calcium and phosphatidylinositol increase, isolating the TJ, thus increase the permeability of BBTB, finally lead to the higher drug delivery efficiency of chemotherapeutic drugs to brain tumors. However, there are many problems that remain to be solved, such as how to effectively use and control the time dependence, how to selectively turn on BBTB without causing additional damage to BBB and so on.

Xie et al. designed a reverse bradykinin (RI-BK) similar to bradykinin, which can resist protease hydrolysis and have a high binding activity to B2 receptor. After RI-BK binds to B2 receptor, it can induce the expression of ZO-1, depolymerize F-actin causing a selectively turn on of BBTB. The affinity of RI-BK toward cells is related to the level of B2 receptor expressed in different types of cells. Due to the lower expression level of B2 receptor in BMECs than in glioma cells, low dose of RI-BK does not cause the change of BBB permeability, thus has no negative effect on BBB. The combination of RI-BK and PTX-loaded NPs improves the efficacy in the treatment of glioma. These findings suggest that RI-BK can be used as an auxiliary method in the treatment of malignant brain tumors.

Cytokine

Endothelial-monocyte-activating polypeptide-II

Endothelial-monocyte-activating polypeptide-II (EMAP-II) is a potential antitumor substance isolated from methylcholanthrene A transformed fibrosarcoma, which
FIGURE 1 (A) Kaplan–Meier survival curves of glioma-bearing nude mice (n = 10). (B) Overview of how RI-BK selectively opens the BBTB to nanocarriers, resulting in antitumor drug delivery to the glioma. Reproduced with permission from Ref. [29]. Copyright © 2015, Elsevier Ireland Ltd

has the dual effects of opening BBTB and inhibiting tumor growth. EMAP-II induces apoptosis of BMECs and inhibits tumor angiogenesis. It significantly down-regulates the expression of ZO-1, occluding and claudin-5 by activating RhoA/Rock signaling pathway, phosphorylating cofilin, and myosin light chain, which mediated TJ disintegration and stress fiber formation, selectively increase the permeability of BBTB. The mechanism of EMAP-II increasing BBTB permeability is also related to the regulation of protein kinase C (PKC) Zeta/phosphatase 2A, PKC-α/β and cyclic adenosine monophosphate-PKA-dependent signaling pathways. The combined application of EMAP-II and other antitumor drugs provides a new way to improve the chemotherapy effect of tumor cells. Liu et al. found that low-dose EMAP-II could increase BBTB permeability by inhibiting the expression of miR-330-3p, up-regulating the expression and activity of PKC-α, and finally decreasing the expression of TJ proteins. Hence, the combination of EMAP-II, anti-miR-330-3p and PKC-α activators may enhance the antitumor effect of Dox on glioma cells. In addition, studies have shown that the combined treatment of EMAP-II and photodynamic therapy can improve the therapeutic effect of tumor as well.

2.5 | Non-coding RNA

Studies have shown that some noncoding RNA can regulate the expression of TJ proteins through the corresponding pathway, which is closely related to the permeability of BBTB. Related non-coding RNA includes long-chain non-coding RNA (lncRNAs) and short-chain non-coding RNA. The latter involves microRNAs (miRNAs), small interference RNAs, piwi-interaction RNAs (piRNAs) and small nucleolus RNAs.

In tumor cells, LncRNA TUG1 was overexpressed and miR-144 was targeted to inhibit the expression of miR-144. The binding of heat shock transcription factor to TJ proteins promoter increased, which increased the expression of occludin, ZO-1 and claudin-5 and then decreased the permeability of BBTB. Therefore, inhibiting the pathway by LncRNA TUG1 silencing can increase the permeability of BBTB. In addition, MALAT/miRNA-140/NFYA/TJ proteins, XIST/miR-137/FOXC1/ZO-2, NEAT1/miR-181d-5p/SOX5, HOTAIR/miR-148b-3p/USF1 and Lnc00462717/miR-186-5p/Occludin pathways were all related to BBTB permeability. Researchers confirmed that knockdown Lnc00462717 or overexpress miR-186-5p could increase the accumulation of Dox in glioma and
decrease the volume of intracranial glioma in BALB/c nude mice.\textsuperscript{47}

