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Strategies to overcome/penetrate the BBB for systemic nanoparticle delivery to the brain/brain tumor

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Abstract

Despite its prevalence in the management of peripheral tumors, compared to surgery and radiation therapy, chemotherapy is still a suboptimal intervention in fighting against brain cancer and cancer brain metastases. This discrepancy is mainly derived from the complicatedly physiological characteristic of intracranial tumors, including the presence of blood-brain barrier (BBB) and limited enhanced permeability and retention (EPR) effect attributed to blood-brain tumor barrier (BBTB), which largely lead to insufficient therapeutics penetrating to tumor lesions to produce pharmacological effects. Therefore, dependable methodologies that can boost the efficacy of chemotherapy for brain tumors are urgently needed. Recently, nanomedicines have shown great therapeutic potential in brain tumors by employing various transcellular strategies, paracellular strategies, and their hybrids, such as adsorptive-mediated transcytosis, receptor-mediated transcytosis, BBB disruption technology, and so on. It is compulsory to comprehensively summarize these practices to shed light on future directions in developing therapeutic regimens for brain tumors. In this review, the biological and pathological characteristics of brain tumors, including BBB and BBTB, are illustrated. After that, the emerging delivery strategies for brain tumor management are summarized into different classifications and supported with detailed examples. Finally, the potential challenges and prospects for developing and clinical application of brain tumor-oriented nanomedicine are discussed.

Keywords

nanoparticle; blood-brain barrier; transport; transcytosis; brain tumor

1. Introduction

Brain tumor, referring to both a primary brain tumor and a metastatic brain tumor, remains one of the most lethal cancers, with median survival between 4 and 15 months after diagnosis [1, 2]. Glioblastoma multiforme (GBM), a grade IV astrocytoma classified by

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WHO, is the most common and aggressive primary brain tumor with a 5-year survival rate of less than 5% and accounts for around 80 % of new diagnosed malignant central nervous system (CNS) tumors [3, 4]. A metastatic brain tumor is a devastating complication of periphery tumors with a high propensity to metastasize to the brain, such as lung cancer (50%), breast cancer (15%), and melanoma (6–11%) [5, 6]. Despite tremendous efforts devoted in the past decades, at present, the standard care for newly diagnosed GBM is surgery, followed by adjuvant radiation therapy and adjuvant chemotherapy with alkylating agent temozolomide (TMZ) [1]. Similar to primary brain tumors, although surgery and radiation therapy are typically adopted as first-line treatments for metastatic brain tumors, there are no specific chemotherapeutics proven effective for brain metastasis therapy, including TMZ [7]. The biggest challenge for surgery is that, due to heterogeneous and invasive growth, it is hard to remove the tumor tissue completely without compromising healthy neurological tissue due to the lack of finely defined glioma boundary. Thus, the residue of neoplastic tissue would lead to rapid glioma recurrence and poor prognosis [8]. Radiation therapy, especially whole-brain radiation therapy (WBRT), is restrained by significant side effects, such as cognitive impairment [9, 10]. Meanwhile, the improved overall survival is conservative and heterogeneous [11].

Compared to significant progress in managing peripheral tumors, chemotherapy is still a suboptimal intervention, which serves as an adjuvant role in a state-of-art regimen for brain tumor treatment. Although most tumoricidal agents for peripheral tumors exhibit comparable pharmacological effects to brain tumor cells *in vitro*, they barely yield a plausible anti-tumor outcome in preclinical brain tumor models and clinical trials. This discrepancy mainly derives from the insufficient accumulation of therapeutic agents in the brain tumor to produce a pharmacological effect, attributing to the presence of brain and tumor-related physioanatomical barriers, such as blood-brain barrier (BBB) and blood-brain tumor barrier (BBTB) to form a pharmacological sanctuary. The intrinsic characteristic of brain tumors, especially metastatic brain tumors, including invasive and aggressive growth, substantial heterogeneity, and readily acquired drug resistance, further reinforce their refractory [12, 13]. During drug research and development for brain tumors, it is of equal importance to develop some novel and practicable approaches, including biological, chemical, and physical strategies to improve drug delivery to intracranial tumors, rather than merely devoted to the discovery and development of the first in class drugs, considering the vast cost in labor, finance, and time. Thus, it is crucial to have a complete picture of the biological and pathological characteristics of intracranial tumors, including BBB and BBTB. Hereby, we review and discuss recent emerging strategies for overcoming the BBB and increasing theranostic agents penetrating BBB (Figure 1), including detailed examples of different categories. Finally, we also summarize the potential challenges and prospects for the design and clinic application of nanomedicine for brain tumors.

2. The physiological barriers of brain tumor: BBB and BBTB

Despite marvelous efforts that have been devoted to the development of the diagnosis and therapeutic avenues, the theranostic of brain tumors remains a significant challenge. This obstacle mainly comes from the presence of the blood-brain barrier (BBB), restricting the access of diagnostic and/or therapeutic agents to brain tumors. BBB is a distinct

physiological structure of microvessels in the brain related to the peripheral ones, which exerts critical functions on precisely regulating the agents' transport between the luminol blood and brain tissue and maintaining the dynamic balance of the brain microenvironment in the CNS. The BBB (Figure 1) is composed of endothelial cells (ECs), pericytes (PCs), basement membrane, and end-feet of astrocytes. ECs are held together interactionally through intermolecular tight junctions (TJs) through a number of transmembrane proteins (such as occludin, claudins, and junction adhesion molecule (JAM) proteins), making up the substantial wall of brain capillaries. A discontinuous layer of PCs surrounds the abluminal surface of ECs. The ECs and PCs are further covered by a stroma-like basement membrane, which is tightly ensheathed by astrocytes' end feet. On the one hand, this nest-like structure of BBB serves as a physical barrier that strictly controls the passage of specific nutrients, such as amino acids, glucose, and fatty acids, from the bloodstream to the CNS via passive diffusion, as well as restricts the passage of potentially harmful substances such as pathogens and neurotoxic compounds. On the other hand, ECs express a bulk of active efflux proteins such as P-glycoprotein and multidrug resistance-associated proteins (MRPs) on the luminal side of the ECs, which have a broad spectrum of substrates to efflux them back to circulation and minimize the substance transportation across the BBB. As a result, a substantially higher electrical resistance was observed between the BBB compared to that of peripheral ones, which would restrict polar and ionic substances transporting to the brain liberally. All these barriers preclude the access of most large molecule drugs and more than 98% of small molecular agents to the brain tissue [14].

Like most peripheral tumors, the original primary brain tumor may derive from the excess "embryonic rests" activation in a particular microenvironment and the imbalance between oncogenes and tumor suppressors [15]. At the early stage of the development of a primary brain tumor, the surrounding nutrients and oxygen can orchestrate inceptive brain cancer cells to grow and develop until the tumor size expands to 2–3 mm (in diameter). After that, the tumor will progress to angiogenesis-dependent nutrient supply mode due to the explosive mass expansion, which leads to the loss of balance between pro- and anti-angiogenic factors [16], resulting in neovascularization. The angiogenetic vessels possess an intact BBB at the initial stage or on the invasive margin. Following the primary tumor's growth, the neovascularization and intratumor vasculature would deteriorate to form a compromised BBB, whose structure and function display a vast difference from the BBB, named blood-brain tumor barrier (BBTB). There are three populations of micro-vessels in a brain tumor, including continuous and non-fenestrated capillaries, continuous and fenestrated capillaries, and discontinuous (with or without fenestrations) capillaries, based on their orientation and identification of structure and organization [17]. The first group is unique to brain tumors, while the other two groups are similar to vessels found in peripheric tumors. Although no apparent difference in the component, BBTB holds differentiated permeability compared to BBB, deriving from functional compromise of intratumor vasculature, including disrupted endothelial lining, disrupted TJs, and snatchy basement membrane, and so on [18]. However, the leakage structure and the penetrating efficiency of BBTB for macromolecule and nanoparticles are highly heterogenous, not limited to different grades of brain tumor but also different regions within a particular brain tumor. This heterogeneity is prominent among different grades of brain tumors. For example, contrast-enhanced MRI exhibited limited

ability in delineating the margin of most of low-grade gliomas (grade II) and more than 40% of high-grade gliomas (grade III), even sometimes failed to accurately classify malignant gliomas due to the heterogeneous BBTB induced limited and un-uniform distribution of paramagnetic contrast agent in the tumor [19]. Moreover, the permeability of capillaries progressively increases from minor to moderate, along with the progression of gliomas [17]. The intertumoral difference in permeability is attributed to the heterogeneous distribution of variable microvessel and spatial variables, such as relatively intact and continuous lipid layer at the margin of brain tumor and enlarged lumen inside the tumor [20]. Even so, BBTB still holds some original characteristics of BBB and its permeability for achieving an ideal leakage for theranostics agents is still significantly different from that of peripheral tumor.

In contrast to the internal germination pattern of primary brain tumor, brain metastasis formation is mainly due to hematogenous dissemination from a peripheral tumor into the brain, followed by the progress of a series of the metastatic cascade, including escape and intravasation into the bloodstream from the primary tumor site, navigation and arrest in the cerebral microcapillaries, and extravasation and proliferation in the brain [21]. It was found that this sowing is always of co-blossom of seed-like cancer cells with their soil-stromal components, such as tumor-associated fibroblast, which can create a modest circumstance for tumor cells colonization and proliferation at the metastatic foci in the early stage [22]. Generally, after the spread of tumor cells to the brain, these progenitor like-tumor cells mainly display a co-opted pattern for proliferation and growth, along with the BBB basement membrane depending on the existing blood vessel at the early stage, and the BBB integrity is maintained until the size of the tumor expands to 3 mm [21]. At the following advanced stage, the co-opting metastatic tumor will evolve to angiogenesis-dependent growth as the aforementioned primary brain tumor and compromise the integrity of BBB. Moreover, due to the original malignant and aggressive nature of metastatic cells, cancer brain metastasis always exhibits multi-nodules and invasive progression, heterogeneity, and poor prognosis [23].

3. Strategies for enhanced BBB penetration

Brain is the most complex system in the body. The accurate controlling passage of various endogenous and exogenous agents from the vasculature into the CNS, and vice versa, is critical for maintaining the homeostasis of the CNS. Due to the presence of a unique physiologic barrier, BBB, endogenous molecules penetrating across the BBB are precisely controlled by diffusion and transcytosis, while most external agents (95%) are blocked by this physical and physiological barrier. Therefore, how to make theranostic agents overcome the BBB barrier and be delivered to the brain without disturbing the homeostasis of the CNS is highly desired. Recently, nanoparticle-based drug delivery systems have been widely investigated to provide therapeutic agents into the brain for the treatment of CNS diseases, including brain tumors, due to their distinctive physiochemical properties, such as high drug loading, prolonged blood circulation time, enhanced stability, facile surface modification for active targeting and controlled drug release. Some nanoparticles based strategies for the diagnosis and treatment of brain tumors have been advanced to different clinical phases (Table 1). The blooming development of nanoparticles would lead to new opportunities for managing brain tumors in both diagnosis and therapy.

3.1 Passive targeting

Enhanced permeability and retention (EPR) effect is the theoretical basis of nanoparticles based passive targeting therapy in most tumors, especial peripheral ones, in which nanoparticles of the size range of 1 to 200 nm utilize the unique structure of tumors, including impaired lymphatic drainage system, hyper-vasculature, and defective vascular architecture to realize nanoparticles entry and accumulation within the tumor mass [34]. The EPR effect is extensively size dependent, referring to the size of the particles and pore dimensions of the vasculature within the tumor, whereas it exhibits little impact on the distribution of small molecular weight compounds such as chemotherapeutic agents, which mainly enter tumor mass by free diffusion [35]. However, the EPR effect displays a significant heterogeneity. Different types of tumors may have different pore dimensions. A given tumor could also have a vast difference in pore size at different locations and in various stages. Attributing to the existence of BBB, the EPR effect in brain tumors may be somewhat different from that of periphery tumors, which is ambiguous and controversial. Brain tumors are divided into four levels based mainly on the histologic progress by World Health Organization standard (WHO) [36], in which tumor microvessel population is an important parameter [17]. As aforementioned, continuous endothelial cells and inter-endothelial gaps are the primary aspects of the microvessels population. It is easy to comprehend that EPR-based passive targeting to access the intratumoral space is associated with the grade of brain tumor. For instance, glioblastoma (WHO grade is IV) has a tortuous and disorganized vessel wall, and its vessel pore size is as large as 550 nm in some regions [37], which gives the reasonability for passive targeting. However, this kind of gap-dependent permeability merely exists in WHO-defined grade II astrocytomas and oligodendrogliomas vessels [19], which leads to low permeability of contrast agents for CT and MRI and following faint contrast signals in the tumor. Similar to most peripheral tumors, only a small fraction of administered nanoparticles will be located in brain tumors via the EPR effect. Most nanoparticles will be susceptible to opsonized by the reticuloendothelial system (RES, such as liver and spleen). This tumor aggravative progress and grade-dependent permeability in brain tumors are heterogenous and may yield enormous challenges for EPR base nanomedicine, which could be an excellent opportunity for personalized medicine.

3.2 Nanoparticle-BBB interaction mediated BBB penetration

Although the inherent features of BBB and BBTB prevent macromolecule and nanoparticles from shuttling freely between the peripheric circulation system and brain tumor, the large blood-brain interface of BBB (around 20 m^2) and the exuberant blood [38], some inevitable, widespread and/or special interactions between circulatory nanoparticles with BBB, including biological interfacial interaction (such as receptor-mediated transcytosis and transporter-mediated transcytosis) and physicochemical interaction (such as absorption-mediated transcytosis and BBB opening) and can be fully utilized to realize nanoparticle delivery to brain tumors. Here we mainly review and discuss the recent progress in developing nanotechnology-based treatments for brain tumors.`

3.2.1 Receptor-mediated transcytosis (RMT)—RMT is considered as the most promising method and is widely employed for realizing intracerebral delivery of

nanoparticles with the characterization of high specificity, selectivity, and affinity, especially for brain tumors [39]. Most endogenous macromolecules are transported into brain parenchyma via RMT, usually involving the following three essential steps, including (1) interactive binding of a ligand to its cognate receptor on the luminal membrane of capillary endothelial cells mediated the formation of corpuscles at the luminal wall; (2) intracellular trafficking from luminal side to abluminal side; (3) exocytosis of the endocytic agent from abluminal side to brain parenchyma [40]. RMT-based transport is usually energy-dependent and with relatively high efficiency. To take advantage of this strategy, the surface of nanoparticles can be decorated with specific ligands to improve the intracerebral transport of loaded therapeutic agents. Theoretically, to achieve an optimal delivery efficiency of therapeutic to a brain tumor, the target receptor should be highly expressed in the endothelial cells of the brain tumor, whereas minimally expressed in other vascular endothelial cells to minimize the off-target effect induced safety concern. However, to date, nearly no receptor can meet this criterion. Meanwhile, an endogenous ligand may exert a competitive bind to the receptor, which will compromise the target delivery efficiency of RMT. Over the past decades, most receptors served as targets for CNS-associated diseases, including brain tumors, are upregulated on the endothelial cells of the brain capillary. Recent advancements in biomedical sciences and nanotechnology provide a better resource for the design of nanoparticle-based RMT. The following section will summarize several typical receptors utilized in nanoparticle-based drug delivery for brain tumors.

