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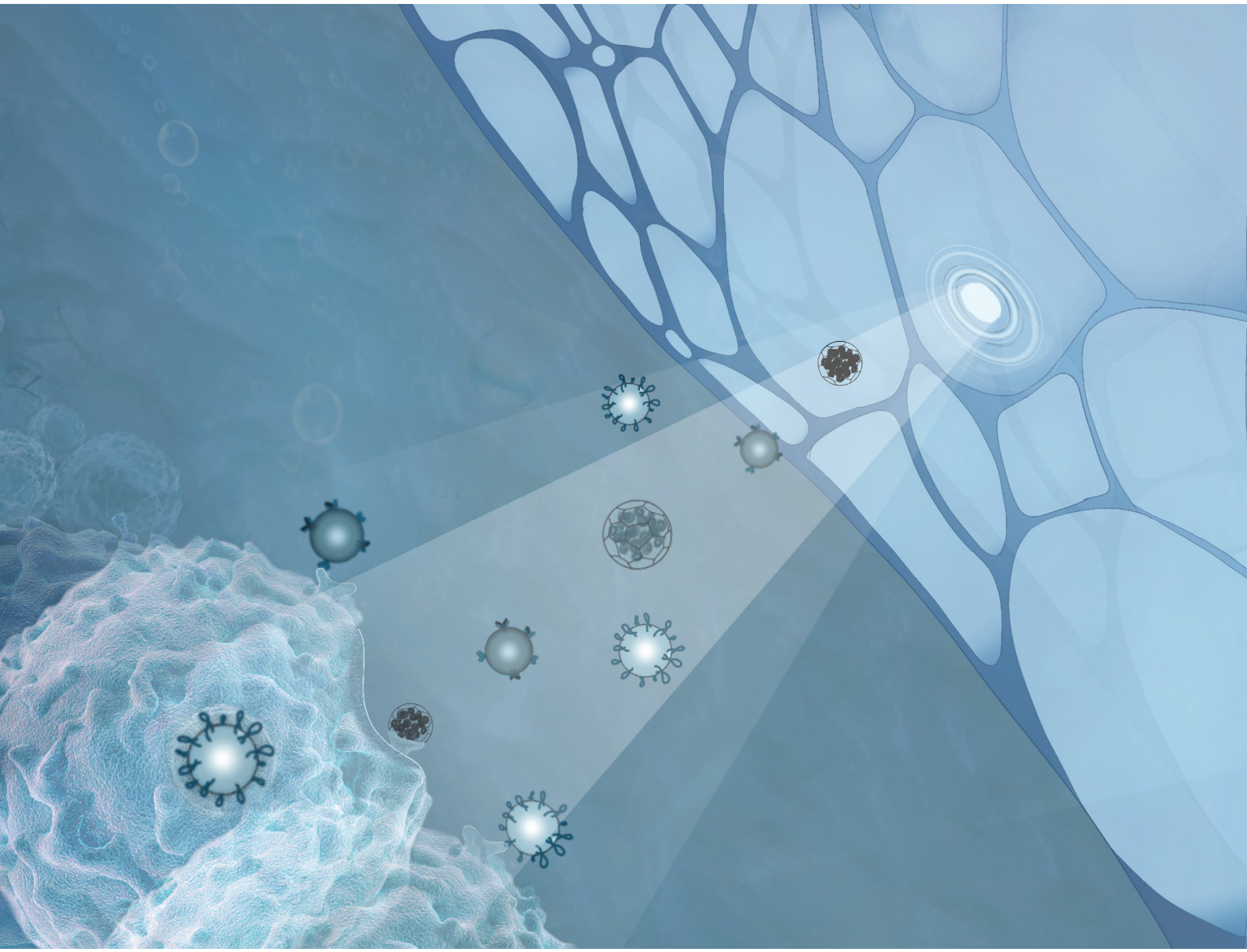

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REVIEW

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Transferrin receptor 1 targeted nanomedicine for brain tumor therapy

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Achieving effective drug delivery to traverse the blood–brain barrier (BBB) and target tumor cells remains the greatest challenge for brain tumor therapy. Importantly, the overexpressed membrane receptors on the brain endothelial cells, especially transferrin receptor 1 (TfR1), which mediate their ligands/antibodies to overcome the BBB by transcytosis, have been emerging as promising targets for brain tumor therapy. By employing ligands (*e.g.*, transferrin, H-ferritin), antibodies or targeting peptides of TfR1 or aptamers, various functional nano-formulations have been developed in the last decade. These agents showed great potential for the treatment of brain diseases due to their ideal size, high loading capacity, controlled drug release and suitable pharmacokinetics. Herein, we summarize the latest advances on TfR1-targeted nanomedicine for brain tumor therapy. Moreover, we also discuss the strategies of improving stability, targeting ability and accumulation of nano-formulations in brain tumors for better outcomes. In this review, we hope to provide inspiration for the rational design of TfR1-targeted nanomedicine against brain tumors.

1 Introduction

Brain tumor refers to a life-threatening disease for public health, usually being considered as an incurable and metastatic tumor in the central nervous system (CNS).[1] There are three major characteristics of brain tumors. (i) The higher incidence and malignant behavior affect thousands of people. According to the World Health Organization (WHO) GLOBOCAN 2020 project, the global incidence of malignant brain tumors is up to about 3.5/100,000.[2,3] (ii) Frequent recurrence and infiltration into surroundings account for the poor prognosis, with a 36% survival rate at 5 years.[4,5] Patients with brain tumors suffer from complications, such as seizures, headaches, increasing intracranial pressure, *etc.*,[6] which decreases the quality of life and life expectancy. (iii) The damage of brain tumors also manifests as brain metastases from other tumors, which is the symbol of the late stage of malignant tumors. Up to 30% of adults with systemic cancers develop brain metastases.

For brain tumor therapy, invasive surgery and combined treatment such as radiotherapy and chemotherapy are clinical

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standard strategies. Invasive surgery and postoperative radiotherapy possess the most direct efficiency, while great trauma and a long-time recovery remain.[7,8] Chemotherapy as an adjuvant or major treatment is also a common way of brain tumor therapy, the mechanism of which induces direct cell death, antiangiogenesis, pro-differentiation, disruption of the tumor microenvironment and the inhibition of tumor invasion. However, the blood–brain barrier (BBB) blocks the majority of drugs from crossing and accumulating at tumor sites, resulting in a higher dosage of drugs needed to be administered to achieve effective therapeutic outcomes. At the same time, this also brings strong systemic toxicity, so we need new treatment strategies. Direct intrathecal infusion or intense physical approaches, such as ultrasound, are commonly used to traverse the BBB, which may cause severe leakage and unexpected damage to the CNS. Non-invasive strategies, such as systemic delivery or oral medication, attract researchers to conduct more extensive and in-depth studies on them due to the safety and the sufficient therapeutic effects. Brain tumor-targeted therapy with lower side effects and superior efficacy has been developed over the years, making it possible to overcome the BBB without damage. Considering the receptor-mediated endocytosis, it is feasible to employ specific carriers for targeting the receptors.

Transferrin receptor 1 (TfR1), which is also known as CD71, is a type II transmembrane glycoprotein functioning in maintaining iron balance and mediating iron metabolism. Studies have shown that TfR1 is highly expressed in both vascular endothelial cells and brain tumor cells.[9] Moreover, TfR1 plays an important role in tumor cytopathology and is positively correlated with the poor prognosis. Taking the above reasons together, TfR1 is considered to be a potent anti-tumor drug transport receptor, especially in brain tumor therapy. In this review, we take TfR1 as a biological target and focus on nanomedicines conjugated with TfR1-targeted molecules including transferrin, H-ferritin (HFn), TfR1-targeted antibodies, TfR1-targeted peptides, *etc.* to directly or indirectly kill brain tumor cells. Importantly, these strategies contribute to higher drug concentration in the brain and lower toxicity for untargeted tissues so as to overcome the obstacles of traditional treatment.

1.1 Challenges of brain tumor therapy

There are many factors that contribute to insufficient chemotherapeutic effects on brain tumors, such as the instability in blood, the low concentration at tumor sites, drug resistance and the obstruction of the BBB and the blood–brain tumor barrier (BBTB). For brain tumor therapy, traversing the barriers and targeting tumor cells is the most challenging.

As an important protective barrier, the BBB is composed of pericytes, astrocytes and endothelial cells connected by tight junctions.[10] On the one hand, the trans-endothelial electrical resistance (TEER) of the intact BBB is approximately 8000 Ω cm^{-2},[11] blocking vascular leakage of most molecules larger than 180 Da.[12] The molecular weights of many chemotherapeutic drugs exceed 400 Da (vincristine, vinblastine, paclitaxel, etoposide, *etc.*) so that most of the therapeutic drugs are inaccessible to the brain. On the other hand, many drugs, such as chlorambucil and etoposide, bind to plasma proteins at a ratio of 90%, reducing the free parts in plasma and being unable to cross the BBB.[13] Another major obstacle of traversing the BBB is the efflux activity. There are many efflux proteins, such as p-glycoprotein (Pgp), breast cancer resistance protein (BCRP) and other drug resistance transporters on the luminal endothelial cell membrane, which actively remove drugs from the brain. Research has shown that inhibiting active efflux proteins could contribute to the remarkable amelioration in brain uptake[14] and significantly enhance the therapeutic effects. In addition, how to promote the selective accumulation in tumor cells remains a problem for cancer therapy. As brain tumors deteriorate, tumor cells start to invade the surrounding normal brain tissues, leading to BBB damage and vascular disruption.[15] The

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Xiyun Yan

Xiyun Yan is a professor at the Institute of Biophysics, a member of the Chinese Academy of Sciences, and the president of the Asian Biophysics Association. Her research interests include studying tumor biology, finding novel targets and developing new methods for tumor theranostics. Dr Yan introduced the concept of "nanozymes" (nanomaterials with enzyme-like characteristics) and used nanozymes for tumor diagnosis in combination with ferritin. Her work has been well recognized through honors, such as the National Prize for Natural Science and the Atlas Award by Elsevier.

