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Nucleic acid-based therapy for brain cancer: Challenges and strategies

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ABSTRACT

Nucleic acid-based therapy emerges as a powerful weapon for the treatment of tumors thanks to its direct, effective, and lasting therapeutic effect. Encouragingly, continuous nucleic acid-based drugs have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Despite the tremendous progress, there are few nucleic acid-based drugs for brain tumors in clinic. The most challenging problems lie on the instability of nucleic acids, difficulty in traversing the biological barriers, and the off-target effect. Herein, nucleic acid-based therapy for brain tumor is summarized considering three aspects: (i) the therapeutic nucleic acids and their applications in clinical trials; (ii) the various administration routes for nucleic acid delivery and the respective advantages and drawbacks. (iii) the strategies and carriers for improving stability and targeting ability of nucleic acid drugs. This review provides thorough knowledge for the rational design of nucleic acid-based drugs against brain tumor.

1. Introduction

Despite the continuous efforts in developing efficient therapies, brain tumors and other central nervous system (CNS) tumors remain a fatal threat to human health over the decades, with an estimated thirty thousand new cases worldwide in 2020 [1,2]. A general treatment for brain tumor is surgery and postoperative radiotherapy combined with chemotherapy, which typically exhibits severe side effects and poor prognosis. To improve the therapeutic efficiency and reduce the toxicity of chemotherapy, several medicine with different mechanisms have been developed [3]. Among them, nucleic acid drugs, due to the simple structures and direct therapeutic mechanisms, offer a promising option for brain tumor therapy. Since Formivirsen® was approved for clinical use [4], several breakthroughs have been made in tremendous artificially designed DNA or RNA technologies and drugs to achieve gene repair and regulate expression (Fig. 1a). In this review, we mainly focused on antisense oligonucleotides (ASO), small interfering RNA (siRNA), microRNA (miRNA), aptamers, message RNA (mRNA), DNA and gene editing and their applications in the treatment of brain tumors. Although these nucleic acid drugs act in different mechanisms, they share some common advantages, such as designable sequence and structure to function in different conditions and small size providing the potential of penetrating the biological barrier [5,6]. (See Table 1.)

As shown in Fig. 1, there has been a great boom of nucleic acid drugs, however, their applications are still limited by the lack of stability, off-target effects, and poor cellular uptake. In the clinic, common delivery routes can be roughly divided into local and systemic delivery [7]. Local delivery avoids long-term blood circulation and instant elimination caused by the small size of the systems, enabling greater accumulation, and minimizing side effects and overcoming biological barriers. Various strategies have been established to realize local delivery such as intrathecal injection, which delivers a high concentration of nucleic acids into the brain, *via* the spinal canal or into the subarachnoid space. As such, a proper drug dosage and administration site should be carefully

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addressed. Systemic delivery, known as the most used treatment in the clinic, administers drugs through the systemic circulation to finally accumulate at the target site. The challenge remains to endow nucleic acids with adequate stability to keep their biological activity to reach the targeting site and reduce toxic side effects. Considering the biological barriers [8] and nucleases in serum [9], it is even harder to achieve efficient accumulation in brain tumors.

There are many related studies dedicated to overcoming the biological barriers and other obstacles in brain tumor therapy. Thus, we summarize the current progress of nucleic acid-based therapy for brain tumor and provide an overview of clinical drugs currently approved by FDA and EMA. We further highlight different routes of administration and various carriers capable of protecting nucleic acids or targeting to the brain tumor effectively. In the last section, challenges are presented for better clinical applications and future perspectives.

2. The biological barriers

The biological barriers blocking nucleic acid drugs mainly include the blood-brain barrier (BBB) and the blood-brain tumor barrier (BBTB).

2.1. The blood-brain barrier

The BBB is defined by the combination of several histological and physiological properties that turns it into one of the hardest *in vivo* barriers to cross (Fig. 2). The intricated interactions of the neural, endothelial, and immune cells that compose the BBB are essential to regulate the movement of molecules and cells between the blood and the brain, ensuring the homeostasis and protection of the CNS [10,11]. Endothelial cells (ECs) in the BBB are non-fenestrated and interconnected by specialized tight junctions, forming a high transepithelial/transendothelial electrical resistance (TEER), leading to a higher integrity and permeability of the cellular monolayer. Additionally, these brain ECs also have a continuous and dense glycocalyx toward the lumen of blood vessels, which prevents large molecules from interacting directly with them [12]. The angiogenesis and vascular remodeling are regulated by the pericytes of the BBB. Both the ECs and the pericytes are supported by the basement membrane (BM), which is composed by two layers: the inner layer secreted by the EC and the pericytes, and the glial membranes secreted by the end-feet astrocytes that are parts of the BBB. The BM is composed of collagen, fibronectin, proteoglycans, and laminin, which produces the passage of molecules and assists the binding of some migratory cells and molecules [13].