3.2.1.1 Transferrin receptor mediated transcytosis: Transferrin receptor (TfR) has been extensively investigated for RMT for brain delivery due to its over-expression in the endothelial cells of brain capillaries [40]. In the brain, TfRs are mainly responsible for the transport of iron ions into the brain parenchyma in the complex form of transferrin-Fe (Tf-Fe) to maintain the homeostasis of iron, which is vital for metabolism, neural signal transduction, and brain function [41]. Transferrin, TfR targeted peptides, anti-TfRs antibodies, and its fragments (such as Fab, sFabs, and scFv) consist of the targeting ligands of TfRs. Moreover, as in most peripheral tumors, due to the proliferation of cancer cells induced exponential demand for iron and iron metabolism, the expression of TfR can rise to 100 folds compared to normal cells [42]. Therefore, those ligand molecules can be engineered onto the surface of nanoparticles via physical, chemical, and biological manners to initiate the RMT [43], realizing a TfR receptor mediated sequential dual-targeted delivery [43]. For example, Dixit et al. prepared transferrin-modified zoledronic acid (ZOL) loaded liposomes (NPs-ZOL-Tf), and evaluated the *in vitro* and *in vivo* anti-brain tumor efficiency [44]. It was demonstrated that NPs-ZOL-Tf exhibited a higher *in vitro* anti-proliferative activity than free ZOL and non-targeted NPs-ZOL, which was attributed to an enhanced receptor-mediated intracellular internalization. Moreover, this TfR-mediated superiority was further validated in intratumor localization and antitumor efficiency in orthotropic and heterotopic brain tumor models [44]. Adopting a similar strategy, Cui et al. developed a dual-targeting PLGA nanoparticles (MNP/T7-PLGA NPs), which combined with magnetic guidance and peptide T7 mediated active targeting, for co-delivery of paclitaxel and curcumin for brain tumor therapy [45]. T7 (sequence HAIYPRH), a human TfR binding peptide, was modified on the nanoparticles surface to mediate the active targeted delivery of NPs through RMT. Since T7 and transferrin bind to distinct sites on TfR, there is

no competitive binding between them. The integration of magnetic iron oxide into the delivery system endowed the nanoparticles magnetic-guided targeting ability by favoring particles-endothelial cells and receptor-ligand (TfR-T7) interaction and attenuating sheering stress applied on the BBB, thus achieving an improved active targeting effect [45]. The following Table 2 give a brief summarization of systems utilizing TfR mediated RMT.

Although TfR has been extensively utilized as a targeting receptor, it is worth noting that TfR-based RMT is still full of debate. One scenario is that the unique receptor-ligand interaction between TfR and TfR's ligands decorated on the surface of nanocarriers may be severely compromised by the competed binding of TfR with endogenous ligand, which will reduce the chance of nanocarrier crossing the BBB. In another case, the efficiency of TfR-mediated transcytosis is relatively low. Some early research in cultured cells and in situ brain perfusion models found that nearly 90 percent of transferrin was recycled back to the luminal side after being endocytosed by brain endothelial cells, and only 10 percent of transferrin was perfused into the brain [56, 57]. More recent follow-up studies revealed that the affinity and the valency of TfR antibody and the density of the ligand decorated on the nanoparticles could have a significant influence on the intracellular TfR trafficking and transport of the nanoparticles [58–60]. Therefore, the microstructure and function of the Tf-TfR complex and its efficiency in TfR-mediated transcytosis should be further explored and improved.

3.2.1.2 Low-density lipoprotein receptor-mediated transcytosis: Low-density lipoprotein receptors related protein (LRP, such as LRP1, LRP2, and LRP8) is another common family of receptors, which have been widely reported to mediate the transport of various nanocarriers across BBB through RMT. Among them, LRP1 and its targeting ligands are extensively investigated and employed for the diagnosis and therapy of CNS diseases, including brain tumors. LRP1, a membrane of the LDL receptor family, is involved in numerous essential processes in the brain under both physiological and pathological conditions [61]. LRP1 is usually expressed at a high level at BBB, participates in the transport of amyloid-beta peptide in the CNS together with its ligands, such as alpha-2-macroglulin and apoliliprotein [61], controls the degradation of extracellular matrix by regulating the level of matrix-degradation proteases, such as matrix metalloproteinases (MMPs) and plasminogen activators (PAs) [62]. Angiopep 2, a 19-amnino acid length peptide derived from aprotinin, a natural ligand of LRP1, is widely used to mediate brain-targeted drug delivery. Sun et al. reported that the modification of Angiopep on the cationic liposome could realize effective codelivery of genetic agent and paclitaxel to brain tumors [63]. After treatment with these liposomes, the median survival time of brain tumor-bearing mice was significantly longer than their non-targeted counterpart (69.5 days vs 35.5 days), even substantially longer than the standard clinical administration of temozolomide (47 days). Thus, this Angiopep-functionalized liposome holds great promise for clinical translation. Apart from the application on the surface modification for nanoplatforms, Angiopep 2 also serves as the key guiding agent for some Engineered Peptide Compound (EPiC) [64], including ANG1005, a novel paclitaxel derivative, consisting of three molecules paclitaxel and one 19-amino-acid peptide Angiopep-2 via cleavable ester bond [65]. It was founded that reducing the activity of LRP1 on U87

glioblastoma cells through gene silencing or corresponding competitors could reduce the cellular uptake of ANG1005, and on the other hand, increase the internalization of ANG1005 into U87 cells under the microenvironment of hypoxic and acidic condition mimicking aggressive tumor [65]. Moreover, in vivo imaging and immunolocalization studies demonstrated that ANG1005 and Cy5.5-Angiopep-2 boosted accumulation in the intracranial Mu87MG.EGFRvII glioblastoma tumor model compared to surrounding or contralateral tissue, mainly attributing to the over-expression of LRP-1 and LRP-1 RMT [65].

Tween 80 is a surfactant agent that can increase nanoparticle's brain delivery by coating the surface of nanoparticles and is considered the "gold standard" coating for brain delivery [66, 67]. Gulyaev et al. found that 1% Tween 80 coating on the surface of doxorubicin-loaded poly(butyl cyanoacrylate) nanoparticles achieved 60-fold brain concertation of doxorubicin as compared to bare nanoparticles and free drug in mice [68]. Wang et al. also verified that 1% Tween 80 coating on the gemcitabine-loaded Polybutylcyanoacrylate (PBCA) nanoparticles could significantly inhibit the growth of C6 glioma cells in vitro and extend the survival time of corresponding brain tumor model mice in vivo, thus realizing a preferable antitumor efficiency [69]. Although the exact mechanism of Tween 80 assisting nanoparticles in crossing the BBB is unclear, it is speculated that the interaction between brush like Tween 80 and LDL receptor is the driving force to initiate the RMT [70]. Another possible explanation is that the Tween 80 may increase the adsorption of plasma-derived apolipoproteins E and B to the surface of nanoparticles [71], which have been confirmed to interact with LDL receptors and trigger receptor-mediated endocytosis [72–74].

3.2.1.3. Insulin receptor-mediated transcytosis: Similarly, insulin receptor expressed on the endothelial cells of brain capillary has also been employed for RMT-based drug delivery to the brain [75, 76]. It was founded that a strong binding force between insulin and insulin-like growth factor I (IGF-I) in the brain tumor, which provides evidence that insulin receptor could be a promising candidate for insulin as a ligand for brain tumor target drug delivery [77]. The 83–14 murine mAb is an insulin peptidomimetic mAb, which has a high affinity with the exofacial epitope of the insulin receptor. Moreover, 83–14 murine mAb could initiate an RMT through the BBB in Rhesus monkeys in vivo [78], holding a great promise as a vector or ligand for delivering drugs to brain tumors. Because of this property, Dieu et al. grafted 83–14 murine mAb on the surface of polymersomes and realized enhanced cellular binding and uptake by the endothelial cells of brain capillary [79]. Employing a similar strategy by modifying 83–14 mAb on the surface of nanoparticles, Kuo et al. achieved the transport of solid lipid nanoparticles carrying chemotherapeutic carmustine across the BBB and brain-targeting delivery with the help of insulin receptor [80]. It was worth noting that the role of insulin receptors in mediating the transport of insulin across BBB in vivo was receptor type dependent and sometimes controversial. For instance, researchers revealed that the transport of insulin across the BBB was independent of insulin receptors, especially for signaling-related insulin receptors [81]. Another looming concern is that potential hypoglycemia may be present when insulin is acted as a targeting ligand, which should be monitored carefully, or prophylactic measures should be taken first, such as co-administration of dextrose [82]. Thus, as an alternative to insulin, peptidomimetic

antibodies, insulin-derived peptides and its derivatives, which have similar binding ability to the exofacial epitope of the insulin receptor, could be a great choice.

3.2.1.4 Nicotinic acetylcholine receptor-mediated transcytosis: Nicotinic acetylcholine receptors (nAChRs), a ligand-gated ion channel widely expressed on the brain capillary endothelial cell, were recently widely investigated as a targeted receptor to facilitate BBB crossing and intracranial transporting of nanocarriers [83, 84]. One of the prominent features of nAChRs is their super susceptibility to inhibition, such as peptide neurotoxins and neurotropic viral proteins, which endowed their ability to mediate various agents crossing into the brain. So far, some synthetic and nature-derived peptides have been explored as nAChRs targeting ligand to facilitate therapeutic agent delivery to the brain, such as RVG-9R [85, 86], a 29-amino-acid peptide derived from rabies virus glycoprotein (RVG), CDX [83], a 16-residue peptide derived from the loop II region of the snake neurotoxin candoxin, and KC2S [87], a synthetic peptide similar to the loop 2 segments of three-finger snake neurotoxins. More recently, adopting similar tactics, Lu's group utilized 2-methacryloyloxyethyl phosphorylcholine (MPC) as a dual targeting ligand to deliver protein therapeutics for the treatment of central nervous system diseases, such as primary brain tumor, metastatic brain tumor and neural degeneration [84, 88–90]. Wherein, MPC, a molecule containing a choline and an acetylcholine analogues, interacts with nAChRs and choline transporter (ChT) via hydrogen bonding and electrostatic interaction, respectively [84], to initiate RMT and realize brain drug delivery. In these systems, MPC monomer and crosslinker were polymerized around a therapeutical protein to yield a nanocapsule shell, which can assist the protein in penetrating the BBB via RMT. One of the representative examples was the systemic delivery of monoclonal antibodies to the CNS for the treatment of brain tumors (Figure 2) [84]. The MPC monomer and matrix metalloproteinase-2 (MMP-2) cleavable peptide crosslinker were assembled around the nimotuzumab (Nimo) or trastuzumab (Tras) to form nanocapsules of antibodies. By virtual of the choline transporters or acetylcholine receptors expressed on the luminal side of BBB, MPC decoration enables the nanocapsules to penetrate through the BBB effectively via RMT. Subsequently, MMP-2, highly expressed in the brain tumor environment, cleaved the peptide linker, broke the shell, and released the encapsulated monoclonal antibodies to realize their pharmacological function [84]. In vivo assay on an orthotopic U87-EGFRwt glioma model demonstrated the effective and safe delivery of the therapeutic protein for brain tumor treatment. It was more interesting to note that excepting for brain targeting capacity, the MPC shell can also prevent the core protein from being phagocytosed by macrophages, which is a key mechanism of nanoparticles clearance during in vivo application, and extend the circulation time of encapsuled protein [91]. Moreover, the polymer shell could prevent the protein from being identified by immune cells, therefore reducing the immunogenicity derived from the therapeutic proteins [91].

3.2.2 Transporter-mediated transcytosis (TMT)—TMT, also known as carrier-mediated transcytosis, is a vital strategy for transporting low molecular weight nutrients from the bloodstream into the brain. Many transporters have been discovered on the BBB [38,39]. Among them, glucose transporter and glutathione transporter are the two

most explored transporters for facilitating nanoparticles crossing the BBB in brain tumor treatment.

3.2.2.1 Glucose transporter-mediated transcytosis: Glucose transporter (such as GLUT1), which is found highly expressed on BBB cells and various tumors, including brain tumors, are mainly responsible for the transport of glucose from the blood to the brain and tumor, the primary energy source for their high metabolism. Firstly, abundant blood flow and hypermetabolic characterization of the brain is the prerequisite for its acting as the central role of CNS, which may partly account for the high expression of GLUT1 on endothelial cells for high and uninterrupted energy requirement. Secondly, the characterization of rapid differentiation, renewal, and metabolism of brain tumors further reinforces the expression of GLUT1 on BBB, as well as the brain tumor itself. Moreover, it seems that the BBB penetration is closely associated with the GLUT1, whose building up glycosylation is a meaningful methodology for enhancing biodistribution to the brain [92, 93]. All of them render GLUT1 an excellent target for brain-targeted drug delivery research. It was revealed that glycemia control boosts the BBB crossing and brain accumulation of glucosylated nanocarrier via GLUT1 (Figure 2) [93]. The surface of self-assembled supramolecular nanocarriers is featured with properly configured glucose, which can regulate the nanocarriers' distribution in the brain by mimicking the glucose transporting BBB through the GLUT1, especially in the circumvent of an external trigger of a glycaemic increase after a fasting state. It was founded that 25% Glu(6/m), the optimal density of glucose on the surface of nanocarriers had the highest facilitating accumulation rate to 6% dose/g-brain [93], which was significantly higher than a previous report [94]. By fully utilizing the over-expression of GLUT1 on both BBB and glioma cells, Jiang et al. employed glucose as a ligand to develop a 2-deoxy-D-glucose modified poly(ethylene glycol)-co-poly(trimethylene carbonate) nanosystem (DGlu-NP) to realize a dual-targeting strategy, enhanced BBB penetration via GLUT-mediated transcytosis and improved drug accumulation in the intracranial tumor via GLUT-mediated endocytosis [95]. All in vitro and in vivo assays validated the feasibility and potential of GLUT-1 as a targeting receptor for enhanced drug delivery to the brain tissue, especially for brain tumors. It is worth mentioning that due to the abundant expression of GLUT-1 (approximately 100 fold higher than transferrin receptor) and its facilitative function [76], the possible toxicity, especially neurotoxicity, should be considered when adopting this strategy. Both acute toxicity and long-term toxicity should be monitored and evaluated.in case a GLUT-mediated transcytosis is employed.