miRNAs negatively regulate the gene expression in a sequence-specific manner by binding to the 3-untranslated region of the target mRNA. MiR-181a can target Kruppel-like factor 6 (KFL6) and negatively regulate KFL6 by overexpression; MiR-34c target and negatively regulate Myc-associated zinc-finger protein; MiR-18a target and negatively regulate MEF2D; MiR-429 target and negatively regulate \textit{P70S6K}. All these pathways given above downregulate the expression of TJ proteins \textit{ZO-1}, claudin-5 and occludin, disrupt the TJ, then finally increase the permeability of BBTB.\textsuperscript{48–51} MiR-200b increases the permeability of BBTB by directly targeting \textit{RhoA} and ROCK, leading to the rearrangement of F-actin, the redistribution of occludin and claudin-5, and eventually opening the TJ.\textsuperscript{52} Some lincRNAs and piRNAs also play a critical role in the regulation of BBTB permeability.\textsuperscript{41,53}

The above non-coding RNAs may be used as potential targets of BBTB for the treatment of brain tumors. However, it is still necessary to consider the specificity of the target, that is, whether it can specifically open BBTB without affecting BBB.

### 2.6 RNA-binding proteins

RNA-binding proteins have also been found to play a vital role in mediating BBTB permeability. The upregulation or silencing of RNA-binding proteins can affect the permeability of BBTB by regulating the expression of TJ proteins. Researchers found that the upregulated \textit{KHDREBS3} in tumor cells increased its stability by binding to c\textit{DENND4C}. CDENND4C, as a miR-577 sponge, affects the permeability of BBTB by binding and inhibiting the negative regulation of miR-577 on TJ proteins \textit{ZO-1}, occludin and claudin-1. Therefore, \textit{KHDREBS3}, c\textit{DENND4C} and miR-577 alone or in combination can effectively promote the antitumor effect of Dox.\textsuperscript{54} In addition, R2FOX1/LINC00673/MAFF pathway and IGF2BP2/FBXL19-AS1/ZNF765 feedback axis also contribute to the regulation of BBTB permeability.\textsuperscript{55,56} Therefore, it has a great potential to consider relevant RNA-binding proteins and related factors in their regulatory pathways as targets to increase drug delivery efficiency.

### 2.7 Calcium-activated potassium channel and ATP-sensitive potassium channel

The change of vascular potassium (K) channels activity can regulate the tension of blood vessels. The activation of K channels can regulate the permeability of BBTB by causing the hyperpolarization of cell membrane, the closure of voltage-dependent calcium channels, the decrease of intracellular calcium ions, and the relaxation of blood vessels.\textsuperscript{57} Calcium-activated potassium (\textit{K}_{\text{Ca}}) channel increases the permeability of BBTB through the accelerated formation of pinocytotic vesicles, which can transport drugs across BBTB.\textsuperscript{58} ATP-sensitive potassium (\textit{K}_{\text{ATP}}) channel modulates the relaxation mechanism of cerebral vessels by coupling intracellular metabolic changes with plasma membrane electrical activity.\textsuperscript{59}

The advantage of modulating BBTB by regulating vascular \textit{K}_{\text{Ca}} and \textit{K}_{\text{ATP}} channels is that they only overexpress on brain tumor microvessels, and can hardly be detected on normal brain vessels. So \textit{K}_{\text{Ca}} and \textit{K}_{\text{ATP}} channels can be used as potential and unique targets of BBTB and increase the permeability of BBTB.\textsuperscript{58–62} Therefore, \textit{K}_{\text{Ca}} and \textit{K}_{\text{ATP}} channels agonists can only increase the permeability of blood vessels in the tumor site, but have no effect on the permeability of normal brain tissue.\textsuperscript{59} This finding is important to improve the targeted delivery of antitumor drugs to brain tumor areas, but does not affect normal brain function to increase the safety of this treatment.\textsuperscript{58}