Table 1 The contrast of common cell membrane receptors

Name	Distribution	Physiological functions	Biomolecular ligands	Differential expression in GBM	Functions in disease treatment	Ref.
TfR1	Lungs, brain, kidneys, *etc.*	•Mediating iron transport; •Maintaining iron homeostasis.	Tf, Ferritin	Up regulation	•sTf for the diagnosis of iron-deficiency anemia; •Receptor-mediated endocytosis in drug delivery.	18 and 19
IR	Ovaries, pancreas, kidneys, *etc.*	•Belonging to the receptor tyrosine kinase; • Maintaining glucose homeostasis; •Phosphorylation and activating downstream effectors.	Insulin, IGF-1, IGF-2	Up regulation	•The diagnosis of diabetes mellitus type 2; •Receptor-mediated endocytosis in insulin delivery.	20 and 21
EGFR	Breasts, lung, esophagus, *etc.*	•Belonging to the receptor tyrosine kinase; •Phosphorylation and activating downstream effectors; •Promoting cell proliferation.	EGF, TGFα	Up regulation	•Drug target; •Receptor-mediated endocytosis in drug delivery.	22–24
LDLR	Adrenal glands, lungs, esophagus, *etc.*	•Mediating cholesterol transport.	LDL, Apolipoprotein, VLDL	Up regulation	•The diagnosis of atherosclerosis and hyperthyroidism; •Drug target; •Receptor-mediated endocytosis in drug delivery.	25–27
GLUT	Esophagus, bladder, kidneys, *etc*.	•Facilitating the transport of glucose across the plasma membrane.	Glucose	Up regulation	•Drug target; •Receptor-mediated endocytosis in glycosylated drug delivery.	28 and 29

Abbreviations: GBM, glioblastoma; sTf, soluble transferrin receptor; IGF, insulin-like growth factor; EGF, endothelial growth factor; TGFα, transforming growth factor α; VLDL, very low-density lipoprotein.

BBTB exists between tumor tissues of the brain and capillary vessels, and has an abnormal distribution of pericytes, endothelial cells with efflux transport proteins and some receptors. Only spherical nanoparticles less than 12 nm can pass through the fenestrated microvessels, making it a great challenge to deliver drugs to the brain.[16,17] Thus, relevant research mainly focuses on the design and development of drugs with BBB/BBTB-penetrating ability and tumor-targeting ability.

Kelong Fan

Kelong Fan received his PhD degree in cell biology from the Institute of Biophysics, Chinese Academy of Sciences, in 2014. After this period, he stayed there to further pursue 3 years of postdoc training and 2 years of associate professor work experience before attaining a full professor position in 2019. He is interested in exploring the novel functions and applications of nanozymes in biomedicine, with a top priority to design functional nanozymes by learning from nature, and to develop novel strategies for disease theranostics. He now serves as an Associate Editor of Exploration and Frontiers in Chemistry.

1.2 Potential targets for brain tumor therapy

Receptor-mediated endocytosis can be employed to deliver therapeutic drugs to the brain. Various receptors and transporters can be used as potential targets for brain tumor therapy (Table 1), including TfR1,[18,19] insulin receptor (IR),[20,21] endothelial growth factor receptor (EGFR),[22–24] low-density lipoprotein receptor (LDLR)[25–27] and glucose transporter (GLUT).[28,29] Macromolecules and NPs cross the BBB in a specific receptor-mediated way, mediated specifically or nonspecifically by vesicular mechanisms.[30–34] IR is one of the earliest proteins demonstrated to undergo transcytosis through the BBB. However, insulin is unsuitable as a targeting ligand due to the hypoglycemic effect and short half-life. Many anti-IR monoclonal antibodies and engineered peptides have been designed, but the clinical therapeutic effects are minimal.[35] LDLR, mediating the endocytosis of low-density lipoproteins (LDLs) such as cholesterol and apolipoprotein, is another common target used for crossing the BBB. As is the case for EGFR, IR, GLUT, *etc.*, most of the molecules targeting LDLR are synthetic antibodies and engineered peptides. By contrast, TfR1 represents a higher expression level on brain capillary endothelial cells. It also motivates TfR1-targeted proteins,

including transferrin and the heavy chain of human ferritin (HFn), to be directly used to traverse the BBB and easily combine with other therapeutic drugs for brain tumors. TfR1-mediated endocytosis has been proven to be effective in most conditions and is widely used in drug delivery.

2 TfR1 biology

2.1 TfR1 expression in the brain

TfR1, a type II transmembrane glycoprotein, is widely expressed in organs, while TfR2 is mainly expressed in the liver.[36–38] TfR1 consists of two homodimeric subunits linked by disulfide bonds, and each monomer consists of an extracellular C-terminal domain, a transmembrane domain and an intracellular N-terminal domain. The cellular expression levels of TfR1 are generally low,[37–40] while highly proliferative cells and some immune cells exhibit higher expression levels.[38] A similar phenomenon of highly expressed TfR1 is also observed on cells with higher iron requirements for heme synthesis. Brain capillary endothelial cells composing the BBB (Fig. 1a) also express high levels of TfR1, which is found to be essential in maintaining iron balance.[38] Usually, the iron regulatory proteins (IRPs) bind to the iron response elements (IRE) in the 3′ untranslated region to initiate gene transcription or directly bind to relevant mRNA to regulate the expression of TfR1.[38,41–43] Numerous studies have shown that the expression levels of TfR1 in cancer cells improve by almost an order of magnitude compared to those in normal cells (Fig. 1b).[38–40] Subsequently, TfR1 in serum is identified as a universal tumor marker in clinical settings.[44] Higher levels of TfR1 are associated with malignant solid tumors, including glioblastoma (GBM), breast cancer, lung cancer, cervical cancer, *etc.*,[45–50] and some hematopoietic malignancies. Therefore, TfR1 exhibits multiple roles in brain tumor therapy. On the one hand, TfR1 expressed in brain capillary endothelial cells allows drugs with targeting ligands to traverse the BBB. On the other hand, a higher expression of TfR1 enables drugs to specifically accumulate at tumor sites, thereby reducing toxicity to normal cells.

2.2 TfR1 as a potential target for brain tumor therapy

In general, endothelial cells of the BBB are tightly connected, and passage of only very small lipophilic drugs is possible. However, most therapeutic agents are non-small lipophilic molecules. Therefore, endogenous transporters, which could avoid an invasive change of the BBB, are required to transport these drugs. In spite of the fact that TfR1, IR and LDLR are highly expressed in BBB endothelial cells, TfR1 is expressed at high levels in both the BBB and brain tumor cells, and correlates with the pathological grading of brain tumors.[50,51] A higher expression on the membrane of tumor cells, along with its internalization capacity and indispensable function, enables the potential of TfR1 to be an attractive target for brain tumor therapy. For example, employing antibodies can specifically target TfR1 not only for drug delivery but also for the direct activation of relevant signaling pathways, such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cytotoxicity, antibody-dependent cell-mediated phagocytosis (ADCP) and complement-dependent cell-mediated cytotoxicity (CDC).

The relevance of using TfR1 as a therapeutic or delivery target has been unchallenged for decades. After the discovery of TfR1 expressed on brain endothelial cells, researchers have constantly been committed to investigate the TfR1-mediated BBB crossing. It has been proven that TfR1-targeted substances exhibited preferential accumulation in the brain.[52] Friden and his colleagues revealed that TfR1-targeted molecules could efficiently pass through the endothelial layer and be absorbed into the brain tissue, accompanied by brain capillaries aggregating at the site of brain tumors.[53–55] These results attracted more researchers to conduct further extensive and in-depth studies on TfR1, making it a promising target to cross the BBB and inspiring the development of targeting drugs that are delivered into the brain in a "Trojan horse"-like fashion.[56] Since these discoveries, the field of research interested in TfR1 as a target for brain drug delivery has grown significantly. Of these, most indicate that the system can work efficiently. In the case of HFn, after proving that the HFn can bind to TfR1 specially in 2010,[57] we used an immunofluorescence assay to observe

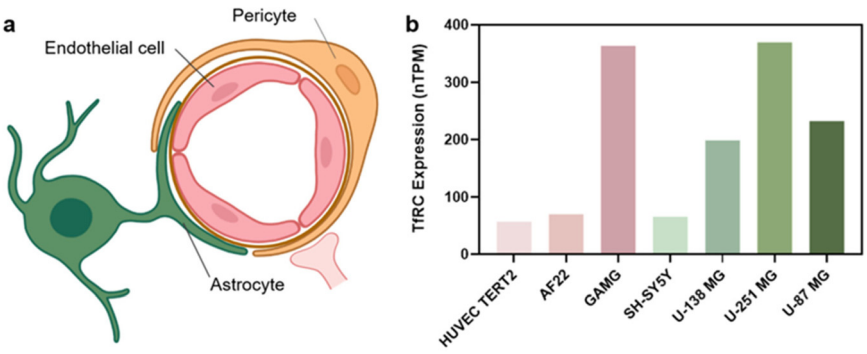

Fig. 1 Profiles of the neurovascular unit and TfR1 expression. (a) Structure of the neurovascular unit. (b) RNA expression data of TfR1 as normalized transcript per million (nTPM) values of endothelial cells, neuroepithelium cells, neuroglial cells and glioma cells. Data from the RNA-seq sub-catalogue of brain cancer cell lines in the Human Protein Atlas database (https://www.proteinatlas.org/). Search term is TFRC.

the TfR1-mediated endocytosis of HFn, which includes TfR1 binding, internalization and intracellular sorting in mouse bEnd.3 cells.[58] In this study, we clearly found that HFn started to localize on the cell membrane just after incubation and was then located in the vesicles in cytoplasm. This phenomenon significantly demonstrates that TfR1 can act as an endogenous receptor to mediate ligands across the BBB and into the tumor.