3.3.2.2 Glutathione transporter-mediated transcytosis: Glutathione (GSH) is an endogenous antioxidant that can minimize oxidative stress within the brain and protect neurons from oxidative stress-induced damage. It was revealed that GSH concentration within the brain is much higher than in the blood and other tissues. Recent studies found that GSH transporters (MRP1) are highly expressed on the endothelia of the BBB and are vital for GSH homeostasis in the brain [96]. Thus, several GSH-conjugated nanoparticles have been developed to boost the delivery of paclitaxel and Doxil to the brain through a glutathione transporter-mediated transcytosis route [97, 98]. Tellingen et al. utilized GSH-conjugated PEGylated liposomes, named G-Technology™, to fabricate (GSH-Doxil) for

the treatment of intracranial xenograft brain tumors [97]. After three consecutive weekly treatments of GSH-Doxil at the 5 mg/kg of doxorubicin equivalents dose, two animals showed complete regression, which was not observed in the Doxil treatment group. In addition, the GSH-Doxil treatment was well tolerated.

3.3.2.3 Other transporter-mediated transcytosis: Apart from the aforementioned two prevalent and widely-adopted transporters as the target for transcytosis, some other transporters, such as monocarboxylate transporters (MCTs) and L-type amino transporters (LATs) expressed on the luminal membrane of brain endothelial cells also drawn some attention in drug delivery. MCTs are mainly responsible for the transport of endogenous short chain monocarboxylate solutes, such as lactate, pyruvate, and acetoacetic acid, which could serve as an alternative energy source and play an important role in energy metabolism [99]. They are also responsible for some exogenous acid drugs' intra-brain transport, such as salicylic acid, valproic acid, benzoic acid, etc [100]. Therefore, some drug delivery systems have leveraged the specific substrates of MCTa as the target ligand for high efficient brain delivery of therapeutic agents. For example, β-Hydroxybutyric acid (HBA), as a novel ligand of MCT1, was grafted on the surface of docetaxel, carmustine and temozolomide loaded solid lipid nanoparticles (HD-SLNs) to improve their delivery to brain [101, 102]. However, due to the universal expression of MCTs almost in all tissues, including kidney, liver, intestine, heart, muscle, etc., the strategy of targeting MCTs may encounter fluctuated disposition of delivery system in different organs and compromise the brain targeting efficiency. Yet it is worth noting that the different isoform of MCTs has a inequable distribution in the different subcellular regions of the brain and types of intracerebral cell population [100, 103], which provides a meaningful guideline for the design of MCTs targeting drug delivery system for tumor located in a different region of the brain.

LATs mainly assist the internalization of neutral amino acids into cells and are commonly upregulated in most tumors [104], including brain tumors, and serve as a great potential molecular target for brain tumor treatment [105, 106]. It was also found that LATs are highly expressed on the luminal and abluminal membranes of capillary endothelial cells of BBB [107]. All of this makes LATs great potential targets for brain tumor dual drug targeted delivery by rational design of the drugs/delivery system mimicking the structure of LATs substrates [108]. One prominent representative methodology is coupling neutral amino acids or analogs to a therapeutic agent or on the surface of a drug delivery system to produce targeted pro-drugs and targeted delivery systems. For example, Parul Kharya et al. grafted L-phenylalanine (PA), a most common BBB penetrating neutral amino acid, on the surface of solid lipid nanoparticles for targeted delivery of DOX to brain tumors [109]. However, one of the critical aspects that should be carefully considered is avoiding the loss of affinity of modified amino acids to LATs after their modification to a drug delivery system. It was revealed that the presence of a free amino group and a free carboxyl group on the α-carbon atom of the amino acid is critical for the specific interaction between amino acid and LATs [110]. So, the biggest challenge and verification is how to maintain the conformational consistency of amino acids. For example, Li et al. induced γ-carboxyl of glutamate to the surface of liposomes through the side chain linkage as the targeted ligand to retain the intact

structure of a free amino group and a free carboxyl group on the α-carbon atom to preserve the active targeting capacity [111].

3.3.3 Adsorptive mediated transcytosis (AMT)—Adsorptive mediated transcytosis (AMT) is another widely explored approach for delivering drugs across the BBB. AMT is triggered by the electrostatic interaction between positively charged agents and negatively charged luminal membranes of brain endothelial cells. Similar to the RMT, the tour of agents from capillary to brain parenchyma also go through a three-step procedure: positively charged agents firstly interact with negatively charged molecules or region on the luminal surface of endothelial cells via electrostatic interaction, thus triggering the formation of transcytotic vesicles; The transcytotic vesicles navigate in the cytoplasm from luminal side to the abluminal membrane of BBB cells; At last, transcytotic vesicles fuse with the membrane and release the carried agent into the brain [40]. Cationic bovine serum albumin (CBSA) and cell-penetration peptides are two kinds of the most frequently used moieties conjugated on NP to trigger NPs across the BBB through AMT [112]. For instance, Lu et al. conjugated CBSA to pegylated PLA nanoparticles to yield CBSA-NP for brain-targeted drug delivery [113]. In an in vitro co-culture BBB model, it was found that the modification of CBSA on the surface of nanoparticles could more effectively facilitate the NP transport across the BBB compared to the parental bovine serum albumin (BSA) modified NP (7.76 times higher). Moreover, the incorporation of CBAS had no impact on BBB's permeability and integrity, ensuring the carrier system's safety [113]. Through encapsulating cytotoxic plasmid pORF-hTRAIL, CBSA-NP-hTRAIL was employed to realize malignant gliomas gene therapy. It was demonstrated that at 30 min after administration, there were some distributions of CBSA-NP-hTRAIL in the brain and brain tumor, main colocalized with the glycoproteins. Subsequently, hTRAIL mRNA and hTRAIL protein were detected in normal brain and tumors at 24 and 48 hour later [114]. Furthermore, the repeated administration of CBSA-NP-hTRAIL in vivo realized the prospective apoptosis of C6 glioma and significantly delayed tumor growth [114, 115].

Cell-penetrating peptides (CPPs), a type of short positively charged peptide heterogeneous in size and sequence, show outstanding ability in assisting various therapeutic agents, such as proteins, oligonucleotide, antibodies, imaging agents, and drug-loaded nanoparticles, crossing the lipophilic barrier of cellular membranes, including BBB [116]. The first discovered and mostly investigated CPPs is the transactivating transcription factor TAT (AYGRKKRRQRRR), a polypeptide motif derived from the surface protein of human immunodeficiency virus-1 (HIV-1) [117]. By integrating TAT into the Angiopep-2 decorated docetaxel-loaded nanoparticles with a rational ratio, Zhong et al. developed co-functional tandem nanomicelles, termed ANG20/TAT10-Ms (Figure 4) [118]. In vivo imaging found that ANG20/TAT10-Ms had a significant higher glioma accumulation compared to the parent nanoparticles (including only Angiopep-2 decorated nanomicelles and no ligand modified ones) in an orthotopic U87MG glioma-bearing mouse model. Furthermore, DTX-loaded ANG20/TAT10-Ms exhibited the strongest glioma inhibitory effect and extend the survival time of U87MG glioma-bearing mice [118]. It was of convince that ANG20/TAT10-Ms not only hold a high glioma cell selectivity via Angiopep-2 peptide mediated targeting recognition but also displayed a markedly enhanced BBB permeation with the help of TAT.

Although many potential pathways for CPPs mediated brain tumor-targeted delivery have been proposed, the cellular membrane transport mechanism of CPPs is still unclear. Earlier research found that around 0.126 % of intravenously injected TAT peptide could cross the BBB and accumulate in the CNS, probably through a nonsaturable mechanism with a unidirectional influx rate of about 0.490 ul/g/min [117]. More overly, it was speculated the CPPs either directly initiate the BBB penetration though RMT as a result of electrostatic interaction between its positive charge and negatively charged luminal surface of BBB or interacted with some particular receptors/proteins to trigger endocytosis/transcytosis [40]. It is worth noticing that although the travel route of AMT is similar to RMT, there is a significant difference between these two pathways. The prominent feature of AMT is its high binding capacity to BBB cells and associated with poor tissue selectivity due to non-special binging. It was striking that the binding potential of AMT between cargoes and endothelial cells via positive and negative electrostatic interaction could be a thousand times higher than that of RMT, which is the main reason for the high transcytosis efficacy of AMT [119]. Likewise, due to the non-special interaction between opposite charges, limited tissue selectivity, and high background noise, system toxicity is the primary shortcoming that should be taken into consideration when employing this approach.

3.4 The temporary BBB opening and disrupting facilitated penetration

Another straightforward, effective, and extensively explored strategy for brain drug delivery is temporary opening and disruption of BBB by using biochemical reagents or physical approaches. Different from interaction-mediated transport, of which there is nearly no influence on the integrity of BBB, the disruption of BBB by physicochemical method is usually referred to as compromising the integrity of BBB to a certain degree temporarily and reversibly. Subsequently, therapeutic agents transport into the brain parenchyma by diffusion. The commonly adopted disruption methods include hyperosmotic agents induced osmotic disruption, ultrasound disruption, magnetic disruption, and their orthogonal combination.

3.4.1 Chemical agents mediated disruption—Common hyperosmotic agents, mannitol, glycerol, and arabinose, may induce a high osmotic pressure and result in the BBB opening temporarily. The primary mechanism of hyperosmotic agents induced the BBB opening is that the osmotic pressure difference leads to the shrinking of vascular endothelial cells and disruption of the tight junction, which results in the broadening of the paracellular space within BBB and subsequent transportation of drugs into the brain parenchyma by diffusion. Moreover, this kind of disruption-induced transportation efficiency can be controlled by regulating the type of hyperosmotic agent, its concentration, injection speed, and retention time [120, 121]. Riina et al. reported that there was no dosed-limiting toxicity from a single dose of super-selective intraarterial cerebral infusion (SIACI) of bevacizumab up to 15 mg/kg after osmotic BBB disruption with mannitol in patients with recurrent malignant glioma [121], demonstrating the safety and toleration of SIACI of mannitol followed by bevacizumab. One month later, magnetic resonance imaging furtherly showed the efficiency of SIACI treatment with bevacizumab for recurrent malignant glioma, referring to the reduction in the tumor area, volume, perfusion and T2-weighted/FLAIR signal, no matter whether the patients previously received the intravenous bevacizumab

exposure or not [121]. It was noted that osmotic-induced BBB disruption might also allow some other macromolecules, toxic and harmful agents, to enter the CNS, which may result in neuropathological changes and dysfunction. Moreover, this osmotic BBB disruption is an invasive technique and requires the collaboration of highly trained neurosurgeons to achieve a plausible therapeutic benefit.

Apart from hyperosmotic agents induced BBB temporary shrinking, biological agents-mediated integrity alternation of BBB/BBTB is another alternative for BBB disruption. These chemical agents are mainly comprised of vasoactive compounds [122], such as histamine and bradykinin, which could selectively target B2 receptor expressed on the endothelium, followed by triggering transiently intracellular Ca^{2+} increase and sequentially leading to tight junctions (TJs) disruption and increased the drug permeability [123, 124]. It was revealed that some chemical agents, such as alkyl glycerols (AGs) [125], exhibited bioactivity similar to vasoactive compounds and could disrupt the BBB. However, it was independent of the alteration of TJs integrity, suggesting the complexity of chemical meditated BBB disruption and their promising research potential [122]. Zhou et al. developed a panel of nano-drug delivery systems by leveraging BBB modulating molecules to increase the accumulation of pharmaceutical agents in brain tumors [126–128]. One representative work utilizes an autocatalytic approach for improving the transport of nanoparticles into brain tumor for a theranostic purpose [126]. The autocatalytic nanoparticles (ABTT NPs) were composed of a biodegradable poly(amine-co-ester) terpolymer, a 36-amino acid peptide (chlorotoxin, CTX), a BBB disruption agent lexiscan, and a chemotherapeutic agent paclitaxel (PTX). Firstly, a small portion of ABTT NPs entered the brain tumor microenvironment through a traditional mechanism, such as active targeting mediated transcytosis (RMT). After penetrating the BBB, ABTT NPs could locally release the loaded BBB modulators lexiscan, which in turn transiently enhanced the BBB permeability to allow more ABTT NPs accumulation in the tumor microenvironment. This cascade amplification triggered by autocatalysis driving positive feedback loop would make nanoparticles readily cross the BBB and preferentially accumulate in the brain tumor [126]. Impressively, it was founded that the accumulation of ABTT NPs in the brain tumor region was 4.3 and 94.0 folds higher than that in the liver and in the non-tumor regions of brain, respectively [126]. Adopting a similar strategy, more recently, they developed an autocatalytic nano-delivery system, LANPs, integrated with breast cancer brain metastasis targeting, tumor microenvironment responsive size shrinking, and BBB disrupting ability [128]. The enhanced brain penetration of LANPs mainly derived from the encapsulated lexiscan, an adenosine receptor agonist, could pharmacologically modulate the permeability of the BBB [129]. It was founded that the enhancement of BBB permeability was dose-dependent, and the discrete TJs would rapidly recover flawlessly [129]. Considering the FDA's approvement and practice in the clinical application of lexiscan and combining with the superiority property of nanomedicine, LANPs hold great potential of translating into clinical application for the treatment of brain tumors, including metastatic brain tumors [128, 129]. Similarly, minoxidil sulfate (MS) could serve as a BBB modulator by upregulating the expression of caveolin-1 on endothelial cells and downregulating the expression of tight junction proteins to ameliorate BBTB permeability, following boosting

the transport of nanoparticles across the BBB and entering brain tumor nidus through transcellular and paracellular pathways [130].