Studies also showed that \textit{K}_{\text{ATP}} channels can be activated by intravenous infusion of minoxidil sulfate (MS).\textsuperscript{63} MS as a molecular targeted \textit{K}_{\text{ATP}} regulator, can selectively activate \textit{K}_{\text{ATP}} channel on BBTB and specifically improve the permeability of BBTB without affecting the normal BBB. It has been reported that MS improves the permeability of BBTB by activating \textit{K}_{\text{ATP}} channel, and increases the delivery efficiency of carbohydrates, temozolamine, adenoviral vector and tratozumab to brain tumors.\textsuperscript{60,63} Researchers found that minoxidil-loaded hyaluronic acid-tethered NPs (M@H-NPs) can effectively and specifically cross BBTB, by the coordination of hyaluronic acid and CD44 receptors, selectively transport drug-loaded NPs to brain metastases, at the same time reduce the damage to normal brain cells. The results showed that M@H-NPs/Dox could significantly prolong the median survival time of mice with brain metastasis (Figure 2).\textsuperscript{64} Whether other types of K channels play a certain role in BBTB permeability regulation remains to be further studied.\textsuperscript{58} Increasing the permeability of BBTB by activating \textit{K}_{\text{Ca}} and \textit{K}_{\text{ATP}} channels can be considered as a very potential mean to increase the efficiency of drug delivery by changing the structure of BBTB.

### 3 REGULATE THE TRANSPORT PROCESS OF BBTB

There are three main mechanisms in the transcellular transport system of BBB/BBTB, including receptor-mediated transport (RMT), carrier-mediated transport
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Figure 2 (A) Schematic illustration of NPs’ in vivo behavior in brain with metastatic tumors. NPs reach blood vessels around brain metastatic tumor lesions through the leaky BBTB. The released MS can specially act on especially expressed mitochondrial K\textsubscript{ATP} channels in BBTB endothelial cells to induce downregulation of TJ proteins and upregulation of transcytosis-related caveolin-1, therefore, increase NPs’ BBTB penetration and accumulation in brain metastases. (B, C) Results of cytotoxicity and apoptosis experiments. Error bars represent SD (n = 3). (D) Kaplan–Meier survival curves for mice bearing brain metastases with indicated treatments. (E) The effect of various treatments on the number and size of brain metastases. Reproduced with permission from Ref. [64]. Copyright © 2015, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

(CMT), and active efflux transport (AET).\textsuperscript{65} RMT is a special endocytosis mechanism. Some macromolecules (for example, antitumor proteins) can be transported non-invasively through BBB/BBTB via RMT. Receptors widely used include insulin-like growth factor 1 receptors, low-density lipoprotein receptor-related protein 1 (LRP1), and transferrin receptor (TfR). CMT is mediated by a series of solute carrier (SLC) transporters, which transport sugar, amino acids, organic cations or anions, nutrients into the brain. Major SLCs include glucose transporters (GLUTs), monocarboxylate transporters, organic ion (cationic and anion) transporters and nucleosides transporters. AET is an ATP-driven mechanism, as the most important transcellular transport pathway, it not only hinders the entry of foreign substances (potential toxic substances and even therapeutic drugs) into the brain, but also transports compounds that have crossed the BBB back into the circulation, which plays a detoxification role. The main drug efflux transporter is the ATP-binding cassette (ABC) transporter superfamily.\textsuperscript{65-67} The above three transport mechanisms can be fully utilized to increase the transport of corresponding transporters to therapeutic drugs, so as to increase the drug crossing BBTB and improve the therapeutic effect.

