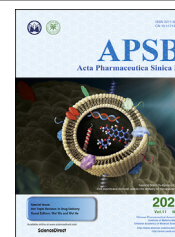




Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Evolution of blood–brain barrier in brain diseases and related systemic nanoscale brain-targeting drug delivery strategies



Liang Han^{a,*}, Chen Jiang^b

^aJiangsu Key Laboratory of Neuropsychiatric Diseases Research, College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, China

^bKey Laboratory of Smart Drug Delivery, Ministry of Education, State Key Laboratory of Medical Neurobiology, Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai 200032, China

Received 17 August 2020; received in revised form 30 September 2020; accepted 9 October 2020

KEY WORDS

Blood–brain barrier;
Brain diseases;
Brain-targeting;
Drug delivery systems;
Nanoparticles

Abstract Blood–brain barrier (BBB) strictly controls matter exchange between blood and brain, and severely limits brain penetration of systemically administered drugs, resulting in ineffective drug therapy of brain diseases. However, during the onset and progression of brain diseases, BBB alterations evolve inevitably. In this review, we focus on nanoscale brain-targeting drug delivery strategies designed based on BBB evolutions and related applications in various brain diseases including Alzheimer's disease, Parkinson's disease, epilepsy, stroke, traumatic brain injury and brain tumor. The advances on optimization of small molecules for BBB crossing and non-systemic administration routes (*e.g.*, intranasal treatment) for BBB bypassing are not included in this review.

Abbreviations: A β , amyloid beta; AD, Alzheimer's disease; AMT, alpha-methyl-L-tryptophan; BACE1, β -secretase 1; BBB, blood–brain barrier; BDNF, brain derived neurotrophic factor; BTB, blood–brain tumor barrier; CMT, carrier-mediated transportation; DTPA-Gd, Gd-diethyltriaminepentaacetic acid; EPR, enhanced permeability and retention; Gd, gadolinium; GLUT1, glucose transporter-1; ICAM-1, intercellular adhesion molecule-1; K_{ATP}, ATP-sensitive potassium channels; K_{Ca}, calcium-dependent potassium channels; LAT1, L-type amino acid transporter 1; LDL, low density lipoprotein; LDLR, LDL receptor; LFA-1, lymphocyte function associated antigen-1; LRP1, LDLR-related protein 1; MFSD2A, major facilitator superfamily domain-containing protein 2a; MMP9, metalloproteinase-9; MRI, magnetic resonance imaging; NPs, nanoparticles; PD, Parkinson's disease; PEG, polyethyleneglycol; PEG-PLGA, polyethyleneglycol-poly(lactic-co-glycolic acid); P-gp, P-glycoprotein; PLGA, poly(lactic-co-glycolic acid); PSMA, prostate-specific membrane antigen; RAGE, receptor for advanced glycosylation end products; RBC, red blood cell; RMT, receptor-mediated transcytosis; ROS, reactive oxygen species; siRNA, short interfering RNA; TBI, traumatic brain injury; TfR, transferrin receptor; TJ, tight junction; tPA, tissue plasminogen activator; VEGF, vascular endothelial growth factor; ZO1, zona occludens 1.

*Corresponding author. Tel./fax: +86 512 65882089.

E-mail address: hanliang@suda.edu.cn (Liang Han).

Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2020.11.023>

2211-3835 © 2021 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

The blood–brain barrier (BBB) strictly controls the transport of various substances from blood to brain^{1,2}. Most drugs are ineffective for brain diseases because their extremely low BBB penetration efficiency is hard to reach therapeutic concentration in brain parenchyma for effective treatment. Various kinds of highly-expressed receptors and transporters on the BBB play the critical roles in supplying brain with essential energy and nutrients^{3–7}. This physiologic function endows receptor-mediated transcytosis (RMT) and carrier-mediated transportation (CMT) with the potential promising role in brain-targeting drug delivery^{7,8}. Nanotechnology has been widely and extensively applied in drug delivery because of the exceptional features in increasing drug loading and stability and reducing side effects and so on^{9–11}. Combining nanotechnology with RMT and CMT holds the potential to boost BBB penetration for systemic brain-targeting drug delivery. In the past two decades, specific ligands and substrates of these receptors and transporters have been extensively used to enhance brain-targeting delivery efficiency^{3,4}.

Pathological BBB breakdown with structural and functional changes, happens in brain regions affected by almost all brain diseases, such as chronic Alzheimer's disease (AD) and Parkinson's disease (PD), acute ischemic stroke and traumatic brain injury (TBI), epileptic seizures and brain tumor^{12,13}. These vascular changes often include up-regulation of luminal adhesion molecules, increased adhesion and transmigration of leukocytes, reduced expression of tight junction (TJ), increased endothelial transcytosis, disrupted expression of receptor and transporter, endothelial degeneration, pericyte degeneration, perivascular accumulation of toxic products, inflammation and immune response^{12,14}. It's remarkable that BBB evolutions are often throughout the onset and progression of brain diseases¹⁵. However, it remains uncertain whether BBB dysfunction is one of the initial events that lead to the brain diseases, or a downstream consequence¹³. For example, because the BBB plays a critical role in maintaining brain homeostasis, the disturbance of proper BBB functioning is increasingly recognized as a potential contributor in pathogenesis of neurodegenerative neurological diseases, including AD and PD¹⁶. On the one hand, BBB disruption allows influx into the brain of neurotoxic blood-derived debris, cells and microbial pathogens and is associated with inflammatory and immune responses, which can initiate multiple pathways of neurodegeneration¹². On the other hand, inflammatory cytokines and chemokines are involved in cleavage, downregulation and spatial redistribution of junction proteins, leading to the function loss of TJ. The BBB evolutions or alterations affect most drug's brain accumulation and intracranial distribution. Disease-initiated BBB breakdown might present an opportunity to deliver treatments to affected neurons. Therefore, drug delivery through the diseased BBB for brain diseases are different and more complex than through the normal BBB. An attempt has been made in this review to briefly introduce the BBB evolutions in various brain diseases and the

related strategies for designing nanoscale brain-targeting drug delivery systems.

2. Normal BBB and diseased BBB

2.1. Normal BBB

The BBB is a selective barrier between blood circulation and brain and formed by a monolayer of tightly sealed endothelial cells, together with pericytes and astrocytic perivascular endfeet^{17–19} (Fig. 1). In contrast to the highly permeable peripheral capillaries, brain capillary endothelial cells or BBB endothelial cells express various TJ proteins and major facilitator superfamily domain-containing protein 2a (MFS2A). On the one hand, TJ proteins impede the paracellular diffusion of most molecules, creating a paracellular physical barrier and extremely low paracellular permeability¹⁷. On the other hand, MFS2A-regulated low transcytosis rate^{20–24}, endows the BBB with the transcellular transcytosis barrier. Through both paracellular and transcellular barrier, the BBB strictly regulates both paracellular and transcellular transfer of various substances from blood to brain^{1,2}. Transport systems at the BBB include free diffusion, absorptive transcytosis, RMT and CMT. Taking free diffusion as an example, BBB is only permeable to small molecules with strictly specified structure, *e.g.*, MW < 400 Da threshold and high lipid solubility^{25,26}. Through these precisely controlled transport pathways together with intra-endothelial enzymolysis and active efflux^{27,28}, BBB tightly controls the chemical composition of brain interstitial fluid for proper synaptic functioning, information processing and neuronal connectivity¹². Besides neurotoxic plasma components, blood cells and pathogens¹⁸, BBB also prevents the entry of most drug molecules to protect the brain^{25,29}. Therefore, most drug treatments are ineffective for brain diseases because of the unavailability of efficient BBB penetration to reach therapeutic concentration in brain parenchyma.

Nevertheless, brain is the most energy-consuming organ in the body. The human brain receives 20% of cardiac output and consumes 20% of total oxygen, glucose and energy under physiological conditions for proper functioning of the brain^{30–32}, although representing only 2% of total body mass. The human brain contains ~644 km of blood vessels in total¹². Capillaries account for approximately 85% of cerebral vessel length and provide ~12 m² of the endothelial surface area available for transport exchanges. The mean intercapillary distance in human brain is about 40 μm³³, making the equilibration of solutes almost instantaneous throughout the brain interstitial space once they cross the BBB. BBB plays the lion's share in providing the energy and nutrients including amino acids, peptides, sugars, vitamins, ferric ion, nicotinic acetylcholine and low-density lipoprotein (LDL) to the brain^{7,34,35}. Various kinds of highly expressed receptors and transporters on the BBB play the critical roles in supplying brain with these essential nutrients^{3–7}. These highly enriched receptors and transporters at the BBB include transferrin receptor (TfR), LDL receptor (LDLR) and glucose transporter-1

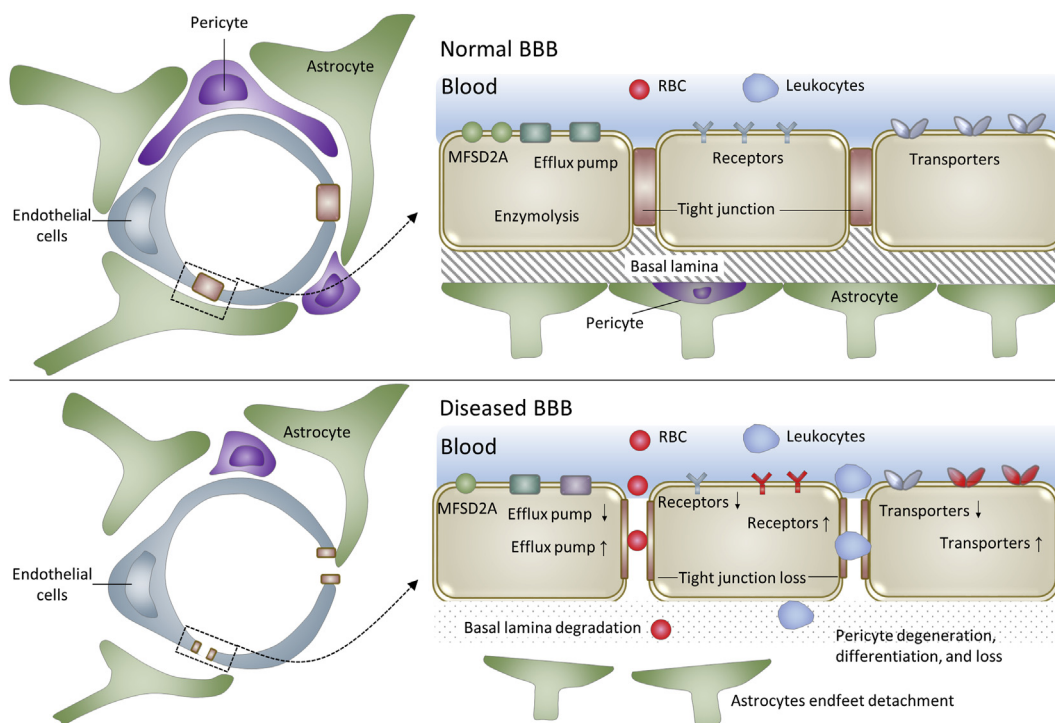


Figure 1 Schematic drawings of the blood–brain barrier (BBB) in normal brain and the diseased BBB in most brain diseases.

(GLUT1) and so on. These receptors and transporters use active transport and facilitative transport to transfer nutrients and energy^{34,36}. Therefore, these receptors and transporters are promising for brain-targeting drug delivery^{7,8}.

2.2. Diseased BBB

Generally, the BBB dysfunctions in various brain diseases, include increased permeability from loss of TJ and enhanced transcytosis rate from loss of MFS2A and/or the increase of caveolin-1 expression (Fig. 1)^{20,37,38}, weaken or strengthened CMT and RMT functions [e.g., impaired glucose transport and active efflux from reduction of GLUT1 and P-glycoprotein (P-gp) and dysregulated blood-to-brain transport of amyloid beta ($A\beta$) from increased receptor for advanced glycosylation end products (RAGE) in AD], microbleeds, perivascular deposits of blood-derived products, cellular infiltration and degeneration of pericytes and endothelial cells¹².

Drug delivery across the diseased BBB is different and more complex than across the normal BBB. BBB alterations affect drugs' brain accumulation and intracranial distribution in both stimulative and suppressive ways. From the stimulative perspective, BBB alterations, especially increased paracellular permeability from loss of junction proteins, present an opportunity to deliver drugs, which would normally be excluded by healthy BBB, to the diseased brain regions and affected neurons^{12,15}. However, from the suppressive perspective, influx of blood-borne molecules and cells interferes with the normal solute diffusion in brain interstitial fluid, resulting in impaired drug distribution throughout the brain. Therapeutic agents are likely to get trapped in pathologically altered and enlarged perivascular spaces, preventing them from reaching a little distant neuronal targets¹². Solute diffusion in regions with BBB disruptions would become

poor, preventing drugs from reaching areas of the brain more than a few hundred microns from the lesion³⁹. Paradoxically, BBB disruptions even retard brain retention of some drugs³⁹, because the efflux transporter for potassium is so robust that BBB disruption does not alter its concentration in the cerebrospinal fluid⁴⁰. Therefore, BBB dysfunction can improve or impede drug delivery across the BBB to brain.

2.2.1. AD

AD is the most common neurodegenerative disorder and contributes to ~70% of dementia cases^{15,41}. Cortical and cerebrovascular extracellular deposits of $A\beta$ and the accumulation of intracellular neurofibrillary tangles which are formed by the aggregation of hyperphosphorylated microtubule-associated tau protein are the main histopathological features of AD⁴². In behavior, AD patients display loss of spatial and temporal orientation and verbal fluency.

Under physiological conditions, endogenous $A\beta$ is transported bidirectionally across the BBB and has the ability to promote memory³⁹. Influx receptor RAGE, efflux receptor LRP1 and transporter P-gp act in tandem to maintain the brain levels of $A\beta$ at its most optimal concentration^{43–47}. $A\beta$ accumulation in brain with AD is implicated in BBB evolutions. Under AD conditions, both increased blood-to-brain transport by up-regulated RAGE and decreased efflux by reduced LRP1 and P-gp enhance $A\beta$ accumulation in brain with AD³⁹. It has been proved in animal models that BBB evolutions occur predominantly in microvessels that are surrounded by plaques⁴⁸. In addition, reduced GLUT1 at the BBB leads to GLUT1 deficiency, early cerebral microvascular degeneration, blood flow reductions and further BBB breakdown, which can worsen AD cerebrovascular degeneration, neuropathology and cognitive function⁴⁹. And the transcytosis rate increases in AD by the loss of pericyte coverage^{12,14}, through the

possible MFSD2A decrease or caveolin-1 increase on BBB endothelial cells. Therefore, BBB evolutions are involved with the pathogenesis and pathology of AD^{12,15,16,18,39,50–52}. The transport-related BBB evolutions include but are not limited to 1) RAGE up-regulation, 2) LRP1 reduction, 3) GLUT1 reduction, 4) TJ loss, 5) possible MFSD2A decrease or caveolin-1 increase, 6) P-gp reduction and 7) basal lamina degradation by matrix metalloproteinase-9 (MMP9)¹². In addition, the expression of adhesion molecules may be increased for diapedesis of leukocytes (Fig. 2).