3.4.2 Ultrasound-mediated disruption—Apart from its clinical application in imaging modality, recently, ultrasound, especially focused ultrasound (FUS), is also widely explored as a physical tool to reversibly disrupt BBB and shows great potential for enhancing the bioavailability of therapeutics for the treatment of CNS diseases, such as brain tumor [131]. Mechanically, by combined with microbubbles (MB), FUS can concentrate acoustic energy to trigger the cavitation activity and produce shear stress in endothelial cells, induce transient and reversible opening of TJs, and/or activate the signaling pathway, which in turn leads to the disruption of BBB [132]. As a non-invasive and readily repeatable therapeutic modality, FUS has been widely used to improve various chemotherapeutics delivery to brain tumors, including doxorubicin [133], temozolomide [134], and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) [135]. Due to the advantage of focused and defined BBB disruption by FUS, the combination of nanoparticles-based drug delivery system (nanomedicine) coupled with MB-facilitated FUS has attracted tremendous attention in brain tumor-targeted therapy and achieved superior pharmacokinetics (PK) properties in contrast to its free drug [136]. An early study investigated the marriage between FUS and liposomal doxorubicin for enhanced brain targeted drug delivery and antitumor effect in glioblastoma multiforme (Figure 5) [137]. The study tested a human atherosclerotic plaque-specific peptide-1 (AP-1)-conjugated liposomes containing doxorubicin (AP-1 Lipo-Dox) for the targeted drug delivery to the tumor highly expressing interleukin-4 receptors (IL-4R). AP-1 Lipo-Dox was administered intravenously in the experimental brain tumor model, followed by pulsed FUS. It was founded that, compared to control mice treated with AP-1 Lipo-Dox or unconjugated Lipo-Dox, the mice that received liposome coupled with FUS showed an enhanced accumulation of doxorubicin in the tumor region. Moreover, AP-1 Lipo-Dox coupled with FUS increased the doxorubicin ratio of the tumor-to-normal brain by two-fold compared to that in the control group [137]. Consequently, the combination treatment yielded an enhanced antitumor efficiency and reduced side effects on normal brain tissue. Based on a similar approach, Price et al. utilized magnetic resonance image (MRI)–guided focused ultrasound (FUS) together with circulating microbubbles to realize brain tumor gene transfection with the help of systemically administrated “brain-penetrating” nanoparticle (BPN) gene vectors [138]. Besides enhancing nanoparticle accumulation in brain tumors via transient and reversible BBB disruption, FUS could also serve as an external stimulus to control drug release from nanoparticle/MBs. Multifunctional MBs not only acted as an ultrasound contrast agent to facilitate the FUS-induced BBB disruption but also served as drug-carrying vehicles that were responsive to ultrasound for drug release [139]. In this case, the microbubble (BCNU-MBs) had a super high 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) loading capability (68.01 % loading efficiency) and free of premature release. Upon FUS, the intravenous administrated BCNU-MBs readily facilitated local BBB disruption, permeated into the brain tumor, and released BCNU at the targeted site [139]. Attributed to the high BBB penetration and tumor-specific release effect of BCNU-MBs, the progression of glioblastoma multiforme was significantly suppressed (39.6%), and the median survival was extended to 32.5 days [139]. It is of worthy noting that the potential adverse effects from BBB disruption induced by FUS, such as hemorrhage, brain damage,

obnubilation, and brain inflammation, should be closely monitored. To balance between achieving the maximum efficiency for brain tumor and minimizing the potential side effects of the treatment, the parameters of FUS, such as frequency and power, should be carefully optimized [67, 140].

3.4.3 Magnetic resonance-mediated disruption—Apart from severing as an imaging modality for brain tumors, magnetic resonance can also be used to increase the BBB permeability locally [115]. When magnetic nanoparticles are exposed to a gradient/alternating magnetic field, local hyperthermia will be generated from the magnetic nanoparticles as a local heat source through a mechanism named Néel relaxation [141], to induce a substantial but reversible opening of the BBB, which is sensitivity to physiologically relevant temperature change (38–39 °C) [142, 143]. Martel et al. validated the concept of brain-targeted drug delivery by remotely controlling the permeability of BBB through magnetic hyperthermia [143]. In the study, poly (maleic acid-co-olefin)-coated Fe_3O_4 (PMO-MNPs) was injected via a microcatheter, which was inserted into the External Carotid Artery (ECA) and advanced to the Internal Carotid Artery (ICA) using a cannulation technique. Subsequently, the brain was exposed to a low radio frequency (RF) field for 30 min. Evans blue (EB) staining revealed that EB has distributed into the brain parenchyma in the magnetic hyperthermia treated groups, indicating the BBB opening by magnetic heating. Immunohistochemistry analysis of CD68 showed no other trace of CD68 was found in the parenchyma of the magnetic heating treated brain, indicating no apparent immune response to magnetic heating in the brain tissue. Moreover, the amount of fluorescent units counted after 2 h of recovery from magnetic heating was substantially lower than hyperthermia and normothermia. In addition, transmission electron microscopy (TEM) imaging did not reveal abnormal brain structure after 2 hours of recovery. Taken together, the author concluded that magnetic hyperthermia could transiently induce BBB opening, and the opening could be recovered within 2 hours [143]. Noticeably, although EB had a substantive extravascular infiltration into brain parenchyma after magnetic hyperthermia, there was no significant appearance of PMO-MNPs (5–20 nm) in the brain parenchyma, suggesting the disruption of BBB by magnetic hyperthermia might have a limitation of size cut-off. However, in another research, Jin et al. found that MNPs (~100 nm) could permeate the BBB and accumulate in the perivascular zone of the brain parenchyma through a transcellular trafficking mechanism when subjected to an external magnetic field without apparent toxicity [144]. Although the paradoxicality in permeability of MNPs into the brain, all these results proved the practicality, relative safety, and recovery of magnetic hyperthermia-mediated BBB opening. Therefore, further investigation about the size limitation of magnetic hyperthermia-induced BBB opening should be systematically performed. And the parameter of magnetic resonance, such as the strength and frequency of alternating magnetic field (AMF), the size and concentration of MNPs, and the time and region of AMF application, should be optimized to maximize the benefits of magnetic hyperthermia-induced disruption of BBB and minimize the risk of systemic toxicities [145].

3.4.4 Multimodality-mediated disruption—Integrating different BBB disruption modalities for brain tumor treatment could be a promising strategy, which not only can accurately change the permeability of BBB for theranostics agents across the brain but

also can real-time display their penetration efficiency and monitor the treatment outcome. Among them, the integration of MRI with FUS is one of the most commonly explored combinations. Usually, FUS with microbubble is performed to induce the increase of BBB permeability, of which enhanced BBB permeability would be facilely characterized by MRI whenever the MRI contract agents transport across the disrupted BBB and retained in brain tumor, which is similar to FUS induced accumulation of chemotherapy agents or nanoparticles [133]. More significantly, MRI contrast agents can be coupled with the FUS microbubble to serve as an integrated and multifunctional contrast agent for theranostic purposes. Lammers et al. embedded ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles into the shell of poly(butyl cyanoacrylate)-based microbubbles (MB) to yield USPIO-MB, which was used to mediate and monitor BBB permeation (Figure 6) [146]. Upon encountering transcranial ultrasound pulse, USPIO-MB induced vessel permeability through acoustic force. Simultaneously, the structure of the microbubbles was destroyed to release the trapped USPIO. Thus, the available and small size of USPIO (around 5.5 nm) would easily cross the disrupted BBB and accumulate in the parenchyma, which could be evidence of BBB opening and be verified by MRI [146]. [146]. Moreover, by leveraging the high spatial resolution, MRI can also be employed as an invasive method to guide FUS to targeted open the BBB at a specific localization, named MR-guided focused ultrasound (MRgFUS) [147]. Attributing to the fine spatial control over the treatment field and real-time image guidance of transcranial non-invasive MRgFUS, a low-intensity ultrasound could meet the requirement of temporary disruption of the BBB, the region of BBB disruption could also be customized with a special shape and location in the intracranial vault [147], emphasizing its safety and feasibility. Using an MRI-based transport analysis, it was further revealed that apart from BBB/BBTB opening, FUS could also significantly augment brain tumor interstitial flow velocity and change “per voxel” flow direction, which might also play a critical role in enhancing nanoparticles transporting through BBB [138].

Photoacoustic (PA) imaging is a nonionizing imaging technique with a superior imaging capability for deep tissue with high resolution by integrating optical excitation with ultrasound detection. By taking advantage of deep tissue imaging, PA holds great potential for imaging theranostic agents penetrating BBB and entering brain parenchyma by overcoming the skull-led light and ultrasound attenuation [148]. Zheng et al. prepared a multifunctional theranostic nanosystem, named as DOX-HCu, by integrating ultrasmall Cu2–xSe and doxorubicin (DOX)-loaded organic-inorganic hybrid hollow mesoporous organosilica nanoparticles (HMONs) [149]. Upon FUS, DOX-HCu could be accurately delivered into glioblastoma multiforme (GBM) and penetrated the tumor tissue to execute its antitumor mission. More importantly, this process could be visualized through PA imaging [149], realizing real-time triggered drug delivery and monitoring of antitumor outcome.

Another promising strategy for enhancing theranostic agents penetrating BBB and delivery to brain tumors is the joint of BBB disruption and receptor-mediated active targeting. The transient and reversible opening of BBB would pave the way for theranostic agents to enter the brain parenchyma. Whereas receptor-mediated active targeting would guide the theranostic agents, especially nanoparticles, to precisely navigate to brain tumor tissue, thus alleviating non-specific distribution-induced background signal enhancement and side effects. For instance, the combination of FUS and interleukin-4 receptor-targeted

liposomal doxorubicin realized enhanced targeted drug delivery and antitumor effect in glioblastoma multiforme [137]. In the presence of MB, pulsed focused ultrasound waves could transcranially disrupt the BBB, creating the condition for the leakage of pre-injected human atherosclerotic plaque-specific peptide-1 (AP-1)-conjugated liposomes containing doxorubicin (AP-1 Lipo-Dox) into brain tumor, similar as EPR effect. Subsequently, AP-1 Lipo-Dox was internalized by glioblastoma multiforme (GBM) 8401, which highly expressed IL-4 receptors, through receptor-mediated endocytosis [137]. Compared to the control tumor, pulsed FUS could increase liposome distribution in the brain tumor, including AP-1 Lipo-Dox and control Lipo-Dox. In addition, the tumor-to-normal brain doxorubicin of pulsed FUS treated tumor was increased twofold compared to that of control tumors, indicating FUS mediated the disruption of BBB. Moreover, the tumor-to-normal brain ratio is the highest in the mice treated with AP-1 Lipo-Dox followed by pulsed FUS. Consequently, combining sonication with AP-1 Lipo-Dox significantly inhibited GBM growth and increased median survival [137].

3.5 Living cell and its membrane-mediated BBB crossing

Recently, cells with intrinsic tropism towards tumors, such as mesenchymal stem cells (MSCs), neural stem cells (NSCs), macrophages, and neutrophils (NEs) have been explored as “Trojan Horse” for the delivery of cargo across the BBB through cell-mediated transportation [150]. The tumor tropism nature of these cells is mainly attributed to their innate bio-interfacing property or genetic modification. The expression of some receptors or markers on the membrane, such as CXCR4, CX3CR1, and L-selectin, could perceive tumor cells and orchestrate the tumor microenvironment (TME). The TME is featured of chemoattractant gradients of various tumor tissue derived chemokines, such as stromal cell-derived factor-1 (SDF-1), colony-stimulating factor 1 (CSF-1), tumor necrosis factor-alpha (TNF-α), hypoxia-inducible factor-1α (HIF-1α), and interleukin 8 (IL-8) and so on [150]. Although the exact mechanisms of these carrier cells trafficking to the brain tumor is fully understood, and different kind of cells might have different trafficking mechanisms, they may still share several general stages [150–152], (1) capture: the navigating carrier cells in circulation system are recruited to tumor region by chemokines (inflammatory factor) as paracrine by tumor tissue; (2) contact: the carrier cells are tethered to the endothelial surface through the specific interaction between receptor and ligand expressed on the membrane of carrier cells and inflamed endothelium, respectively; (3) rolling, the carrier cells roll along the endothelium and eventually adhere them totally along with elevated interaction; (4) extravasation: under the attraction of chemokine or cytokine gradient, the adhered carrier cells adaptively emigrate to the tumor perivascular region and penetrate tumor mass (Figure 7). Because of this intrinsic homing property, many cargos, such as therapeutic agents loaded nanoparticles, liposomes, immunomodulators, imaging contrast agents, and oncolytic viruses, could be efficiently delivered to brain tumors [150]. Generally, Trojan horse and backpacker are the two main approaches that are used by carrier cells to deliver specific payloads to brain tumors [153]. The former indicates that the cargos are internalized into the carrier cells first and protected inside the cell carriers before they are delivered to the destination, followed by being released from the carriers to excrete their function [153, 154]. The backpacker means that the payloads are attached to the surface of carrier cells through a covalent bond, electrostatic interaction, and/or biological interaction [155]. Similar to a

trojan horse, a package like cargo also should be unloaded from the backpacker-like carrier cells to perform their task. Moreover, cell carriers can be genetically modified to express specific therapeutic agents and directly target, regulate, and kill cancer cells. These secreted therapeutic agents could be cytotoxic proteins, such as TRAIL [156], immunomodulatory-associated cytokines, human interleukin-2 (IL-2) [157], and specific enzymes, such as rabbit CE [158].

Despite living cell-based carriers holding promising potential in brain tumor application, several concerns should be well taken into consideration. The first and foremost is that the loading of theranostic cargos should have negligible influence on the cell viability and their tropism toward brain tumor, which means that the ratio between the number of carrier cells and the cargos concentration, or loading content, is critical for desired delivery efficiency, and should be optimized [159], especially for cytotoxic drugs. Another important aspect is the loaded cargos need to be released from cell carriers in a controlled manner. The desired drug release profile of a cell carrier system is a limited cargo premature release in the circulation system and effective drug unload in the destination, which would achieve a preferable theranostic effect [160]. Moreover, much attention should be paid to the final fate of carrier cells in the targeted destination and throughout the organism after their delivery, especially for the potential adverse effects [150].