3.1 Receptor-mediated transport

The binding of targeting ligands (such as monoclonal antibodies) to receptors leads to endocytosis and the transport of complexes to the abluminal surface by vesicular trafficking machinery. Drugs and particles can be connected to ligands through specific molecules, thereby enabling passing through the BBTB.\textsuperscript{8} TfR is probably the most famous RMT system, which mediates iron transfer to the brain by binding to transferrin (Tf). Tf has been widely used in NPs, liposomes, and other targeted delivery systems.\textsuperscript{66,68-71} Angiopep-2 is a peptide against the LRP1. The covalent combination of Angiopep-2 and PTX can greatly increase the delivery to the brain metastases and improve the median survival time in pre-clinical and clinical studies.\textsuperscript{8,72,73} Angiopep-2 can also modify micelle and carbon nanotube to transport drugs into the brain.\textsuperscript{66} L57, as the first synthetic LRP1 binding peptide, shows higher permeability than other LRP binding peptides (Angiopep-7, Angiopep-2), which can be used as a more effective LRP1 ligand to deliver drugs to the brain after modification.\textsuperscript{72}

Although RMT is a potential strategy, it still has shortcomings. The widespread expression of these receptors in other tissues may cause off-target effect and potential toxicity in the practical use.\textsuperscript{8,74} Off-target effect can be mitigated by targeting proteins specifically expressed in the brain. AccumuBrain, a new technology, can achieve both high antibody concentration in the brain (ten times or more) and a long-circulating half-life by binding to myelin oligodendrocyte glycoprotein, a protein specifically expressed in oligodendrocytes.\textsuperscript{75} Off-target effect can also be addressed in combination with environment-mediated targeting (EMT). Study showed that a dual-targeting polypeptide nanogel loaded with phenylboric acid and morpholine can specifically recognize sialic acid (SA).
epitopes and extracellular acidic environments at the same time. This nanogel successfully solved the off-target effect caused by SA and proved to be highly potent in inhibiting tumor growth. The potential toxicity to normal tissue can be reduced by specific acid response. For example, adding an acid-cleavable linker between Tf and the gold NP core increased the dissociation and entrance rate of the drug delivery system into the tumor site.

### 3.2 Carrier-mediated transport

CMT system is considered to be one of the most promising methods to promote drug transport into the brain. SLC transporters regulate the intake and/or efflux of a wide variety of essential molecules through cell membranes such as sugar, amino acids, inorganic ions, neurotransmitters, hormones, vitamins and drugs. One of the major SLCs, GLUT1, is expressed on the surface of BMECs. As one of the most effective transport systems, GLUT1 can effectively transport glucose through BBB to the brain. Cancer cells frequently exhibit changes in glucose metabolism and an increased glucose demand. This leads to an overexpression of GLUTs in malignant tissues, especially GLUT1, which is related to the survival time of patients.

Due to GLUT1 is highly expressed on BMECs, targeting GLUT1 can greatly improve the penetration of antitumor-targeted drugs through brain barriers and enhance the uptake of drugs by tumor cells, which can be used as a promising strategy for the development of brain-specific drug delivery system. Inhibiting GLUT1 is an effective way to reduce the growth of tumor by reducing the glucose supply. Studies showed that GLUT1 inhibitor resveratrol (RSV) controlled tumor growth by regulating glucose uptake, metabolism, and signaling pathways. And research has also found that RSV-loaded solid lipid NPs significantly increased the brain concentration of RSV in tumor areas. However, due to the off-target effect and low anticancer efficiency, GLUT1 inhibitors are not successful as a single therapy. They can be used in combination with traditional anticancer drugs, immunotherapy or radiotherapy to reduce side-effects and achieve maximum therapeutic effect. Furthermore, glucose-modified liposomes were designed and synthesized as ligand for liposomes. Through GLUT1 transport, the liposomes effectively deliver PTX pass the brain barriers and target glioma. The relative uptake efficiency and concentration efficiency were enhanced significantly compared to that of free PTX. The glucose-modified liposomes also displayed the maximum accumulation of DiD-loaded liposomes at tumor sites compared to the other groups in vivo imaging. All these results suggest that glucose-modified-lipids targeted to the glucose transporter GLUT1 would be a potential delivery system for improving the penetration of antitumor-targeted drugs through brain barriers.

