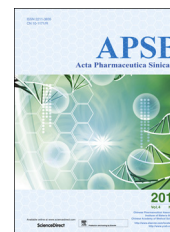




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REVIEW

Brain tumor-targeted drug delivery strategies



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Abstract Despite the application of aggressive surgery, radiotherapy and chemotherapy in clinics, brain tumors are still a difficult health challenge due to their fast development and poor prognosis. Brain tumor-targeted drug delivery systems, which increase drug accumulation in the tumor region and reduce toxicity in normal brain and peripheral tissue, are a promising new approach to brain tumor treatments. Since brain tumors exhibit many distinctive characteristics relative to tumors growing in peripheral tissues, potential targets based on continuously changing vascular characteristics and the microenvironment can be utilized to facilitate effective brain tumor-targeted drug delivery. In this review, we briefly describe the physiological characteristics of brain tumors, including blood–brain/brain tumor barriers, the tumor microenvironment, and tumor stem cells. We also review targeted delivery strategies and introduce a systematic targeted drug delivery strategy to overcome the challenges.

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1. Introduction

According to the GLOBOCAN 2008, the worldwide cancer incidence of malignant brain tumors is 3.5 per 100,000 people and about 650 people are diagnosed with malignant brain tumors every day¹. Brain tumors severely threaten human health due to their fast development and poor prognosis. Glioma, the most frequent primary brain cancer, accounts for 29% of all primary brain and CNS tumors and 80% of malignant brain tumors². The median overall survival of patients with glioblastoma (GBM) is only 14.6 months after current multimodal treatment—aggressive surgical resection followed by concurrent or sequential radiation and temozolomide chemotherapies³. One reason why poor prognosis and rapid recurrence are associated with this standard therapy is that the infiltrate growth of gliomas makes it difficult for the surgeon to completely remove pathologic or cancer-infiltrated tissues without affecting normal brain functions⁴. Furthermore, the failure is also ascribed to the side effects of radiotherapy and poor outcome of usual chemotherapy. For many years, researchers have endeavored to deliver therapeutic agents to the tumor region effectively and reduce unnecessary drug accumulation in normal brain and peripheral tissues. For brain tumors, active targeted drug delivery systems have attracted extensive attention in recent decades. Since brain tumors possess many distinctive characteristics from peripheral tumors due to their complicated oncogenesis, many factors must be taken into consideration for effective brain tumor-targeted drug delivery, such as the barriers included in the whole process, the tumor microenvironment, and tumor cells. Now various targets have been exploited to achieve the targeting therapy using nanocarriers. Herein we provide a brief review of several possible targeting delivery strategies for brain tumors.

2. Barriers to targeted drug delivery strategies

The oncogenesis of gliomas is complicated, with various barriers preventing drug from reaching the tumor sites. There are three main barriers for brain tumor treatment: the blood–brain barrier (BBB), the blood–brain tumor barrier (BBTB), and a relatively weak EPR effect. Specific brain tumor development stages require corresponding barrier targeting treatment strategies.

2.1. BBB targeting strategies and related drug delivery systems

At the early stage of brain tumor development and at the infiltration growth region of the tumor, the blood–brain barrier remains intact. The blood–brain barrier, which acts as a natural guard to protect the brain from harmful substances in the blood stream while supplying the brain with the necessary nutrients for proper function, is the key challenge for delivering drugs to brain tumor⁵. The BBB is a specialized system of capillary endothelial cells which are partially covered by pericytes and basement membrane, and almost fully surrounded by the end feet of astrocytes, preventing approximately 98% of the small molecules and nearly 100% of large molecules including recombinant proteins and genes from being transported into the brain and reaching the tumor sites^{6,7}. The BBB strictly limits drug transport into the brain by serving as a physical (tight junctions), metabolic (enzymes) and immunological barrier⁸.

To tackle this challenge, many kinds of active targeting strategies were adopted for developing effective drug delivery systems to the brain. The active targeting systems are mainly divided into absorptive-mediated transcytosis (AMT), transporter-mediated transcytosis, and receptor-mediated endocytosis (RMT)⁸.

2.1.1. Absorptive-mediated transcytosis

Absorptive-mediated transcytosis provides a means for the delivery of drugs across the BBB by cationic proteins or cell-penetrating peptides (CPPs). It is triggered by electrostatic interactions between the positively charged moieties of the proteins and negatively charged membrane surface regions on the brain endothelial cells⁹. Typical cationic bovine serum albumin-conjugated, pegylated nanoparticles (CBSA-NP) were prepared by Lu et al.¹⁰ for brain targeting. They demonstrated that the permeability of CBSA-NP was about 7.76 times higher than that of BSA-NP, which offered the possibility of delivering therapeutic agents to CNS. It was reported that plasmid pORF-hTRAIL (pDNA)-incorporated CBSA-NP (CBSA-NP-hTRAIL) colocalized with glycoproteins in brain and tumor microvasculature and accumulated in tumor cells at 30 min after i.v. administration to C6 glioma bearing nude mice, *via* absorptive-mediated transcytosis¹¹. Aclarubicin (ACL)-loaded cationic albumin-conjugated pegylated nanoparticles (CBSA-NP-ACL) could significantly prolong the survival of the intracranial glioblastoma-bearing mice¹². Du et al.¹³ adopted another cationic protein, wheat germ agglutinin (WGA) conjugated to the surface of liposomes and also demonstrated enhanced BBB transport.