The traffic of molecules through the BBB is further regulated by specific enzymes, receptors, and transporters. The luminal membrane of EC is highly enriched in efflux ATP-dependent transporters, which moves small molecules to the back to the blood stream against their concentration gradient. These efflux proteins, such as the P-glycoprotein (P-gp), are frequently associated with mechanisms of drug resistance. On the other hand, the movement of solutes down the concentration gradient is mediated by solute transporters, such as glucose, amino acids, and fatty acids transporters. Several receptors as the transferrin receptor (TfR) [14] or the insulin-like growth factor receptor (IGFR) [15] are involved in receptor-mediated vesicular transport through the BBB. In some regions, such as the pineal gland, or the median eminence of the hypothalamus, the capillaries are fenestrated and allow direct communication between the blood and the brain. Small (<500 Da) or lipid-soluble molecules can passively enter the CNS and they are then moved back to the blood by efflux transporters. However, polar or larger molecules movement into the brain is still highly restricted unless mediated by specific transporters [16].

2.2. The blood-brain tumor barrier

Besides the BBB, there is an additional BBTB, whose vascularity can vary with the tumor. In low-grade brain tumors, for instance, the BBTB is usually continuous with non-fenestrated capillaries that resemble normal brain capillaries [3]. However, high-grade brain tumors are generally associated with disruption of the normal vasculature. The abnormal vasculature of the BBTB is caused by the increased expression of the vascular endothelial growth factor (VEGF) and triggered by the high metabolic demands of the tumor. Higher grade gliomas may have continuous fenestrated capillaries or even capillaries with interendothelial gaps [3,17]. Moreover, brain tumor capillaries can express drug efflux transporters, as those found in the BBB, while some tumors express ATP-binding cassette (ABC) transporters. Altogether, these transporters constitute an extra obstacle to drug delivery and can compromise the treatments [18]. On the other hand, the endothelial cells of the capillaries of the brain tumor can also overexpress receptors, such as those of the BBB, that can also be used to increase the delivery

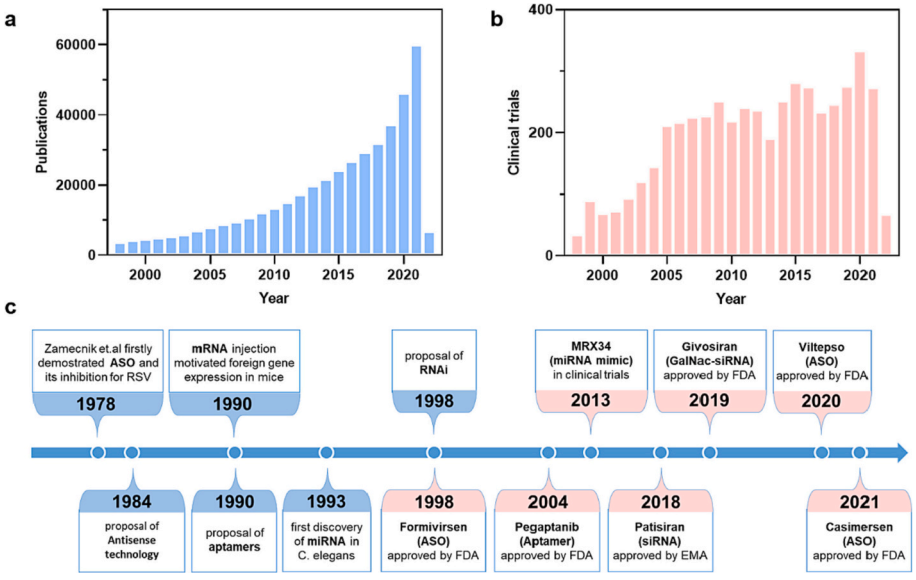

Fig. 1. A summary of nucleic acid-based therapy. (a) Nucleic acid-based therapy related publications. Data based on Scopus. (b) Nucleic acid-related clinical trials. Data based on Clinicaltrials.gov. (c) Timeline of breakthroughs in the nucleic acid field and several drugs approved by the Food and Drug Administration (FDA) or the European Medicines Agency (EMA).

and enable targeted treatments [14,15].