TfR1-targeted tumor therapy has been comprehensively reviewed. However, TfR1-targeted nanodrugs for brain tumor therapy have not been systematically summarized. Recently, much effort has been devoted into developing therapeutic platforms based on transferrin, ferritin and TfR1-specific antibodies, peptides or aptamers to realize targeted drug delivery or direct cell death. Based on the natural selectivity of the receptor, drugs are carried across the BBB and achieve targeted delivery to the tumor site. Herein, we summarize the recent studies of these five delivery modalities.

3 TfR1-targeted biomolecules and nanomedicine

TfR1 has been widely explored as the target of brain tumor therapy, exhibiting effective BBB penetration and a sufficient concentration of drugs at tumor sites. The common TfR1-targeted biomolecules mainly include transferrin (Fig. 2a), HFn (Fig. 2b), TfR1-targeting antibody/peptides and aptamers. Their biological characteristics have been proven to guarantee biosafety, high affinity and specific target. Nanomedicines are drugs at the nanoscale that exhibit a higher bioavailability, like liposome-based drugs and therapeutic nanoparticles. Hence, a universal strategy for overcoming the BBB is utilizing TfR1-targeted proteins combined with nanomedicine. Herein, we elaborate on the targeting strategies by discussing the design concept, current developments and future perspectives of nanomedicine for brain tumor therapy.

3.1 Tf

3.1.1 Tf biology. Tf, an iron-containing protein existing in the blood, plays an important role in iron transport and iron metabolism. The two lobes of Tf, N- and C-lobes, are responsible for reversibly binding to multivalent metal ions.[37,59] The iron-free form of Tf, apo-Tf, can bind iron ions efficiently in the blood to form holo-Tf, interact with TfR1 to form the diferroic Tf/TfR1 complex and transport into cells through the clathrin-coated pits mechanism. Subsequent iron release occurs in the lysosome, as the interaction of iron and Tf is pH dependent: combination occurring in neutral environments and dissociation in acidic environments. The protons pump in and thus induce lysosome acidification, realizing conformational changes in Tf and iron release. Iron is then transported by the divalent metal transporter 1 (DMT1). However, the Tf/TfR1 complex persists until the endosome reaches the cell surface and then realizes TfR1 recycling.[38]

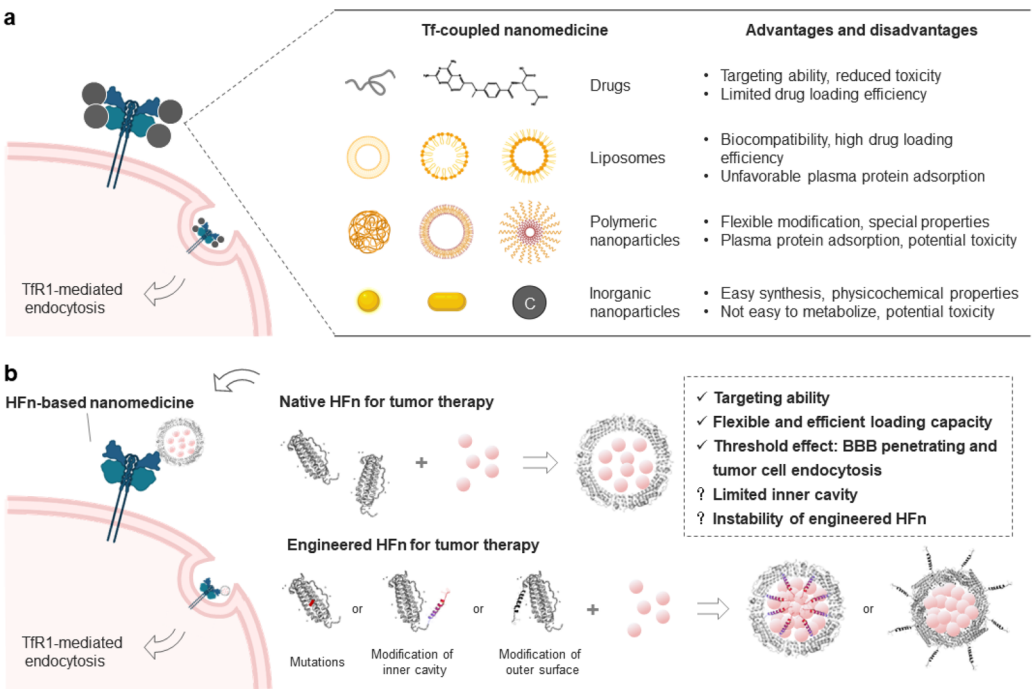

Fig. 2 Overview of Tf-coupled and HFn-based nanomedicine. (a) Tf binds to TfR1 and internalizes to realize drug delivery. Drugs, liposomes, polymeric nanoparticles and inorganic nanoparticles, in combination with Tf coating, exhibit enhanced BBB crossing and tumor targeting ability. Their advantages and disadvantages are summarized. (b) HFn binds to another site of TfR1 to realize endocytosis. Native HFn can be an ideal drug carrier because of the targeting ability, flexible and efficient loading capacity and threshold effect. To further improve the characteristics, numerous engineered HFn variants for tumor therapy have been developed.

3.1.2 Tf-coupled drugs. Based on the combination of Tf and TfR1 and the high expression of TfR1 in the endothelial cells and the surface of tumor cells, Tf is expected to be a promising targeting ligand, which has been proven to be effective to treat brain tumors in both animal models and clinical trials.[60] Thus, Tf-coupled drugs contribute to better tissue distribution, BBB penetration and tumor targeting ability (Table 2). To alleviate the systemic toxicity and enhance brain accumulation, Douglas W. *et al.* investigated the conjugation of Tf to a recombinant diphtheria toxin derivative, CRM107, which possesses stronger antiproliferative activity. The Tf-CRM107 conjugates crossed the BBB with the affinity of Tf for TfR1, and then selectively killed the tumor cells expressing high levels of TfR1 by the toxicity of CRM107. The results showed at least a 50–60% reduction in tumor volume and a complete remission of two patients.[61] The therapeutic effect of Tf-CRM107 in brain tumors has been explored in animal models and patients with malignant glioma. The results of Phase I clinical studies demonstrate that Tf-CRM107 can lead to tumor responses without severe side effects in patients suffering from malignant brain tumors. In the Phase II clinical trials, no serious toxicity was demonstrated in 35% of evaluable patients.[62] As a supplement, more studies can focus on enhancing the therapeutic effect of Tf-coupled nanomedicines.[63]

3.1.3 Tf-coupled liposomes. Lipid-based nanocarriers have been widely utilized in the delivery platform owing to their superior properties, such as efficient loading capacity, biosafety, biocompatibility, prevention of drug degradation, stability and suitability of various routes of administration. Tf modification represents a potential method to enable liposomes to cross the BBB, enhance the targeting ability of liposomes and develop nanomedicine for brain tumor therapy. In a recent study, Tf moiety was displayed on the surface of PEGylated liposomes to traverse the BBB and target to glioma. Aditi *et al.* loaded resveratrol (RES) into PEGylated liposome to overcome the shortcomings as a free drug. Compared with free RES and RES-loaded liposomes (RES-Ls), Tf-RES-Ls showed the most significant cytotoxicity and induced higher levels of apoptosis in GBM cells. In the glioma-bearing mice model, Tf-RES-Ls exhibited a more effective therapeutic effect in inhibiting tumor growth and improving mouse survival (Fig. 3a and b).[64] Vladimir's group further investigated the implications for targeting tumor-initiating cells or tumor stem cells. The neurosphere-derived cells from GBM cell lines highly expressed TfR1s, which allows the Tf-RES-Ls to show a significantly higher association with neurospheres *in vitro*.[65] These results advocated further explorations with Tf to target the tumor-initiating cells and emphasized the effect of Tf-coating that binds to TfR1 and activates the receptor-mediated endocytic pathway on the uptake of medicine. Other targeting ligands, such as cell-penetrating peptides (CPP), are also employed to enhance the endocytic process and have a high delivery efficiency across the BBB.[66–68] Sushant and Jagdish modified the surface of the liposome with Tf and proved that Tf-liposome successfully bound TfR1 on vascular endothelial cells to help in crossing the BBB. Meanwhile, the cell transmembrane peptide PFVYLI (PFV) was used to promote the entry of chemotherapy drugs into brain tumor cells (Fig. 3c). Experiments *in vitro* found that the cell uptake of drugs was significantly increased in the PFV-loaded group compared to the group without PFV (Fig. 3d).[68] Sushant *et al.* accomplished an