Furthermore, alternative AMT-type cell-penetrating peptide (CPP)-based delivery systems show great ability in BBB transport. CPPs have been used to overcome the lipophilic barrier of cellular membranes and deliver a large variety of cargoes, including peptide/proteins, DNA/oligonucleotide, antibodies, imaging agents, toxins, and nanodrug carriers such as liposomes and micelles¹⁴. CPPs are heterogeneous in size and sequence and are positively charged. Some share common features such as an amphipathic sequence and the ability to interact with lipid membranes. The CPPs are always derived from natural proteins including the transcription-activating factor Tat, penetratin, and the Syn-B vectors, among which Tat might be the most frequently used¹⁵. Qin et al.¹⁶ covalently conjugated cell-penetrating peptide TAT (AYGRKKRRQRRR) to cholesterol for preparing doxorubicin-loaded liposomes for glioma therapy. The biodistribution in the brain and heart demonstrated higher efficiency of brain delivery and lower cardiotoxicity. The survival time of the glioma-bearing rats treated with TAT-modified liposomes was significant prolonged¹⁶. Moreover, self-assembled polymeric micelles modified with transcriptional activator TAT peptide (TAT-PEG-*b*-Col) were constructed by Liu et al.¹⁷ to successfully deliver antibiotics across the BBB. These studies show the potential of Tat-modified nanoparticle transport into the brain for the diagnosis and treatment of brain diseases¹⁸.

2.1.2. Transporter mediated transcytosis

Since there are many kinds of transport systems in the cerebral endothelium that provide the brain with the necessary nutrients and endogenous substances, transporter mediated transcytosis takes advantage of these transport systems as a promising brain targeting strategy. Transporter mediated transcytosis is substrate-selective, so only drugs that closely mimic the endogenous substrates will be taken up and transported into the brain⁶.

Glucose transporters (GLUT), which facilitate the transport of glucose from the blood to the brain, have a broad prospective use in brain targeting. Liposomes that incorporated a mannose derivative were able to cross the BBB *via* the glucose transporter GLUT1 in mouse brain¹⁹. Qin et al.²⁰ synthesized a new glycosyl derivative of cholesterol as a material for preparing novel liposomes to overcome the ineffective delivery of normal drug formulations to brain by targeting the glucose transporters on the BBB. Pharmacokinetic and distribution experiments demonstrated

that this novel brain drug delivery system possessed good brain targeting ability. Another important transport system is the choline transporter which binds positively charged quaternary ammonium groups or simple cations²¹. Fenart et al.²² examined the ability of 60 nm nanoparticles coated with quaternary ammonium ligands to cross a cell culture model of the blood–brain barrier (BBB) formed by a coculture of bovine brain capillary endothelial cells and rat astrocytes. The coated nanoparticles were able to cross an *in vitro* model of the BBB (bovine BCEC). Their passage through the endothelial cell monolayer was three- or four-fold higher than that of uncoated nanoparticles, without any modification of paracellular permeability, suggesting that the enhancement was mediated by the BBB choline transporter.

2.1.3. Receptor mediated transcytosis

Generally, for absorptive-mediated transcytosis the specificity of this BBB targeting strategy is poor since the cationic proteins or CPPs can bind with any negatively charged cell membrane constituents. Moreover, the potential toxicity and immunogenicity of cationic proteins or CPPs limit their use. The transporter-mediated transcytosis is substrate selective, in that only drugs that closely mimic the endogenous carrier substrates will be taken up and transported into the brain. Receptor-mediated transcytosis is considered one of the most mature strategies for brain targeted drug delivery with the characteristics of high specificity, selectivity and affinity, although the ligand may have an effect on homeostasis and natural ligands may compete with the drug ligand to reduce targeting efficiency. Since many kinds of receptors are expressed on the capillary endothelium of the brain, such as transferrin receptor (TfR), the low density lipoprotein receptor (LDLR), the insulin receptor and nicotinic acetylcholine receptors, targeting ligands, including endogenous ligands and ligands based on phage display or structure-guided design, have been exploited to facilitate receptor-mediated BBB transport of drug delivery systems.

One of the most widely characterized receptor-mediated transcytosis systems for brain targeting is the transferrin receptor (TfR), which is highly expressed on endothelial cells of the BBB²³. A transferrin-conjugated drug delivery system for BBB delivery has been developed. Zhang et al.²⁴ prepared transferrin-modified paclitaxel-loaded polyphosphoester hybrid micelles (TPM), and *in vitro* and *in vivo* brain-targeting efficiencies were evaluated. It was demonstrated that TPM exhibited stronger anti-glioma activity and the mean survival time of mice bearing intracranial U87 MG glioma was significantly prolonged. However, Tf is likely not an ideal brain delivery ligand since the Tf-modified targeted drug delivery system would have to compete with the natural ligand²⁵. As an alternative, a mouse monoclonal antibody (MAb) against the rat TfR, OX26, has been extensively studied. When OX26 is coupled with the liposomes, transferrin receptor-mediated targeting of daunomycin to the rat brain was achieved by using an immunoliposome-based drug delivery system²⁶.

Another common receptor, the low-density lipoprotein (LDL) receptor-related protein (LRP), has been reported to mediate transport of various ligands conjugated to nanocarriers across the BBB. Aprotinin is a LRP ligand and its BBB transport ability was evaluated using an *in vitro* model of the BBB and *in situ* brain perfusion. Its transcytosis across bovine brain capillary endothelial cell monolayers was at least 10-fold greater than that of holo-transferrin²⁷. Angiopep, derived from aprotinin with the Kunitz domains of human proteins, exhibited even higher transcytosis capacity and parenchymal accumulation. Sun et al.²⁸ reported angiopep-2 modified cationic

liposomes for the efficient co-delivery of a therapeutic gene with paclitaxel to the brain. After treatment with liposomes, the median survival time of brain tumor-bearing mice was significantly longer than that of other groups, making it a promising drug delivery strategy against glioma.