As the extension of living cell-mediated BBB crossing, recently, cell membrane cloaked nanoparticles have drawn mounting attention in the diagnosis and therapy of brain tumors [150]. As an aforementioned statement, contact interaction between carrier cells with tropism toward brain tumor cells and tumor surrounding environment is the prerequisite for BBB penetration into the brain. This interaction is mainly modulated by carrier cell-surface receptors and adhesions molecules on the endothelial cells, involving multiple cell-surface and secreted proteins. It does not depend exclusively on the expression of a single protein or ‘receptor’ [158]. Therefore, it was postulated that the membrane-associated biological functions, such as tumor tropism, would be preserved after they are isolated and followed by cloaking onto nanoparticles if their membrane proteins are retained. In 2011, for the first time, Zhang et al. designed this strategy and put it into practice by coating plasma membrane derived from red blood cell (RBC) onto the surface of PLGA nanoparticles [161], creating the precedent of biological membrane mimetic nanoparticles. By integrating membranes from different types of cells, core nanoparticles, and loaded agents according to different objectives, this biomimetic strategy would create a variety of new cell membrane-camouflaged nanocarriers with different functions. This coating generally confers the biomimetic nanocarriers with the capacity to evade confiscation by the RES, prolong the circulation time in the body, cross biological barriers, and actively target disease tissue [162–165]. Inspired by this biomimetic concept, some research works have adopted this strategy for the treatment of brain-related diseases, including glioblastoma multiforme [166, 167], ischemic stock [168, 169], Parkinson’s disease [170], and brain metastases tumor [166]. As to brain tumors, the first research work that adopted this biomimetic strategy was carried out in 2017. Thereof, apart from the coating of RBC membrane on the surface of DOX-loaded PLGA nanoparticles (RBCNP), a CDX peptide, which had a high binding affinity with nicotinic acetylcholine receptors (nAChRs) highly expressed on the surface of brain tumor ECs, was grafted on the surface of RBCNP to

increase the brain tumor targeting ability to yield ^{D}CDX-RBCNPs [167]. By integrating the active targeting effect of CDX peptide and the merits of RBCs membrane coating, such as prolonged blood circulation, good biocompatibility, and low immunogenicity, DOX-loaded ^{D}CDX-RBCNPs exhibited superior therapeutic efficacy with limited toxicity to normal cells in comparison to non-targeting counterpart [167]. Different from RBCs membrane coated nanocarriers integrated with targeted ligand, Liu et al. directly employed brain metastatic tumor cell membranes to camouflage nanoparticles [166]. By inheriting the intrinsic brain-homing property of the brain metastatic tumor cell, the coating of brain metastatic tumor cell membranes would bestow the nanoparticles with BBB penetrating capacity, as well as target brain tumor by a homotypic binding mechanism [171, 172], which offers a new perspective for brain tumor diagnosis and therapy. Recently, our group employed a similar Trojan horse strategy to combat the early-stage brain metastasis of breast cancer (BMBC) with the cell membrane of BMBC cells (Figure 8) [173]. Compared to other brain tumors, the BBB of early-stage BMBC is intact and has not yet deteriorated to form BBTB. Therefore, minimal drugs, including nanomedicines, can penetrate the BBB and reach metastasis lesions, which partially leads to the exclusion of chemotherapy from the standard care for BMBC [174]. In our study, we collected and purified brain-homing breast cancer cells (MDA-MB-231/Br) after several rounds of intracardiac injection and isolation from the brain. It was founded that after camouflaging the cell membrane of MDA-MB-231/Br onto the surface of DOX loaded poly (D, L-lactic-co-glycolic acid) (PLGA) nanoparticles (DOX-PLGA@CM), the DOX-PLGA@CM was endowed the magical ability to penetrate the BBB of normal mice and homing to early-stage of BMBC behind an intact BBB. In vivo study found that DOX-PLGA@CM could significantly slow down the progression of BMBC, reduce tumor burden, and extend the survival time for the mice with BMBC [173]. This study offered a paradigm of integrating drug-loaded nanoparticles and the cell membrane of brain homing cancer cells, which opened a new window for the treatment of BMBC and other brain metastatic cancers.

4. The influence of nanoparticles fabrication parameters on the BBB penetration

In addition to the aforementioned pathological and physiological characteristics of brain tumors, as well as the interaction between BBB and nanoparticles, the parameters of nanoparticles also influence their crossing the BBB and entering brain tumors, including surface modification, size, morphology, and even the loaded cargo. To maximize BBB penetrating efficacy and subsequent therapeutic effect of nanoparticles on brain tumors, those parameters should be optimized during the design and construction of nanoparticles. The following sections briefly summarize the influence of nanoparticles parameters on the BBB transcytosis behavior.

4.1 Surface functionalization

Since both RMT and AMT involve the interaction between nanoparticles and BBB, the primary aim of the surface functionality of nanoparticles is to increase their interaction with BBB to further facilitate them across the BBB into brain parenchyma. These functionalizations include specifical ligand decoration to trigger RMT, surface chemistry

modification to improve pharmacokinetics [175, 176], and surface charge optimization to facilitate AMT, etc.

As for ligand decoration, except for the choice of ligands as discussed above, another important aspect is the ligand density decorated on the surface of the nanoparticles [177]. Unsurprisingly, it is easy to understand that a low density of ligand decoration would not be sufficient to trigger a strong multivalent binding for RMT. In contrast, a too high density of ligand decoration might trap nanoparticles on the luminal side of BBB due to too strong binding and preventing the release of nanoparticles inside the brain [178]. For instance, by tuning the transferrin (Tf) density on gold nanoparticles to optimize their binding to transferrin receptor on brain endothelial cells, Wiley et al. found that 30 Tf and 20 Tf molecules per nanoparticles decoration were the optimized densities for gold nanoparticles with a size of 45 nm and 80 nm, respectively, to realize their best traversing into the brain parenchyma [178]. It is noted that there are no universal principles to define the best number or density of ligands for nanoparticle surface decoration, the optimal ligand density for a brain-targeted nanoparticle is ligand and nanoparticle specific. Another scenario is decorating multiple types of targeting ligands on the surface of nanoparticles, which would make the nanoparticles interact with different receptors simultaneously, increasing BBB penetrating efficacy and brain tissue selectivity [118, 179, 180]. However, the use of a combination of different ligands onto the nanoparticles is an artistic and specialized skill, referring to the choice of ligands, the ratio optimization of different ligands, and the ligand specific grafting technology.

Pegylation is one of the most common surface chemistry functionalizations for nanoparticles [181]. The coating of polyethylene glycol (PEG) on the surface of nanoparticles can prevent them from aggregation, opsonization and phagocytosis, and improve the systemic circulation time by forming a hydrophilic layer on the surface, which subsequently increases the interacting opportunity between nanoparticles and brain vascular endothelial cells [182]. Moreover, the grafted PEG can also serve as a linker between ligand molecules and nanoparticles. However, many parameters of PEG, such as its molecular weight, content, density, and conformation, influence the function and should be optimized during the fabrication of brain tumor-targeted nanoparticles [181]. In addition, some potential drawbacks of Pegylation, such as shielding the exposure of targeting ligands, interfering with the interaction between nanoparticle and targeted cells, and possible activation of anti-PEG immune response, should be thoroughly considered. Except for PEG, others polymers, such as hyperbranched glycerol (HPG) [183], polysaccharide [184], vitamin E TPGS [185], polyvinyl alcohol (PVA) [186] and polyethylene oxide (PEO) [187], etc., are also used as alternative surface chemistry functionalization approaches to improve nanoparticles in vivo pharmacokinetics and brain tumor targeting.

The surface charge of nanoparticles also has a significant influence on their brain tumor targeting capacity and pharmacokinetic properties [188]. It is well known that nanoparticles with positive charges could readily interact with negatively charged cell membranes through electrostatic interaction, followed by triggering AMT [188, 189]. However, highly positively and negatively charged nanoparticles could alter and disrupt the integrity and permeability of BBB [190], facilitate the formation of protein coronas that would shield and compromise the

functional modification of the surface [191], and be cleared from blood circulation system [192]. Another main aim of surface functionality, including the Pegylation mentioned above, is to optimize the surface zeta potentials of nanoparticles to increase their stability and limit their interaction with RES.

4.2 Size

Size is a fundamental feature of nanoparticles. Due to the existence of the inter-endothelial gap (IEG) of BBTB, the size of the nanoparticles is one of the most vital parameters affecting nanoparticles penetrating across the BBB, especially for paracellular leakage pathways [23, 130]. Along with the aggravation of brain tumors, IEG gradually appears and increases, reaching 550 nm in some regions of glioblastoma [37]. Moreover, the IEG is usually of full heterogeneity, driven by different pore sizes of IEG in various types of brain tumors, different IEG in different grades of brain tumors, and the differentiated distribution of IEG within other regions of the same brain tumor. All these heterogeneities of IEG lead to the heterogeneous distribution of theranostics agents in tumors [23, 193]. Sarin et al. used different size Gd labeled polyamidoamine dendrimers to systematically investigate the physiologic upper limit of pore size of rodent malignant gliomas through dynamic contrast-enhanced MRI [194]. It was revealed that along with the increase of particles size from generation 5 (G5) to generation 8 (G8) (approximately 2 nm increase), there was a significant decrease in nanoparticles penetrating across the BBTB to accumulation in the orthotopic RG-2 glioma tumor, and nearly no extravasation of G8 dendrimers across the BBTB. Therefore, the upper limit of pore size within the BBTB of orthotopic RG-2 rat gliomas was approximately 12 nm. Meanwhile, it was founded that the fibrous glycocalyx coated on the surface of the pore in the BBTB was the major obstacle to the transvascular extravasation of particles across the BBTB of brain tumors [194]. It was noted that although G1-G4 dendrimers with smaller sizes could traverse the BBTB, their signal-to-background ratios were weak and attenuated rapidly due to their blood half-time derived from the fast excretion via renal filtration [195]. These investigations indicated that when employing paracellular leakage pathways, the size of nanoparticles should be optimized with the consideration of balancing between their extravasation across the BBTB and their filtration through the kidney. Different from the influence of nanoparticles size on IEG depending on paracellular extravasation, the influence of nanoparticle size on the transcytosis penetration is moderated. The nanoparticles systemically administrated usually have a diameter between 30–200 nm, which can realize the coordination of “Trojan horse” delivery and long half-life [177].

4.3 Shape

Currently, most nanoparticles developed for brain-targeted delivery are spherical. However, several recent studies showed that nanoparticles’ morphology, including nanosphere, nanostar, nanorod, and nanocage, would greatly influence their BBB passage by affecting nanoparticle-cell interaction [196, 197], which is a critical step for transcytosis. A previous study found that particles with a high aspect ratio showed a higher cellular uptake by tumor cells due to their high surface area, which facilitates the multivalent interaction between nanoparticles and cell membranes [198]. Specific to transcytosis of endothelium cells, Poornima et al. systemically probed the influence of ligand-displaying nanoparticles’

shape (nanorod vs nanosphere) on their specificity to lung and brain endothelial [199]. It was revealed that, in the microfluidic system that mimics the vasculature in vitro, nanorod displayed a higher selectivity and efficacy than that of nanosphere and exhibited high specific and lower nonspecific accumulation in the targeted region. In vivo study in mice further confirmed that nanorods showed a seven-fold enhanced vascular accumulation compared to that of nanosphere [199]. A similar phenomenon was also observed on gold nanorods (AuNRs) [200]. Lee et al. developed an interesting gold nanorod, RVG-PEG-AuRNs@SiO2, by mimicking the rabies virus in terms of size, surface glycoprotein property, and especially the shape with an aspect ratio of 2.34, which was very close to that of a live rabies virus (2.4) [200]. It found that the rod shape of AuNRs played a crucial role in increasing RVG29 mediated cellular uptake, BBB active targeting, and BBB penetration. Ruling out the influence of RVG29 ligand, AuNRs were easily entering N2a cells in vitro, and this merit was more prominent in the orthotopic glioma tumor model. It was emphasized that although the RVG29 ligand decorated on the surface of AuNRs could trigger the receptor (nicotinic acetylcholine receptor, AchR) mediated active targeting, the elongated shape of AuNR would increase the chance of interaction between the ligand on the nanoparticle surface and its receptor on the cell membrane due to a greater surface area [176, 199]. In addition, the shape of the nanoparticles may also have a significant influence on their pharmacokinetics in vivo [175, 201]. These results suggested that the shape of nanoparticles should also be carefully considered when utilizing nanoparticles to realize the diagnosis and therapy of brain tumors.

4.4 Cargo

Apart from being used for tumor diagnosis, imaging, and therapeutic agents, many BBB regulator agents can also be loaded into the nanoparticles as an auxiliary approach to promoting the nanoparticle penetrating the BBB to brain tumor through pharmacological and physical modulation strategies with or without external stimulus [136, 202]. In the strict sense, the cargo loaded in the nanoparticles is not a parameter of the nanoparticles. Whereas, because of its flexibility and accessibility, the loaded BBB regulator could be an auxiliary parameter of the delivery system. These disrupting cargos are divided into two categories, chemical disruption agents and physical disruption agents[136]. Universal chemical disrupting agents include osmotic agents [203], vasodilator [130, 166], and efflux pump inhibitors [136], etc.. One prominent feature of these disrupting cargos is their enhanced and selected BBB disruption and reduced systemic side effects by leveraging the nanoparticles. For example, lexiscan, an adenosine receptor agonist with a definite capacity for modulating the permeability of BBB, was loaded into active breast cancer brain metastases (BCBMs) and shrinkable nanoparticles (LANPs) [128]. LANPs were decorated with AMD3100, a ligand that can actively recognize and bind the overexpressed CXCR4 on tumor cells and could respond to the tumor microenvironment (enriched neutrophil elastase) to shrink their size. These two prominent characterizations of LANPs made lexiscan efficiently open the BBB without remarkable side effects, cooperatively inhibiting BCBMs growth and prolonging the survival of BCBMs bearing mice [128]. A similar strategy was also applied to minoxidil, which could boost transcytosis and down-regulated the tight junction proteins, to endow the nanoparticles (M@HNPs) with enhanced transcellular and paracellular BBB surmounting capacity [130]. As to physical disruption

agents, most of the nanoparticles themselves play the role of BBB disruption and vehicle simultaneously, represented as magnetic iron oxide nanoparticles (IONP). IONP can not only act as the classic magnetic imaging contrast for T2 MR imaging, but also leverage the low radiofrequency magnetic field to produce hyperthermia, which had been verified to transiently increase BBB permeability and drug penetration to brain tumor [67]. Another representative agent is microbubble, one of widely adopted focused ultrasound (FUS) contrasts. The microbubble can not only execute cavitation activity mediated mechanical stress to increase the transcytosis activity and induce BBB temporary open [204, 205] but also can serve as the reliable vehicle of ultrasmall nanoparticles [206] and small molecular drug [207] to realize the FUS augmented brain tumor imaging and treatment.

Besides promoting nanoparticles penetrating the BBB through tuning the permeability of BBB, some loaded cargoes (or detachable blocks) could change the properties of the nanocarriers themselves, such as the charge, size, shape, and surface geomorphology, by leveraging the pathophysiological features of the brain and/or external stimulus, to facilitate the nanocarriers penetrate brain tumors [208, 209]. Therefore, the investigations involving potentially multifunctioal cargoes, adaptive nanocarriers and their rational mechanism would pioneer a new methodology of cargo-driving nanocarriers penetrating the brain and flourishing drug delivery systems-assisted theranostic for brain tumor.

5. The potential challenges and future directions

Unlike peripheral tumors, the major challenge for the diagnosis and therapy of brain tumors is the presence of the substantive physiological barrier, such as BBB, which severely prevents the access of diagnostic and therapeutic agents to brain tumors. Although various BBB crossing/disturbing strategies with different advantages have been explored, they also encounter some inevitable limitations (Table 3). Nevertheless the BBB would deteriorate to yield BBTB as the progress of brain tumor, in which the integrity of the physiological barrier is partially broken, the resulted fenestration remains an obstacle for the permeation of most agents, especially for nanoparticles. Therefore, nanoparticle-mediated drug delivery systems represent a promising approach for improving brain tumor chemotherapy. On the one hand, compared with free drugs, NPs are endowed with unique pharmaceutical properties, such as high drug loading, controlled drug release features, and prolonged half-life. On the other hand, attributed to the exclusive recognition between ligand decorated on the surface of NPs and the corresponding receptor expressed on the surface of brain endothelial cells, an improved cargo delivery to the brain would be realized by RMT [188]. Even so, due to the competitive binding with a high concentration of endogenous ligands [189] and reduced targeting efficacy induced by the rapid formation of plasma protein corona on the nanoparticle surface in the circulation system [210], the accumulation of most NPs in the brain via RMT is still lower than 1% [211], which may not be adequate to yield a desired therapeutic response. The multiple targeting and/or multistage targeting by multi-ligands mediated active targeting may not only enhance BBB penetration of nanoparticles but also distinguish the normal brain tissue and brain tumor, thus achieving enhanced antitumor efficiency and reducing potential side effects.