### 3.3 Active efflux transport

The ABC transporter superfamily exists in all animal species and requires energy from ATP hydrolysis to actively remove compounds from the cell. When the efflux transporters are missing or suppressed, a significant increase in brain permeability would be expected. Therefore, it seems to be an attractive strategy to inhibit the efflux transporters by specific inhibitors, leading to the increase of drug penetration across BBTB without damaging BMECs and the TJ integrity.

P-gp, as a member of the ABC transporter superfamily, is one of the most widely studied efflux transporters. The gene encoding is MDR1 or ABCB1. P-gp is expressed in the lumen and choroid plexus of BMECs, which limits the passage of drugs through BBB/BBTB, and plays a key role in the entry of drugs into the brain. Two different signaling pathways are demonstrated that can rapidly reduce the activity of P-gp on BBTB. The first signaling pathway is related to proinflammatory factors, sheath lipids and protein kinases. PKCβ1 and sphingosine-1-phosphate receptor 1 play an essential role in this process and can be used as targets for reducing P-gp activity on BBTB. The second signaling pathway is related to VEGF. Under the pathological condition of increased level of VEGF, the activity of P-gp decreased significantly. Based on this, the downstream elements of VEGF signaling pathway have the potential to become targets to regulate P-gp activity, thus improving the delivery of drugs to the brain. Researchers found that quercetin (Qu) can inhibit the drug efflux through binding with P-gp. Qu-loaded NPs showed a stronger antitumor effect and better biosafety. This is because when Qu was released, it combined with P-gp, then inhibited the drug efflux triggered by P-gp (Figure 3A-C). Another study demonstrated that compared with Imatinib (IMM)-loaded NPs, NPs functionalized with P-gp inhibitor Pluronic P84 showed a significantly increased cell uptake in P-gp overexpressed cells (U251MG and C6) and dramatically reduced cytotoxicity (Figure 3D-F).

### 3.4 Mfsd2a inhibition causing leaky BBB

Major facilitator superfamily domain containing protein-2a (Mfsd2a), considered as an orphan transporter, has gained increasing attention for its regulatory role in the
maintenance of a proper functioning of BBB. Specifically expressed on the cell membrane of BMECs, Mfsd2a is implicated in the delivery of some substances across the brain barriers.\textsuperscript{87,88} Mfsd2a is the first inhibitor of the transcytosis and the first transporter for lysophosphatidylcholine (LPC)-docosahexaenoic acid in BMECs.\textsuperscript{66} The absence of Mfsd2a resulted in a leaky barrier due to increased transcytosis across the endothelial cytoplasm. The lipid transport function of Mfsd2a is considered to be the key to the inhibition of endocytosis.\textsuperscript{87–89} Targeting the transcytosis is considered as a promising therapeutic approach because up to now there have been no known restrictions on the molecular mass or physicochemical properties of a substrate for its transportation by this route.

However, there are also limitations on regulating BBTB by targeting Mfsd2a. Currently, no known effective inhibitor of Mfsd2a is available.\textsuperscript{88} There are several pharmacological strategies of Mfsd2a-based drug delivery system. The reversible inhibition of Mfsd2a may temporarily induce a general disinhibition of the transcytosis in BMECs, thereby, transporting macromolecular drugs across the BBTB. However, this strategy is risky, as enhanced transcytosis might facilitate the absorption of substances other than the target drug and might exacerbate further damage to the brain tissue. Hence, coupling drugs with LPC appears to be a safer choice. Second, Mfsd2a can be used for the transport of some small-molecule drugs chemically conjugated to LPC across the BBTB. Third, Mfsd2a uniquely transports lipids, so the conjugation of drugs with higher lipophilicity with Mfsd2a will facilitate their Mfsd2a-mediated transport since increasing the lipophilicity of drugs enhances their penetration through the BBTB.\textsuperscript{88,90}