Nicotinic acetylcholine receptors (nAChRs) are a kind of ligand-gated ion channel that is widely expressed in the brain including the brain capillary endothelial cells²⁹. Since they bind the second loop of the three-finger snake toxin with high affinity and selectivity, nAChRs could be exploited to facilitate BBB crossing and intracranial transport of drug delivery systems. Kumar et al.³⁰ reported that a 29-amino acid peptide derived from rabies virus glycoprotein-RVG29 enabled transvascular siRNA delivery to the brain *via* nAChRs and provided a safe and noninvasive approach for the delivery of therapeutic agents across the BBB. Therefore, nAChR-mediated brain targeting may be a promising strategy for the intracranial transport of drug delivery systems. It was previously reported that a 16 amino acid peptide CDX, which was derived from the loop II region of the snake neurotoxin candoxin, exhibited high binding affinity to nicotinic acetylcholine receptors. It was shown that this peptide enabled brain-targeted drug delivery when conjugated to micelles and significantly prolonged the survival time of intracranial glioblastoma-bearing mice³¹.

2.2. BBTB targeting strategies and related drug delivery systems

The blood–brain tumor barrier (BBTB), similar to blood–brain barrier (BBB), is located between brain tumor tissues and microvessels formed by highly specialized endothelial cells (ECs), limiting the paracellular delivery of most hydrophilic molecules to tumor tissue³². With the development of brain tumors, tumor cells begin to invade the surrounding normal brain tissue. Only when the tumor cell clusters grow to a certain volume will BBB be damaged and BBTB be formed. The blood–tumor barrier (BTB) of malignant solid tumors growing in peripheral tissues is typically more permeable than the BBTB of similar malignant solid tumors growing in the brain^{33,34}. The physiologic upper limit of pore size in the BTB of malignant solid tumor-orthotopic RG-2 rat gliomas microvasculature is approximately 12 nm³⁵.

With the deterioration of brain tumors, angiogenesis and gradual impairment of BBB, BBTB becomes the main obstacle of drug delivery nanosystems. At this stage, tumor neovasculature has formed to support the growth of gliomas. The abnormality of microvessels enhances the permeability of BBTB, whereas the cranial microenvironment and/or the gliomas specificity make malignant gliomas less permeable^{36,37}. Even though the BBB is compromised in malignant gliomas, the permeability differs from other regions. The infiltrating gliomas, especially around the tumor edge, still utilize the available brain vasculature and the BBB still limits glioma-targeted transport of chemotherapeutic agents³⁷. Hence, the receptors present on the BBB/BBTB provide a chance for gliomas-targeted drug delivery at this stage. The strategies proposed for BBTB targeting are mainly based on receptors high expressed on the tumor such as epidermal growth factor receptors and integrin.

The adhesion receptor integrin, which is overexpressed on tumor neovasculature and glioblastoma U87 cells, was identified as a marker of angiogenic vascular tissue^{38–40}. Particularly, integrin $\alpha\beta3$ expression is prominent in malignant glioma but not overexpressed on normal brain cells. As the ligands for integrin, cyclic arginine–glycine–aspartic acid (RGD) peptide and its analogs have

been widely investigated for the glioma-targeted drug delivery. Herein, the integrin–RGD interaction still provides a promising BBTB-targeted drug delivery strategy. Our group has designed c(RGDyK)-modified polyethylene glycol–polyethylenimine (PEG–PEI) nanoparticles for intracranial glioblastoma-targeted gene delivery. They exhibited high binding affinity with U87 cells and facilitated targeted gene delivery against intracranial glioblastoma *in vivo* compared to the PEG–PEI gene carrier without RGD modification. It was demonstrated that the therapeutic efficacy of pORF-hTRAIL of this gene carrier was also enhanced as evidenced by a significantly prolonged survival of intracranial glioblastoma-bearing nude mice. These results demonstrated the therapeutic feasibility of this gene delivery system for brain glioblastoma treatment through mediation of integrin $\alpha v\beta 3$ ⁴¹. Zhan et al.⁴² reported a cyclic RGD peptide conjugated PEG–PLA micelle for the intracranial gliomas targeted chemotherapy, and the median survival time of mice bearing intracranial U87MG tumor xenografts was significantly prolonged after treated by c(RGDyK)–PEG–PLA–PTX micelles, suggesting that RGD motif is efficient for targeted drug delivery to integrin $\alpha v\beta 3$ -overexpressed glioblastoma.

EGFR, a member of ErbB family of receptors, is a transmembrane tyrosine kinase normally expressed in epithelial, mesenchymal, and neuronal tissues^{43,44}. Overexpression of the epidermal growth factor receptor (EGFR) on the BBTB makes it a promising target for therapy⁴⁵. EGF and anti-EGFR monoclonal antibody are the commonly used EGFR ligands mediating glioma-targeted therapy. Fondell et al.⁴⁶ adopted epidermal growth factor to target the EGFR and developed a new two-step targeting strategy to transport a recently synthesized daunorubicin derivative into cancer cell nuclei using PEG-stabilized targeting liposomes named “Nuclisome-particles”. Tsutsui et al.⁴⁷ constructed a novel drug delivery system using hybrid bionanocapsules (BNCs) conjugated with anti-human EGFR antibody and confirmed the specific delivery of these BNCs to the brain tumor in the *in vivo* brain tumor model. Anti-human EGFR antibody-conjugated immunoliposomes also were prepared by Feng et al.⁴⁸ and successfully delivered sodium borocaptate into EGFR-overexpressing gliomas in an animal model of brain tumors.

2.3. EPR effect-based strategies and related drug delivery systems

As the brain tumor develops, the enhanced permeability and retention effect (EPR effect) appears even though it is significantly weaker in the cranial microenvironment than in peripheral tumors, which enables nanosystems with suitable particle size to enter the brain tumor *via* endothelial gaps on the microvessels of brain tumors^{42,49,50}. Some of the nanoscale drug delivery systems have been developed to make use of the glioma EPR effect for tumor targeting. Huang et al.⁵¹ have developed a tumor-targeting nanoparticle system which possesses passive tumor target ability *via* the enhanced permeability and retention effect. This system was able to prolong the survival time of intracranial U87MG tumor-bearing nude mice.