2.2.2. PD

PD is the second most common neurodegenerative disorder. PD is characterized by accumulation of α -synuclein and degeneration of dopaminergic neurons¹². The α -synuclein is pathologically aggregated into Lewy bodies or cytoplasmic eosinophilic inclusions⁵³. The formation of Lewy bodies is firstly at the brain stem and olfactory bulb and later at cortex and nigra and can be observed in most neuronal populations in PD patients. This ultimately leads to the dysfunctioning of neurotransmitter systems such as adrenergic, cholinergic and serotonergic. The loss of dopamine happens mainly in regions from the substantia nigra pars compacta to the corpus striatum^{41,50}, resulting in bradykinesia, rigidity and resting tremor, as dopamine is involved in the transmission of electric signals to the normal physical motion.

BBB evolutions are a critical feature of PD pathology and occur throughout the basal ganglia of PD patients¹⁸. Vascular dysfunction consisting of BBB breakdown and dysfunction, leads to neurotoxic accumulation of fibrin (ogen), thrombin, plasmin (ogen) and red blood cell (RBC) extravasation, and release of hemoglobin and iron causing reactive oxygen species (ROS), which all can injure dopaminergic neurons¹⁸. Lee et al.⁵⁴ reviewed how BBB evolutions promote the onset and development of PD and how PD compromise BBB. The pathway for mediating the

BBB crossing of the α -synuclein and for contributing the α -synuclein pool in the brain may be increased¹⁸. Endothelial LRP1 is a potential efflux transporter for α -synuclein and may be similarly downregulated in PD as in AD^{55–57}. Oxidative stress has been identified as a putative molecular contributor to PD pathogenesis and implicated as harmful to BBB by apoptosis inducing and MMP9 activity increasing⁵⁴. Chen et al.⁵⁸ revealed reduced TJ proteins zona occludens 1 (ZO1) and occludin in the striatum in PD mouse model. Korteckaas et al.⁵⁹ found higher accumulation of verapamil by reduced P-gp in PD brain. Several other studies reported angiogenic activity in PD-affected regions (*e.g.*, basal ganglia)⁵⁴. By summarizing current existing reports, transport-related BBB evolutions in PD include but are not limited to 1) up-regulation of unknown pathway for BBB crossing of α -synuclein, 2) LRP1 reduction, 3) TJ loss, 4) P-gp reduction, 5) possible integrin increase for angiogenesis, 6) the possible increased expression of adhesion molecules for diapedesis of leukocytes and 7) basal lamina degradation by MMP9 (Fig. 3).

2.2.3. Epilepsy

Epilepsy is a chronic neurological disorder manifesting as spontaneous, recurrent and unpredictable seizures resulting from transient abnormal electrical activity in the brain^{15,60}. The main causes of epilepsy include infection progression in the brain, reduced oxygen supply, head trauma, arteriovenous malformations, cerebrovascular diseases and perinatal injuries⁵⁰. The impact of epilepsy is multidimensional and extends far beyond the harm induced by seizures themselves⁶¹. The unpredictability of seizures imposes severe lifestyle restrictions on patients with epilepsy, resulting in significant impairments in psychological, emotional, economical and/or social spheres⁶¹.

L-Type amino acid transporter 1 (LAT1) is up-regulated due to the huge consumption for tryptophan in epileptogenic focus for the aberrant activation of kynurenine pathway to synthesize

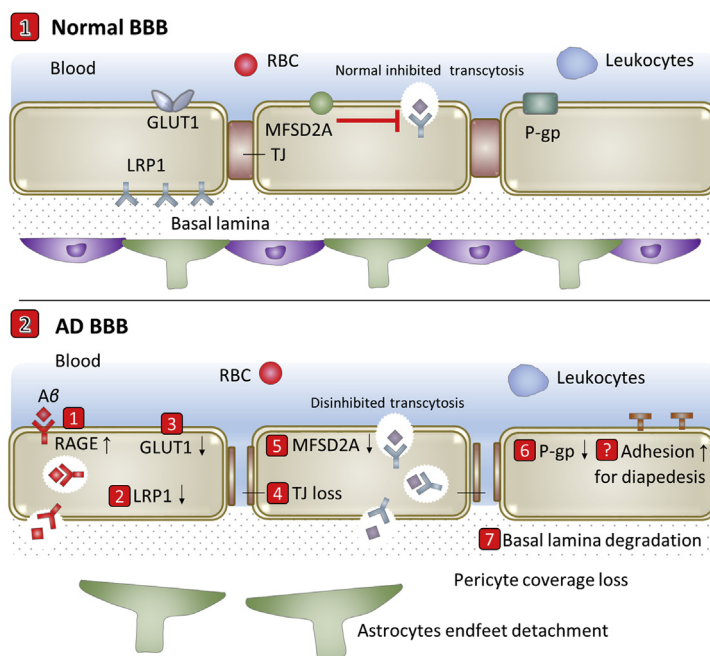


Figure 2 Scheme of transport-related BBB evolutions in Alzheimer's disease (AD).

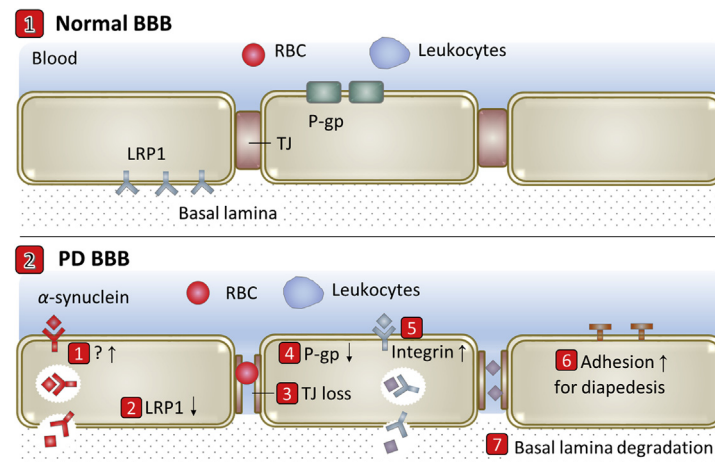


Figure 3 Scheme of transport-related BBB evolutions in Parkinson's disease (PD).

kynurenic acid and quinolinic acid⁶². Specific up-regulation of multidrug efflux transporter P-gp is another important BBB evolution in epilepsy^{61–64}. Loss of TJ and increased microvascular density were also observed in temporal lobe epilepsy¹⁸. These BBB dysfunctions are localized to epileptic regions and positively correlates with seizure frequency, suggesting that BBB plays a contributory role in epileptic disorders. Neuroinflammation is also a hallmark of epileptogenic brain tissue⁶⁵, during which leukocytes are extensively recruited and trafficked into the brain parenchyma by chemotaxis and diapedesis⁶⁶. By summarizing current existing reports, transport-related BBB evolutions in epilepsy include but are not limited to 1) LAT1 up-regulation, 2) P-gp up-regulation, 3) TJ loss, 4) possible integrin increase for angiogenesis and 5) increased expression of adhesion molecules for diapedesis of leukocytes (Fig. 4).

2.2.4. Stroke

Stroke, secondary to occlusion of cerebral vessels, is a leading cause of death and disability worldwide⁶⁷. The ischemic cascade involves necrotic cell death, apoptotic cell death and inflammation because neurons are very vulnerable to reduction in supply of oxygen and glucose from reduced cerebral blood flow. Reperfusion of blood to ischemic brain is the only option to reverse brain

damage after a stroke. At present, restoration of blood flow *via* fibrinolytic tissue plasminogen activator (tPA) is the only approved clinical treatment for stroke. However, besides the short therapeutic window of 3–4.5 h post-onset⁶⁸, tPA activates different pathways for detachment of astrocytes endfeet, MMP9-mediated degradation of basal lamina¹⁵, and TJ loss, contributing to BBB alterations.

Biphasic BBB breakdown before neurological damage has been proved in both human and animal ischemia models⁶⁹. As shown in Fig. 5, the most accepted model of this biphasic BBB hyperpermeability is characterized by (a) an early phase (occurring a few hours poststroke) of enhanced transcellular transport mediated by caveolin-1 increase²⁰ and possible MFSD2A decrease, followed by (b) a delayed phase of hyperpermeability (~2 days poststroke) in which both enhanced transcellular transport and TJ protein disassembly contribute to the loss of BBB integrity^{20,67}. The ischemia-induced BBB degradation leads to blood shift to the ischemic brain, with the risk of developing edema and hemorrhagic transformation^{70,71}. The reperfusion also causes neuroinflammation response⁷², during which the up-regulated adhesion molecules at brain capillary endothelial cells strongly interact with activated leukocytes, leading their entry into brain¹⁵. The processes involve capture, rolling, activation, adhesion, crawling and *trans-* or *para-*cellular diapedesis. In addition,

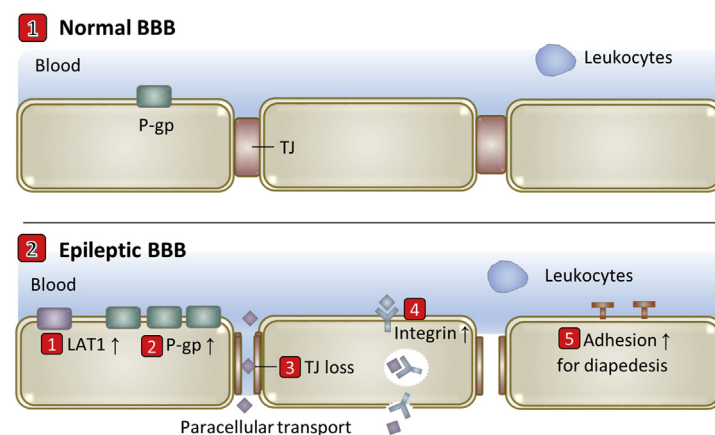


Figure 4 Scheme of transport-related BBB evolutions in epilepsy.

integrin $\alpha v\beta 3$ is up-regulated on reactive brain capillary endothelial cells for angiogenesis after ischemia⁷³.

2.2.5. TBI

TBI produces primary physical damage to neurons and vessels, leading to loss of neuron function and hemorrhage. The release of intracellular contents from disrupted neurons and the influx of blood components cause secondary injury including ionic imbalance, cytotoxicity, free radical production, oxidative stress, inflammation and further BBB breakdown. These responses lead to vasogenic/osmotic edema for hours and days, resulting in lifelong physical, cognitive and psychosocial impairments^{74,75}. The primary injury appears to be irreversible, making the focus of therapeutic strategies on neuroprotective approaches that suppress progressive cell death and tissue damages during the secondary injury⁷⁴.

Besides the direct physical damage to vessels by TBI, the hemorrhage and edema lead to further damages to BBB at the lesion site and neighboring regions⁷⁴. The transcytosis rate increases after TBI, through caveolin-1 increase and possible MFSD2A decrease from pericyte deficiency (Fig. 6). Neuroinflammation involves release of cytokines and chemokines, which activate endothelial cells to up-regulate adhesion molecules to recruit neutrophils through the decreased TJ and degraded extracellular matrix. Changes of molecular expression undergone on neurovascular cells (*e.g.*, down-regulation of P-gp) have been extensively reviewed^{37,38,76}. Oxidative stress and neuroinflammation induce expression of MMPs, which degrade extracellular matrix and TJ³⁷. Neuroinflammation promotes the upregulation of the water channel aquaporin 4 and

contributes to swelling of endothelial cells and astrocytic endfeet^{37,74}. It is noteworthy that most of these processes are interconnected and happen simultaneously.

It is noteworthy that endothelial cells recover vascular integrity to brain and angiogenesis occurs to support the newly formed scar and connective tissues after astrocytes reestablish glial limitans surrounding the injury tissue. The recovery reestablishes the BBB in the injured brain that revives the inherent problems of drug delivery in the chronic phase following TBI⁷⁴. Studies in rats with controlled cortical impact have shown that claudin-5 was decreased early at three days after injury but increased when P-gp was upregulated one week after injury³⁸. In addition, the recovery of disrupted BBB appears to be highly variable depending on injury types, severity and complexity.

2.2.6. Brain tumor

Brain tumor is a life-threatening brain disease with a relatively low survival rate⁷⁷. Glioblastoma, the most invasive brain tumor, is characterized by the highest mortality rate, short lifetime and poor prognosis with a high tendency of recurrence. Surgery is often applied to excise the tumor bulk, but the infiltrating tumor cells inside the normal brain parenchyma cannot be completely removed. On the other hand, these residual tumor cells are protected against drug therapy by the BBB, leading to the tumor recurrence⁷⁸.

The progression of brain tumor leads to BBB changes including astrocyte endfeet displacement and heterogenous subpopulation of pericyte and astrocyte, forming highly

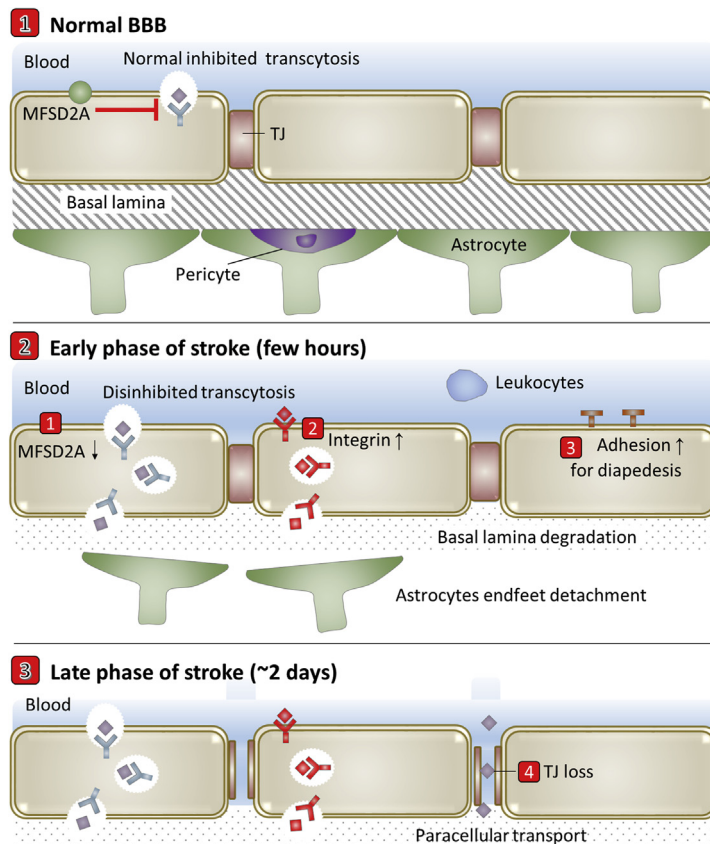


Figure 5 Scheme of transport-related BBB evolutions in stroke.

heterogeneous and compromised BBB⁷⁹. The BBB in brain tumor is often termed as blood–brain tumor barrier (BTB), with different structural and functional characteristics to the BBB (Fig. 7). The transcytosis rate increases on BTB through the decrease expression of MFSD2A⁸⁰. Permeability of the BTB to drugs or contrast agents was higher but more heterogeneous than that of the BBB⁸¹. ATP-sensitive potassium channels (K_{ATP}) and calcium-dependent potassium channels (K_{Ca}) are specially up-regulated on BTB endothelial cells to regulate BTB permeability⁸². As a transmembrane protein with internalization function, prostate-specific membrane antigen (PSMA) is specifically expressed on "breast cancer brain metastases-associated" BTB endothelial cells^{83–86}. Downregulation of ZO1 and vascular-endothelial cell adhesion molecule (VE-CAM) leads to the loss of continuous endothelial cell adhesions, creating abnormal molecular permeation channels⁸¹. The BTB was also found to differ from the BBB by: the presence of swollen capillary endothelial cells; abundant vascular endothelial growth factor (VEGF); a prominent local neuroinflammatory milieu consisting of activated microglia, astrocytes and immune cells; an altered basement membrane composition; shifts in subpopulations of pericytes; and loss of astrocyte endfoot polarization of aquaporin channels⁸¹. Other molecular alterations reported in BTB endothelial cells that contribute to increased permeability include altered expression of membrane transporters (GLUT1 and breast cancer resistance protein⁸⁷), cytokine receptors such as the receptor for tumor necrosis factor and membrane proteins and growth factors including claudin-5 and angiopoietin 2⁸¹.