Recent studies show that Mfsd2a is essential for the transport of sphingosinol-1 phosphate (S1P) during the formation and maintenance of BBB.\textsuperscript{91} Mfsd2a and Spin-\textsuperscript{ster homolog 2 form a protein complex to ensure the efficient transport of Astrocytic S1P3. S1P3 is overexpressed in patients’ brain metastases. It elevates BBTB permeability by producing interleukin-6 and chemokine (C-C motif) Ligand 2 to relax BMECs adhesion.\textsuperscript{91,92} As outlined above, Mfsd2a and S1P3 can be potential targets for drug delivery to the brain and an adjuvant treatment to standard clinical therapy.

4 CONCLUSIONS AND PROSPECT

Due to the high incidence of brain tumors, malignant brain tumors have become the main cause of cancer-related
death in children and young people. The survival rate of patients has not been greatly improved by a single standard treatment, such as surgery, radiotherapy, or chemotherapy, which is largely due to the barrier effect of BBB/BBTB.67

In this review, we focused on the strategies for increasing the permeability of BBTB. Specifically, the penetration of BBTB can be regulated by changing the structure, and the transport of BBTB can be modulated through utilizing and adjusting transporters in the process of RMT, CMT and AET. However, problems remain with the above approaches. Under ideal circumstances, methods for regulating BBTB were only specific to BBTB, but the specificity of most strategies is not sufficient to distinguish BBB from BBTB, which may lead to adverse effects on normal brain tissues. The choice of the optimal interval of administration, the dose, the location and the transition time of combination treatments need to be carefully investigated.

Despite the aforementioned problems, the methods reviewed in this article successfully improved the penetration of therapeutics across the BBTB. However, in-depth studies need to be performed. For example, animal models that can mimic the patient’s condition need to be established to evaluate the biological distribution and pharmacokinetics of the drug systems. Monitoring and adjusting the changes of BBTB are recommended to optimize the treatment scheme in time and determine the optimal solution. Using innovative ideas and multidisciplinary methods, the standard treatments, such as radiotherapy and chemotherapy, may combine with emerging techniques, such as immunotherapy and gene therapy, to enhance the therapeutic efficacy of brain tumor treatment.

AUTHOR CONTRIBUTIONS
Huile Gao, Yujun Song, Chuan Hu made contributions to conception. Yujun Song was responsible for drafting the manuscript. Yao Fu and Huile Gao were involved in revising the manuscript and supervising.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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ORCID
Yujun Song https://orcid.org/0000-0003-4755-0694
Chuan Hu https://orcid.org/0000-0002-8882-3276
Yao Fu https://orcid.org/0000-0002-5855-4706
Huile Gao https://orcid.org/0000-0002-5355-7238

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**AUTHOR BIOGRAPHIES**

**Yujun Song** is an undergraduate student from West China School of Pharmacy in Sichuan University. She is going to pursue her master's degree under the supervision of Professor Huile Gao.

**Yao Fu** obtained a PhD degree in Pharmaceutical Science from the University of Wisconsin-Madison under the supervision of Professor W. John Kao in 2012. After a Postdoctoral experience with Prof. Kristyn Masters and Prof. Pamela Kreeger in the Department of Biomedical Engineering, she joined the West China School of Pharmacy, Sichuan University in 2014. Her research focuses on the interaction between materials and the biological interface, as well as novel biomaterials to achieve targeted drug delivery and controlled drug release.

**Huile Gao** is a Professor at Sichuan University. He obtained his PhD in Pharmaceutics from Fudan University in 2013 and then joined West China School of Pharmacy, Sichuan University as a lecturer. In 2017, Gao was promoted as a Professor. His research focuses on the rational design of drug delivery systems for central nervous system diseases and tumors.

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