3. Tumor microenvironment

Studies in developmental biology and tumor progression have demonstrated that the extracellular microenvironment is not simply a passive structural element in which cells reside, but is an interactive partner that can be altered by and can alter cellular processes and responses⁵².

3.1. Lowered extracellular pH

Lowered extracellular pH (pHe) is a common characteristic of various solid tumors, including brain tumors, which is caused by the high rate of glycolysis in cancer cells, under both aerobic and anaerobic conditions^{53,54}. It has been reported that the tumor pHe is around 5.7–7.2 depending upon tumor histology, tumor volume, and tumor location, and is lower than normal tissue^{55,56}. Low extracellular pH may be an important factor inducing more aggressive cancer phenotypes. During recent decades many approaches have been developed to target various tumors by pHe, including a shielding/deshielding mechanism or a pH-triggered drug release mechanism. Huang and coworkers have developed a tumor targeting nanoparticle system (called dtACCP) that responds to the lowered tumor pHe and unregulated matrix metalloproteinase 2 (MMP2) expression in the tumor microenvironment. The nanoparticles are modified with an activatable cell-penetrating peptide that is quenched by a pH-sensitive masking peptide, linked by a MMP2 substrate. Once the modified nanoparticles reach the glioblastoma microenvironment, the negative charge of the masking peptide will be eliminated due to the lowered pHe, while the linker is cleaved by MMP2, exposing CPP to drive the nanoparticle's internalization into the tumor cells^{51,57}. This shielding/deshielding mechanism makes it feasible to develop a lowered tumor pHe-triggered drug delivery system, increasing target drug accumulation at the tumor site and lowering the cytotoxicity to the normal tissue.

3.2. Angiogenesis

With the deterioration of brain tumors, angiogenesis occurs with new vessels developing from pre-existing ones. Angiogenesis is essential for glioma tumor growth and metastasis. The angiogenesis targeting delivery system is capable of specifically delivering anti-angiogenic therapeutics to the tumor neovasculature while minimizing systematic toxicity. Therefore, receptors highly expressed during tumor angiogenesis, such as epidermal growth factor receptors and integrin, provide tumor-targeted drug delivery strategies as mentioned in the BBTB targeting strategies section. Currently, antiangiogenic therapy has been restricted to targeting endothelial cells. It was shown that endothelial cells–pericytes signaling networks could also contribute to tumor angiogenesis and metastasis, suggesting that pericytes could be another promising complementary target for cancer treatment. The activated pericytes are capable of stabilizing blood vessels, providing EC survival signals and protecting ECs from VEGF withdrawal, leading to pericyte-mediated resistance to antiangiogenic therapies^{58,59}. Guan et al.⁶⁰ developed a pericyte-targeting drug delivery system, TH10 peptide (TAASGVRSMH)-conjugated nanoparticles loaded with docetaxel (TH10-DTX-NP) that can target the NG2 proteoglycan highly expressed in tumor vascular pericytes. Enhanced antitumor effects were achieved, revealing the potency and significance of targeting tumor vascular pericytes using nano-DDS in antiangiogenic cancer therapy. It was reported that doxorubicin-loaded liposomes modified with peptide ligands were constructed for the combined therapy of targeting both tumor pericytes and ECs, displaying enhanced anti-tumor effects and prolonged survival in human neuroblastoma-bearing mice. These results clearly demonstrate that this combined targeting strategy was more effective for destruction of tumor vasculature than either monotherapy⁶¹.

3.3. Vasculogenic mimicry

Vasculogenic mimicry (VM) occurs when highly invasive tumor cells form vascular channels for transporting plasma and red blood cells to provide the supply required for tumor growth and metastasis themselves. The channels formed by VM, which can be stained by Periodic Acid-Schiff (PAS), are composed of basement membranes and lined by tumor cells, and no endothelial cells are found on the inner wall⁶². In 1999, Maniotis and colleagues⁶³ found that tissue sections from aggressive human intraocular (uveal) and metastatic cutaneous melanomas generally lacked evidence of significant necrosis and contained patterned networks of interconnected loops of extracellular matrix. Red blood cells were detected within these vascular channel networks and no endothelial cells were identified by microscopy or an immunohistochemical panel of endothelial cell markers. From then on, research on vasculogenic mimicry had attracted much more attention and provoked the further thinking about its molecular regulatory mechanism and its application in tumor therapy^{64,65}.

With further research, VM has been observed in several malignant tumor types such as breast cancer, liver cancer, glioma, ovarian cancer, melanoma, prostate cancer, and others^{66–69}. Vasculogenic mimicry was previously reported in human glioblastoma tissues and human glioma cell-line xenografts⁷⁰. Yue and Chen⁷¹ examined 45 astrocytomas (including 15 World Health Organization grade II cases, 15 grade III cases, and 15 grade IV cases) for CD34 endothelial cell markers and periodic acid-Schiff (PAS) staining, among which CD34-negative, PAS-positive patterns of VM were observed in malignant astrocytomas. Hence VM provides new insights for the therapeutic intervention of tumor-associated vasculature.

Until now little has been known about the molecular mechanisms involved in VM. It was reported that aggressive VM-positive tumors exhibited higher expression of the basement membrane extracellular matrix (ECM) component laminin 5 γ 2, MMP1, MMP2, MMP9 and membrane type-1-matrix/metalloproteinase⁷². Paulis et al.⁷³ considered the VE-cadherin/EphA2/MMP-2 as one of the key VM signaling pathways. Besides, the ischemia and hypoxia effects of the local tumor microenvironment also have an impact on the formation of VM channels⁶⁹. Many researchers involved in VM studies have been trying to treat tumors based on an anti-VM therapy. But almost all VM targeting therapies have been performed *in vitro*. Hess et al.⁷⁴ adopted general inhibitors of protein tyrosine kinases with transient knockout of EphA2 to abrogate the ability of tumor cells to form tubular structures. Downregulating VE-cadherin, using antibodies against human MMPs and the laminin 5 γ 2 chain are also possible strategies to inhibit VM⁷⁵. Francescone and colleagues⁷⁵ found that vascular channels of VM in GBM were composed of mural-like tumor cells that strongly express VEGF receptor 2 (Flk-1). Therefore, the identification of Flk-1 as a key factor regulating VM could offer a novel therapeutic target for GBM treatment.