2.2.7. MFSD2A decrease in different brain diseases

In the brain, MFSD2A is exclusively expressed at luminal side of BBB^{21,31}. Pericytes are required for MFSD2A expression⁸⁸. MFSD2A transports lysophosphatidylcholine esterified docosahexaenoic acid to supply the brain with the essential omega-3 fatty

acid²². Of particular note is that MFSD2A is required for proper BBB development and functional integrity^{21,31,32}. MFSD2A suppresses caveolae-mediated transcytosis to regulate BBB permeability *via* controlling lipid composition of BBB endothelial cells^{21,31,32}. In addition, pericytes can regulate vesicular transport at the BBB *via* regulating MFSD2A expression³¹.

MFSD2A decrease leads to increase of vesicular transport in the BBB^{79,89}. The response of MFSD2A to pathological processes has been revealed in some brain diseases⁹⁰, for example, MFSD2A decrease in the perihematomal tissue on mice model of intracerebral hemorrhage and subarachnoid hemorrhage^{89,91}. MFSD2A decrease was also shown on disrupted brain vascular endothelium in brain metastatic tumors^{79,80}. In addition, increased transcytosis in the BBB of AD, ischemic stroke and TBI^{12,20,37,38}, suggests the possible MFSD2A decrease at the BBB besides the increase of caveolin-1 expression. For now, there is no report on directly comparing the degree of MFSD2A decrease in different brain diseases. The involvement and decrease extent of MFSD2A in different neurological diseases deserve further investigation⁹².

2.2.8. TJ loss in different brain diseases

Almost all brain diseases are involved with loss of TJ. However, for TJ loss, difference of cause, loss extent, time, and location exists between different brain diseases, especially between acute and chronic diseases of the brain. AD and PD belong to chronic neurodegenerative disorders while TBI and stroke are acute brain insults^{93,94}. For AD, endothelial GLUT1 deficiency initiates early BBB disruption as represented by the reduction in TJ proteins in mice^{16,49}. RAGE-mediated $A\beta$ cytotoxicity contributes to disruption of TJ *via* Ca^{2+} -calcineurin signaling⁵². The length of TJ in mice with 5 familial AD mutations is significantly shorter than that in littermate control mice^{16,18}. In addition, the TJ loss in AD is mainly located in the cortex and hippocampus¹². For PD, α -synuclein accumulation may be an important contributory event in

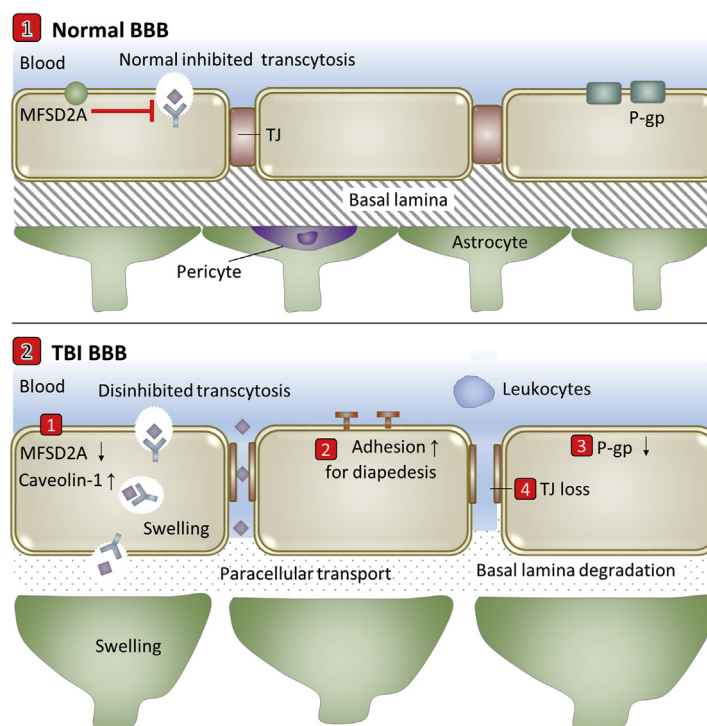


Figure 6 Scheme of transport-related BBB evolutions in traumatic brain injury (TBI).

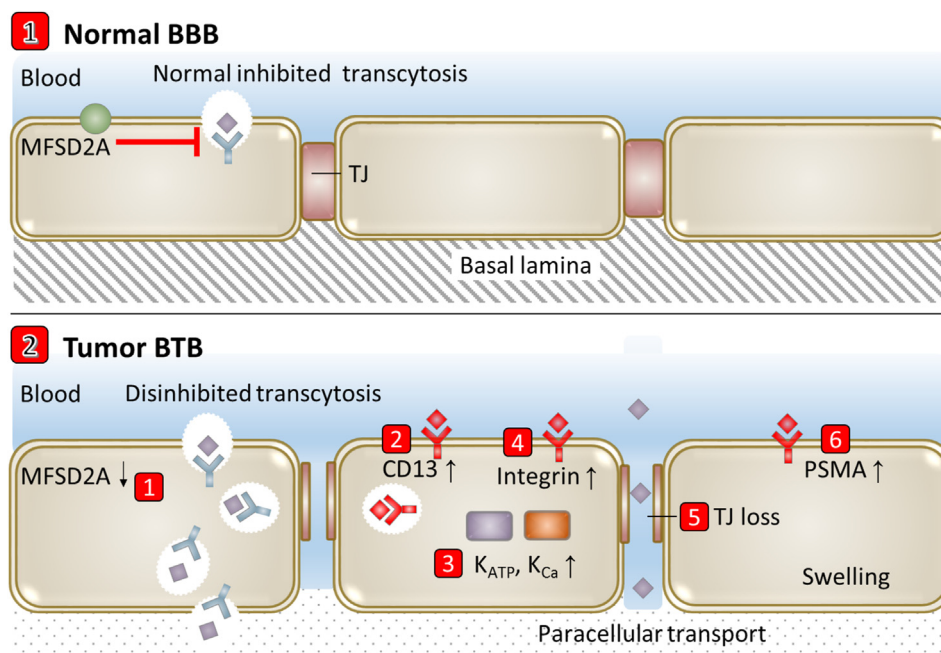


Figure 7 Scheme of transport-related BBB evolutions in brain tumor.

the pathogenesis¹⁸. The reduction of TJ proteins in PD is located in the basal ganglia^{12,18,54}.

Acute brain diseases often display relatively instant loss of TJ from the angle of time. For epilepsy, TJ loss was observed early after status epilepticus as well as during latent and chronic periods, and the permeability increase is positively correlated to seizure frequency⁹⁵. Both similarities and differences on loss of TJ exist between TBI and ischemic stroke⁹⁶. For TBI, the BBB permeability to macromolecules are biphasic on rat TBI models, peaking at 4–6 h and Day 2–3 after injury⁷⁶. The expression of claudin-5 rises again 1–2 weeks after injury and remain elevated as much as 4–8 weeks after initial injury. For stroke, TJ remains normal even after 25 h of cerebral ischemia after embolic middle cerebral artery occlusion and display profound structural defects 2 days after ischemia²⁰. Active remodeling of TJ may occur in the late phase of reperfusion when angiogenesis of brain vessels begins. For brain tumor, tumor cells associate with blood vessels and insert themselves between the endfeet and the endothelial wall of the preexisting blood vessel to alter astrocyte-vascular interactions at the preexisting vasculature. The loss of contact between endfeet and blood vessels leads to loss of TJ. Importantly, single glioma cells are sufficient to locally open the BBB⁹⁷. In addition, VEGF produced in brain tumors, can promote endocytosis of the endothelial cell adhesion molecule VE-cadherin, leading to BBB disruption and increase in endothelial permeability⁹⁸.

The optimized size of nanocarriers based on the opening of BBB gaps for different brain diseases was discussed in “Section 4.2. TJ loss based strategy”. For now, the comprehensive characterization and quantitative comparison of “dynamic” TJ loss between different brain diseases depend on the disease progression stage, the established animal model and the individual difference. Liu et al.¹³ previously compared BBB permeability between ischemic stroke and TBI on animal models and found that the BBB dysfunction in cold-injury-induced TBI was more serious than that in ischemic stroke. However, the related studies

are still in their infancy and thus need to be extensively and intensively researched. The design of a kind of effective, acceptable and quantitative index will be helpful.

3. Nanoscale drug delivery systems

Over the past few decades, the rapid expansion of nanotechnology has begun to be developed for biological and medical applications. In drug delivery field, nanoscale drug delivery systems have already started to exhibit immense potential due to many incomparable advantages. Such advantages include but are not limited to decreased side effects, prolonged blood circulation, and increased drug stability, bioavailability and targeting efficiency^{10,11}. Besides, versatile nanocarriers could simultaneously carry imaging agents, therapeutic drugs and targeting ligands, which is beneficial for tracing drug distribution, activating drug release from an external stimulus (*e.g.*, laser light, temperature, or ultrasound), and theoretically detecting potentially dangerous drug accumulation. Based on all these advantages, nanoscale drug delivery systems have found renowned attention in tumor-targeting imaging and drug therapy⁹⁹. Due to the rapid expansion of nanotechnology, various types of nanocarriers have been developed, for example, liposomes, micelles and various nanoparticles (NPs, Fig. 8).

Nanoscale brain-targeting drug delivery systems have great perspectives for drug management of brain diseases. For this application, the nanocarriers should be capable of overcoming the BBB, and improving diagnostics and drug therapy efficiency. With subtle design, the functionalization could assist the translocation of nanocarriers across the BBB and provide satisfactory BBB permeation¹⁰⁰. Endogenous and exogenous specific ligands and substrates have been extensively used to enhance the efficiency of BBB crossing and brain-targeting delivery *via* binding with specific CMT or RMT on the BBB^{3,4,101,102}. For example, transferrin, lactoferrin, glucose, polysorbate 80 and anti-TfR antibodies have been developed as brain targeting ligands and have shown high

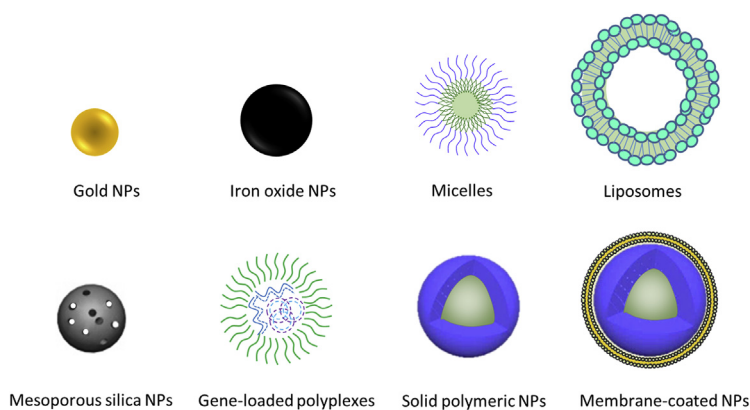


Figure 8 Various types of NPs developed for drug delivery.

efficiency in amplifying BBB penetration and brain delivery of nanocarriers^{41,103–110}. Nanocarriers can also be functionalized by loading BBB permeability modulators (*e.g.*, lexiscan) and P-gp inhibitors and conjugating with cell penetrating ligands, to regulate drug diffusion through paracellular barrier, inhibit active efflux, and utilize adsorptive-mediated transcytosis, respectively^{111–113}. The capacity to cross the BBB offers new ways for drug delivery to brain, which remain much needed in brain diseases. Nazem and Mansoori reviewed the promise of nanotechnology on the diagnosis and therapy of AD and other neurodegenerative diseases¹¹⁴. Kubinova and Sykova¹¹⁵ summarized applications of nanocarriers for therapy of brain stroke and spinal cord injury.

4. Diseased BBB-based brain-targeting delivery strategies

Because of BBB evolutions in brain diseases, drug delivery through the diseased BBB is different and more complex than through the normal BBB. However, BBB crossing strategies can be correspondingly designed against the altered BBB. According to the general BBB alterations in brain diseases, the following strategies have been reported or remain to be explored to specially deliver drug across the diseased BBB.

4.1. Evolutionary RMT and CMT based strategy

In the brain, MFSD2A selectively transports the omega-3 fatty acid docosahexaenoic acid across the BBB^{21,22,80}. More importantly for drug delivery, MFSD2A suppresses caveolin-dependent transcytosis on the BBB, with genetic deletion of murine MFSD2A leading to enhanced transcellular transport and breakdown of the vascular endothelial barrier in the brain^{23,116}. It has been reported that the expression of MFSD2A on brain capillary endothelial cells is significantly reduced in brain regions affected by intracerebral hemorrhage, subarachnoid hemorrhage and metastatic brain tumors^{79,80,89,91}. Therefore, RMT-based ligand-modified brain-targeting delivery strategies should have enhanced crossing efficiency for diseased BBB than normal BBB. In addition, transcytosis of ligand-modified nanocarriers *via* RMT is likely to be trapped within brain capillary endothelial cells due to the high binding affinity of ligand with receptors, which greatly reduces the amount of nanocarriers across BBB^{117,118}. Acid-responsive dissociation of brain-targeting ligands in endo/lysosomes was previously reported with high efficiency in improving escape from endo/lysosome and transcytosis across the

BBB^{117,118}. The increased transcytosis by MFSD2A decrease holds the potential to combine with the promising acid-responsive ligand dissociation strategy for obtaining significantly improved brain-targeting efficiency.

As listed in Table 1, the up-regulated RAGE in AD endows the decoration of RAGE-specific ligand on nanocarriers with the ability of mediating crossing of BBB associated with AD. Integrin receptors for angiogenesis are increased on reactive brain capillary endothelial cells in PD, ischemia and brain tumors^{54,73,119}, which makes it feasible to use related ligands to mediate the crossing of the diseased BBB in these lesions. Epilepsy-specific over-expression of multidrug efflux transporters, such as P-gp, reduces intracranial concentrations of antiepileptic drugs^{62–64}, and should be emphasized when designing drug delivery systems for epilepsy. The reduced GLUT1 and LDLR-related protein 1 (LRP1) on the BBB in AD may need to be reversed to strengthen the BBB crossing efficiency of glucose-modified nanocarriers or angiopep-2-modified nanocarriers. BTB in brain tumors is often induced by tumor to express PSMA, CD13, integrin receptors, K_{ATP} and K_{Ca} ^{119–123}, which can be utilized to mediate the crossing of the barrier in brain tumor regions. Some typical brain-targeting drug delivery systems based on up-regulated targets in different brain diseases are listed in Table 2^{65,73,82,120,124–126}.