Table 2 Currently developed transferrin- and ferritin-based nanomedicine for brain tumor therapy

Delivery system	Therapeutic drug	Study model	Ref.
Transferrin			
Transferrin-CRM107	CRM107	U251MG tumor-bearing mice and phase III clinical trial (discontinued)	62
Transferrin modified liposome	RES	U87MG, U87MG tumor-bearing mice	64
Transferrin-conjugated biodegradable polymersome	Dox	C6, C6 tumor-bearing mice	100
Transferrin conjugated magnetic silica PLGA	Dox & PTX	U87MG, U87MG tumor-bearing mice	101
Polysorbate 80-coated PLGA NPs loaded with methotrexate–transferrin conjugates	MTX	C6, C6 tumor-bearing mice	102
Tf and AS1411 modified mesoporous ruthenium nanoparticles	RBT (PDT)	U87, U251, HBMEC BBB model, glioma-bearing mice	103
Holo-transferrin	ICG (PTT)	U87, bEnd.3, glioma-bearing mice	104
Ferritin			
HFn	Dox	U87MG tumor-bearing mice	83
HFn	PTX	C6, bEnd.3 BBB model, C6 tumor-bearing mice	105
Apoferritin	TMZ and N3P	U373 V, U373M	106
HFn with integrin α2β1-targeting peptide	Dox	U87MG, U87MG tumor-bearing mice	95
HFn with RGE peptide	SR717	GL261, G422, bEnd.3 BBB model, GL261 tumor-bearing mice	107
Apoferritin modified with GKRK peptide	VCR	U87MG, bEnd.3/U87MG co-culture BBB model, U87MG tumor-bearing mice	108
HFn with the hydrophobic peptide motifs on the inner cavity	Cpt & Epi	U87MG, U87MG tumor-bearing mice	109

Abbreviations: RES, resveratrol; Dox, doxorubicin; PTX, paclitaxel; MTX, methotrexate; RBT, $[Ru(bpy)_2(tip)]^{2+}$; PDT, photodynamic therapy; ICG, indocyanine green; PTT, photothermal therapy; TMZ, temozolomide; N_3P, N_3-propargyl imidazotetrazine analog; VCR, vincristine sulfate; Cpt, camptothecin; Epi, epirubicin hydrochloride.

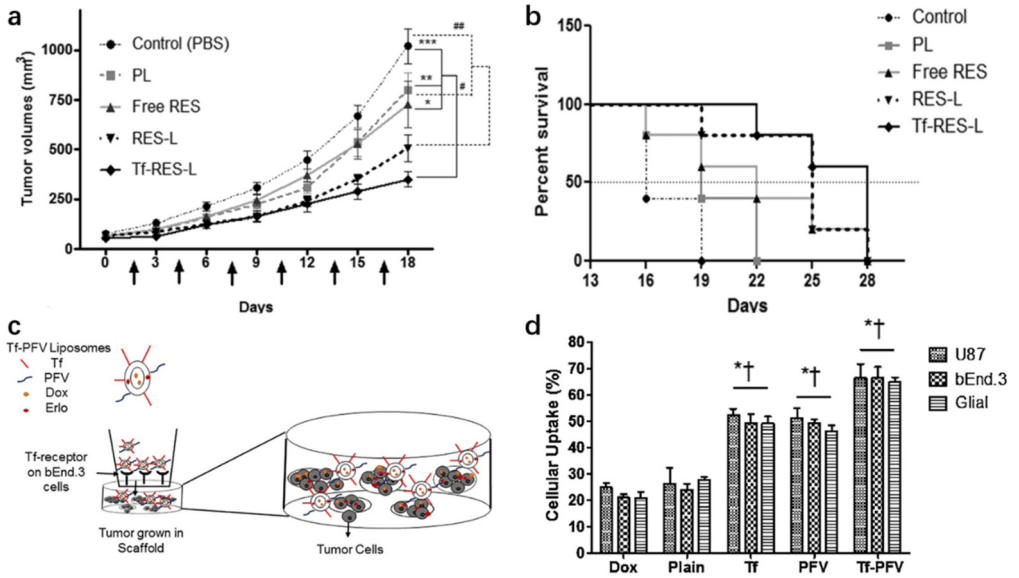

Fig. 3 Tf-coupled liposome medicine of brain tumor therapy. (a) A line-graph for tumor growth inhibition in the tumor-bearing mice. Two-tailed Student's *t*-tests were used (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$). (b) Survival analysis of mice after therapeutic drug treatment. These figures have been reproduced from ref. 64 with permission from the Journal of Controlled Release, copyright 2018. (c) Design of Tf-PFV-liposomes. (d) Cellular uptake of Dox encapsulated liposomes in different cell lines. The Tf-PFV liposomes showed significantly higher cellular uptake than the single ligand or liposomes. Statistically significant ($p < 0.05$) differences are shown as (*) with plain liposomes, and (†) with Dox. These figures have been reproduced from ref. 68 with permission from Colloids and Surfaces B: biointerfaces, copyright 2019.

increase in anticancer drug concentration and survival time in mouse brains by simultaneously expressing penetratin (Pen) on the surface after encapsulation of Dox in liposomes. The results showed that Tf-Pen liposome effectively crossed the BBB mediated by TfR1 and increased cell penetration, leading to a more striking therapeutic effect.[69] Liu *et al.* designed a dual-mediated sterically stabilized liposome named Tf-CPP-SSL, with Tf as a receptor-targeted ligand and CPP for electrostatic adsorption-mediated endocytosis. Data showed significant internalization and lysosomal escape capabilities, ensuring the liposomal contents entering into the cytosol and thus exerting pharmacological effects.[70]

3.1.4 Tf-coupled polymeric nanoparticles. Polymeric nanoparticles are regard as potential alternatives due to their specific properties, improved stability and flexible modification. Materials including poly (lactic-*co*-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), D-α-tocopherol polyethylene glycol succinate (TPGS) and chitosan (CS) have been used to prepare polymeric nanoparticles with different morphologies, such as polysomes, micelles, dendrimers, *etc.* Sonali *et al.* developed Tf-TPGS conjugate micelles to deliver docetaxel (DTX) for glioma therapy. TPGS, a kind of vitamin E derivative, was conjugated with polyethylene glycol (PEG), thus functioning as a biosafe block copolymer and improving the stability and cellular uptake of nanomedicine. The bio-distribution in glioma-bearing rats was carried out to prove that Tf-TPGS is an effective carrier for brain tumor-targeted nanomedicine.[71] Similarly, PCL represents a biodegradable material and is also widely used in nanomedicine preparation. Pang *et al.* prepared Dox-loaded PEG-PCL polymersomes conjugated with Tf on the surface to endow the ability of BBB penetration and tumor targeting. In the glioma-bearing rats, higher intracellular accumulation and tumor cell apoptosis were observed.[72] On the other hand, Tf exhibits unique characteristics in the metabolism pathway that can also be utilized to deliver drugs. Tf decomposition is pH-dependent and occurs inside the lysosome. Based on this, Ruan *et al.* designed an acid-responsive programmed targeted drug delivery system (DD-MCT) (Fig. 4a). The acid-responsive cleavage of Tf could release therapeutic drugs, which promoted the escape of nanoparticles from lysosome. Further, the released drugs could be exocytosed by GLUT, resulting in an extremely enhanced tumor uptake of nanoparticles.[73] The combination of targeted ligand and polymeric nanoparticles offers a potential strategy to deliver therapeutic nanomedicine and treat brain tumors.

3.1.5 Tf-coupled inorganic nanoparticles. Recently, inorganic nanoparticles have attracted considerable interests due to their easy synthesis, flexible morphology, multifunctionality and special physicochemical properties. Carbon- and silicon-based nanoparticles represent the most biosafe materials, such as carbon dots, carbon nanotubes, graphene oxide, silicon dioxide, *etc.*, and are constantly developed as delivery systems. Liu *et al.* synthesized Dox-loaded mesoporous silica nanoparticles decorated with Tf and RGD ligands. By introducing a calcium phosphate coating and pH-triggered chemotherapeutic release, these nanomedicines can realize reduced side effects and enhanced tumor targeting.[75] Gold nanoparticles offer both drug delivery possibilities and powerful advantages in the investigation of nanoparticle behavior. In an other interesting example, Mark's group investigated the