It is necessary to combine both classic anti-angiogenesis and anti-VM in anti-tumor therapies. Tumor cells, which line in the inner surface of VM channels, are highly malignant and have high plasticity. They are directly exposed to blood flow and readily allow tumor cells to migrate and metastasize to other organs. With traditional anti-angiogenesis therapy, the vasculogenic mimicry could also nourish the tumor with nutrients and promote metastasis. As a result, multiple antivascular approaches, including targeting VM and angiogenesis together, may represent the best possible regimen in the treatment of tumors. Since VEGF receptors are highly expressed on angiogenic cells and the VM of GBM, a corresponding ligand-mediated drug delivery system may be utilized for the tumor targeting therapy⁷⁵.

4. Tumor cells

Two alternative models have been put forward to explain how tumors initiate and develop. One is the stochastic model, proposing that tumor cells are heterogeneous, and virtually any of them can function as a tumor-initiating cell. Another one is the hierarchical model, encouraged by increasing experimental data, which hypothesizes that only a small subpopulation of tumor stem cells can induce tumorigenesis^{76,77}. The normal tumor cells, accounting for most of the tumor tissue, are incapable of tumorigenesis and could be killed by common chemotherapeutic drugs, while cancer stem cells have the ability to self-renew, spending most of their time in the G0 phase of the cell division cycle. Cancer stem cells are likely to share many of the properties of normal stem cells that include a long lifespan, relative quiescence, resistance to drugs and toxins through the expression of several ATP-binding cassette (ABC) transporters, an active DNA-repair capacity, and a resistance to apoptosis⁷⁷. In recent years, brain tumor stem cells have been reportedly identified and isolated, and the concept of cancer stem cells was extended to brain tumors^{78,79}. The brain tumor stem cells (BTSC) are capable for self-renewal, multipotency, and induction of tumorigenesis. BTSC exhibited remarkable resistance to chemotherapy due to their relative quiescence, active DNA repair regulatory systems and high expression of ABC transporters^{80,81}. Thus, for brain tumor targeted strategies, cancer stem cell targeting is essential. Recently, several stem cell targeting drug delivery systems have been constructed. Zhang et al.⁸² prepared mitochondrial targeting liposomes showing the strongest efficacy in treating MCF-7 cancer cells and cancer stem cells *in vitro* and in treating relapsed tumor cells in mice. Nevertheless, brain tumor stem cells targeting nanocarriers are still under research.

CD133, a 120 kDa cell-surface protein, is considered as a marker of normal human neuronal precursors and could be used for the enrichment of tumor stem-like cells from brain tumors^{83,84}. Sun and colleagues⁸⁵ obtained a specific mouse CD133 binding peptide named LS7 (LQNAPRS) by phage-displayed peptide library technology, which could be used to target cancer stem cells. Nestin, a 200–240 kDa intermediate filament protein, is also the most commonly used marker for the isolation and study of brain tumor stem cells. Beck et al.⁸⁶ identified an effective Nestin targeted peptide (AQYLNPS) through the phage display technology, providing a promising opportunity to design a BTSC-targeted drug delivery system. BTSC and neuronal stem cells share many characteristics including these two markers. Up to now, research on BTSC-targeted drug delivery system is only in its infancy, and specific targets are still needed for the BTSC targeted drug delivery systems.

5. Conclusions

In actual research practice, several targeting strategies have been combined to achieve better therapeutic effects. One common dual targeting drug delivery system was the combination of trans-BBB targeting and brain tumor cell targeting in two ways. The first was dual-targeting moiety modification such as transferrin (Tf) and wheat germ agglutinin (WGA)⁸⁷, and p-aminophenyl- α -D-mannopyranoside (MAN) and transferrin (Tf)⁸⁸, the second one is a single-targeting moiety that targets both BBB and tumor cells, such as angiopep-2 targeting to LRP overexpressed on both BBB and glioma cells^{89,90}. Another dual targeting drug delivery system was combining trans-BBTB targeting with brain tumor cell

gaps that remain in fully understanding the relationship between the physiological and pathological conditions of brain tumors, providing a basis for various targeted delivery systems. We believed that systematic targeted drug delivery strategies for brain tumors that consider characteristics of brain tumor growth will provide promising ways to treat brain tumor in the future.