4.2. TJ loss-based strategy

If impaired drug diffusion inside the brain was not a consideration, any loss of TJ in the BBB would improve delivery of drugs, especially nanocarriers, to the brain. Nanocarriers allow rapid accumulation in damaged brain regions with TJ loss, while still being large enough to be retained in these regions, not diffusing away as quickly as smaller molecules¹²⁷. This phenomenon is much similar to the enhanced permeability and retention (EPR) effect seen in tumors^{128,129}. More importantly, the size of nanocarriers should be optimized for different brain diseases and diseases at different stages because of the different leaky gaps at the diseased BBB. Based on the TJ loss in different brain diseases, the size of nanocarriers should be between the normal BBB pore size (1.4–1.8 nm) and the opening of TJ in different brain diseases^{66,130}. Generally, the space between TJ is narrower than that between adherent junctions (~ 20 nm)⁸⁸. There is limited quantitative characterization on the extent of BBB opening at different stages in AD, PD and epilepsy. However, it is estimated that even under severe pathological conditions, only nanocarriers with size smaller than 20 nm can penetrate the BBB⁶⁶. Amyloid oligomer-

specific scFv antibody-modified polyethyleneglycol (PEG)ylated superparamagnetic iron oxide NPs with size of 11.8 nm were shown to be able to cross the BBB and specifically bind to the PD area¹³¹. Similar iron oxide NPs with size of 10–20 nm were shown to be able to penetrate the BBB on acute temporal lobe epilepsy model^{132,133}. There exists some controversy about the opening extent of BBB gaps after stroke. On the one hand, up to 30% of TJ were reported to be open at 48–58 h after transient middle cerebral artery occlusion, and the opening of these gaps can increase progressively, reaching gaps of 0.2–1.2 μm ^{20,67}. On the other hand, the pore size upper limit of ischemic vasculatures in mice was determined to be in the range of 10–11 nm¹³⁴. The pore size upper limit in brain tumor vasculatures was reported to be ~ 12 nm^{134,135}. However, nanocarriers with size in the range of 5–40 nm were demonstrated to be able to permeate through the BTB^{136,137}. The size of BBB opening after TBI on animal models depends on the impact types (focal and diffuse) and different stages. On blast-induced TBI mouse model, BBB opening in the acute period was ~ 70 kDa (hydrodynamic diameters ~ 10 nm), followed by recovery of BBB integrity by 1 day post-injury¹³⁸. On controlled cortical impact model, selective influx of 82 nm PEG-liposomes was found at 0–8 h after injury¹²⁸, and both 20 and 40 nm PEGylated polystyrene NPs accumulated significantly in the injury penumbra up to 13 h after injury^{75,139}. On diffuse TBI model, PEGylated polystyrene NPs failed to accumulate in the brain tissue after mild closed-head injury¹⁴⁰. On cryo-lesion TBI model, 100 nm NPs were shown to have greater penetration onto the brain lesion than larger NPs¹⁴¹.

4.3. Neuroinflammation based strategy

The influx of blood-borne molecules and cells into brain due to the BBB disruption in brain diseases, leads to inflammatory and immune responses, making most of brain diseases accompanying with neuroinflammation. In the whole process including initiation, maintenance and duration, blood-borne cells including RBCs, platelets and leukocytes are extensively recruited and trafficked into the brain parenchyma by chemotaxis, diapedesis and precise molecular interactions⁶⁶, which is crucial for the development of inflammatory¹⁵. The up-regulated adhesion molecules at the surface of brain capillary endothelial cells lead to leukocyte entry to brain. Leukocytes infiltrate across the BBB in a multistep sequential process involving capture, rolling, activation, adhesion, crawling and *trans-* or *para-*cellular diapedesis. The recruitment of leukocytes to intracranial lesions offers a unique opportunity for crossing of the diseased BBB and the leukocytes could serve as carriers to deliver drugs across the BBB to inflammation sites inside brain¹⁴². The designed drug-bearing nanocarrier-phagocytosed

leukocytes could directly target pathologically affected regions in the brain⁶⁶.

Cell-membrane-coating nanotechnology has attracted much attention to constructing biomimetic drug delivery systems with synthetic NPs as the core and a layer of natural cell membrane as the shell¹⁴³. The biomimetic nanocarriers inherit the properties of the source cells, possessing a series of favorable functions such as prolonged circulation and disease-relevant targeting¹⁴⁴. Some cell membrane camouflaged nanocarriers have been applied for imaging and photoactivatable therapy in various cancer models^{126,145–152}. Therefore, biomimetic nanocarriers coated by membrane from blood-borne cells can be designed and should be capable of crossing the diseased BBB through the neuroinflammation process.

4.4. Selecting the most favorable transport-related BBB molecular changes for different brain diseases

Different brain diseases display various BBB evolutions throughout the onset and progression. Various BBB crossing strategies can be designed based on these BBB evolutions for brain-targeting drug delivery. For example, for designing AD-targeting nanocarriers, favorable transport-related BBB molecular changes in AD include TJ loss, RAGE up-regulation, adhesion molecule up-regulation and increased transcytosis related possible MFSD2A decrease or caveolin-1 increase. For other brain diseases, transport-related BBB molecular changes can be found in Table 1 and Section 2.2. Diseased BBB.

The most favorable transport-related molecular changes for designing targeted delivery systems for specific brain disease remain to be investigated. TJ loss allows nanocarriers with size less than 20 nm to penetrate the diseased BBB *via* an EPR-like effect. MFSD2A decrease, caveolin-1 increase and receptor up-regulation could improve transcytosis of ligand-modified nanocarriers through the diseased BBB. Based on increased transcytosis, ligand-modified nanocarriers can be several hundred nanometers with relatively high drug loading. It is complicated to determine which transport-related BBB molecular change is most favorable for designing brain-targeting nanocarriers for specific brain diseases. On the one hand, the degree of transport-related BBB molecular changes determines the efficiency of related strategies. Unfortunately, the quantitative characterization on the extent of many transport-related molecular changes remains to be deciphered. On the other hand, physicochemical properties of nanocarrier (*e.g.*, chemical composition, size and zeta potential) may impact the efficiency. It is remarkable that the efficiency of BBB crossing and brain targeting drug delivery may be significantly boosted by engineered strategies integrating different BBB molecular changes

Table 1 Transport-related molecular changes in various brain diseases.

Type of brain diseases	Transport-related BBB molecular change
AD	↓ MFSD2A (possible), ↑ Caveolin-1 (possible), ↑ Adhesion molecules, ↑ RAGE, ↓ LRP1, ↓ GLUT1, ↓ P-gp
PD	↑ α -Synuclein pathway, ↓ LRP1, ↑ Integrin, ↓ P-gp
Epilepsy	↑ LAT1, ↑ P-gp, ↑ Integrin
Stroke	↓ MFSD2A (hemorrhagic), ↑ Caveolin-1, ↑ Integrin
TBI	↓ MFSD2A (possible), ↑ Caveolin-1, ↑ Adhesion molecules, ↓ P-gp
Brain tumor	↓ MFSD2A, ↑ PSMA, ↑ CD13, ↑ Integrin, ↑ K_{ATP} , ↑ K_{Ca}

Table 2 Typical brain-targeting drug delivery systems based on diseased BBB in different brain diseases.

Brain disease	Up-regulated target on diseased BBB	Ligand	Example	Ref.
AD	RAGE	KLVFFAED	A reactive oxygen species (ROS)-responsive curcumin-loaded polymeric micelle to normalize the oxidative and inflammatory microenvironment and reeducate microglia through ROS scavenging and $A\beta$ inhibition.	124
Epilepsy	Adhesion molecules	Monocytes	Monocytes for delivery of magnetite-laden NPs to epileptogenic brain tissue to delineate inflammation in epilepsy.	65
Stroke	Integrin $\alpha v\beta 3$	Cyclo (Arg-Gly-Asp-D-Tyr-Lys) c(RGDyK)	The engineered c(RGDyK)-conjugated exosomes to deliver curcumin to ischemic region to suppress inflammatory response and cellular apoptosis in the lesion region.	73
Stroke	Adhesion molecules (<i>e.g.</i> , P-selectin & ICAM-1)	Neutrophils	Neutrophil membrane-derived nanovesicles loaded with resolvin D2 to enhance resolution of inflammation to protect brain damage during ischemic stroke.	125
Glioma	CD13	Cyclo(CRNGRGPDC) iNGR	The modified complexes between polycation pOEI and siRNA nanospheres for delivery of RNA interference oncotherapy to glioma.	120
Glioma	Integrin $\alpha v\beta 3$	c(RGDyK)	The modified red blood cell membrane-coated drug nanocrystals for effective drug delivery to glioma.	126
Brain metastases	K_{ATP}	Loaded minoxidil	The minoxidil-loaded hyaluronic acid-tethered NPs can boost transcytosis across the BTB through strengthening activation of up-regulated K_{ATP} on BTB to deliver doxorubicin to brain metastases.	82

and impacted by BBB recovery or deterioration after drug intervention of the disease progression.

5. Applications

5.1. AD

5.1.1. RAGE up-regulation

RAGE expression in brain microvasculature is increased in AD parallel to advanced stages of the disease^{153,154}. The up-regulated RAGE and RAGE-mediated transcytosis pathway can be employed for brain drug delivery. Lu et al.¹²⁴ reported a RAGE-targeting ROS-responsive polymeric micelle system to normalize the oxidative and inflammatory microenvironment and reeducate microglia from an early phase of AD based on the crosstalk between microglia and brain microenvironment. Through the RAGE-mediated $A\beta$ transportation pathway, the micelles accumulated into the diseased regions and exert synergistic effects of polymer-based ROS scavenging and cargo-based $A\beta$ inhibition upon microenvironment stimuli. Wu et al.¹⁵⁵ found that the nanocarrier assembled by hierarchical forms of a brain specific phage-derived peptide can target brain capillary endothelial cells through both RAGE and TfR, cross the BBB and reach neurons and microglial cells. The brain accumulation of the nanocarrier can reach 5.7% of the injected dose on normal mice. With up-regulated RAGE on BBB in AD brain, the brain delivery efficiency of the nanocarrier may be able to be further enhanced on AD mice. Through intravenous injection, effective down-regulation of β -secretase 1 (BACE1) in the brain was achieved

by the delivered short interfering RNA (siRNA) against BACE1 to inhibit $A\beta$ production in AD¹⁵⁵.

5.1.2. Reduction of both LRP1 and GLUT1

Under physiological conditions, LRP1, at the abluminal side of the BBB, binds $A\beta$ and initiates $A\beta$ clearance from brain to blood *via* transcytosis across the BBB. Considering the LRP1 reduction in AD, statins had been used to upregulate LRP1 at the BBB to promote $A\beta$ clearance^{44,154,156}. The strategy of up-regulating LRP1 *via* statins can be employed for brain drug delivery. Guo et al.¹⁵⁷ developed simvastatin-loaded angioprep-2-anchored NPs for raising expression of LRP1 at brain capillary endothelial cells to surmount the low transcytosis of BBB. The developed NPs also heightened LRP1 on brain metastatic tumor cells with improved chemotherapy of brain metastases. However, the strategy of up-regulating LRP1 *via* statins has not been yet applied in brain-targeting drug delivery and therapy for AD.

AD is also characterized by reduced GLUT1 at the BBB and reduction of glucose transport⁴⁹. Anraku et al.¹⁵⁸ designed rapid glycaemic increase after fasting and self-assembled supramolecular nanocarriers with surface featuring properly configured glucose to boost BBB crossing and brain accumulation of glucose-functionalized nanocarrier. The putative up-regulation of luminal GLUT1 by hypoglycemia and the migration of GLUT1 from the luminal BBB to the abluminal BBB by the rapid glycaemic increase after fasting boosted the BBB crossing and brain accumulation of the developed nanocarriers. The brain accumulation of glucose-properly-configured nanocarrier can reach at 6% dose/g-brain on normal mice with glycaemic control. Although it has not

been applied for drug delivery and therapy for AD, this strategy holds the potential to overcome the reduced GLUT1 in AD for drug delivery to brain with AD.

5.1.3. TJ loss

It seems that TJ loss would improve paracellular drug delivery to the brain³⁹. However, BBB with TJ loss is still very restrictive in comparison to peripheral tissue beds³⁹. Cheng et al.¹⁵⁹ found that TJ loss in AD animal model was not sufficient to influence brain influx of small molecule drugs. Nanocarriers with prolonged blood circulation possess the potential to improve the leaky vessel-based drug delivery efficiency. Tanifum et al.¹⁶⁰ designed A β -targeting lipid conjugate-incorporated stealth liposomes to treat amyloid plaque deposition in AD. The liposomes successfully extravasated into brain *via* the compromised BBB and bound with A β plaque deposits¹⁶¹. In addition, lexiscan and minoxidil as BBB modulators were respectively loaded into NPs to further enhance paracellular BBB permeability^{82,111,157,162}. The strategy of co-delivery of BBB modulators can be applied to improve drug delivery to AD brain.

5.2. PD

5.2.1. Crossing of diseased BBB in PD

Although TJ loss and possible increased expression of integrin and adhesion molecules for angiogenesis and leukocyte diapedesis have been identified as critical BBB evolutions in PD, very few diseased BBB-based BBB crossing strategies have been reported for systemic brain-targeting drug delivery. Chen et al.¹⁶³ reported polyethyleneglycol-poly(lactic-co-glycolic acid) (PEG-PLGA) NPs for delivery of schisantherin A and proved the effective brain delivery and strong neuroprotective effects in PD zebrafish model. Although transcytosis was thought as the underlying BBB crossing mechanism, TJ loss may also contribute to brain uptake of NPs. The α -synuclein, which forms Lewy bodies in brain with PD, holds the potential to be engineered as safe functionalization for BBB crossing and PD targeting. However, neither α -synuclein nor derived peptide has been used for brain drug delivery and PD therapy. Divalent cation Ca²⁺ is the α -synuclein-specific pathophysiological ligand. Lee et al.¹⁶⁴ designed gold NPs which were cross-linked by coated cysteine-bearing α -synuclein, to coat mesoporous silica NPs for intracellular Ca²⁺-responsive cargo release. In another study, the α -synuclein was used for the mutual connection of gold NPs for protease-sensitive and light-responsive drug release¹⁶⁵. It can be postulated that α -synuclein-functionalized delivery systems hold the potential for brain drug delivery.

5.3. Epilepsy

5.3.1. P-gp up-regulation

Based on the specific up-regulation of P-gp on the BBB in epilepsy, about 35%–40% of epilepsy patients are resistant to drug therapy. Therefore, development of formulation that can modulate P-gp function as well as facilitate brain delivery of antiepileptic drug represents a promising strategy for epilepsy intervention⁶¹. Zybina et al.⁶³ found that P-gp inhibitor verapamil increased the anticonvulsant effect of carbamazepine and reduced its effective dose by \sim 30% in isoniazid-induced rat epilepsy model.

Fang et al.⁶⁴ designed pluronic P85-coated poly(*n*-butylcyanoacrylate) NPs for brain delivery of phenytoin while overcoming the up-regulated P-gp in rats with chronic temporal lobe epilepsy induced by lithium-pilocarpine. The NPs successfully overcame the drug resistance in epilepsy and efficiently delivered phenytoin to rat hippocampus with P-gp overexpression. Liu et al.⁶² designed tryptophan-functionalized pluronic P123/F127 mixed micelles for delivery of antiepileptic lamotrigine to epileptogenic focus. The delivery was based on the combination of transporter-mediated endocytosis and pluronic block copolymer-mediated overcoming of multidrug resistance. The micelles were efficient in delivering lamotrigine to the brain, especially the hippocampus *via* the tryptophan-mediated active targeting as well as P-gp modulation at the epileptogenic focus.