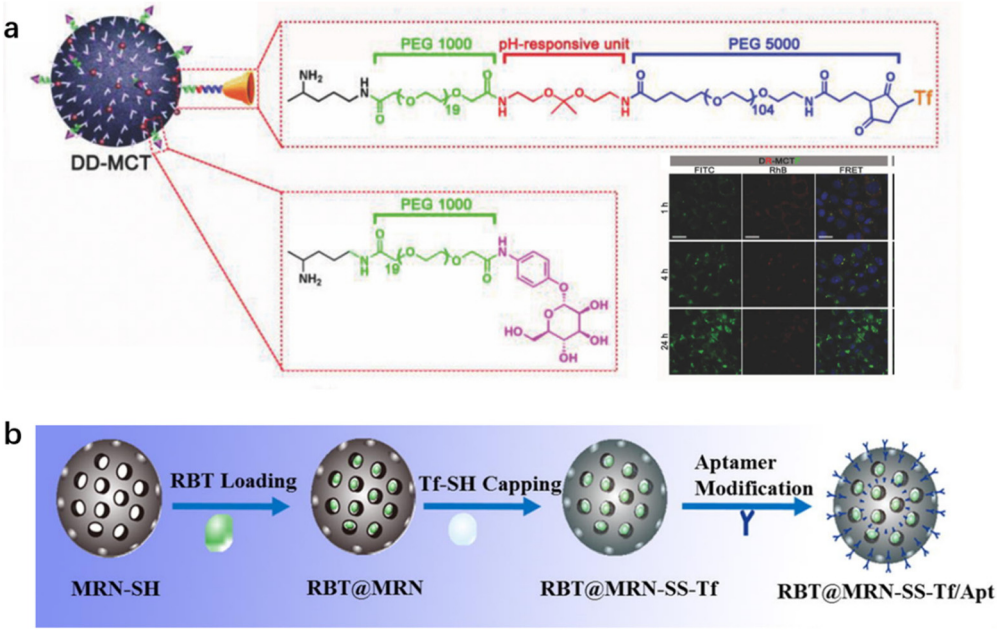

Fig. 4 Tf-coupled polymeric and inorganic nanoparticles of brain tumor therapy. (a) Schematic illustration of the conjugation of DD with the acid-cleavable Tf and MAN moiety. Confocal images of bEnd.3 cells treated with DR-MCTF and DR-MTF demonstrate that the acid-responsive Tf could be cleaved in an acidic environment. This figure has been reproduced from ref. 73 with permission from Advanced Functional Materials, copyright 2018. (b) Schematic illustration of the design of the dual-targeting RBT@MRN-SS-Tf/Apt. This figure has been reproduced from ref. 74 with permission from Acta Biomaterialia, copyright 2018.

cellular uptake behavior and distribution of Tf-Au by mediating the affinity of the material for TfR1. Data showed that TfR1-mediated endocytosis correlates with the size and Tf density on the surface of nanoparticles.[76] They further utilized an acid-cleavable bond to link Tf and Au nanoparticles to realize and facilitate the selective release. Increased accumulation of nanoparticles in the brain has been observed, which suggests that a lower dosage can bring an equally effective therapeutic effect.[77] For combined therapy, Tf can be utilized as the targeting ligand of photosensitizer or photothermic agents. Zhu *et al.* combined a Tf/aptamer with a mesoporous ruthenium nanosystem through a redox-cleavable disulfide bond to achieve selective drug delivery. A type of anti-tumor drug, $[Ru(bpy)_2(tip)]^{2+}$ (RBT), was encapsulated to produce reactive oxygen species (ROS) under laser irradiation (Fig. 4b). After administration, the cooperation of Tf and the aptamer significantly improved the targeting accuracy of this drug delivery system and promoted BBB crossing and drug accumulation in the brain. ROS generated by RBT can also effectively promote drug release and realize photodynamic therapy. Data showed that this nanosystem induced a strong anti-tumor effect both *in vivo* and *in vitro* and significantly prolonged the mice survival (Fig. 4c and d).[74] In another study, Xiaobing Wang's group developed an indocyanine green (ICG) loaded holo-Tf nanosystem for tumor-targeted photothermal therapy.[78] All these studies have fully elaborated the effective BBB penetration and targeting ability to brain tumors mediated by Tf.

3.2 Ferritin

3.2.1 Ferritin biology. Ferritin represents a widely expressed iron storage protein in various organisms including mammals, plants, fungi, bacteria, *etc.*[79,80] Ferritin consists of 24 self-assembled subunits, forming a spherical structure with an external diameter of 12 nm and an inner cavity of 8 nm.[81] Typically, ferritin possesses a conserved structure and components in eukaryotes. HFn contains ferroxidase catalytic sites responsible for the oxidation of Fe(II), while the light chain ferritin (LFn) plays an indispensable role in the nucleation of oxidized iron.[82] In 2010, Seaman's group discovered that HFn binds to TfR1 to realize cellular uptake,[57] providing evidence that ferritin has natural tumor-targeting capabilities. Yan's group was the first and has been committed to applying the specific combination of HFn with TfR1 to realize tumor diagnosis and treatment, attracting researchers to conduct more extensive and in-depth studies on HFn.[83,84] Notably, the recognition region of HFn to TfR1 differs from that of Tf to TfR1. Compared to Tf-based nanomedicine, HFn-based nanomedicine not only ensures that the introduction of exogenous HFn-based nanomedicine does not interfere with normal iron metabolism but can also exploit the threshold effect to achieve a different biological effect.[85] Some typical researches are presented in Table 2. It has been proved that HFn-mediated transcytosis is regulated by the surface density of TfR1. Specifically, HFn nanocarriers are transcytosed into endosomes in cells expressing low levels of TfR1 while being endocytosed into

lysosomes for accumulation in cells in cells expressing high density of TfR1. In a brain tumor model, the data showed that TfR1 expression was 10-fold higher in tumor cells than in endothelial cells.[83] This drives HFn to efficiently penetrate the BBB without remaining in the endothelium while accumulating in the lysosomes of brain tumor cells. This property allows HFn to become a desirable choice in the field of brain tumor-targeted therapy.

3.2.2 Native HFn for tumor therapy. Simsek and Kilic firstly investigated the loading ability of ferritin by encapsulating anti-cancer drugs into its cavity. Depending on the reversible self-assembly ability of apoferritin, Dox was encapsulated in apoferritin by pH-mediated disassembly-reassembly, resulting in a higher loading efficiency than liposomes.[86] However, the severe acidic pH may irreversibly damage the structures and form defects on the surface of the protein, which seriously affects the stability and *in vivo* applications. Other loading methods have been established, such as urea-related, temperature-related, iron ion-depended methods, *etc.*[87] These unique physicochemical characteristics and the special core–shell structure allow ferritin to function as a delivery vehicle. We further designed ferritins without iron cores and only composed of heavy-chains and proposed the concept of ferritin drug carriers (FDC).[88] Compared to the natural ferritin, FDC is an engineered product with a cavity structure composed only of heavy chains. Without iron cores, the inner cavity along with the targeting ability makes FDC a promising drug delivery system, especially in the treatment of brain tumors. Our previous work attempted to load Dox into HFn to overcome the BBB and enhance the drug accumulation in the brain to treat GBM (Fig. 5a). We dissolved HFn in a high urea environment to induce ferritin depolymerization and then replaced it into a low urea environment in a gradient method to realize repolymerization. We used Irdye-800-labeled HFn and mCherry-labeled tumor and found that the two stained region could overlap well (Fig. 5b). This result verified that HFn-Dox could successfully traverse the BBB *via* TfR1-mediated transcytosis and specifically target the tumor site. Further, we obtained the *in vivo* bioluminescence (BLI) images of tumor-bearing mice that were intravenously injected with different formulations (Fig. 5c and d). We found that HFn-Dox treatment leaded to the apparent regression of tumor growth.[58] Similarly, Liu *et al.* prepared HFn-PTX in a pH-dependent disassembly/assembly method.[89] With a more in-depth study, we identified a natural thermal-response drug entry channel on the shell of HFn and developed a simple channel-based drug loading strategy (Fig. 5e), which highlights the potential of ferritin for drug delivery. Data showed a successful loading of Dox. It has been proved that without denaturing agents, the loading efficiency and stability showed significant improvement (Fig. 5f), leading to an extreme therapeutic effect. After being injected into the U87MG-bearing mice, HFn-Dox caused a visible regression of

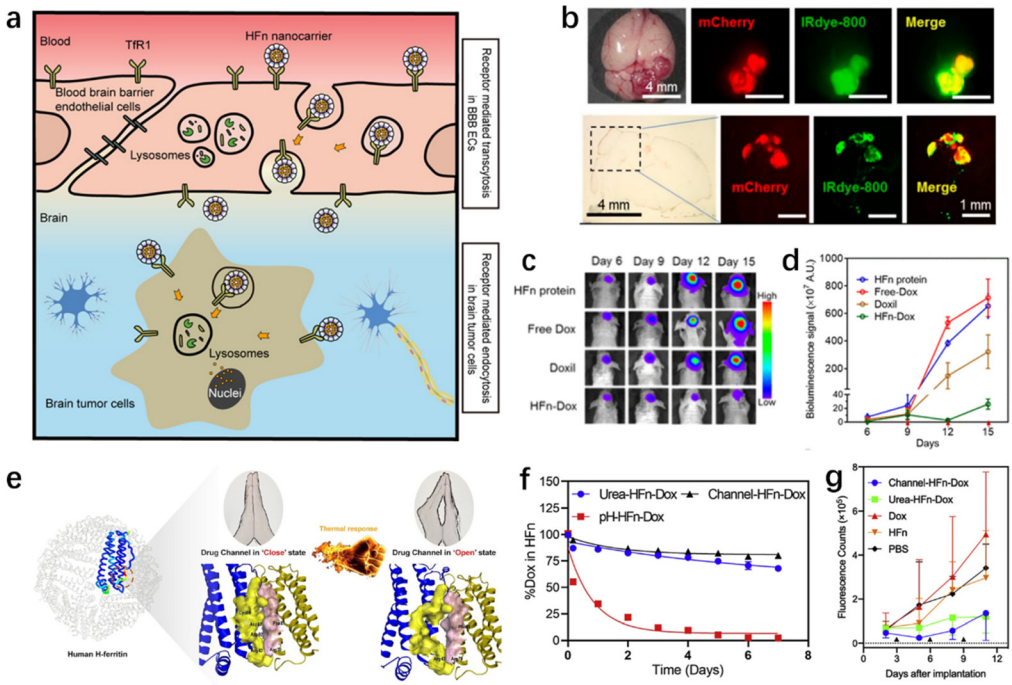

Fig. 5 Native HFn-based nanomedicines for brain tumor therapy. (a) Schematic illustration of HFn crossing the BBB and killing glioma tumor cells. (b) HFn labeled by Irdye-800 overlapping with tumor labeled by mCherry upon intravenous injection of HFn. (c) *In vivo* BLI images of tumor-bearing mice injected with different formulations. (d) Quantitative analysis of the bioluminescence signals of (c). These figures have been reproduced from ref. 58 with permission from ACS Nano, copyright 2018. (e) A natural thermal-response drug entry channel on the shell of HFn. (f) The stability of channel-HFn-Dox was higher than the pH-HFn-Dox and Urea-HFn-Dox in 5% BSA buffers. (g) The anti-tumor activity of channel-HFn-Dox was better than the other groups. These figures have been reproduced from ref. 90 with permission from Nano Today, copyright 2020.