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References

- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010;**127**:2893–917.
- Dolecek TA, Propp JM, Stroup NE, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005–2009. *Neuro Oncol* 2012;**14**:v1–49.
- Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New Engl J Med* 2005;**352**:987–96.
- Sanai N, Berger MS. Glioma extent of resection and its impact on patient outcome. *Neurosurgery* 2008;**62**:753–64.
- Daneman R. The blood–brain barrier in health and disease. *Ann Neurol* 2012;**72**:648–72.
- de Boer AG, Gaillard PJ. Drug targeting to the brain. *Ann Rev Pharmacol Toxicol* 2007;**47**:323–55.
- Wong AD, Ye M, Levy AF, Rothstein JD, Bergles DE, Searson PC. The blood–brain barrier: an engineering perspective. *Front Neuroeng* 2013;**6**:7.
- Pardridge WM. Drug targeting to the brain. *Pharm Res* 2007;**24**:1733–44.
- Hervé F, Ghinea N, Scherrmann JM. CNS delivery via adsorptive transcytosis. *AAPS J* 2008;**10**:455–72.
- Lu W, Tan YZ, Hu KL, Jiang XG. Cationic albumin conjugated pegylated nanoparticle with its transcytosis ability and little toxicity against blood–brain barrier. *Int J Pharm* 2005;**295**:247–60.
- Lu W, Sun Q, Wan J, She ZJ, Jiang XG. Cationic albumin–conjugated pegylated nanoparticles allow gene delivery into brain tumors via intravenous administration. *Cancer Res* 2006;**66**:11878–87.
- Lu W, Wan J, Zhang Q, She ZJ, Jiang XG. Aclarubicin-loaded cationic albumin-conjugated pegylated nanoparticle for glioma chemotherapy in rats. *Int J Cancer* 2007;**120**:420–31.
- Du J, Lu WL, Ying X, Liu Y, Du P, Tian W, et al. Dual-targeting topotecan liposomes modified with tamoxifen and wheat germ agglutinin significantly improve drug transport across the blood–brain barrier and survival of brain tumor-bearing animals. *Mol Pharm* 2009;**6**:905–17.
- Gupta B, Levchenko TS, Torchilin VP. Intracellular delivery of large molecules and small particles by cell-penetrating proteins and peptides. *Adv Drug Deliver Rev* 2005;**57**:637–51.
- Banks WA, Robinson SM, Nath A. Permeability of the blood–brain barrier to HIV-1 Tat. *Exp Neurol* 2005;**193**:218–27.
- Qin Y, Chen HL, Zhang QY, Wang XX, Yuan WM, Kuai R, et al. Liposome formulated with TAT-modified cholesterol for improving brain delivery and therapeutic efficacy on brain glioma in animals. *Int J Pharm* 2011;**420**:304–12.
- Liu LH, Venkatraman SS, Yang YY, Guo K, Lu J, He BP, et al. Polymeric micelles anchored with TAT for delivery of antibiotics across the blood–brain barrier. *Pept Sci* 2008;**90**:617–23.
- Zou LL, Ma JL, Wang T, Yang TB, Liu CB. Cell-penetrating peptide-mediated therapeutic molecule delivery into the central nervous system. *Curr Neuropharmacol* 2013;**11**:197–208.
- Umezawa F, Eto Y. Liposome targeting to mouse brain: mannose as a recognition marker. *Biochem Biophys Res Commun* 1988;**153**:1038–44.
- Qin Y, Fan W, Chen HL, Yao N, Tang WW, Tang J, et al. *In vitro* and *in vivo* investigation of glucose-mediated brain-targeting liposomes. *J Drug Target* 2010;**18**:536–49.
- Lockman PR, Allen DD. The transport of choline. *Drug Dev Ind Pharm* 2002;**28**:749–71.
- Fenart L, Casanova A, Dehouck B, Duhem C, Slupek S, Cecchelli R, et al. Evaluation of effect of charge and lipid coating on ability of 60-nm nanoparticles to cross an *in vitro* model of the blood–brain barrier. *J Pharmacol Exp Ther* 1999;**291**:1017–22.
- Ponka P, Lok CN. The transferrin receptor: role in health and disease. *Int J Biochem Cell B* 1999;**31**:1111–37.
- Zhang PC, Hu LJ, Yin Q, Zhang ZW, Feng LY, Li YP. Transferrin-conjugated polyphosphoester hybrid micelle loading paclitaxel for brain-targeting delivery: synthesis, preparation and *in vivo* evaluation. *J Control Release* 2012;**159**:429–34.
- Qian ZM, Li HY, Sun HZ, Ho K. Targeted drug delivery via the transferrin receptor-mediated endocytosis pathway. *Pharmacol Rev* 2002;**54**:561–87.
- Huwylar J, Wu D, Pardridge WM. Brain drug delivery of small molecules using immunoliposomes. *Proc Natl Acad Sci USA* 1996;**93**:14164–9.
- Demeule M, Régina A, Che C, Poirier J, Nguyen T, Gabathuler R, et al. Identification and design of peptides as a new drug delivery system for the brain. *J Pharmacol Exp Ther* 2008;**324**:1064–72.
- Sun XY, Pang ZQ, Ye HX, Qiu B, Guo LR, Li JW, et al. Co-delivery of pEGFP-hTRAIL and paclitaxel to brain glioma mediated by an angiopep-conjugated liposome. *Biomaterials* 2012;**33**:916–24.
- Abbruscato TJ, Lopez SP, Mark KS, Hawkins BT, Davis TP. Nicotine and cotinine modulate cerebral microvascular permeability and protein expression of ZO-1 through nicotinic acetylcholine receptors expressed on brain endothelial cells. *J Pharm Sci* 2002;**91**:2525–38.
- Kumar P, Wu HQ, McBride JL, Jung KE, Kim MH, Davidson BL, et al. Transvascular delivery of small interfering RNA to the central nervous system. *Nature* 2007;**448**:39–43.
- Zhan CY, Li B, Hu LJ, Wei XL, Feng LY, Fu W, et al. Micelle-based brain-targeted drug delivery enabled by a nicotine acetylcholine receptor ligand. *Angew Chem Int Ed Engl* 2011;**50**:5582–5.
- Ningaraj NS, Rao M, Hashizume K, Asotra K, Black KL. Regulation of blood–brain tumor barrier permeability by calcium-activated potassium channels. *J Pharmacol Exp Ther* 2002;**301**:838–51.
- Monsky WL, Carreira CM, Tsuzuki Y, Gohongi T, Fukumura D, Jain RK. Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: mammary fat pad versus cranial tumors. *Clin Cancer Res* 2002;**8**:1008–13.
- Roberts WG, Delaat J, Nagane M, Huang S, Cavenee WK, Palade GE. Host microvasculature influence on tumor vascular morphology and endothelial gene expression. *Am J Clin Pathol* 1998;**153**:1239–48.
- Sarin H, Kanevsky AS, Wu H, Sousa AA, Wilson CM, Aronova MA, et al. Physiologic upper limit of pore size in the blood–tumor barrier of malignant solid tumors. *J Transl Med* 2009;**7**:51.
- Schlageter KE, Molnar P, Lapin GD, Groothuis DR. Microvessel organization and structure in experimental brain tumors: microvessel populations with distinctive structural and functional properties. *Microvasc Res* 1999;**58**:312–28.
- Zhan CY, Lu WY. The blood–brain/tumor barriers: challenges and chances for malignant gliomas targeted drug delivery. *Curr Pharm Biotechnol* 2012;**13**:2380–7.
- Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin $\alpha\beta 3$ for angiogenesis. *Science* 1994;**264**:569–71.
- Kumar CC, Armstrong L, Yin Z, Malkowski M, Maxwell E, Ling H, et al. Targeting integrins $\alpha\beta 3$ and $\alpha\beta 5$ for blocking tumor-induced angiogenesis. *Adv Exp Med Biol* 2000;**476**:169–80.
- Kim S, Bell K, Mousa SA, Varner JA. Regulation of angiogenesis *in vivo* by ligation of integrin $\alpha 5\beta 1$ with the central cell-binding domain of fibronectin. *Am J Clin Pathol* 2000;**156**:1345–62.