Temporary disruption and opening of BBB by chemical and physical methods is a promising method to enhance therapeutic drugs across the BBB for enhanced diagnosis and therapy of brain tumors. On the one hand, the opening of BBB may be well controlled not only by manipulating the mechanical parameters, such as ultrasound intensity and frequency, magnetic field intensity, and intervention time but also by choosing the optimal time and location to implement the intervention and real-time monitoring the outcome by integrating multimodality imaging into the system [146]. On the other hand, the combination of temporary disruption with other brain active targeting strategies, such as FUS plus RMT, would realize an enhanced targeted drug delivery and achieve boosted anti-cancer efficacy [137]. It is also noted that further study should be performed to uncover the mechanism of BBB opening and recovery, which might be critical for their clinic application [147]. Meanwhile, it is vital to monitor the potential adverse effects associated with the opening of BBB [212].

Although possessing promising potential in treating and detecting brain tumors, living cells and their derivatives-based delivery systems are still in their infancy. There are several concerns that must be taken into careful consideration before their translation to clinic practice. First, the mechanism behind the brain tumor tropism and BBB penetration of living cells should be extensively explored to balance the potential theranostic benefits and side effects associated with the treatment. Second, how to maintain and strengthen the inherent characteristics of the cells during external expansion and cargo loading to achieve a reliable strategy should be investigated. Thus, developing a standard operating protocol for utilizing living cells is well desired. Moreover, living cells-based diagnosis and therapy may bring broad application prospects to individual precision medicine, but how to reduce the time and financial cost of developing a versatile and reliable platform should be considered for general applications in CNS disease therapy.

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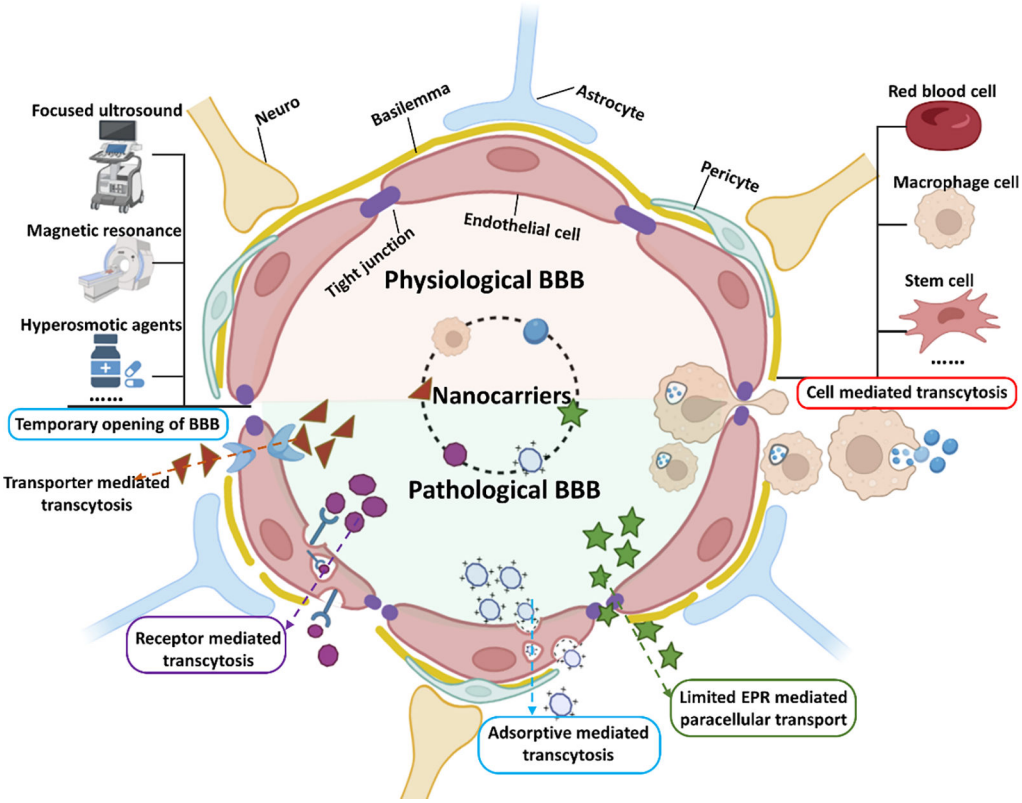

Figure 1.

The structure of BBB and recent emerging strategies for drugs and theranostic agents crossing BBB for treating brain cancer and cancer brain metastases.

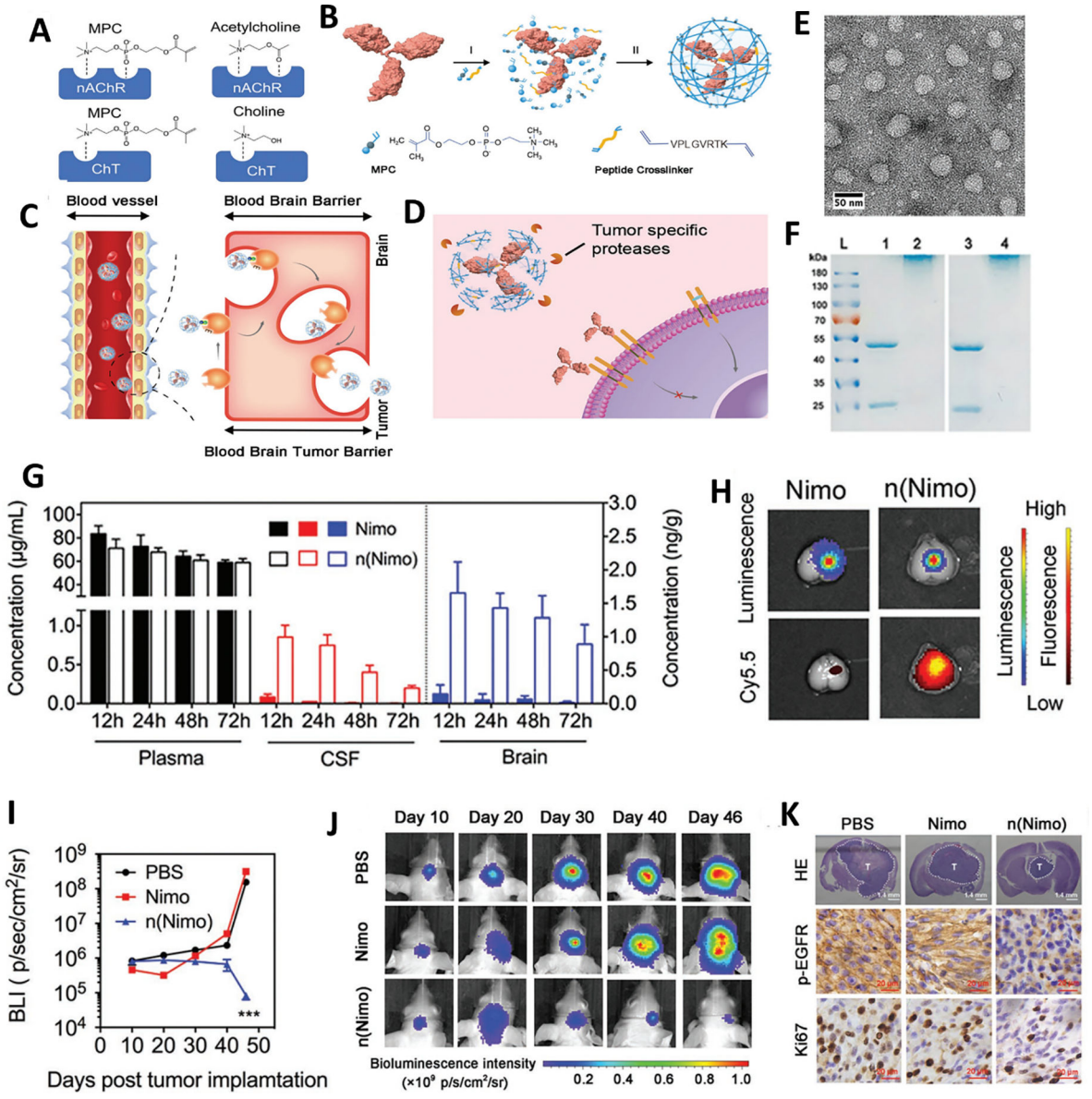

Figure 2.

Nicotinic acetylcholine receptor (nAChR) mediated BBB penetration of nimotuzumab for brain tumor therapy. (A) Schematic illustrating the interaction between nAChRs and MPC. (B) The synthetic schemes of nanocapsules that contain acetylcholine and choline analogs. (C) nAChRs mediated BBB penetration of n(Nimo). (D) Nimotuzumab releases from n(Nimo) with the help of MMP-2. (E) The representative TEM image of n(Nimo). (F) SDS-PAGE of (L) ladder, (1) nimotuzumab, (2) n(Nimo), (3) trastuzumab, and (4) n(Tras). (G) The concentration of free nimotuzumab and n(Nimo) in the plasma, CSF, and brain. (H) Representative ex vivo fluorescence images of cy5.5 labeled nimotuzumab and n(Nimo) distribution in brain tumor-bearing brain. Bioluminescence intensity (I) and representative bioluminescence image (J) of the glioma-bearing mice at different times after receiving different treatments. (K) Representative images of the HE-stained brain,

immunohistochemistry staining of p-EGFR expression and Ki67 in different treatment groups at 46 days. Adapted with permission from ref. [84].

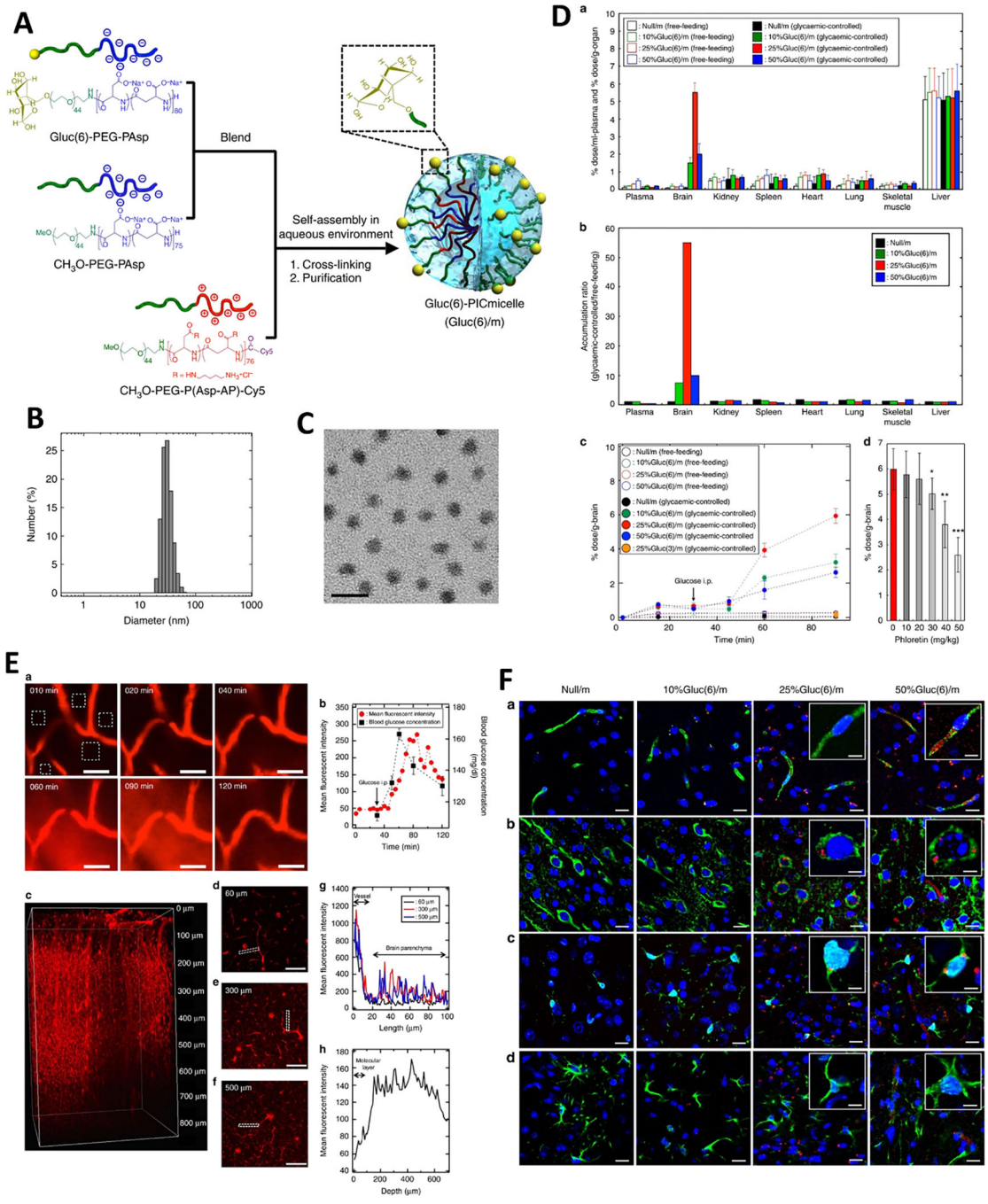

Figure 3.