5.3.2. TJ loss

Alpha-methyl-L-tryptophan (AMT) is a synthetic amino acid and is recognized as a surrogate marker for epilepsy, characterized by high uptake in the epileptic focus. Based on TJ loss in epilepsy, Wang et al.¹³² engineered AMT-modified iron oxide NPs for delivery IL-1 β monoclonal antibody across the BBB in the acute temporal lobe epilepsy for epilepsy treatment. Akhtari et al.¹³³ developed AMT-attached magnetic NPs for magnetic resonance imaging of acute and chronic temporal lobe epilepsy. The magnetic NPs crossed the BBB, entered into brain parenchyma, and localized to epileptogenicity location in both acute and chronic conditions. In another study, magnetic field was used to improve the BBB crossing efficiency of AMT-coated NPs¹⁶⁶. TJ loss was thought as the BBB crossing mechanism of AMT-coated NPs for passive epilepsy targeting.

5.3.3. Neuroinflammation

Neuroinflammation in epilepsy can be targeted by biomimetic delivery systems for BBB crossing through the infiltration mechanisms of leukocytes into the epileptic brain. Han et al.⁶⁵ designed NPs-loaded monocytes for drug delivery to epileptic brain. The distribution of NPs-loaded monocytes in hippocampal CA1 subregion and dentate gyrus in rats with spontaneous seizures was 176- and 380-fold higher than unloaded NPs.

5.4. Stroke

5.4.1. Integrin up-regulation

Integrin receptors, especially $\alpha v \beta 3$, are up-regulated at the BBB in stroke. Tian et al.⁷³ designed c(RGDyK)-conjugated exosomes for delivery of curcumin to brain ischemic lesions in transient middle cerebral artery occlusion mice model. The curcumin-loaded exosomes showed strong suppression of the inflammatory response and cellular apoptosis in the lesion region. In another study, PEGylated ceria NPs was conjugated with biotinylated-LXW7 which is a ligand of integrin $\alpha v \beta 3$. The modified ceria NPs could cross the BBB through the interaction between LXW7 and $\alpha v \beta 3$ which is upregulated on BBB after stroke, and exhibited better effects in reducing infarction size and the degree of BBB breakdown¹⁶⁷.

5.4.2. Neuroinflammation

The recruitment of activated leukocytes to intracranial lesions, a typical feature of neuroinflammation response which occurs in

cerebral ischemia, offers a unique opportunity for drug delivery across the BBB to inflammation sites inside brain¹⁴². The expression of P-selectin and intercellular adhesion molecule-1 (ICAM-1) on brain capillary endothelial cells are up-regulated during stroke. The interaction between P-selectin and P-selectin glycoprotein ligand-1 which are expressed on leukocytes promotes the binding of leukocytes to BBB in stroke. Leukocytes realized firmer adhesion on the vascular wall through the specific binding between the ICAM-1 and lymphocyte function associated antigen-1 (LFA-1), leukocytes subsequently infiltrate into the ischemic brain parenchyma through the damaged BBB. Dong et al.¹²⁵ designed neutrophil membrane-coated NPs for brain delivery of anti-inflammation agent to prevent brain damage led by ischemia/reperfusion. Zhang et al.¹⁴² developed neutrophil-targeting PGP peptide-modified catalase-loaded cross-linked dendrigraft poly-L-lysine NPs. The NPs can be internalized into neutrophils in blood through the binding of PGP with CXCR2 receptor on neutrophils and subsequent receptor-mediated endocytosis. Neutrophils enhanced catalase delivery to ischemic regions and reduced infarct volume in middle cerebral artery occlusion mice. The neutrophil-based strategy is also promising for treatment of other inflammation-related brain diseases because neuroinflammation occurs in many neurological disorders.

5.4.3. TJ loss

TJ loss in stroke allows drug accumulation in stroke region^{39,168,169}. NPs showed further enhanced delivery of therapeutics via the leaky BBB^{74,170}. Various NPs have been reported to passively cross the disrupted BBB in stroke. Al-Ahmady et al.⁶⁷ showed selective recruitment of liposomes into ischemic region in brain in mouse model. The brain liposomal level in ipsilateral side was significantly increased from 0.04% dose/g-brain under healthy condition to 0.24% and 0.1% dose/g-brain when given to mice at 0.5 and 48 h after transient middle cerebral artery occlusion. The recruitment of intravenously injected liposomes was correlated with the biphasic BBB breakdown⁶⁷. The biphasic BBB breakdown precedes neurological damage, endowing NPs with the great potential for neuroprotection. Uptake of liposomes by glial cells in the ischemic region was selectively enhanced, highlighting the potential for blocking inflammatory responses or shifting the polarization of microglia/macrophages toward brain repair.

Based on the ischemia targeting ability of platelet membrane lipids and proteins, Li et al.¹⁷¹ designed platelet membrane-derived nanobubbles for ultrasound imaging of acute ischemic stroke and timely perfusion intervention. The nanobubbles exhibited preferentially accumulation in ischemic region for real-time contrast-enhanced ultrasound imaging to indicate the severity and dynamic development of the stroke and critical microvascular bio-remodeling for recanalization of the obstructed vessels to protect the neural cells around the ischemic region. Adenosine with neuropharmacological activity is often inefficient upon systemic administration because of its fast metabolism and blood clearance. Gaudin et al.¹⁷² conjugated adenosine to lipid squalene for formation of nanoassemblies. The nanoassemblies prolonged circulation of nucleoside for extended interaction of adenosine with the neurovascular unit and provided neuroprotection against stroke in mouse model. Animals with cerebral ischemia showed improved neurologic deficit score after receiving systemic squalenyl adenosine nanoassemblies.

After passively crossing the disrupted BBB in stroke, various NPs have been designed to actively target specific components, *e.g.*, SDF-1, in the ischemic microenvironment. Through various

expressed chemokines, *e.g.*, SDF-1 receptor CXCR4, neural stem cells migrate to the injured brain area. Ma et al.¹⁷³ designed poly(lactic-co-glycolic acid) (PLGA) NPs coated with engineered CXCR4-overexpressed neural stem cell membrane for delivery of anti-edema glyburide to ischemic brain for stroke treatment. RAGE is up-regulated on neurons and glial cells in ischemic brain to induce inflammation¹⁷⁴. Oh et al.¹⁷⁴ developed RAGE-targeting peptide modified NPs for delivery of heme oxygenase-1 plasmid for ischemic stroke gene therapy. The NPs inhibited RAGE-mediated signal transduction and functioned as cytoprotective reagent to decrease inflammation, apoptosis and ROS in hypoxic cells. In light of upregulated ROS in ischemic neurons, Lv et al.¹⁷⁵ designed stroke homing peptide-modified RBC membrane-coated ROS-responsive dextran nanocarrier for delivery of neuroprotective peptide NR2B9C to treat ischemic brain damage. After homing to ischemic brain, the NPs released NR2B9C upon responding to intracellular ROS in ischemic neurons. The NPs showed prolonged systemic circulation, active targeting, and reduced ischemic brain damage by protective effects in the middle cerebral artery occlusion rats^{175–177}.

5.5. TBI

5.5.1. BBB disruption during the early phase after injury

Although the recruitment of neutrophils and the increase of the transcytosis rate are two important characteristics of early stage TBI, the two characteristics were very rarely used to design brain drug delivery systems for treatment of TBI.

Loss of TJ during the early phase after TBI provides an opportunity for passive accumulation of intravenously administered NPs through an enhanced permeation and retention-like effect^{75,178}. Through the leaky BBB, NPs successfully delivered therapeutics such as cerebrolysin and antioxidants to TBI region in brain^{74,140,179,180}. Bharadwaj et al.¹³⁹ showed that, when systemically administered within the critical time window (<12 h) after TBI, PEGylated NPs with different size were significantly accumulated in brain in rodent with moderate/severe TBI. Many papers researched the effect of the size of NPs on the efficiency of crossing the compromised BBB to TBI regions^{75,139–141}. Miller et al.⁷⁵ found that 80 nm PLGA NPs had maximal permeability coefficient in TBI mouse model when intravenously injected 3 h post-injury. Lin et al.¹⁸¹ synthesized poly(*n*-butyl-2-cyanoacrylate) NPs for delivery of large molecules through the BBB in injured brains to treat TBI. NPs were distributed widely near injured sites and hardly detected in normal regions, demonstrating the compromised BBB-based brain-targeting.

TBI can lead to internal bleeding in brain and long-term neurological deficits. Currently, there are no treatments for internal bleeding beyond fluid resuscitation and surgery. Hubbard et al.¹⁸² designed anti-inflammatory dexamethasone-loaded hemostatic PLGA NPs to stop the bleeding and reduce inflammation after TBI. The NPs reduced apoptosis and restored BBB disruption in the amygdala, ameliorated anxiety parameters, improved survival and long-term functional outcomes in rats with induced TBI. Using nucleic acid therapeutics to modulate destructive pathways is promising for intervention during the secondary injury. Kwon et al.¹⁸³ engineered a neuron-targeting NP for intracellular trafficking of siRNA. The engineered NPs infiltrated brain tissue with compromised BBB in TBI animal model after systemic administration, to downregulate potential therapeutic target. As a promising neuroprotective agent, NR2B9c peptide showed extremely low brain penetrability. CAQK was reported to

have high affinity with extracellular matrix of injured brain site¹⁸⁴. Wu et al.¹⁸⁵ designed CAQK-modified thrombin-responsive protein NPs for brain delivery of NR2B9c and demonstrated the enhanced targeted delivery of NR2B9c to the injured brain lesion with TBI and improved therapeutic benefits.

5.6. Brain tumor

5.6.1. CD13 up-regulation

Based on widely overexpressed endothelial CD13 on the surface of glioma neovascular endothelial cells, An et al.¹²⁰ synthesized iNGR-modified polycation with redox-sensitive disulfides to condense siRNA nanospheres for brain siRNA delivery for glioma therapy. The NPs demonstrated stability in circulation, enhanced accumulation in glioma, glutathione-responsive release of siRNA nanospheres in glioma cells, digestion of siRNA nanospheres, and remarkable luciferase knockdown.

5.6.2. K_{ATP} and K_{Ca} up-regulation

K_{ATP} and K_{Ca} are specially up-regulated on BTB endothelial cells to regulate BTB permeability⁸². Minoxidil sulfate, as a molecular targeted K_{ATP} modulator, can selectively activate K_{ATP} channels present in BTB endothelial cells to up-regulate caveolin-1 and down-regulate TJ proteins, to specially raise BTB permeability in both transcellular and paracellular pathways. Minoxidil sulfate-loaded hyaluronic acid-tethered NPs were designed to surmount the BTB and target brain metastases. The NPs can boost transcytosis and downregulate TJ proteins in brain capillary endothelial cells at brain tumor for promoted BTB penetration⁸².

5.6.3. Integrin up-regulation

Integrin receptor $\alpha v \beta 3$ is upregulated on neovasculature of brain tumor¹¹⁹, and holds the potential for BTB crossing. Chai et al.¹²⁶ reported c(RGDyK)-modified RBC membrane-coated docetaxel nanocrystals for brain delivery of docetaxel to treat glioma. The nanocrystals exhibited high drug loading, long-term stability, excellent biocompatibility, prolonged retention time, superior tumor accumulation and therapeutic efficacy in mice bearing subcutaneous tumor or orthotopic glioma. Zhu et al.¹⁸⁶ designed cRGD-functionalized intracellularly shell-sheddable micelles for enhanced doxorubicin delivery to glioma and improved tumor growth inhibition.

5.6.4. TJ loss

With prolonged circulation time, NPs can access brain tumor through the leaky BBB and the enhanced permeability and retention effect^{82,111,157,187–193}. There have been many papers using this strategy for targeted delivery of anti-cancer drugs to brain tumor^{120,194}. The use of celastrol for the treatment of cancer is impeded by its low solubility, poor bioavailability and systemic toxicity¹⁹⁴. Huang et al.¹⁹⁴ prepared liposomal celastrol and assessed the effects in SHG-44 glioma xenografts in mice. Treatment of subcutaneous xenografts with liposomal celastrol induced greater antitumor activity than free celastrol with fewer severe side effects.

Gadolinium (Gd) chelate contrast-enhanced magnetic resonance imaging (MRI) is a preferred method of glioma detection and preoperative localization because it offers high spatial resolution and non-invasive deep tissue penetration¹⁹⁵. However, currently used contrast agents, such as Gd-diethyltri-aminepentaacetic acid (DTPA-Gd, Magnevist), suffer from rapid renal clearance, non-specificity and low contrast

efficiency. Based on the BTB disruption and MMP2 over-expression in glioma, Huang et al.¹⁹⁶ designed chlorotoxin-modified poly-L-lysines for brain delivery of DTPA-Gd to image glioma. Increased glioma accumulation of chlorotoxin-modified conjugate, enhanced and prolonged MRI signal in glioma in nude mice. Huang et al.¹⁹⁷ also designed chlorotoxin-modified polyamidoamine for brain delivery of gene therapy to treat glioma. Based on the BTB disruption and the highly expressed glucose transporter GLUT1 on tumor cells, Shao et al.¹⁹⁸ developed dehydroascorbic acid-decorated intracellular microenvironment-responsive release nanodevice for high integrity in the bloodstream, special binding with tumor cells and intracellular glutathione-triggered drug release in tumor cells.

Based on the BTB disruption and adenosine receptor on brain endothelial cells, an “autocatalytic” approach was designed *via* loading lexiscan (specific agonist of adenosine receptor) inside chlorotoxin-modified NPs, to boost BTB disruption-based brain tumor targeting efficiency¹¹¹. The accumulated NPs in brain tumor through the BTB disruption, release lexiscan to modulate the BBB to allow more NPs to enter brain, especially brain tumor regions. The preferential accumulation in brain tumor can be 4.3- and 94.0-fold greater than that in the liver and in brain regions without tumors, respectively.

6. Conclusions and future directions

Brain diseases are difficult to treat pharmacologically due to the existence of the BBB, which is a formidable barrier for drug delivery to brain. However, many aspects of the BBB evolve during the onset and progression of many brain diseases. Substantial proof-of-concept studies tested the combined use of BBB evolutions (*e.g.*, BBB disruption and regulation of transport systems) and nanocarriers for selectively enhanced drug delivery to diseased brain regions for drug therapy of brain diseases. Although many BBB evolution-based brain-targeting strategies have shown significant efficiency, quantitative brain delivery efficiency data (*e.g.*, percentage of dose/g-brain) on disease animal models are missing in some studies and remain to be further investigated. Some BBB evolutions haven't been tested for mediating brain drug delivery, *e.g.*, increased integrin and adhesion molecules and leukocyte diapedesis in PD. Related studies are still in their infancy and thus need to be further extensively and intensively researched. There are still many issues remain to be solved, *e.g.*, to accurately determine the transition time, the degree, the location and the heterogeneity of various BBB evolutions, and to reveal the connection of BBB evolutions with age and restoration after drug treatment. Real-time quantitative monitoring of various BBB evolutions will also be instructive to choose suitable BBB evolution-based BBB crossing strategies and brain-targeting drug delivery systems and to optimize the therapeutic regimen, especially for personalized medicine. In addition, more thorough discovery and understanding of BBB evolutions will be beneficial for the formation of a drug delivery system with extremely high brain-targeting efficiency, which will be very promising for clinical translation. Cellular neurovascular unit models using patient-derived endothelial cells may contribute to a better understanding of the heterogeneity of the BBB evolutions¹⁹⁹. Such studies with improved BBB models will also help shed light on the cellular interactions and signaling that occur in pathophysiological conditions. Additional studies must be carried out to overcome the safety and toxicity issues against the use of

nanocarriers. Overall, tremendous developments have occurred in diseased-based brain targeting drug delivery during past two decades. Although it is still in development, BBB evolution-based BBB crossing strategies will play an increasingly important role in treating brain diseases.