41. Zhan CY, Meng QG, Li QH, Feng LL, Zhu JH, Lu WY. Cyclic RGD-polyethylene glycol-polyethylenimine for intracranial glioblastoma-targeted gene delivery. *Chem-Asian J* 2012;**7**:91–6.
42. Zhan CY, Gu B, Xie C, Li J, Liu Y, Lu WY. Cyclic RGD conjugated poly(ethylene glycol)-co-poly(lactic acid) micelle enhances paclitaxel anti-glioblastoma effect. *J Control Release* 2010;**143**:136–42.
43. Yano S, Kondo K, Yamaguchi M, Richmond G, Hutchison M, Wakeling A, et al. Distribution and function of EGFR in human tissue and the effect of EGFR tyrosine kinase inhibition. *Anticancer Res* 2002;**23**:3639–50.
44. Spano JP, Fagard R, Soria JC, Rixe O, Khayat D, Milano G. Epidermal growth factor receptor signaling in colorectal cancer: preclinical data and therapeutic perspectives. *Ann Oncol* 2005;**16**:189–94.
45. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. *Am J Clin Pathol* 2007;**170**:1445–53.
46. Fondell A, Edwards K, Ickenstein LM, Sjöberg S, Carlsson J, Gedda L. Nuclisome: a novel concept for radionuclide therapy using targeting liposomes. *Eur J Nucl Med Mol I* 2010;**37**:114–23.
47. Tsutsui Y, Tomizawa K, Nagita M, Michiue H, Nishiki T, Ohmori I, et al. Development of bionanocapsules targeting brain tumors. *J Control Release* 2007;**122**:159–64.
48. Feng B, Tomizawa K, Michiue H, Miyatake S, Han X, Fujimura A, et al. Delivery of sodium borocaptate to glioma cells using immunoliposome conjugated with anti-EGFR antibodies by ZZ-His. *Biomaterials* 2009;**30**:1746–55.
49. Liu Y, Lu WY. Recent advances in brain tumor-targeted nano-drug delivery systems. *Expert Opin Drug Del* 2012;**9**:671–86.
50. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, et al. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc Natl Acad Sci USA* 1998;**95**:4607–12.
51. Huang SX, Shao K, Liu Y, Kuang YY, Li JF, An S, et al. Tumor-targeting and microenvironment-responsive smart nanoparticles for combination therapy of antiangiogenesis and apoptosis. *ACS Nano* 2013;**7**:2860–71.
52. Seftor RE, Hess AR, Seftor EA, Kirschmann DA, Hardy KM, Margaryan NV, et al. Tumor cell vasculogenic mimicry: from controversy to therapeutic promise. *Am J Clin Pathol* 2012;**181**:1115–25.
53. Engin K, Leeper DB, Cater JR, Thistlethwaite AJ, Tupchong L, McFarlane JD. Extracellular pH distribution in human tumours. *Int J Hyperth* 1995;**11**:211–6.
54. van Sluis R, Bhujwalla ZM, Raghunand N, Ballesteros P, Alvarez J, Cerdán S, et al. *In vivo* imaging of extracellular pH using ¹H MRSI. *Magn Reson Med* 1999;**41**:743–50.
55. Zhang XM, Lin YX, Gillies RJ. Tumor pH and its measurement. *J Nucl Med* 2010;**51**:1167–70.
56. Lee ES, Gao ZG, Bae YH. Recent progress in tumor pH targeting nanotechnology. *J Control Release* 2008;**132**:164–70.
57. Huang SX, Shao K, Kuang YY, Liu Y, Li JF, An S, et al. Tumor targeting and microenvironment-responsive nanoparticles for gene delivery. *Biomaterials* 2013;**34**:5294–302.
58. Raza A, Franklin MJ, Dudek AZ. Pericytes and vessel maturation during tumor angiogenesis and metastasis. *Am J Hematol* 2010;**85**:593–8.
59. Erber R, Thumher A, Katsen AD, Groth G, Kerger H, Hammes HP, et al. Combined inhibition of VEGF and PDGF signaling enforces tumor vessel regression by interfering with pericyte-mediated endothelial cell survival mechanisms. *FASEB J* 2004;**18**:338–40.
60. Guan YY, Luan X, Xu JR, Liu YR, Lu Q, Wang C, et al. Selective eradication of tumor vascular pericytes by peptide-conjugated nanoparticles for antiangiogenic therapy of melanoma lung metastasis. *Biomaterials* 2014;**35**:3060–70.
61. Loi M, Marchiò S, Becherini P, Di Paolo D, Soster M, Cumis F, et al. Combined targeting of perivascular and endothelial tumor cells enhances anti-tumor efficacy of liposomal chemotherapy in neuroblastoma. *J Control Release* 2010;**145**:66–73.
62. Folberg R, Maniotis AJ. *Vasculogenic mimicry*. *APMIS*, **112**: 508–25.
63. Maniotis AJ, Folberg R, Hess A, Seftor EA, Gardner LM, Pe'er J, et al. Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. *Am J Clin Pathol* 1999;**155**:739–52.
64. Bissell MJ. Tumor plasticity allows vasculogenic mimicry, a novel form of angiogenic switch: a rose by any other name? *Am J Clin Pathol* 1999;**155**:675–9.
65. Barinaga M. New type of blood vessel found in tumors. *Science* 1999;**285**:1475.
66. Shirakawa K, Tsuda H, Heike Y, Kato K, Asada R, Inomata M, et al. Absence of endothelial cells, central necrosis, and fibrosis are associated with aggressive inflammatory breast cancer. *Cancer Res* 2001;**61**:445–51.
67. Sood AK, Seftor EA, Fletcher MS, Gardner LM, Heidger PM, Buller RE, et al. Molecular determinants of ovarian cancer plasticity. *Am J Clin Pathol* 2001;**158**:1279–88.
68. Sharma N, Seftor REB, Seftor EA, Gruman LM, Heidger PM, Cohen MB, et al. Prostatic tumor cell plasticity involves cooperative interactions of distinct phenotypic subpopulations: role in vasculogenic mimicry. *Prostate* 2002;**50**:189–201.
69. Zhang SW, Zhang DF, Sun BC. Vasculogenic mimicry: current status and future prospects. *Cancer Lett* 2007;**254**:157–64.
70. Niclou SP, Danzeisen C, Eikesdal HP, Wiig H, Brons NHC, Poli AMF, et al. A novel eGFP-expressing immunodeficient mouse model to study tumor-host interactions. *FASEB J* 2008;**22**:3120–8.
71. Yue WY, Chen ZP. Does vasculogenic mimicry exist in astrocytoma? *J Histochem Cytochem* 2005;**53**:997–1002.
72. Seftor RE, Seftor EA, Koshikawa N, Meltzer PS, Gardner LM, Bilban M, et al. Cooperative interactions of laminin 5 gamma2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. *Cancer Res* 2001;**61**:6322–7.
73. Paulis YWJ, Soetekouw PMMB, Verheul HMW, Tjan-Heijnen VCG, Griffioen AW. Signalling pathways in vasculogenic mimicry. *BBA-Rev Cancer* 2010;**1806**:18–28.
74. Hess AR, Seftor EA, Gardner LM, Carles-Kinch K, Schneider GB, Seftor RE, et al. Molecular regulation of tumor cell vasculogenic mimicry by tyrosine phosphorylation: role of epithelial cell kinase (Eck/EphA2). *Cancer Res* 2001;**61**:3250–5.
75. Francescone R, Scully S, Bentley B, Yan W, Taylor SL, Oh D, et al. Glioblastoma-derived tumor cells induce vasculogenic mimicry through Flk-1 protein activation. *J Biol Chem* 2012;**287**:24821–31.
76. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;**414**:105–11.
77. Vescovi AL, Galli R, Reynolds BA. Brain tumour stem cells. *Nat Rev Cancer* 2006;**6**:425–36.
78. Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA. Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers *in vitro*. *Glia* 2002;**39**:193–206.
79. Galli R, Binda E, Orfanelli U, Cipelletti B, Gritti A, de Vitis S, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* 2004;**64**:7011–21.
80. Bao SD, Wu QL, McLendon RE, Hao YL, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 2006;**444**:756–60.
81. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005;**5**:275–84.
82. Zhang L, Yao HJ, Yu Y, Zhang Y, Li RJ, Ju RJ, et al. Mitochondrial targeting liposomes incorporating daunorubicin and quinacrine for treatment of relapsed breast cancer arising from cancer stem cells. *Biomaterials* 2012;**33**:565–82.
83. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. *Nature* 2004;**432**:396–401.

84. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res* 2003;**63**:5821–8.
85. Sun JM, Zhang C, Liu GB, Liu H, Zhou CP, Lu YX, et al. A novel mouse CD133 binding-peptide screened by phage display inhibits cancer cell motility *in vitro*. *Clin Exp Metastasis* 2012;**29**:185–96.
86. Beck S, Jin X, Yin JL, Kim SH, Lee NK, Oh SY, et al. Identification of a peptide that interacts with Nestin protein expressed in brain cancer stem cells. *Biomaterials* 2011;**32**:8518–28.
87. He H, Li Y, Jia XR, Du J, Ying X, Lu WL, et al. PEGylated poly (amidoamine) dendrimer-based dual-targeting carrier for treating brain tumors. *Biomaterials* 2011;**32**:478–87.
88. Ying X, Wen H, Lu WL, Du J, Guo J, Tian W, et al. Dual-targeting daunorubicin liposomes improve the therapeutic efficacy of brain glioma in animals. *J Control Release* 2010;**141**: 183–92.
89. Demeule M, Currie JC, Bertrand Y, Ché C, Nguyen T, Régina A, et al. Involvement of the low-density lipoprotein receptor-related protein in the transcytosis of the brain delivery vector Angiopep-2. *J Neurochem* 2008;**106**:1534–44.
90. Xin HL, Jiang XY, Gu JJ, Sha XY, Chen LC, Law K, et al. Angio-pep-conjugated poly(ethylene glycol)-*co*-poly(epsilon-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma. *Biomaterials* 2011;**32**:4293–305.