Glycaemic control boosts glucosylated nanocarrier crossing the BBB into the brain through glucose transporter-1 (GLUT1). (A) Scheme of Gluc(6)/m preparation. (B) DLS size distribution of 25% Gluc(6)/m. (C) TEM image of 25% Gluc(6)/m, scale bar=50 nm. (D) Da). Biodistribution of different micelles in different organs under different feeding conditions at 48 h after injection. Db) Relative accumulation ratio of glycaemic-controlled micelle/free-feeding micelle in various organs calculated from the value from Da. Dc) Time course of different micelles accumulation in brain under different feeding conditions. Dd)

In vivo inhibition study of the brain accumulation of glycaemic-controlled/free-feeding by phloretin. The data are expressed as the mean ± SEM, n=5, *p<0.05. E Real-time observation of the 25%Gluc(6)/m crossing the BBB. (E) Ea) Representative images of 25%Gluc(6)/m distribution in mouse cerebrum at different time by using IVRT-CLSM. Scale bar is 50 μm. Eb) Time course of blood glucose concentration and the mean fluorescent intensities of the 25%Gluc(6)/m in the region of interest (ROI) in (Ea). Ec) Images of Gluc(6)/m in the cerebrum observed using intravital multiphoton microscopy 48 h after administration. Cross-sections at depths of 60 μm (Ed), 300 μm (Ee) and 500 μm (Ef) are also shown. Scale bar is 100 μm. Eg) Distribution profiles of Gluc(6)/m from blood vessels to the brain parenchyma in the selected region in (Ed), (Ee) and (Ef). Eh) Depth profiles of the mean fluorescent intensities from 0 to 700 μm in the brain parenchymal region. (F) Immunohistochemical analysis of the mouse brains Cerebral sections at 48 h after administration of different polymeric micelles. BCECs (Fa), neurons (Fb), microglia (Fc) and astrocytes (Fd) (green) are stained by corresponding antibodies. Nuclei (blue) are stained with DAPI. Scale bar is 20 μm (10 μm in insets). Adapted with permission from ref. [93].

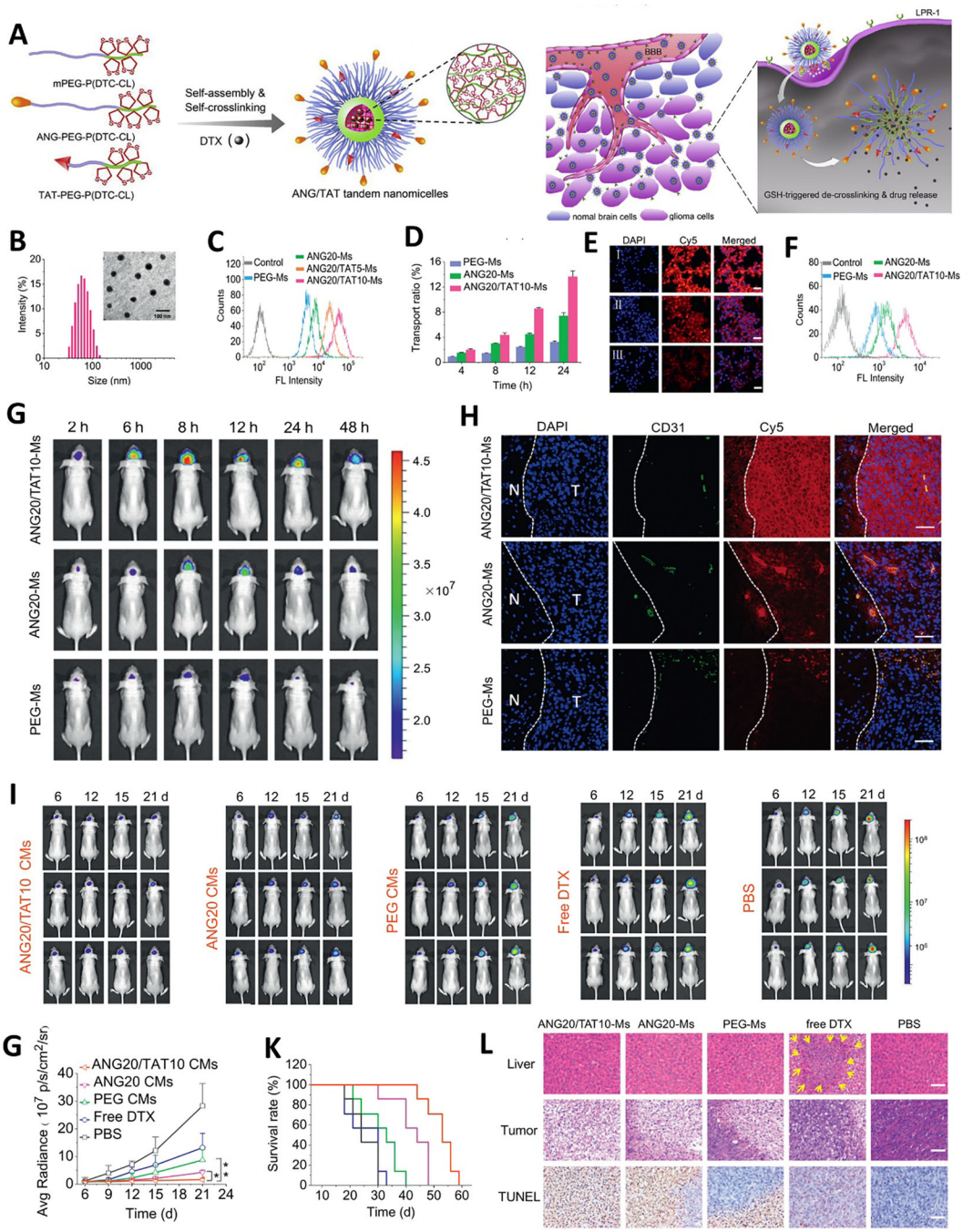

Figure 4.

Cell-penetrating peptides TAT mediated BBB penetration of nanomedicine for brain tumor therapy. (A) Schematic illustrating the preparation of ANG/TAG tandem nanomicelles (ANG/TAT-Ms) and their in vivo anti-glioma pathway. (B) The size distribution of ANG/TAT-Ms by DLS and TEM. (C) Cellular uptake of different nanoparticles in U87MG after 4 h incubation by FCS. (D) Cumulative transport of different particles across in vitro BBB model. Cellular uptake of different nanoparticles after transporting the in vitro BBB model by U87MG by CLSM (E) and FCS (F). (G) In vivo distribution of different nanoparticles

in orthotopic U87MG glioma bearing mice. (H) The penetration behavior of different nanoparticles in orthotopic U87MG glioma observed by CLSM. In vivo anti-orthotopic U87MG glioma evaluation of different treatments monitored by bioluminescence imaging (I) and bioluminescence intensity (G). (K) Kaplan-Meier survival curves of mice in different treatment groups. (L) H&E staining of liver and tumor and TUNEL assay of tumor in different treatment groups. Adapted with permission from ref. [118].

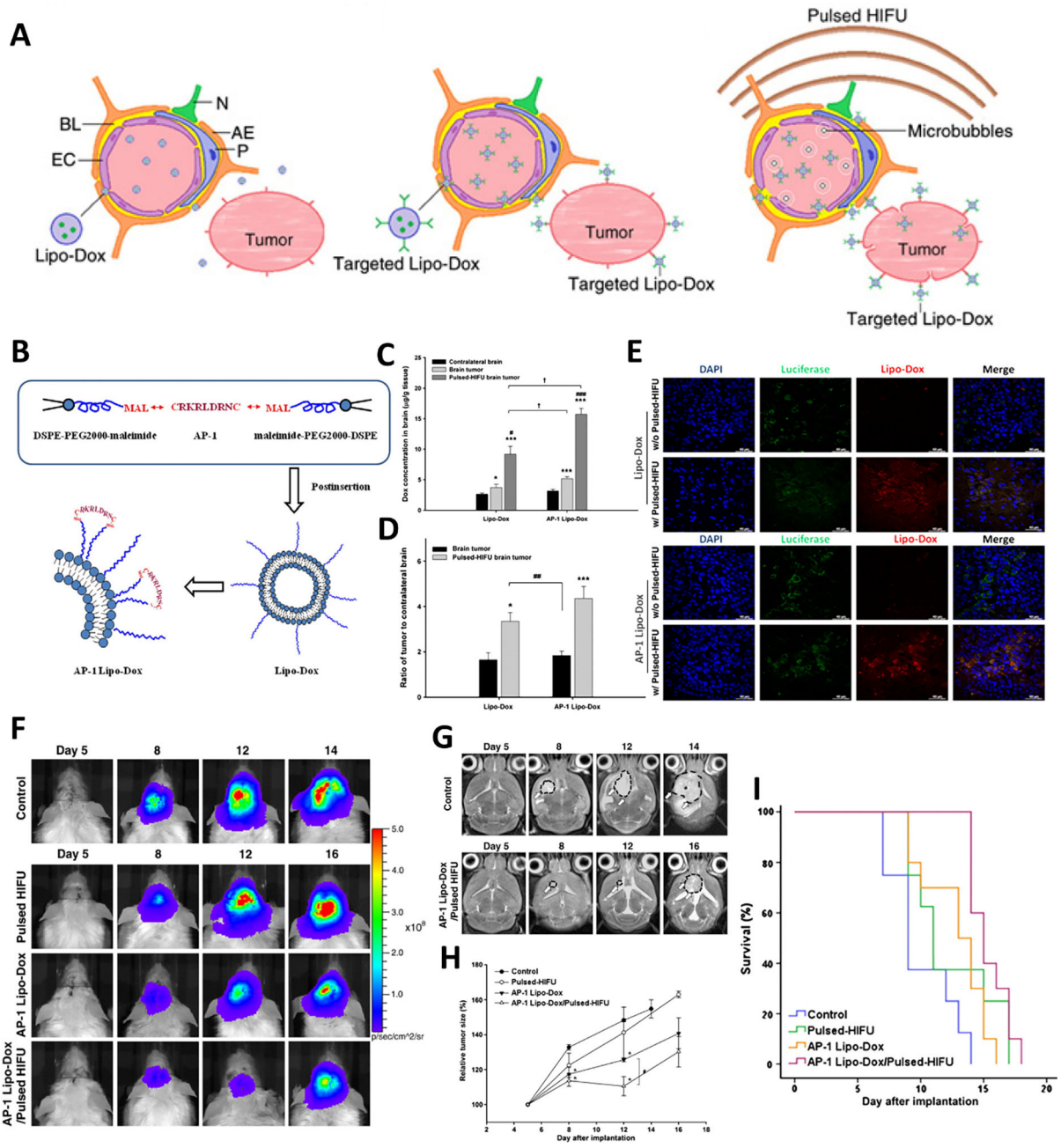

Figure 5.

Focused ultrasound and interleukin-4 receptor mediated BBB penetration of liposomes for glioblastoma multiforme therapy. (A) Schematic illustration of receptor and FUS enhanced BBB penetration of DOX loaded liposome and superior anti-brain tumor. (B) Schematic illustrating the preparation of DOX loaded AP-1 conjugated liposomes. Measurements of Lipo-Dox and AP-1 Lipo-Dox in the brain tumor (C) and contralateral normal brain regions (D) with or without sonication. (E) Immunocytochemistry staining of brain tumor to evaluate the distribution of Lipo-Dox and AP-1 Lipo-Dox in glioma xenografts with/without FUS. (F) Bioluminescence imaging of glioma xenografts in different treatment groups to evaluate the anti-tumor efficiency. (G) Representative T2-weighted magnetic resonance

imaging of glioma xenografts in the brain in different treatment groups. (H) Relative tumor growth trend of GMB 8401 glioma xenografts in different treatment groups (relative to day 5). (I) Kaplan–Meier survival plot of GBM-bearing mice in different treatment groups. Adapted with permission from ref. [137].

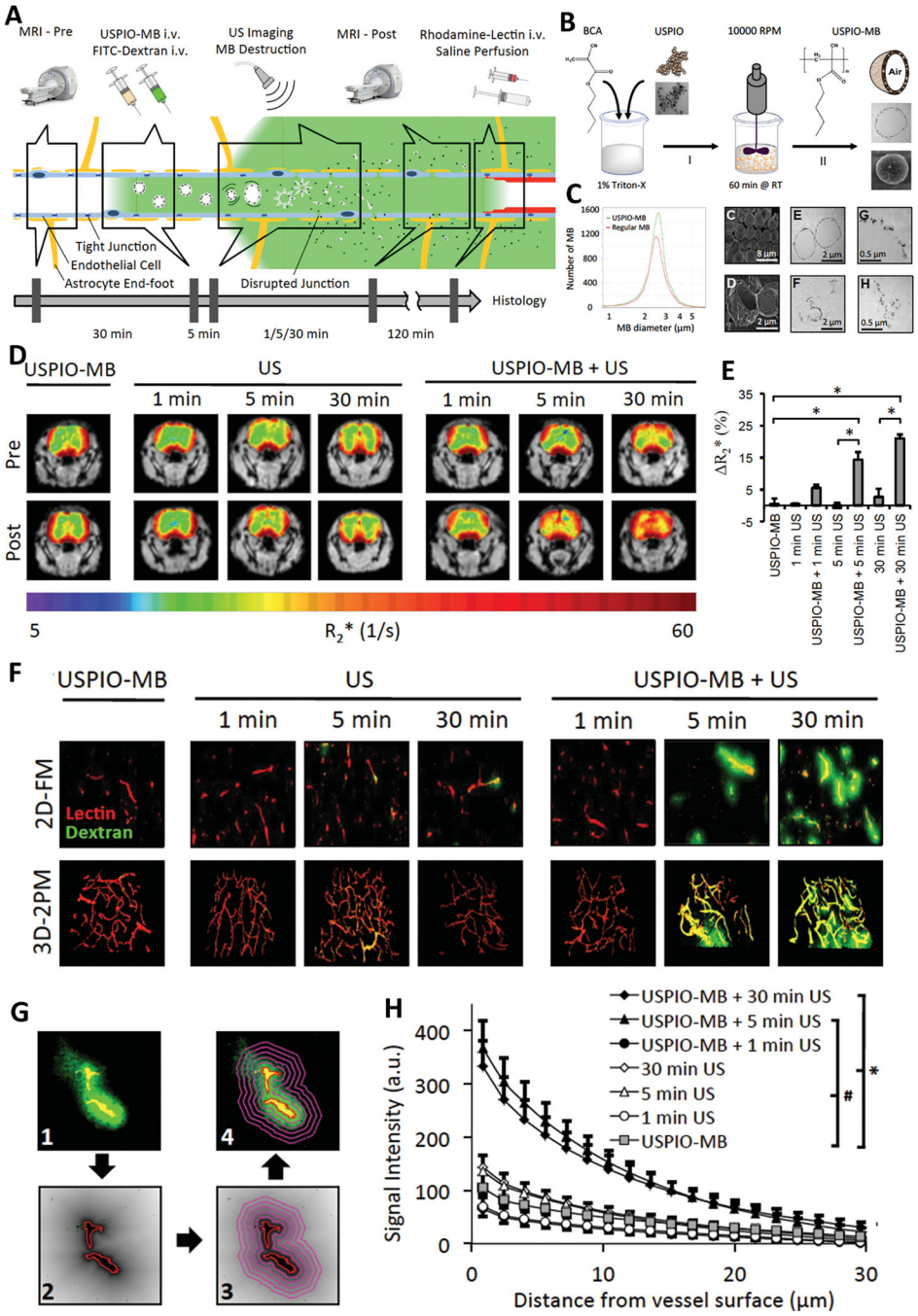

Figure 6.