Acknowledgments

This work was funded by the international cooperative project of the National Key R&D Program of China (No. 2017YFE0126900), the National Natural Science Foundation of China (No. 81703428 and No. 81973254), the Natural Science Foundation of Jiangsu Province (No. BK20191421, China), the Suzhou Science and Technology Development Project (No. SYS2019033, China) and the Priority Academic Program Development of the Jiangsu Higher Education Institutes (PAPD, China).

Author contributions

Liang Han and Chen Jiang designed the review, wrote the manuscript, revised the manuscript and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

- Abbott NJ, Patabendige AA, Dolman DEM, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. *Neurobiol Dis* 2010;**37**:13–25.
- Agrawal M, Ajazuddin, Tripathi DK, Saraf S, Saraf S, Antimisiaris SG, et al. Recent advancements in liposomes targeting strategies to cross blood–brain barrier (BBB) for the treatment of Alzheimer's disease. *J Control Release* 2017;**260**:61–77.
- Mittapalli RK, Manda VK, Adkins CE, Geldenhuys WJ, Lockman PR. Exploiting nutrient transporters at the blood–brain barrier to improve brain distribution of small molecules. *Ther Deliv* 2010;**1**:775–84.
- Bhutia YD, Babu E, Prasad PD, Ganapathy V. The amino acid transporter SLC6A14 in cancer and its potential use in chemotherapy. *Asian J Pharm Sci* 2014;**9**:293–303.
- Geldenhuys WJ, Mohammad AS, Adkins CE, Lockman PR. Molecular determinants of blood–brain barrier permeation. *Ther Deliv* 2015;**6**:961–71.
- Pardridge WM. Blood–brain barrier endogenous transporters as therapeutic targets: a new model for small molecule CNS drug discovery. *Expert Opin Ther Targets* 2015;**19**:1059–72.
- Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, et al. Quantitative targeted absolute proteomics of human blood–brain barrier transporters and receptors. *J Neurochem* 2011;**117**:333–45.
- Geier EG, Chen EC, Webb A, Papp AC, Yee SW, Sadee W, et al. Profiling solute carrier transporters in the human blood–brain barrier. *Clin Pharmacol Ther* 2013;**94**:636–9.
- Singh I, Swami R, Jeengar MK, Khan W, Sistla R. p-Aminophenyl- α -D-mannopyranoside engineered lipidic nanoparticles for effective delivery of docetaxel to brain. *Chem Phys Lipids* 2015;**188**:1–9.
- Wang ZJ, Guo WL, Kuang X, Hou SS, Liu HZ. Nanopreparations for mitochondria targeting drug delivery system: current strategies and future prospective. *Asian J Pharm Sci* 2017;**12**:498–508.
- Shi JJ, Zhang HL, Chen ZY, Xu LH, Zhang ZZ. A multi-functional nanoplatform for efficacy tumor theranostic applications. *Asian J Pharm Sci* 2017;**12**:235–49.
- Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol* 2018;**14**:133–50.
- Liu WY, Wang ZB, Wang Y, Tong LC, Li Y, Wei X, et al. Increasing the permeability of the blood–brain barrier in three different models *in vivo*. *CNS Neurosci Ther* 2015;**21**:568–74.
- Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* 2011;**12**:723–38.
- Krol S, Macrez R, Docagne F, Defer G, Laurent S, Rahman M, et al. Therapeutic benefits from nanoparticles: the potential significance of nanoscience in diseases with compromise to the blood brain barrier. *Chem Rev* 2013;**113**:1877–903.
- Yamazaki Y, Kanekiyo T. Blood–brain barrier dysfunction and the pathogenesis of Alzheimer's disease. *Int J Mol Sci* 2017;**18**:1965.
- Eichler AF, Chung E, Kodack DP, Loeffler JS, Fukumura D, Jain RK. The biology of brain metastases-translation to new therapies. *Nat Rev Clin Oncol* 2011;**8**:344–56.
- Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV. Blood–brain barrier: from physiology to disease and back. *Physiol Rev* 2019;**99**:21–78.
- Kim KS. Mechanisms of microbial traversal of the blood–brain barrier. *Nat Rev Microbiol* 2008;**6**:625–34.
- Knowland D, Arac A, Sekiguchi KJ, Hsu M, Lutz SE, Perrino J, et al. Stepwise recruitment of transcellular and paracellular pathways underlies blood–brain barrier breakdown in stroke. *Neuron* 2014;**82**:603–17.
- Ben-Zvi A, Lacoste B, Kur E, Andreone BJ, Mayshar Y, Yan H, et al. Mfsd2a is critical for the formation and function of the blood–brain barrier. *Nature* 2014;**509**:507–11.
- Nguyen LN, Ma DL, Shui GH, Wong PY, Cazenave-Gassiot A, Zhang XD, et al. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature* 2014;**509**:503–6.
- Andreone BJ, Chow BW, Tata A, Lacoste B, Ben-Zvi A, Bullock K, et al. Blood–brain barrier permeability is regulated by lipid transport-dependent suppression of caveolae-mediated transcytosis. *Neuron* 2017;**94**:581–94.
- Zhao Z, Zlokovic BV. Blood–brain barrier: a dual life of MFSD2A?. *Neuron* 2014;**82**:728–30.
- Pardridge WM. Blood–brain barrier biology and methodology. *J Neurovirol* 1999;**5**:556–69.
- Pardridge WM. Drug transport across the blood–brain barrier. *J Cereb Blood Flow Metab* 2012;**32**:1959–72.
- Tsuji A, Tamai I. Organic anion transporters. *Pharm Biotechnol* 1999;**12**:471–91.
- Tsuji A, Tamai I. Carrier-mediated or specialized transport of drugs across the blood–brain barrier. *Adv Drug Deliv Rev* 1999;**36**:277–90.
- Cardoso FL, Brites D, Brito MA. Looking at the blood–brain barrier: molecular anatomy and possible investigation approaches. *Brain Res Rev* 2010;**64**:328–63.
- Iadecola C. The pathobiology of vascular dementia. *Neuron* 2013;**80**:844–66.
- Pandit R, Chen LY, Gotz J. The blood–brain barrier: physiology and strategies for drug delivery. *Adv Drug Deliv Rev* 2019;**165–166**:1–14.
- Kou LF, Hou YX, Yao Q, Guo WL, Wang G, Wang ML, et al. L-Carnitine-conjugated nanoparticles to promote permeation across blood–brain barrier and to target glioma cells for drug delivery via the novel organic cation/carnitine transporter OCTN2. *Artif Cells Nanomed Biotechnol* 2018;**46**:1605–16.
- Pardridge WM. Targeted delivery of protein and gene medicines through the blood–brain barrier. *Clin Pharmacol Ther* 2015;**97**:347–61.
- Krizbai IA, Nyul-Toth A, Bauer HC, Farkas AE, Traweger A, Hasko J, et al. Pharmaceutical targeting of the brain. *Curr Pharm Des* 2016;**22**:5442–62.

35. Xu D, Wu D, Qin M, Nih LR, Liu CY, Cao Z, et al. Efficient delivery of nerve growth factors to the central nervous system for neural regeneration. *Adv Mater* 2019;**31**:1900727.
36. Khan AR, Yang XY, Fu MF, Zhai GX. Recent progress of drug nanoformulations targeting to brain. *J Control Release* 2018;**291**: 37–64.
37. Rodriguez-Grande B, Ichkova A, Lemarchant S, Badaut J. Early to long-term alterations of CNS barriers after traumatic brain injury: considerations for drug development. *AAPS J* 2017;**19**:1615–25.
38. Badaut J, Ajao DO, Sorensen DW, Fukuda AM, Pellerin L. Caveolin expression changes in the neurovascular unit after juvenile traumatic brain injury: signs of blood–brain barrier healing?. *Neuroscience* 2015;**285**:215–26.
39. Banks WA. Drug delivery to the brain in Alzheimer's disease: consideration of the blood–brain barrier. *Adv Drug Deliv Rev* 2012;**64**:629–39.
40. Somjen GG, Segal MB, Herreras O. Osmotic-hypertensive opening of the blood–brain barrier in rats does not necessarily provide access for potassium to cerebral interstitial fluid. *Exp Physiol* 1991;**76**: 507–14.
41. Niu XQ, Chen JJ, Gao JQ. Nanocarriers as a powerful vehicle to overcome blood–brain barrier in treating neurodegenerative diseases: focus on recent advances. *Asian J Pharm Sci* 2019;**14**:480–96.
42. Baker SK, Chen ZL, Norris EH, Revenko AS, MacLeod AR, Strickland S. Blood-derived plasminogen drives brain inflammation and plaque deposition in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A* 2018;**115**:9687–96.
43. Deane R, Yan SD, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, et al. RAGE mediates amyloid-beta peptide transport across the blood–brain barrier and accumulation in brain. *Nat Med* 2003;**9**: 907–13.
44. Deane R, Wu ZH, Zlokovic BV. RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood–brain barrier. *Stroke* 2004;**35**:2628–31.
45. Vogelgesang S, Cascorbi I, Schroeder E, Pahnke J, Kroemer HK, Siegmund W, et al. Deposition of Alzheimer's beta-amyloid is inversely correlated with P-glycoprotein expression in the brains of elderly non-demented humans. *Pharmacogenetics* 2002;**12**: 535–41.
46. Vogelgesang S, Jedlitschky G, Brenn A, Walker LC. The role of the ATP-binding cassette transporter P-glycoprotein in the transport of β -amyloid across the blood–brain barrier. *Curr Pharm Des* 2011;**17**: 2778–86.
47. Cirrito JR, Deane R, Fagan AM, Spinner ML, Parsadanian M, Finn MB, et al. P-glycoprotein deficiency at the blood–brain barrier increases amyloid-beta deposition in an Alzheimer disease mouse model. *J Clin Invest* 2005;**115**:3285–90.
48. Wisniewski HM, Vorbrodt AW, Wegiel J. Amyloid angiopathy and blood–brain barrier changes in Alzheimer's disease. *Ann N Y Acad Sci* 1997;**826**:161–72.
49. Winkler EA, Nishida Y, Sagare AP, Rege SV, Bell RD, Perlmutter D, et al. GLUT1 reductions exacerbate Alzheimer's disease vasculo-neuronal dysfunction and degeneration. *Nat Neurosci* 2015;**18**: 521–30.
50. Ramanathan S, Archunan G, Sivakumar M, Selvan ST, Fred AL, Kumar S, et al. Theranostic applications of nanoparticles in neurodegenerative disorders. *Int J Nanomedicine* 2018;**13**:5561–76.
51. Zenaro E, Piacentino G, Constantin G. The blood–brain barrier in Alzheimer's disease. *Neurobiol Dis* 2017;**107**:41–56.
52. Cai ZY, Qiao PF, Wan CQ, Cai M, Zhou NK, Li Q. Role of blood–brain barrier in Alzheimer's disease. *J Alzheimers Dis* 2018;**63**:1223–34.
53. Braak H, Tredici KD, Rub U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003;**24**:197–211.
54. Lee H, Pienaar IS. Disruption of the blood–brain barrier in Parkinson's disease: curse or route to a cure?. *Front Biosci* 2014;**19**:272–80.
55. Sui YT, Bullock KM, Erickson MA, Zhang J, Banks WA. Alpha synuclein is transported into and out of the brain by the blood–brain barrier. *Peptides* 2014;**62**:197–202.
56. Storck SE, Meister S, Nahrath J, Meissner JN, Schubert N, Di Spiezio A, et al. Endothelial LRP1 transports amyloid- β (1-42) across the blood–brain barrier. *J Clin Invest* 2016;**126**:123–36.
57. Deane R, Wu ZH, Sagare A, Davis J, Yan SD, Hamm K, et al. LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. *Neuron* 2004;**43**:333–44.
58. Chen XS, Lan X, Roche I, Liu RG, Geiger JD. Caffeine protects against MPTP-induced blood–brain barrier dysfunction in mouse striatum. *J Neurochem* 2008;**107**:1147–57.
59. Kortekaas R, Leenders KL, van Oostrom JCH, Vaalburg W, Bart J, Willemsen ATM, et al. Blood–brain barrier dysfunction in parkinsonian midbrain *in vivo*. *Ann Neurol* 2005;**57**:176–9.
60. Meenu M, Reeta KH, Dinda AK, Kottarath SK, Gupta YK. Evaluation of sodium valproate loaded nanoparticles in acute and chronic pentylenetetrazole induced seizure models. *Epilepsy Res* 2019;**158**: 106219.
61. Rosillo-de la Torre A, Luna-Barcenas G, Orozco-Suarez S, Salgado-Ceballos H, Garcia P, Lazarowski A, et al. Pharmacoresistant epilepsy and nanotechnology. *Front Biosci* 2014;**6**:329–40.
62. Liu JS, He YJ, Zhang J, Li JJ, Yu XR, Cao ZL, et al. Functionalized nanocarrier combined seizure-specific vector with P-glycoprotein modulation property for antiepileptic drug delivery. *Biomaterials* 2015;**74**:64–76.
63. Zybina A, Anshakova A, Malinovskaya J, Melnikov P, Baklaushev V, Chekhonin V, et al. Nanoparticle-based delivery of carbamazepine: a promising approach for the treatment of refractory epilepsy. *Int J Pharm* 2018;**547**:10–23.
64. Fang ZY, Chen SD, Qin JM, Chen B, Ni GZ, Chen ZY, et al. Pluronic P85-coated poly(butylcyanoacrylate) nanoparticles overcome phenytoin resistance in P-glycoprotein overexpressing rats with lithium-pilocarpine-induced chronic temporal lobe epilepsy. *Biomaterials* 2016;**97**:110–21.
65. Han H, Eyal S, Portnoy E, Mann A, Shmuel M, Benifla M, et al. Monocytes as carriers of magnetic nanoparticles for tracking inflammation in the epileptic rat brain. *Curr Drug Deliv* 2019;**16**:637–44.
66. Furtado D, Björnalm M, Ayton S, Bush AI, Kempe K, Caruso F. Overcoming the blood–brain barrier: the role of nanomaterials in treating neurological diseases. *Adv Mater* 2018;**30**:1801362.
67. Al-Ahmady ZS, Jasim D, Ahmad SS, Wong R, Haley M, Coutts G, et al. Selective liposomal transport through blood brain barrier disruption in ischemic stroke reveals two distinct therapeutic opportunities. *ACS Nano* 2019;**13**:12470–86.
68. Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008;**359**:1317–29.
69. Cunningham LA, Wetzel M, Rosenberg GA. Multiple roles for MMPs and TIMPs in cerebral ischemia. *Glia* 2005;**50**:329–39.
70. del Zoppo GJ, Mabuchi T. Cerebral microvessel responses to focal ischemia. *J Cereb Blood Flow Metab* 2003;**23**:879–94.
71. Fukuda S, Fini CA, Mabuchi T, Koziol JA, Eggleston Jr LL, del Zoppo GJ. Focal cerebral ischemia induces active proteases that degrade microvascular matrix. *Stroke* 2004;**35**:998–1004.
72. Gao J, Dong XY, Wang ZJ. Generation, purification and engineering of extracellular vesicles and their biomedical applications. *Methods* 2020;**177**:114–25.
73. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials* 2018;**150**:137–49.
74. Lee HJ, Ryu JS, Vig PJ. Current strategies for therapeutic drug delivery after traumatic CNS injury. *Ther Deliv* 2019;**10**:251–63.
75. Miller HA, Magsam AW, Tarudji AW, Romanova S, Weber L, Gee CC, et al. Evaluating differential nanoparticle accumulation and retention kinetics in a mouse model of traumatic brain injury *via* K_{trans} mapping with MRI. *Sci Rep* 2019;**9**:16099.