3. Current progress of nucleic acid-based therapy for brain tumor

3.1. Nucleic acid-based therapy

3.1.1. Antisense oligonucleotides

Antisense technology has been developed to inhibit the gene expression by using short artificial DNA or RNA strands known as ASOs. Since the first ASO has been explored to inhibit viral replication *in vitro* [19], increasing studies have been conducted to study the functional mechanism and therapeutic potential. ASOs modulate gene expression through different molecular mechanisms [20]. On the one hand, ASO binds to mRNA in an occupancy-only mechanism to modulate protein translation without any degradation, such as Eteplirsen and Nusinersen approved by the FDA [21,22]. On the other hand, it can be designed to cause mRNA cleavage mediated by RNase-H1. For glioblastoma multiforme (GBM) therapy, Teplyuk et al. used anti-miR-10b ASOs to inhibit the expression of miR-10b that plays an important role in tumor growth and metastasis. ASOs combined with 5′-untranslated regions of miR-10b and caused changes of cell cycle and relative mRNA network. In a series of *in vivo* experiments, it has shown a down-regulation of multiple splicing factors and components of spliceosome, which contributes to gene suppression and significant inhibition of GBM. Additionally, different routes of administration including intratumoral injection, osmotic delivery and intravenous injection were performed. Despite the evident therapeutic effect in all conditions, only a small portion of anti-miR-10b ASOs was delivered to GBM [23]. Recent researches have focused on chemical modification such as phosphorothioate (PS) modification of the phosphodiester backbone to enhance tumor uptake, which enabled improved base-pairing affinity and specificity as well as increased resistance to nucleases [24].

3.1.2. siRNA

In 1998, Andrew Fire and Craig Mello first revealed and named the RNA interference (RNAi) phenomenon in *Caenorhabditis elegans* (*C. elegans*) [25]. After the small interfering RNA (siRNA) binding to RNA-induced silencing complex (RISC), the active complex promotes the degradation of mRNA and achieves inhibition of translation and regulation of gene expression. Thus, exogenous siRNA can motivate RNAi pathway to realize gene therapy, such as Patisiran approved by the EMA [26]. There have been continuous studies on siRNA drugs for brain tumor therapy. A study showed that the expression of HER2 in glioblastoma cells treated with HER2-siRNA had an obvious reduction by 40%–65%, resulting in increased radiosensitivity and inhibition of tumor migration [27]. However, there is no supporting *in vivo* evidence to further assess the immunogenicity, stability, and therapeutic effect which are usually obstacles in the applications of siRNA. Chemical modifications have been employed for better stability and lower off-target effect. Bohong Cen et al. prepared a methoxy-modified siRNA that selectively inhibiting phosphatidylinositol-4,5-biphosphate 3-kinase catalytic subunit β (PIK3CB). A remarkable suppression of GBM cell growth with less cytotoxicity to astrocytes has been reported *in vivo* and *in vitro* [28]. Brain penetrant RNAi-based spherical nucleic acids have shown promising results in recurrent GBM patients, in a phase 0 first-in-human trial. These results provide evidence that systemic administration of the nanoconjugates leads to uptake by tumor cells, as well as endothelial and immune cells, with favorable safety profiles and correlation with decreased target protein expression in GBM patients [29].

3.1.3. miRNA

The first miRNA was found in *C. elegans*, which plays an important role in multiple paths of post-transcriptional regulation [30–32]. After that, the successful expression of exogenous proteins under miRNA mimic injection demonstrated that miRNA mimics represent a promising therapeutic strategy [33]. Ondrej Slaby's group applied miR-338-5p mimics in potentiating radiotherapy of GBM therapy, which participated in DNA damage response, proliferation and cell cycle regulation [34]. Similarly, miR-26b mimics could reduce the expression of matrix metalloproteinase-2 (MMP-2), MMP-9 and cyclooxygenase-2 (COX-2), inhibiting the glioma cells growth and proliferation [35]. The effect caused by exogenous miRNA may be mild, requiring a higher therapeutic dosage and thus a severe off-target effect. Combination of related miRNAs provides more efficient ways to target tumor cells. Adam Kosti et al. used miR-124, miR-128 and miR-137 combination to disrupt cancer phenotypes and glioma stem cell growth. Transcriptomic analyses indicated that biological processes affected by transfection of this miRNA combination were more significant and the miRNAs on shared and related targets produced stronger regulatory effects. The prominent therapeutic effects is mainly due to the strong inhibition of common targets, increased effects on neuronal differentiation, and the broad and powerful effect on oncogenic pathways [36]. Therefore, the synergistic methods work with lower side-effect as well as a better therapeutic outcome and indicate a promising kind of nucleic acid drugs for future tumor therapy.