tumor growth and an extended survival time of mice (Fig. 5g).[90]

Since the finding that TfR1 was overexpressed in tumors in contrast to normal tissues, it has been identified as a general target for tumor diagnosis and treatment. HFn loaded with diagnostic molecules has been widely used in tumor detection. We developed an HFn-based nanomedicine loaded with iron oxide nanoparticles named magnetoferritin (M-HFn). With the high affinity between HFn and TfR1 on tumor cells, the specific identification of tumors and subsequent endocytosis could be achieved. After internalization and an additional DAB substrate and H_2O_2, there was a color response that visualized the tumor cells on the basis of intrinsic peroxidase-like activity of M-HFn.[91,92] Through specific recognition of HFn to TfR1, this nanomedicine can target to the tumor cells and exert catalytic function, leading to a color reaction to visualize tumor tissue. This can be applied for MRI, SPECT/MRI and other multi-modal imaging. It should be noted that the HFn-mediated delivery of antibodies can mediate killing and may represent a promising combination strategy for immunotherapy. Rizzuto *et al.* employed recombinant forms of human apoferritin to deliver mAb to activate the ADCC responses *via* a TfR1-mediated strategy.[93] These results highlight the potential of ferritin for clinical applications and demonstrate FDC as an ideal platform in the TfR1-mediated BBB crossing strategy and targeted tumor therapy.

3.2.3 Engineered HFn for tumor therapy. In general, ferritin is usually obtained by biosynthetic methods, which makes ferritin-based nanomedicine low-cost and easy to design and modify. The exterior interface of the HFn nanocage possesses several reactive amino acids that can be easily modified with functional components. Zhai *et al.* modified the GKRK peptide, targeting glioma through binding with the heparan sulfate proteoglycan (HSPG), on the surface of apoferritin to realize the dual-targeting delivery of vincristine sulfate (VCR). This delivery system showed a favorable anti-tumor effect *in vitro* and *in vivo.*[94] Huang *et al.* developed a unique nanomedicine named 2D-HFn by adding integrin α2β1-targeted peptide to the N-terminus of HFn and loading Dox in the cavity. Compared with the native HFn, 2D-HFn significantly improved not only the loading capacity, but also the transcytosis into glioma cells.[95] Various therapeutic drugs have been loaded into HFn as targeted nanomedicine for brain tumor therapy, but challenges remain in loading hydrophobic drugs due to their hydrophilic nature. Recently, we designed an amphiphilic protein nanocage (Am-PNCage) by replacing the fifth helix of the HFn subunit with a functional motif consisting of a hydrophobic-hydrophilic-RGD peptide (Fig. 6a). Therefore, hydrophilic drugs can be introduced into the lumen through channels of HFn nanocages. At the same time, hydrophobic peptides displayed on the outer surface can be used to bind hydrophobic drugs. Data showed an effective and stereoscopical loading of hydrophilic and hydrophobic drugs, and the dual-targeting properties endowed Am-PNCage with selectivity that facilitated the penetration and accumulation at tumor sites (Fig. 6b). This kind of bioengineered protein nanocage exhibited an alleviated toxicity and a remarkably improved therapeutic effect against brain tumors (Fig. 6c and d).[96] Fusing the C-terminal of HFn with the hydrophobic peptides to re-engineer the inner cavity is also a common method (called ins-FDC).[97] In addition, the redesign of naturally existing cage-like proteins is a newly developed technology to prepare protein nanocage analogs. Zhao's group designed a 16-mer lenticular ferritin cage by inserting amino acid residue into native ferritin and thus reengineering the key subunit interfaces. This smaller ferritin cage retained the ability of loading drugs and exhibited an enhanced cellular uptake compared to natural ferritin,[98] as well as a successful penetration of the BBB.[99] However, more studies are still required on the bioengineered protein nanocage for brain tumor therapy.

3.3 Antibodies

3.3.1 Antibody biology. Antibodies are dimeric structures linked by disulfide bonds, typically composed of two light chains and heavy chains both with variable regions (Fv) and crystallizable regions (Fc).[110,111] The Fv fragments specifically bind with antigens, whereas the Fc fragments contribute to the subsequent biological effects, antigen presentation and pharmacokinetic behaviors. Because of the specific recognition of antibody to antigen, the high expression of TfR1 in brain tumor cells means that we can use TfR1 antibody for drug delivery as it plays a good targeting role. At the same time, antibodies can be further genetically engineered, such as scFv and humanized antibodies, to be better tolerated and to enable higher efficacy in patients. These TfR1 targeting antibodies can be divided into antagonistic and non-antagonistic antibodies, as well as certain antibody-like molecules.[112] In this chapter, we summarize the currently developed antibody-based nanomedicine (Table 3).

3.3.2 Antagonistic antibody-based nanomedicine. Antagonistic antibodies or antibody fragments have been designed to target TfR1 because of their specificity and high affinity. Through competition or spatial site blockage, they can prevent transferrin from binding directly to TfR1. At the same time, they affect the physiological function of cells by making them deficient in Tf-bound iron. The first monoclonal antibody specifically targeting TfR1 was employed to inhibit tumor growth. In 1981, a mouse IgG1 named B3/25 was synthesized to antagonize the binding of transferrin to TfR1.[113] Since then, various TfR1-targeted antibodies have been developed and exert cytotoxic effects. A24 is a typical antagonistic antibody that binds TfR1 competitively with Tf and induces TfR1 degradation. A24 was found to prevent the proliferation of acute and chronic ATL (Adult T-cell leukemia) forms of malignant T cells *in vitro* by inducing programmed cell death.[114] In addition, the widely studied antibodies also include 42/6,[115] E2.3,[116] CH128.1,[117] *etc.* Some univalent scFv have undergone significant research in addition to conventional antibodies like IgG.[118]

3.3.3 Non-antagonistic antibody-based nanomedicine. Non-antagonistic antibodies are those that bind with TfR1 while causing no influences on Tf combination and other

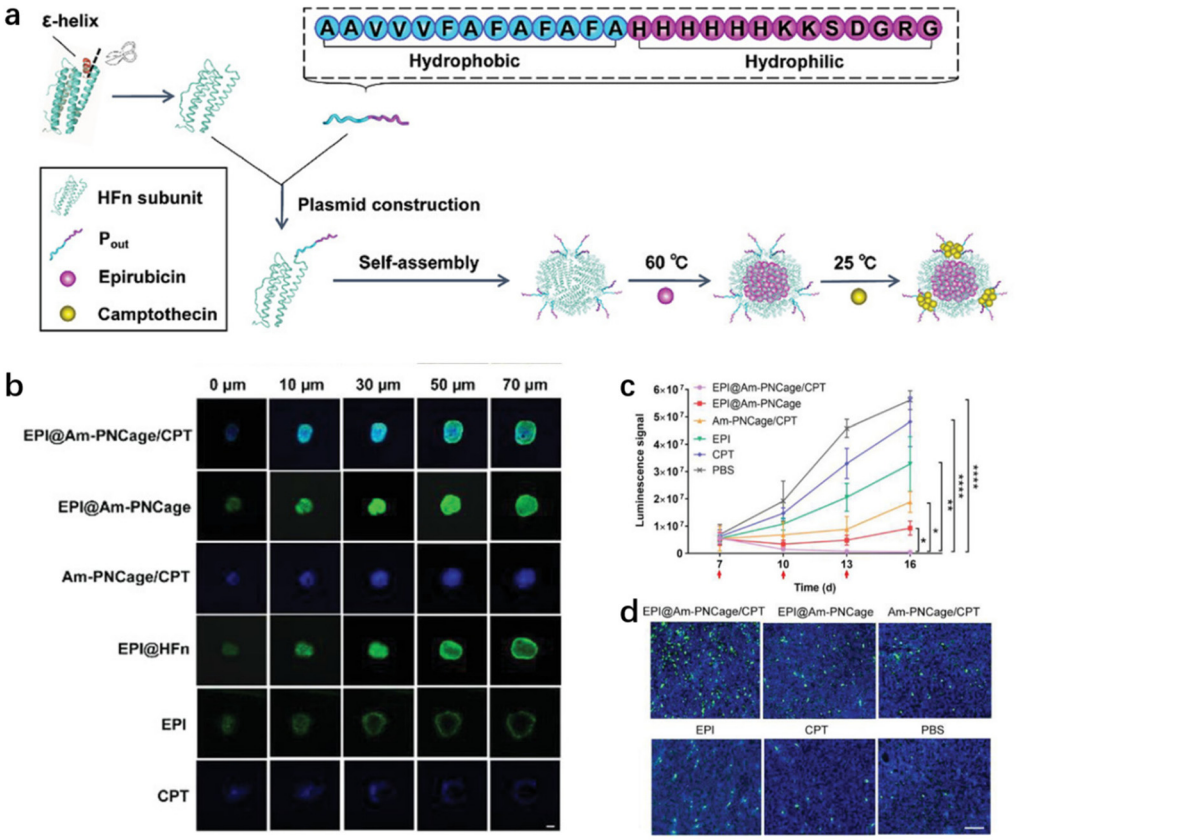

Fig. 6 Engineered HFn-based nanomedicines for brain tumor therapy (a) Design of the Am-PNCage. (b) The Am-PNCage significantly increased drug penetration into the tumor *in vitro*. (c) Tumor growth curves after different drug treatment. Data are shown as means ± SD (n = 5–6; *p < 0.05, **p < 0.01, ****p < 0.0001). (d) Am-PNCage treatment induced more apoptosis in the tumor compared to that of either drug alone *in vivo*. These figures have been reproduced from ref. 96 with permission from Advanced Functional Materials, copyright 2021.