Theranostic USPIO-MB for mediating and monitoring BBB permeation. (A) Schematic illustration of FUS-mediated disruption of BBB and MRI-based monitoring of BBB permeation and drug delivery across BBB by using USPIO-MB. (B) Schematic illustrating the preparation of USPIO-MB. (C) Characterization of USPIO-MB with DLS, TEM, and SEM. (D) MRI imaging of USPIO deposition across the BBB upon USPIO-MB destruction. (E) Quantification of R2* values in D. (F) 2D fluorescence (2D-FM) and 3D two-photon microscopy (3D-2PM) imaging of FITC-dextran (green) and Rhodamine-lectin-stained

blood vessels (red) to evaluate the BBB permeation. (G) Schematic illustrating the protocol of quantifying the extravasation and penetration of FITC-dextran. (H) Signal intensity of extravasated FITC-dextran as a function of distance to vessel surface. Adapted with permission from ref. [146].

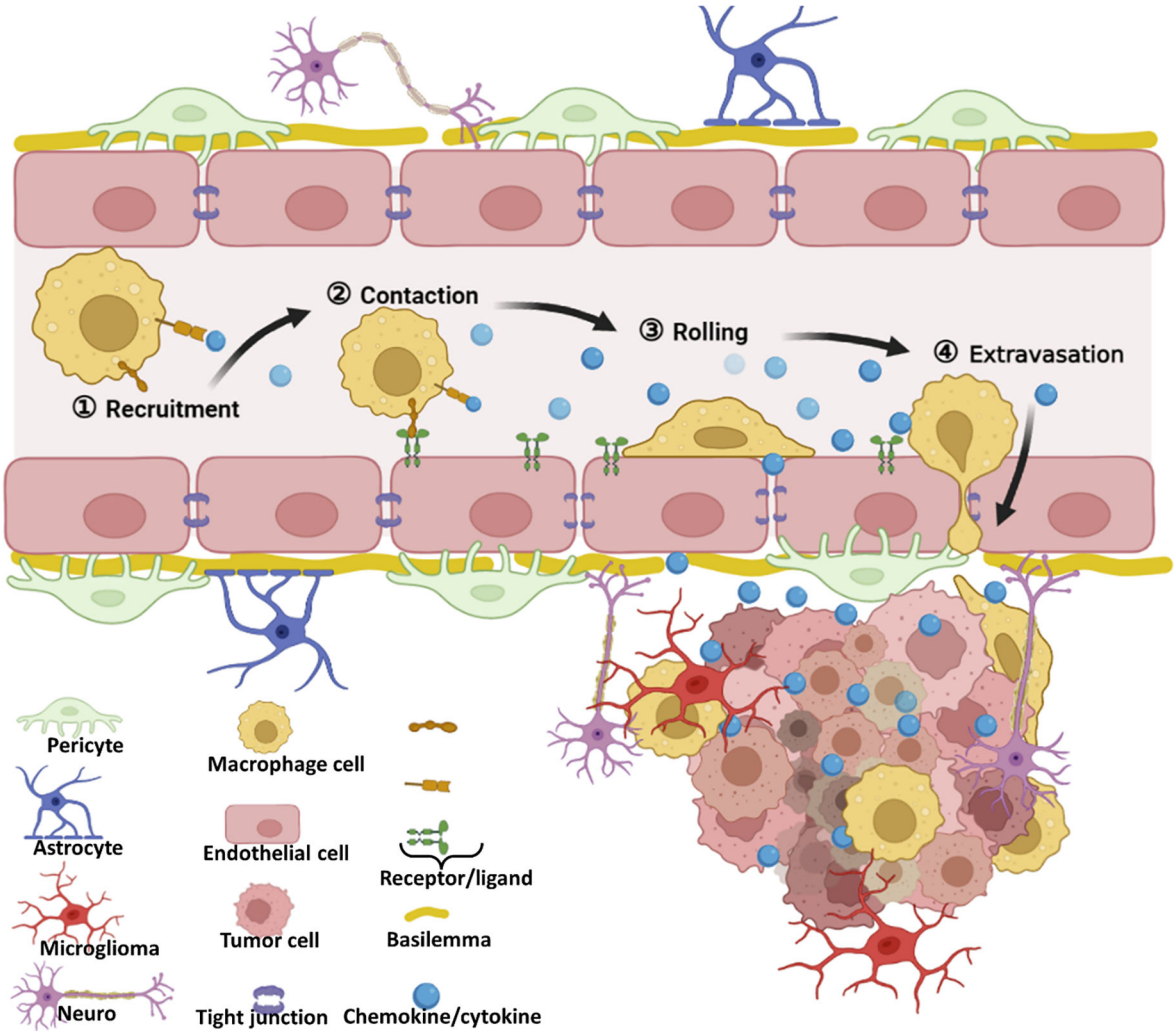

Figure 7.
The potential pathway of living cell-mediated BBB crossing.

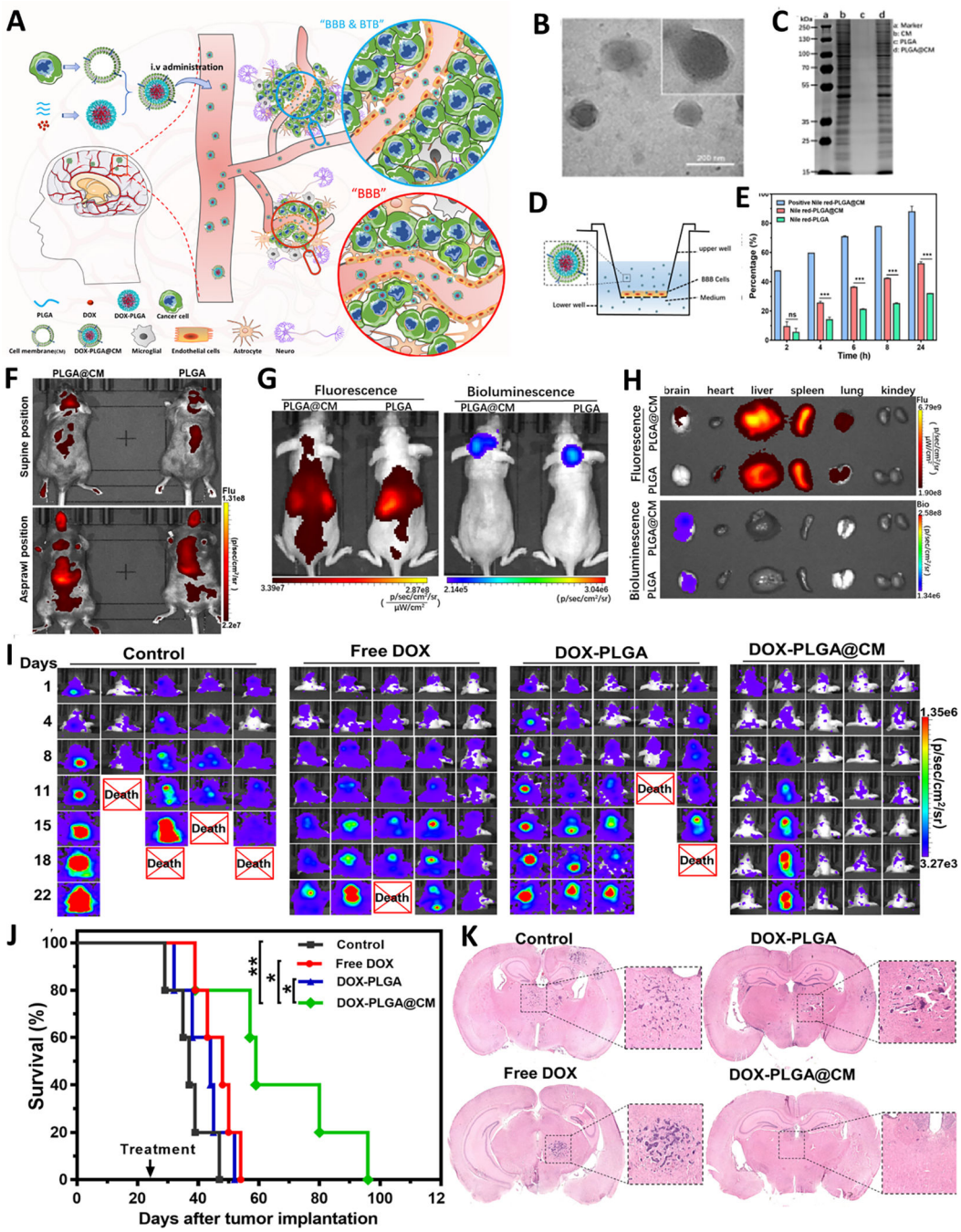

Figure 8.

Brain metastatic tumor cell membrane mediated nanoparticles BBB crossing for the treatment of brain metastasis of breast cancer. (A) The schematic illustration for the fabrication of DOX@PLGA@CM and its BBB penetration. B The TEM of DOX@PLGA@CM. (C) The protein analysis of CM on the surface of PLGA@CM by SDS-PAGE. (D) The scheme of BBB crossing of PLGA@CM in vitro BBB model. (E) Quantitative analysis of transcytosis efficiency of PLGA@CM in vitro BBB model. (F) In vivo fluorescence imaging indicating the distribution of DIR labeled PLGA@CM in

normal C57BL/6J mice. (G) In vivo fluorescence imaging indicating the distribution of DIR labeled PLGA@CM in BMBC model. (H) Ex vivo fluorescence imaging of DIR labeled PLGA@CM in different organs of BMBC model mice. (I) In vivo bioluminescence imaging of BMBC in different groups at the indicated time. (J) Kaplan–Meier survival curves of BMBC bearing mice in different treatment groups. n=5. (K) Representative whole brain H&E staining of BMBC bearing mice in different treatment groups. Adapted with permission from ref. BMBC [173].

Table1

Summary of clinical trials of nanoparticles based strategies for the diagnosis and treatment of brain tumors

Name	Vehicle	Cargo	Brain tumor	Objective	Status of Clinical trial	Delivery target and ligand
Caelyx [24, 25] (liposomal doxorubicin)	Liposome	Doxorubicin	Recurrent malignant glioma	Chemotherapy	Marketed (off-label employment)	Passive targeting (EPR)
Ferumoxytol [26]	Ultra-small superparamagnetic iron oxide	/	Recurrent and progressed malignant glioma	MRI imaging	Phase I	EPR
Nab paclitaxel (Abraxane) [27]	Albumin nanoparticles	Paclitaxel	Breast cancer, brain metastasis of breast cancer	Chemotherapy	Marketed (off-label employment)	EPR
AGuIX [28]	Gadolinium-based nanoparticle	/	Multiple brain metastases	Radiosensitizer Imaging enhance	Phase II	EPR
MTX110 [29]	Gold nanoparticle	Panobinostat	Diffuse Intrinsic Pontine Glioma	Antineoplastic activity	Phase II	Interventional (Convection-Enhanced Delivery)
CPT-11 [30]	Liposome	Irinotecan	Recurrent High-Grade Gliomas	Chemotherapy	Phase I	EPR
SGT-53 [31]	Cationic liposome	p53 cDNA	Recurrent Glioblastoma	Gene therapy	Phase II	Transferrin receptor; anti-transferrin receptor single chain antibody fragment
EGFR(V)-EDV-Dox [32]	Nanocells	Doxorubicin	Recurrent Glioblastoma Multiforme	Chemotherapy	Phase I	Epidermal growth factor receptor (EGFR); bispecific antibodies
2B3-101 [33]	Liposome	Doxorubicin	Breast Cancer and Leptomeningeal Metastases	Chemotherapy	Phase II	GSH transport; Glutathione

Table 2

Nanocarriers utilize Transferrin receptor mediated transcytosis.

Ligands	Nanoparticles	Cargoes	Cell lines	application
T7 peptide (HAIYPRH)	Liposomes	Anti-EGFR siRNA	U87	Decrease EGFR, anti-glioma[46]
Transferrin, cell penetrating peptide PFVYLI (PFV)	Liposomes	doxorubicin (Dox) and Erlotinib (Erlo)	U87, brain endothelial (bEnd.3)	Anti- glioblastoma tumor [47]
T7 peptide	PLGA nanoparticles, magnetic nanoparticles	Paclitaxel, curcumin	U87, bEnd.3	Anti- glioblastoma tumor [45]
Transferrin peptide (Tf_{pep})	gold nanoparticles	photodynamic pro-drug, Pc 4	LN229, U87	Fluorescence imaging [48]
Transferrin	Carbon dots	Dox	SJGBM2, CHLA266	No in vivo data [49]
Transferrin, transferrin receptor monoclonal antibodies (OX26 or R17217)	Human serum albumin	Loperamide	No data	Anti-nociceptive effects [50]
Y-shaped ligands (with Tat peptide and transferrin)	iron oxide (Fe3O4) particles	Chlorin e6	U87-MG	Photodynamic glioblastoma tumor therapy [51]
Transferrin	magnetic silica PLGA nanoparticles	Dox, paclitaxel (PTX)	U87-MG	Anti-brain glioma [52]
EGF peptide (YHWYGYTPQNVI), Transferrin peptide (HAIYPRH)	gold nanoparticles	photosensitizer phthalocyanine 4 (Pc 4)	LN229	Photodynamic glioblastoma tumor therapy [53]
Transferrin	carbon dots	Epirubicin, temozolomide	SJGBM2, CHLA266, CHLA200 U87	Anti- glioblastoma tumor [54]
D-T7 peptide	PEGylated bilirubin nanoparticles (BRNPs)	PTX, cediranib	bEnd.3, C6 cells	Antiangiogenesis and Chemotherapy of Glioma [55]

Table 3

Pros and cons of different BBB crossing/disturbing strategies

Strategies	Methodology	Pros	Cons
Passive targeting	/	Simple structure of nanoparticle; High specificity (lesion dependent distribution); safety	Heterogeneous distribution; low distribution; brain tumor type and stage dependent
Biological interfacial interaction	Receptor/ Transporter-mediated transcytosis	Receptor/transporter mediated high efficiency and relative high distribution; Active targeting and multiple targeting mediated highly specific distribution in brain tumor	Relative complicated and high-cost nanoparticles; Off-target distribution deriving from non-exclusive distribution of receptor/ transporter; Compromised target ability due to the competition from endogenous ligand and the formation of protein corona
Physicochemical interaction	Adsorptive mediated transcytosis	High transcytosis efficacy; High distribution in brain tumor;	No-specific high distribution in brain; limited tissue selectivity; Potential system toxicity
	Temporary BBB opening and disrupting	Highly efficient, enables focus on targeted region; temporary and reversible BBB opening; most method approved in the clinic;	Potential side effects, such as increased intracranial pressure, chronic inflammation; Non-specific opening of the BBB;
Living cell	/	High specificity; High efficiency; High carry capacity; Decreased drug immunogenicity	Complicated operation; High cost; Heterogeneous outcome; Potential system toxicity; Poor spatial and temporal control of cargoes release