3.1.4. Aptamers

Aptamers are single-strand DNA or RNA oligonucleotides completely designed and synthesized artificially with a special three-dimensional structure and capacity of identifying molecules using systematic evolution of ligands by exponential enrichment (SELEX) technology [37,38]. Unlike other nucleic acid-based therapy, aptamer-based drugs target not

Table 1
Summary of nucleic acid-based therapy.

Type	Definition	Mechanism	Reference
Antisense oligonucleotides (ASOs)	Short single-stranded nucleic acids (~5–40 base pairs)	Binding to target mRNA in an occupancy-only mechanism or cause cleavage	[20]
Small interfering RNAs (siRNAs)	Short double-stranded nucleotides in RNAi pathways	Gene silencing	[25]
microRNAs (miRNAs)	Small single-stranded non-coding RNA molecules	Modulating multiple pathways in combination with mRNA	[30]
Aptamers	Single-stranded oligonucleotides screened through systematic evolution of ligands by exponential enrichment (SELEX)	Targeting specific molecules, including proteins, peptides by 3D structure	[37,38]
Message RNA (mRNA)	Small single-stranded RNA guiding protein synthesis	Being translated into exogenous proteins or acting as vaccine	[45,47]
DNA	Plasmid DNA or small DNA molecules	Expressing exogenous genes or initiating biological process	[49,50]
Gene editing system	Site directed modification of the genome	Cause DNA cleavage and initiate DNA repair mechanism based on endonuclease	[52]
Peptide nucleic acids (PNA)	synthetic analogs of DNA/RNA with 2-([2-aminoethyl] amino) acetic acid backbone	Bind to RNA/DNA complementarily to inhibit translation and transcription	[60]