76. Price L, Wilson C, Grant G. Blood–brain barrier pathophysiology following traumatic brain injury. In: Laskowitz D, Grant G, editors. *Translational research in traumatic brain injury*. Boca Raton (FL): CRC Press/Taylor and Francis Group; 2016. Chapter 4.
77. Chen YX, Wei CX, Lyu YQ, Chen HZ, Jiang G, Gao XL. Biomimetic drug-delivery systems for the management of brain diseases. *Biomater Sci* 2020;**8**:1073–88.
78. Xue JW, Zhao ZK, Zhang L, Xue LJ, Shen SY, Wen YJ, et al. Neutrophil-mediated anticancer drug delivery for suppression of postoperative malignant glioma recurrence. *Nat Nanotechnol* 2017;**12**:692–700.
79. Arvanitis CD, Ferraro GB, Jain RK. The blood–brain barrier and blood–tumour barrier in brain tumours and metastases. *Nat Rev Cancer* 2020;**20**:26–41.
80. Tiwary S, Morales JE, Kwiatkowski SC, Lang FF, Rao G, McCarty JH. Metastatic brain tumors disrupt the blood–brain barrier and alter lipid metabolism by inhibiting expression of the endothelial cell fatty acid transporter mfsd2a. *Sci Rep* 2018;**8**:8267.
81. Achrol AS, Rennert RC, Anders C, Soffietti R, Ahluwalia MS, Nayak L, et al. Brain metastases. *Nat Rev Dis Primers* 2019;**5**:5.
82. Miao TT, Ju XF, Zhu QN, Wang YM, Guo Q, Sun T, et al. Nanoparticles surmounting blood–brain tumor barrier through both transcellular and paracellular pathways to target brain metastases. *Adv Funct Mater* 2019;**29**:1900259.
83. Kasoha M, Unger C, Solomayer EF, Bohle RM, Zaharia C, Khreich F, et al. Prostate-specific membrane antigen (PSMA) expression in breast cancer and its metastases. *Clin Exp Metastasis* 2017;**34**:479–90.
84. Wernicke AG, Varma S, Greenwood EA, Christos PJ, Chao KSC, Liu H, et al. Prostate-specific membrane antigen expression in tumor-associated vasculature of breast cancers. *APMIS* 2014;**122**:482–9.
85. Nomura N, Pastorino S, Jiang PF, Lambert G, Crawford JR, Gymnopoulos M, et al. Prostate specific membrane antigen (PSMA) expression in primary gliomas and breast cancer brain metastases. *Cancer Cell Int* 2014;**14**:26.
86. Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res* 1997;**3**:81–5.
87. Yonemori K, Tsuta K, Ono M, Shimizu C, Hirakawa A, Hasegawa T, et al. Disruption of the blood brain barrier by brain metastases of triple-negative and basal-type breast cancer but not HER2/neu-positive breast cancer. *Cancer* 2010;**116**:302–8.
88. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and dysfunction of the blood–brain barrier. *Cell* 2015;**163**:1064–78.
89. Zhao CS, Ma JW, Wang Z, Li HY, Shen HT, Li X, et al. Mfsd2a attenuates blood–brain barrier disruption after sub-arachnoid hemorrhage by inhibiting caveolae-mediated transcellular transport in rats. *Transl Stroke Res* 2020;**11**:1012–27.
90. Eser Ocak P, Ocak U, Sherchan P, Zhang JH, Tang JP. Insights into major facilitator superfamily domain-containing protein-2a (Mfsd2a) in physiology and pathophysiology. What do we know so far?. *J Neurosci Res* 2020;**98**:29–41.
91. Yang YR, Xiong XY, Liu J, Wu LR, Zhong Q, Zhou K, et al. Mfsd2a (major facilitator superfamily domain containing 2a) attenuates intracerebral hemorrhage-induced blood–brain barrier disruption by inhibiting vesicular transcytosis. *J Am Heart Assoc* 2017;**6**:005811.
92. Zhang WT, Chen RY, Yang T, Xu N, Chen J, Gao YQ, et al. Fatty acid transporting proteins: roles in brain development, aging, and stroke. *Prostaglandins Leukot Essent Fatty Acids* 2018;**136**:35–45.
93. Zlokovic BV. The blood–brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2008;**57**:178–201.
94. Yuan XN, Fu ZS, Ji PF, Guo LB, Al-Ghamdy AO, Alkandiri A, et al. Selenium nanoparticles pre-treatment reverse behavioral, oxidative damage, neuronal loss and neurochemical alterations in pentylentetrazole-induced epileptic seizures in mice. *Int J Nanomedicine* 2020;**15**:6339–53.
95. Lochhead JJ, Yang JZ, Ronaldson PT, Davis TP. Structure, function, and regulation of the blood–brain barrier tight junction in central nervous system disorders. *Front Physiol* 2020;**11**:914.
96. Lo EH, Singhal AB, Torchilin VP, Abbott NJ. Drug delivery to damaged brain. *Brain Res Brain Res Rev* 2001;**38**:140–8.
97. Watkins S, Robel S, Kimbrough IF, Robert SM, Ellis-Davies G, Sontheimer H. Disruption of astrocyte-vascular coupling and the blood–brain barrier by invading glioma cells. *Nat Commun* 2014;**5**:4196.
98. Weiss N, Miller F, Cazaubon S, Couraud PO. The blood–brain barrier in brain homeostasis and neurological diseases. *Biochim Biophys Acta* 2009;**1788**:842–57.
99. Singh I, Swami R, Khan W, Sisla R. Lymphatic system: a prospective area for advanced targeting of particulate drug carriers. *Expert Opin Drug Deliv* 2014;**11**:211–29.
100. 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.
101. Petri B, Bootz A, Khalansky A, Hekmatara T, Muller R, Uhl R, et al. Chemotherapy of brain tumour using doxorubicin bound to surfactant-coated poly(butyl cyanoacrylate) nanoparticles: revisiting the role of surfactants. *J Control Release* 2007;**117**:51–8.
102. Gao HL. Progress and perspectives on targeting nanoparticles for brain drug delivery. *Acta Pharm Sin B* 2016;**6**:268–86.
103. Ji B, Maeda J, Higuchi M, Inoue K, Akita H, Harashima H, et al. Pharmacokinetics and brain uptake of lactoferrin in rats. *Life Sci* 2006;**78**:851–5.
104. Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. *Nat Rev Drug Discov* 2002;**1**:131–9.
105. Olivier JC. Drug transport to brain with targeted nanoparticles. *NeuroRx* 2005;**2**:108–19.
106. Li J, Cai P, Shalviri A, Henderson JT, He CS, Foltz WD, et al. A multifunctional polymeric nanotheranostic system delivers doxorubicin and imaging agents across the blood–brain barrier targeting brain metastases of breast cancer. *ACS Nano* 2014;**8**:9925–40.
107. He CS, Ping C, Li J, Tian Z, Lin L, Abbasi AZ, et al. Blood–brain barrier-penetrating amphiphilic polymer nanoparticles deliver docetaxel for the treatment of brain metastases of triple negative breast cancer. *J Control Release* 2017;**246**:98–109.
108. Kreuter J. Nanoparticulate systems for brain delivery of drugs. *Adv Drug Deliv Rev* 2001;**47**:65–81.
109. Deeken JF, Loscher W. The blood–brain barrier and cancer: transporters, treatment, and Trojan horses. *Clin Cancer Res* 2007;**13**:1663–74.
110. Liu G, Men P, Harris PLR, Rolston RK, Perry G, Smith MA. Nanoparticle iron chelators: a new therapeutic approach in Alzheimer disease and other neurologic disorders associated with trace metal imbalance. *Neurosci Lett* 2006;**406**:189–93.
111. Han L, Kong DK, Zheng MQ, Murikinati S, Ma C, Yuan P, et al. Increased nanoparticle delivery to brain tumors by autocatalytic priming for improved treatment and imaging. *ACS Nano* 2016;**10**:4209–18.
112. Yang TZ, Ferrill L, Gallant L, McGillicuddy S, Fernandes T, Shields N, et al. Verapamil and riluzole cocktail liposomes overcome pharmacoresistance by inhibiting P-glycoprotein in brain endothelial and astrocyte cells: a potent approach to treat amyotrophic lateral sclerosis. *Eur J Pharm Sci* 2018;**120**:30–9.
113. Pinzon-Daza ML, Garzon R, Couraud PO, Romero IA, Weksler B, Ghigo D, et al. The association of statins plus LDL receptor-targeted liposome-encapsulated doxorubicin increases *in vitro* drug delivery across blood–brain barrier cells. *Br J Pharmacol* 2012;**167**:1431–47.
114. Nazem A, Mansoori GA. Nanotechnology solutions for Alzheimer's disease: advances in research tools, diagnostic methods and therapeutic agents. *J Alzheimers Dis* 2008;**13**:199–223.

115. Kubinova S, Sykova E. Nanotechnology for treatment of stroke and spinal cord injury. *Nanomedicine* 2010;**5**:99–108 (Lond).
116. Chow BW, Gu CH. Gradual suppression of transcytosis governs functional blood-retinal barrier formation. *Neuron* 2017;**93**:1325–33.
117. Ruan SB, Qin L, Xiao W, Hu C, Zhou Y, Wang RR, et al. Acid-responsive transferrin dissociation and GLUT mediated exocytosis for increased blood–brain barrier transcytosis and programmed glioma targeting delivery. *Adv Funct Mater* 2018;**28**:1802227.
118. Cai LL, Yang CY, Jia WF, Liu YW, Xie R, Lei T, et al. Endo/lysosome-escapable delivery depot for improving BBB transcytosis and neuron targeted therapy of Alzheimer's disease. *Adv Funct Mater* 2020;**30**:1909999.
119. Zhang T, Lip H, He CS, Cai P, Wang ZG, Henderson JT, et al. Multitargeted nanoparticles deliver synergistic drugs across the blood–brain barrier to brain metastases of triple negative breast cancer cells and tumor-associated macrophages. *Adv Healthc Mater* 2019;**8**:1900543.
120. An S, Jiang XT, Shi JS, He X, Li JF, Guo YB, et al. Single-component self-assembled RNAi nanoparticles functionalized with tumor-targeting iNGR delivering abundant siRNA for efficient glioma therapy. *Biomaterials* 2015;**53**:330–40.
121. Ningaraj NS, Rao M, Black KL. Calcium-dependent potassium channels as a target protein for modulation of the blood–brain tumor barrier. *Drug News Perspect* 2003;**16**:291–8.
122. 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.
123. Ningaraj NS, Rao MK, Black KL. Adenosine 5'-triphosphate-sensitive potassium channel-mediated blood–brain tumor barrier permeability increase in a rat brain tumor model. *Cancer Res* 2003;**63**:8899–911.
124. Lu YF, Guo ZY, Zhang YJ, Li C, Zhang Y, Guo Q, et al. Microenvironment remodeling micelles for Alzheimer's disease therapy by early modulation of activated microglia. *Adv Sci* 2018;**6**:1801586.
125. Dong XY, Gao J, Zhang CY, Hayworth C, Frank M, Wang ZJ. Neutrophil membrane-derived nanovesicles alleviate inflammation to protect mouse brain injury from ischemic stroke. *ACS Nano* 2019;**13**:1272–83.
126. Chai ZL, Ran DN, Lu LW, Zhan CY, Ruan HT, Hu XF, et al. Ligand-modified cell membrane enables the targeted delivery of drug nanocrystals to glioma. *ACS Nano* 2019;**13**:5591–601.
127. Nance E, Zhang F, Mishra MK, Zhang Z, Kambhampati SP, Kannan RM, et al. Nanoscale effects in dendrimer-mediated targeting of neuroinflammation. *Biomaterials* 2016;**101**:96–107.
128. Boyd BJ, Galle A, Daglas M, Rosenfeld JV, Medcalf R. Traumatic brain injury opens blood–brain barrier to stealth liposomes via an enhanced permeability and retention (EPR)-like effect. *J Drug Target* 2015;**23**:847–53.
129. Greish K. Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. *Methods Mol Biol* 2010;**624**:25–37.
130. Zhang TT, Li W, Meng GM, Wang P, Liao WZ. Strategies for transporting nanoparticles across the blood–brain barrier. *Biomater Sci* 2016;**4**:219–29.
131. Liu XG, Lu S, Liu DQ, Zhang L, Zhang LX, Yu XL, et al. ScFv-conjugated superparamagnetic iron oxide nanoparticles for MRI-based diagnosis in transgenic mouse models of Parkinson's and Huntington's diseases. *Brain Res* 2019;**1707**:141–53.
132. Wang YL, Wang YL, Sun R, Wu XR, Chu X, Zhou SH, et al. The treatment value of IL-1 β monoclonal antibody under the targeting location of alpha-methyl-L-tryptophan and superparamagnetic iron oxide nanoparticles in an acute temporal lobe epilepsy model. *J Transl Med* 2018;**16**:337.
133. Akhtari M, Bragin A, Cohen M, Moats R, Brenker F, Lynch MD, et al. Functionalized magnetonanoparticles for MRI diagnosis and localization in epilepsy. *Epilepsia* 2008;**49**:1419–30.
134. Zheng SY, Bai YY, Changyi YZ, Gao XH, Zhang WQ, Wang YC, et al. Multimodal nanoprobe evaluating physiological pore size of brain vasculatures in ischemic stroke models. *Adv Healthc Mater* 2014;**3**:1909–18.
135. Sarin H, Kanevsky AS, Wu HT, Brimacombe KR, Fung SH, Sousa AA, et al. Effective transvascular delivery of nanoparticles across the blood–brain tumor barrier into malignant glioma cells. *J Transl Med* 2008;**6**:80.
136. Kang JH, Cho J, Ko YT. Investigation on the effect of nanoparticle size on the blood–brain tumor barrier permeability by *in situ* perfusion via internal carotid artery in mice. *J Drug Target* 2019;**27**:103–10.
137. Su BX, Wang RF, Xie ZX, Ruan HT, Li JC, Xie C, et al. Effect of retro-inverso isomer of bradykinin on size-dependent penetration of blood–brain tumor barrier. *Small* 2018;**14**:1702331.
138. Hue CD, Cho FS, Cao SQ, Nicholls RE, Vogel Iii EW, Sibindi C, et al. Time course and size of blood–brain barrier opening in a mouse model of blast-induced traumatic brain injury. *J Neurotrauma* 2016;**33**:1202–11.
139. Bharadwaj VN, Lifshitz J, Adelson PD, Kodibagkar VD, Stabenfeldt SE. Temporal assessment of nanoparticle accumulation after experimental brain injury: effect of particle size. *Sci Rep* 2016;**6**:29988.
140. Bharadwaj VN, Rowe RK, Harrison J, Wu C, Anderson TR, Lifshitz J, et al. Blood–brain barrier disruption dictates nanoparticle accumulation following experimental brain injury. *Nanomedicine* 2018;**14**:2155–66.
141. Cruz LJ, Stammes MA, Que I, van Beek ER, Knol-Blanckevoort VT, Snoeks TJ, et al. Effect of PLGA NP size on efficiency to target traumatic brain injury. *J Control Release* 2016;**223**:31–41.
142. Zhang C, Ling CL, Pang L, Wang Q, Liu JX, Wang BS, et al. Direct macromolecular drug delivery to cerebral ischemia area using neutrophil-mediated nanoparticles. *Theranostics* 2017;**7**:3260–75.
143. Fang RH, Kroll AV, Gao WW, Zhang LF. Cell membrane coating nanotechnology. *Adv Mater* 2018;**30**:1706759.
144. Wang CX, Wu B, Wu YT, Song XY, Zhang SH, Liu ZH. Camouflaging nanoparticles with brain metastatic tumor cell membranes: a new strategy to traverse blood–brain barrier for imaging and therapy of brain tumors. *Adv Funct Mater* 2020;**30**:1909369.
145. Wang DD, Dong HF, Li M, Cao Y, Yang F, Zhang K, et al. Erythrocyte-cancer hybrid membrane camouflaged hollow copper sulfide nanoparticles for prolonged circulation life and homotypic-targeting photothermal/chemotherapy of melanoma. *ACS Nano* 2018;**12**:5241–52.
146. Chai ZL, Hu XF, Wei XL, Zhan CY, Lu LW, Jiang K, et al. A facile approach to functionalizing cell membrane-coated nanoparticles with neurotoxin-derived peptide for brain-targeted drug delivery. *J Control Release* 2017;**264**:102–11.
147. Sun HP, Su JH, Meng QS, Yin Q, Chen LL, Gu WW, et al. Cancer cell membrane-coated gold nanocages with hyperthermia-triggered drug release and homotypic target inhibit growth and metastasis of breast cancer. *Adv Funct Mater* 2017;**27**:1604300.
148. Rao L, Bu LL, Cai B, Xu JH, Li A, Zhang WF, et al. Cancer cell membrane-coated upconversion nanoprobe for highly specific tumor imaging. *Adv Mater* 2016;**28**:3460–6.
149. Li SY, Cheng H, Qiu WX, Zhang L, Wan SS, Zeng JY, et al. Cancer cell membrane-coated biomimetic platform for tumor targeted photodynamic therapy and hypoxia-amplified bioreductive therapy. *Biomaterials* 2017;**142**:149–61.
150. Yang R, Xu J, Xu LG, Sun XQ, Chen Q, Zhao YH, et al. Cancer cell membrane-coated adjuvant nanoparticles with mannose modification for effective anticancer vaccination. *ACS Nano* 2018;**12**:5121–9.
151. Fang RH, Hu CMJ, Luk BT, Gao WW, Copp JA, Tai YY, et al. Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. *Nano Lett* 2014;**14**:2181–8.
152. Zhang Y, Cai KM, Li C, Guo Q, Chen QJ, He X, et al. Macrophage-membrane-coated nanoparticles for tumor-targeted chemotherapy. *Nano Lett* 2018;**18**:1908–15.