metabolic pathways. As a result, they are less toxic compared with antagonistic antibodies. The high specificity of anti-TfR1 antibodies is thought to be an optimal candidate to promote the intracellular uptake of therapeutic drugs, enhancing the anti-tumor effect, reducing healthy cell exposure, and improving overall therapeutic efficacy. Similar to endogenous Tf, TfR1 antibody has been utilized as a targeting molecule to co-deliver with drugs and nanocarriers. The main types of therapeutic agents include nanodrugs targeting TfR1 and other agents such as antibody drug conjugates (ADCs) or precursor drug adjuvants (PDCs). A widely used TfR1-targeted molecule is OX26, introduced by Jefferies and his colleagues.[119,120] Over the next few years, several studies were conducted to verify the BBB-penetrating ability of OX26.[55] For several preclinical models of brain diseases, OX26-related agents have yielded therapeutic effects,[121] notably for a type of brain cancer, glioblastoma multiforme (GBM).[122–124]

Moreover, antibody-decorated liposomes, also named immunoliposomes, have recently shown potential for drug delivery and targeted therapy. The carrier combines liposome technology, PEGylation technology and BBB targeting technology along with plasmid-based therapeutic gene technologies. Polymer nanoparticles can pass through the BBB or BBTB passively or through an active endocytic mechanism. Tempol-loaded PLGA NPs conjugated with OX26 have been shown to possess brain targeting capacity and can protect against oxidative damage.[125] In 2018, Ramalho *et al.* developed PLGA NPs functionalized with OX26 mAb on the surface and TMZ encapsulation inside the particles (Fig. 7). After the loading of mAb, TMZ-mAb-PLGA showed a stronger killing effect on tumor cells and a significantly reduced toxicity to normal cells compared to free TMZ.[126] In addition to being able to carry small molecules in liposomes, it is also possible to carry smaller molecules, such as nucleic acids, including antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs).[127] Zhang *et al.* used PEGylated immunoliposomes to target brain tumors with receptor-specific mAbs to deliver plasmids expressing siRNA, resulting in an 88% amelioration in the survival of mice with advanced intracranial brain cancer.[123,128]

3.4 Targeting peptide-based nanomedicine

Peptides can form a variety of secondary structures or self-assemble into multiple structures through non-covalent supramolecular interactions. By rational design, amphiphilic proteins or polypeptides of any sequence or with desired specific secondary structures can be obtained and thus peptide-based

Table 3 Currently developed antibody-based nanomedicine

Name		Delivery system	Study model	Effect of antibody	Ref.
Antagonistic antibody					
7579 mAb	IgG1	—	U251, U87MG	Inducing S phase accumulation and apoptosis of tumor cells	129
42/6	IgG1	—	Normal and malignant myeloid cells	Inhibiting combination of transferrin to its receptor and cell growth	115
A24	IgG2b	—	Adult T-cell leukemia/ lymphoma cells	Inhibiting Fe-transferrin uptake, blocking T-cell proliferation, reducing TfR expression and recycling and inducing programmed cell death	114
E2.3	IgG1	—	Myeloma cells	Inhibition of myeloma cell growth	116
A27.15	IgG1				
ch128.1	IgG3	ch128.1, ch128.1Av	Disseminated multiple myeloma xenograft mice	Limited cytotoxicity and remarkable anticancer activity	117
3TF12	Univalent scFv	—	Hematopoietic tumor cell lines	Antiproliferative effects	118
3GH7	Univalent scFv				
F12CH	Bivalent scFv	—	Hematopoietic tumor cell lines	Enhanced antiproliferative effects, inducing intracellular iron depletion and cell death	
H7CH	Bivalent scFv				
Non-antagonistic antibody					
OX26 mAb		PEGylated liposomes	C6, F98, BMVEC BBB model, C6 tumor-bearing rats	BBB penetration	130
		Cisplatin-loaded PEGylated liposome	C6, BCEC BBB model, C6 tumor-bearing rats	BBB penetration and higher cellular uptake	131
		PLGA nanoparticles loading TMZ	U251, U87, NHA	Higher cellular uptake	126
RVS10 mAb		pH sensitive PMLA nanoparticles loading hydrazided TMZ	U87MG, T98G	Tumor targeting and higher cellular uptake	132
8D3 mAb		8D3-AuNP	Healthy mice	BBB penetration, TfR-mediated and clathrin-dependent endocytosis process	133
		Pegylated immunoliposomes encoding a siRNA of EGFR	U87	Tumor targeting and longer survival time	128
B3/25		HPMA copolymer conjugated to B3/25	Fibroblasts	Higher total uptake and cellular accumulation	134
RI7217		RI7217 and muscone co-modified DTX long-circulating liposomes	U87MG, hCMEC/D3, U87MG tumor-bearing mice	BBB penetration and targeting to GBM	135
TfR scFv		Cationic liposome loading TMZ	U87, U251, U87R, U87 tumor-bearing mice	BBB penetration and targeting to GBM and cancer stem cells	136
		Cationic liposome loading p53 cDNA	U87, U251, U87 tumor-bearing mice, Phase I clinical trials	BBB penetration and targeting to GBM	137
Antibody-like molecules					
Dual-variable-domain immunoglobulin		Dual-variable-domain immunoglobulin	HEK293, healthy mice	BBB penetration and higher cellular uptake	112

Abbreviations: TMZ, temozolomide; PMLA, poly(β-L-malic acid); HPMA, *N*-(2-hydroxypropyl) methacrylamide; DTX, docetaxel.

nanomedicine with targeting ability can be constructed. Compared with other self-assembled biomolecules, peptide-based nanomedicine presents distinct stability against enzymatic degradation and subsequent longer half-life. Nowadays, an increasing amount of research has focused on their biomedical applications, especially in the field of tumor therapy, including TfR1-targeted treatment of brain tumors.

A variety of peptides have been found target the recognition of TfR1and Tf (Table 4). A T7 peptide selected in the phage display library exhibited a strong affinity for TfR1. Wei *et al.* used T7-LPC/siRNA NPs to efficiently deliver siEGFR into U87 glioma cells *via* TfR1-mediated internalization to induce EGFR downregulation to inhibit tumor proliferation.[138] However, T7 peptide is easily degraded by proteases *in vivo* and loses its activity. It was found that, compared to L-T7, D-T7 showed a superior TfR1 binding capacity and serum stability.[139,140] In order to improve the performance of the targeted peptide, Li *et al.* developed a red blood cell membrane-coated solid lipid nanoparticle (RBCSLN). The design of RBSCLN is based on solid lipid nanoparticles of native lipids. The investigators formed the complete RBCSLN by wrapping erythrocyte membranes modified with a T7 peptide to bind TfR1 to cross the BBB and an NGR peptide to target tumor-highly expressed CD13 and vincristine inside. This ingenious design demonstrated effective brain targeting by dual targeting in zebrafish and mouse orthotropic models and reduced

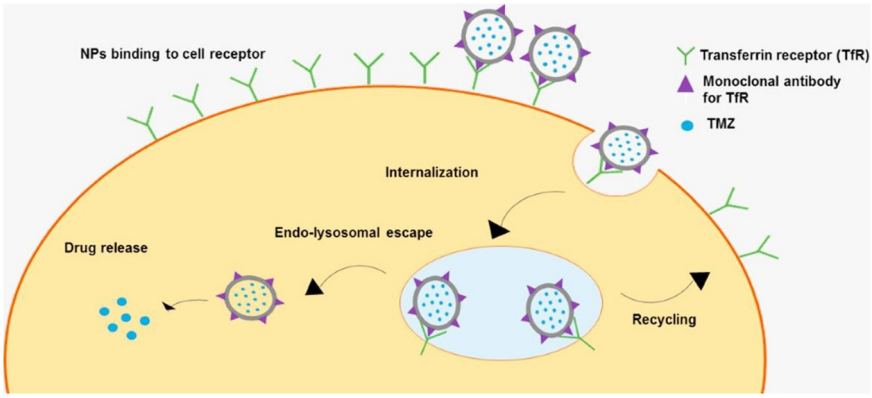

Fig. 7 PLGA NPs functionalized with an mAb of TfR1 could help the delivery of drugs into brain tumor cells. This figure has been reproduced from ref. 126 with permission from the International Journal of Pharmaceutics, copyright 2018.

Table 4 Currently developed targeting peptides for brain tumor therapy

Name	Sequence	Target	Affinity	Effect of targeting peptide	Study model	Ref.
B6	GHKAKGPRK	TfR1	—	•Binding with TfR1 •Realizing BBB penetration and anti-glioma	GL261, hCMEC/D3 BBB model, healthy mice	142 and 143
B18	SPRPRHTLRLSL	TfR1	—	•Binding with TfR1 positive cell	SMMC-7721	144
T7	HAIYPRH	TfR1	10 nM	•Binding with TfR1 •Realizing BBB penetration and glioma targeting	C6, bEnd.3 BBB model, glioma-bearing mice	141
T12	THRPPMWSPVWP	TfR1	15 ± 7 nM	•Binding with TfR1 •Realizing glioma targeting and enhanced cellular uptake	U87MG, HUVECs/U87MG co-culture BBB model, U87MG tumor-bearing mice	145 and 146
D-T7	HRPYIAHC	TfR1	22 ± 1 nM	•Binding with TfR •Realizing BBB penetration and glioma targeting	C6, bEnd.3/C6 BBB model, C6 tumor-bearing mice	139 and 140
CRT	CRTIGPSVC	Tf	—	•Binding with endogenous Tf •Realizing TfR-mediated BBB penetration	C6, BCEC BBB model, C6 tumor-bearing mice	147, 148 and 149
T10	CGGGHKYLRW	Tf	0.9 ± 0.25 μM	•Binding with endogenous Tf •Realizing TfR-mediated BBB penetration	U87, C6, bEnd.3 BBB model, D rats, U87 tumor-bearing mice	150

tumor growth by 50%. Red blood cell (RBC) membranes are used to carry nanoparticles and endow them with targeting function. In this way, the physicochemical properties of nanoparticles can be preserved and the biological functions of endogenous materials can be achieved.[141]

In addition to the T7 peptide, there are many other TfR1-targeting peptides. The B18 peptide was identified by Qin *et al.* through the phage display method, which also binds to TfR1 and specifically targets to TfR1-positive tumor tissues.[144] CRT peptide is a Tf binding peptide that can be conjugated with polymeric nanoparticles (CRT-NPs) to inhibit the tumor cell growth and prolong the median survival time in mice.[147]

3.5 Aptamers

Aptamers refer to short oligonucleotides that can be designed to bind with different types of nucleic acids or biomolecules through their unique three-dimensional (3D) interactions. Typically, they are composed of short single-stranded DNA or RNA molecules, thus possessing a high degree of specificity towards nucleic acids. As a result, they are particularly amenable to binding as delivery vehicles to other RNA therapeutics, such as siRNA, miRNA, shRNA, long-stranded non-coding RNA (lncRNA), ASO, and circulating RNA (circ RNA). On the other hand, the designable 3D structures make it possible to target to organic molecules, proteins, cells, *etc*. The general targeting ability enables aptamers to be considered as "chemical antibodies", along with the advantages of lower immunogenicity and bioavailability. Since the first RNA aptamer was isolated and discovered in 1990,[151] many aptamers and aptamer–drug couples have been developed and tested for clinical drug delivery. To date, several aptamers have been reported for TfR1. Chen *et al.* reported two aptamers specifically targeting mouse TfR1.[152] Wilner *et al.* reported a 2′-F-modified RNA aptamer that recognizes human TfR1.[153] Another RNA aptamer was later reported for siRNA delivery and the inhibition of New World hemorrhagic fever mammary virus (NWAs) entry.[154] Compared to RNA aptamers, DNA aptamers are more stable, less costly, and easier to modify. Tan's group reported a DNA

aptamer for TfR1 that has been tested for coupled drug delivery in pancreatic cancer.[155,156]

However, few aptamers have been applied in brain tumor therapy. Zhang *et al.* used the drug-resistant colon cancer cell line HCT-8T as a target to produce a DNA aptamer HG1-9, which was shown to bind to TfR1 with a comparable affinity. Data showed that there was a significant penetration in the epithelial barrier through TfR1-mediated endocytosis.[157] Macdonald *et al.* developed an aptamer with dual-targeting ability, binding to TfR1 along with the epithelial cell adhesion molecule (EpCAM). Both *in vivo* and *in vitro* experiments have shown that this aptamer could mediate BBB-penetration and target to tumor cells.[158] Furthermore, they designed an aptamer–Dox conjugate to realize both the selective accumulation and drug delivery. It has been proved that the conjugates could penetrate the BBB and deliver therapeutic drugs specifically to EpCAM-positive brain tissues, which improved drug payload and treatment efficiency.[159]

4 Conclusion and perspectives

Despite proactive clinical applications of surgery, radiotherapy and chemotherapy, brain tumors remain a threat due to their rapid progression and poor prognosis. As a physical and immunological barrier, the BBB blocks most of the drugs transported into the brain. Approaches targeting BBB-related receptor proteins have been extensively studied for decades. Years of research have found that drugs modified with specific targeting ligands can distinctly increase drug accumulation in the brain and alleviate systemic toxicity. Therefore, it could be a promising new approach for brain tumor treatment. Among the different receptors in the field of targeted therapy, TfR1 remains one of the most important receptors used to deliver brain-targeted nanomedicine.

In this review, we summarize the five major classes of TfR1-targeted nanomedicine currently used to traverse the BBB and target brain tumors, including nanocarriers composed of transferrin, antibodies, ferritin and targeting peptides, respectively. Conjugates of Tf-coupled drugs presented BBB-crossing ability, demonstrating the reliability of Tf as a targeting molecule in brain tumor therapy. Although limited drug loading efficiency detracts from their appeal, more attention has been paid to the combined carriers, including liposomes, polymeric nanoparticles and inorganic materials. A considerable number of studies have proved the outstanding ability of Tf-coupled nanomedicine to target and bind TfR1. However, Tf-based strategies exhibit some limitations, including that Tf-coupled nanomedicine can reduce the affinity of endogenous Tf molecules for TfR1 through competitive bindings, leading to a reduced effect and abnormal influence on normal metabolism. To avoid these challenges, alternative strategies have been explored by using ligands that do not competitively bind receptor alternative sites. At the same time, the uncontrollable and non-reproducible synthesis of monoclonal antibodies limits their clinical applications. How to improve the uptake of therapeutic antibodies by the brain is a current challenge that should be addressed. One of the popular strategies is to use antibodies or peptides from the brain receptor, TfR1, to synergistically cross the BBB and improve drug uptake. It is also possible to employ the classical bispecific antibody approach with the TfR1-targeting antibody as one of the specific antibodies. Furthermore, scFv against TfR1 has been utilized, as it has both the selectively targeting ability and greater tissue penetration with faster blood/tissue clearance. In addition, because circulating proteases can induce the proteolysis of targeted peptides, the common drawbacks of targeted peptides are a high clearance and poor pharmacokinetics. Regarding the aptamer, it has the advantages of a low immunogenicity, rapid tissue penetration and cellular internalization, along with stability towards enzymatic degradation and renal filtration effects. Both of these aspects can be achieved by chemical modifications, such as replacing the 2′ position with fluorine (F), amino (NH_2) or *O*-methyl (OCH_3) groups, respectively, and increasing nuclease resistance by capping the 3′ end with reverse thymidine or formulating with bulky fractions, such as polyethylene glycol and cholesterol, which significantly enhances the binding affinity as well.

Ferritin can provide a substantial cavity, which solves both problems to some extent. In addition, recent studies have shown that TfR1-positive cells bind ferritin-based nanomedicine with a threshold effect,[85] indicating superior tumor selectivity *in vivo*. Moreover, Salvati *et al.* found that the protein corona can affect the targeting capabilities of Tf nanoparticles.[160] In addition, the Tf-mediated BBB targeting and transcytosis ability are strongly weakened after coating with protein corona.[161] Theoretically, in addition to Tf conjugated nanoparticles, other conjugated nanoparticles, such as antibodies, peptides and aptamers, would form the protein corona after entering the body. As a result, these protein coronas can affect the binding of TfR1 and the targeting of BBB. Certainly, these need to be studied in detail. In this regard, HFn has the natural advantage of being endogenous, and its targeting will be much less affected by the protein corona *in vivo* than the other carries. Since the first report on human HFn possessing intrinsic tumor targeting ability in 2012, HFn has been widely investigated in the field of targeted therapy functioning as a targeting molecule and delivery platform for diagnostic agents and therapeutic drugs. Meanwhile, to meet the rapidly growing demand for precision medicine, ferritin can be further developed as dynamic nanoassembly-based ferritin drug carriers (DNFDCs). As a multi-module platform, DNFDCs can directly integrate desired functions into the nanocage. Among various modules, the response modules enable DNFDCs to specifically exert therapeutic function upon stimuli for precise therapy.[162] Nevertheless, the limited inner cavity and negative charge make the loading large and negative molecules a challenge. In the current studies, there is a minority of drugs being loaded into HFn successfully, though several methods and hydrophilic/hydrophobic channels have been developed. Therefore, the expansion of the inner space and the change of the charge environment to evaluate loading poten-

tial of ferritin deserve to be explored. Some studies have demonstrated the availability of engineered HFn, having the potential to bring forth the breakthrough of FDCs and therapeutic applications.

Overall, the drug delivery patterns of different nanoparticles significantly increase drug concentration in the brain tissue by employing TfR1 as a brain tumor target. There are various methods for its targeting process. These methods can still be rationally designed and modified to further transport the drug from brain capillary endothelial cells to the brain parenchyma for better tumor treatment. Strategies for TfR1-mediated drug delivery across the BBB to the brain are rapidly evolving. New inventions have inspired further developments. It is hoped that this therapeutic concept will lead to further clinical advances.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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