153. Miller MC, Tavares R, Johanson CE, Hovanesian V, Donahue JE, Gonzalez L, et al. Hippocampal RAGE immunoreactivity in early and advanced Alzheimer's disease. *Brain Res* 2008;**1230**:273–80.
154. András IE, Eum SY, Huang W, Zhong Y, Hennig B, Toborek M. HIV-1-induced amyloid beta accumulation in brain endothelial cells is attenuated by simvastatin. *Mol Cell Neurosci* 2010;**43**:232–43.
155. Wu LP, Ahmadvand D, Su JA, Hall A, Tan XL, Farhangrazi ZS, et al. Crossing the blood–brain-barrier with nanoligand drug carriers self-assembled from a phage display peptide. *Nat Commun* 2019;**10**:4635.
156. Zandl-Lang M, Fanaee-Danesh E, Sun YD, Albrecher NM, Gali CC, Čančar I, et al. Regulatory effects of simvastatin and apoJ on APP processing and amyloid- β clearance in blood–brain barrier endothelial cells. *Biochim Biophys Acta Mol Cell Biol Lipids* 2018;**1863**:40–60.
157. Guo Q, Zhu QN, Miao TT, Tao J, Ju XF, Sun ZL, et al. LRP1-upregulated nanoparticles for efficiently conquering the blood–brain barrier and targetedly suppressing multifocal and infiltrative brain metastases. *J Control Release* 2019;**303**:117–29.
158. Anraku Y, Kuwahara H, Fukusato Y, Mizoguchi A, Ishii T, Nitta K, et al. Glycaemic control boosts glucosylated nanocarrier crossing the BBB into the brain. *Nat Commun* 2017;**8**:1001.
159. Cheng ZQ, Zhang JQ, Liu HF, Li Y, Zhao YG, Yang E. Central nervous system penetration for small molecule therapeutic agents does not increase in multiple sclerosis- and Alzheimer's disease-related animal models despite reported blood–brain barrier disruption. *Drug Metab Dispos* 2010;**38**:1355–61.
160. Tanifum EA, Dasgupta I, Srivastava M, Bhavane RC, Sun L, Berridge J, et al. Intravenous delivery of targeted liposomes to amyloid- β pathology in APP/PSEN1 transgenic mice. *PLoS One* 2012;**7**:48515.
161. Hettiarachchi SD, Zhou YQ, Seven E, Lakshmana MK, Kaushik AK, Chand HS, et al. Nanoparticle-mediated approaches for Alzheimer's disease pathogenesis, diagnosis, and therapeutics. *J Control Release* 2019;**314**:125–40.
162. Han L, Cai Q, Tian DF, Kong DK, Gou XC, Chen ZM, et al. Targeted drug delivery to ischemic stroke via chlorotoxin-anchored, lexiscan-loaded nanoparticles. *Nanomedicine* 2016;**12**:1833–42.
163. Chen TK, Li CW, Li Y, Yi X, Wang RB, Lee SMY, et al. Small-sized MPEG-PLGA nanoparticles of schisantherin A with sustained release for enhanced brain uptake and anti-parkinsonian activity. *ACS Appl Mater Interfaces* 2017;**9**:9516–27.
164. Lee D, Hong JW, Park C, Lee H, Lee JE, Hyeon T, et al. Ca²⁺-dependent intracellular drug delivery system developed with "raspberry-type" particles-on-a-particle comprising mesoporous silica core and α -synuclein-coated gold nanoparticles. *ACS Nano* 2014;**8**:8887–95.
165. Hong CS, Park JH, Lee S, Rhoo KY, Lee JT, Paik SR. Fabrication of protease-sensitive and light-responsive microcapsules encompassed with single layer of gold nanoparticles by using self-assembly protein of α -synuclein. *ACS Appl Mater Interfaces* 2018;**10**:26628–40.
166. Pedram MZ, Shamloo A, Alasty A, Ghafar-Zadeh E. MRI-guided epilepsy detection. *Conf Proc IEEE Eng Med Biol Soc* 2015;**2015**:4001–4.
167. Zhang T, Li CY, Jia JJ, Chi JS, Zhou D, Li JZ, et al. Combination therapy with LXW7 and ceria nanoparticles protects against acute cerebral ischemia/reperfusion injury in rats. *Curr Med Sci* 2018;**38**:144–52.
168. Kroli RA, Neuwelt EA. Outwitting the blood–brain barrier for therapeutic purposes: osmotic opening and other means. *Neurosurgery* 1998;**42**:1083–99.
169. Greig NH, Fredericks WR, Holloway HW, Soncrant TT, Rapoport SI. Delivery of human interferon-alpha to brain by transient osmotic blood–brain barrier modification in the rat. *J Pharmacol Exp Ther* 1988;**245**:581–6.
170. Ishii T, Asai T, Oyama D, Fukuta T, Yasuda N, Shimizu K, et al. Amelioration of cerebral ischemia-reperfusion injury based on liposomal drug delivery system with asialo-erythropoietin. *J Control Release* 2012;**160**:81–7.
171. Li MX, Liu Y, Chen JP, Liu TT, Gu ZX, Zhang JQ, et al. Platelet bio-nanobubbles as microvascular recanalization nanof ormulation for acute ischemic stroke lesion theranostics. *Theranostics* 2018;**8**:4870–83.
172. Gaudin A, Yemisci M, Eroglu H, Lepetre-Mouelhi S, Turkoglu OF, Donmez-Demir B, et al. Squalenoyl adenosine nanoparticles provide neuroprotection after stroke and spinal cord injury. *Nat Nanotechnol* 2014;**9**:1054–62.
173. Ma JN, Zhang SQ, Liu J, Liu FY, Du FY, Li M, et al. Targeted drug delivery to stroke via chemotactic recruitment of nanoparticles coated with membrane of engineered neural stem cells. *Small* 2019;**15**:1902011.
174. Oh J, Lee J, Piao CX, Jeong JH, Lee M. A self-assembled DNA-nanoparticle with a targeting peptide for hypoxia-inducible gene therapy of ischemic stroke. *Biomater Sci* 2019;**7**:2174–90.
175. Lv W, Xu JP, Wang XQ, Li XR, Xu QW, Xin HL. Bioengineered boronic ester modified dextran polymer nanoparticles as reactive oxygen species responsive nanocarrier for ischemic stroke treatment. *ACS Nano* 2018;**12**:5417–26.
176. Zhao Y, Jiang Y, Lv W, Wang ZY, Lv LY, Wang BY, et al. Dual targeted nanocarrier for brain ischemic stroke treatment. *J Control Release* 2016;**233**:64–71.
177. Xu JP, Wang XQ, Yin HY, Cao X, Hu QY, Lv W, et al. Sequentially site-specific delivery of thrombolytics and neuroprotectant for enhanced treatment of ischemic stroke. *ACS Nano* 2019;**13**:8577–88.
178. Smith NM, Gachulincova I, Ho D, Bailey C, Bartlett CA, Norret M, et al. An unexpected transient breakdown of the blood brain barrier triggers passage of large intravenously administered nanoparticles. *Sci Rep* 2016;**6**:22595.
179. Ruozzi B, Belletti D, Sharma HS, Sharma A, Muresanu DF, Mossler H, et al. PLGA nanoparticles loaded cerebrolysin: studies on their preparation and investigation of the effect of storage and serum stability with reference to traumatic brain injury. *Mol Neurobiol* 2015;**52**:899–912.
180. Bailey ZS, Nilson E, Bates JA, Oyalowo A, Hockey KS, Sajja VSSS, et al. Cerium oxide nanoparticles improve outcome after *in vitro* and *in vivo* mild traumatic brain injury. *J Neurotrauma* 2020;**37**:1452–62.
181. Lin Y, Pan YH, Shi YF, Huang XJ, Jia NQ, Jiang JY. Delivery of large molecules via poly(butyl cyanoacrylate) nanoparticles into the injured rat brain. *Nanotechnology* 2012;**23**:165101.
182. Hubbard WB, Lashof-Sullivan M, Greenberg S, Norris C, Eck J, Lavik E, et al. Hemostatic nanoparticles increase survival, mitigate neuropathology and alleviate anxiety in a rodent blast trauma model. *Sci Rep* 2018;**8**:10622.
183. Kwon EJ, Skalak M, Lo Bu R, Bhatia SN. Neuron-targeted nanoparticle for siRNA delivery to traumatic brain injuries. *ACS Nano* 2016;**10**:7926–33.
184. Mann AP, Scodeller P, Hussain S, Joo J, Kwon E, Braun GB, et al. A peptide for targeted, systemic delivery of imaging and therapeutic compounds into acute brain injuries. *Nat Commun* 2016;**7**:11980.
185. Wu P, Zhao HT, Gou XC, Wu XW, Zhang SQ, Deng G, et al. Targeted delivery of polypeptide nanoparticle for treatment of traumatic brain injury. *Int J Nanomedicine* 2019;**14**:4059–69.
186. Zhu YQ, Zhang J, Meng FH, Deng C, Cheng R, Feijen J, et al. cRGD-functionalized reduction-sensitive shell-sheddable biodegradable micelles mediate enhanced doxorubicin delivery to human glioma xenografts *in vivo*. *J Control Release* 2016;**233**:29–38.
187. Huang SX, Li JF, Han L, Liu SH, Ma HJ, Huang RQ, et al. Dual targeting effect of Angiopep-2-modified, DNA-loaded nanoparticles for glioma. *Biomaterials* 2011;**32**:6832–8.
188. Yu X, Gou XC, Wu P, Han L, Tian DF, Du FY, et al. Activatable protein nanoparticles for targeted delivery of therapeutic peptides. *Adv Mater* 2018;**30**:1705383.
189. Guo Q, Chang ZY, Khan NU, Miao TT, Ju XF, Feng HX, et al. Nanosizing noncrystalline and porous silica material-naturally occurring opal shale for systemic tumor targeting drug delivery. *ACS Appl Mater Interfaces* 2018;**10**:25994–6004.

190. Han L, Li JF, Huang SX, Huang RQ, Liu SH, Hu X, et al. Peptide-conjugated polyamidoamine dendrimer as a nanoscale tumor-targeted T1 magnetic resonance imaging contrast agent. *Biomaterials* 2011;**32**:2989–98.
191. Han L, Huang RQ, Li JF, Liu SH, Huang SX, Jiang C. Plasmid pORF-hTRAIL and doxorubicin co-delivery targeting to tumor using peptide-conjugated polyamidoamine dendrimer. *Biomaterials* 2011;**32**:1242–52.
192. Han L, Guo YB, Ma HJ, He X, Kuang YY, Zhang N, et al. Acid active receptor-specific peptide ligand for *in vivo* tumor-targeted delivery. *Small* 2013;**9**:3647–58.
193. Han L, Ma HJ, Guo YB, Kuang YY, He X, Jiang C. pH-controlled delivery of nanoparticles into tumor cells. *Adv Healthc Mater* 2013;**2**:1435–9.
194. Huang YL, Zhou D, Hang TJ, Wu ZH, Liu JG, Xu QN, et al. Preparation, characterization, and assessment of the antiglioma effects of liposomal celastrol. *Anticancer Drugs* 2012;**23**:515–24.
195. Li JF, Huang SX, Shao K, Liu Y, An S, Kuang YY, et al. A choline derivate-modified nanoprobe for glioma diagnosis using MRI. *Sci Rep* 2013;**3**:1623.
196. Huang RQ, Han L, Li JF, Liu SH, Shao K, Kuang YY, et al. Chlorotoxin-modified macromolecular contrast agent for MRI tumor diagnosis. *Biomaterials* 2011;**32**:5177–86.
197. Huang RQ, Ke WL, Han L, Li JF, Liu SH, Jiang C. Targeted delivery of chlorotoxin-modified DNA-loaded nanoparticles to glioma *via* intravenous administration. *Biomaterials* 2011;**32**:2399–406.
198. Shao K, Ding N, Huang SX, Ren SM, Zhang Y, Kuang YY, et al. Smart nanodevice combined tumor-specific vector with cellular microenvironment-triggered property for highly effective antiglioma therapy. *ACS Nano* 2014;**8**:1191–203.
199. Kaiser MA, Sajja RK, Prasad S, Abhyankar VV, Liles T, Cucullo L. New experimental models of the blood–brain barrier for CNS drug discovery. *Expert Opin Drug Discov* 2017;**12**:89–103.