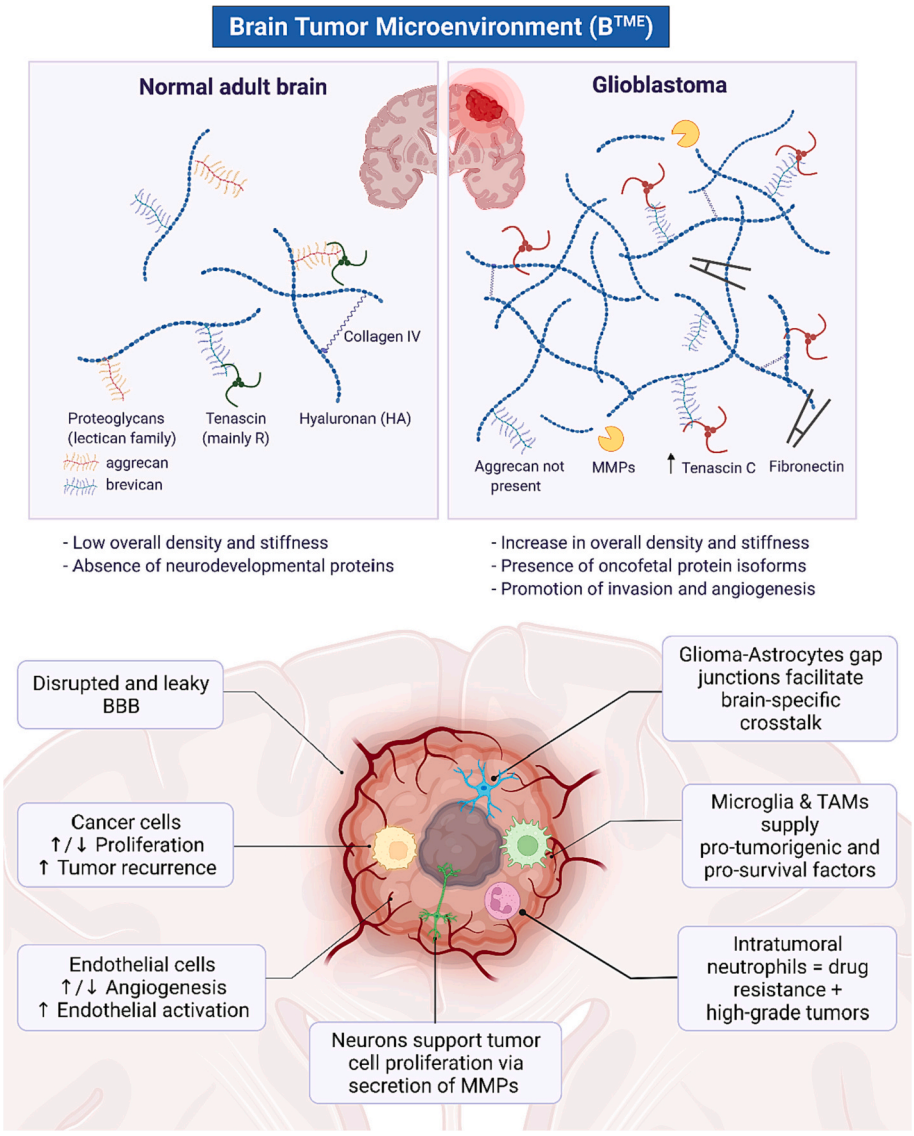

Fig. 2. Biology of brain tumor microenvironment. Histological and physiological differences in the properties of brain tumor microenvironment between normal adult brain and glioblastoma.

only nucleic acids but proteins due to the characteristic structure. Ye Cheng et al. developed a targeting aptamer named AS1411 and investigated the mechanisms by which AS1411 kills glioma cells. It has been confirmed that AS1411 bound nucleolin (NCL) that was overexpressed in glioma cells, inducing up-regulation of p53 and down-regulation of Bcl-2 [39]. The subsequent cell apoptosis and cycle arrest were detected, revealing a promising treatment and specific identification of glioma cells. Similarly, other RNA aptamers with high affinity like PDR3 have also been developed [40]. Thanks to their special structures, aptamers are mainly used as targeting ligands. An AS1411 aptamer/hyaluronic acid-bifunctionalized microemulsion was developed to penetrate the BBB, target to glioma and deliver shikonin and docetaxel [41]. This platform selectively accumulated in U87 glioma cells and inhibited tumor growth and the formation of cancer stem cells. Similarly, AS1411-modified platform was widely used in the treatment and imaging of glioma [42–44].

3.1.5. *mRNA*

As an important translation regulator, mRNA plays a vital role in protein synthesis. However, tumor is typically characterized by genetic mutations and abnormal protein expression. Synthetic mRNA can be translated into therapeutic proteins in the cytoplasm and has no influences on genome, providing a promising method for tumor therapy. Hao Peng et al. delivered mRNA of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by intracranial injection to treat glioma. With TransIT-mRNA reagent-assisted synthetic mRNA delivery, caspase signal pathway has been activated and glioma growth was inhibited [45]. In another research, Zhaogang Yang et al. prepared mRNA-encapsulated exosomes from the cells with a focal and transient electrical stimulus. Exosomes obtained in this way displayed targeting peptide on the surface and thus endowed mRNA with enhanced stability and targeting ability. Suppression of tumor and increased survival were detected in orthotopic phosphatase and tensin homologue (PTEN)-deficient glioma mouse [46]. Owing to the immunogenicity, exogenous mRNA is widely used in immunotherapy. There are multiple mRNA vaccines approved by FDA under an Emergency Use Authorization during the pandemic of SARS-CoV-2, such as mRNA-1273 and BNT162b2. Some mRNA-pulsed dendritic cell (DC) vaccines or mRNA-nanoparticle vaccines have been explored for glioma therapy [47,48], while the development of tumor vaccines is still challenging.

3.1.6. DNA

DNA therapy refers to deliver specific DNA strands or plasmid DNA to function in the nucleus, being a promising candidate in the treatment of malignant tumor. Devika's group investigated the transfection efficiency of plasmid DNA with gene segment encoding the interferon-beta1 (IFN-β1). Encapsulated in 2-Methacryloxyethyl phosphorylcholine (MPC)-based copolymers, this complex induced a significant apoptosis of U-87MG cells and inhibited tumor growth and migration without any non-specific toxicity [49]. Some small DNA molecules like Dbait have been designed to activate H2AX phosphorylation, thus preventing DNA repair and making cells being sensitized to irradiation, which has been demonstrated in U251 cells [50]. Immunogenicity is a hurdle in the application of nucleic acid-based drugs, leading to a severe side effect, but is also a chance to prepare vaccine. Tyrosinase-related protein-2 (TRP2)-encoded DNA vaccine has been designed to combine with glioblastoma-targeted chemotherapy, showing a markedly enhanced overall survivability of orthotopic glioblastoma-bearing mice [51]. Nevertheless, the side effects caused by genetic changes limit the application of DNA therapy.

3.1.7. Gene editing system

Since the discovery of CRISPR/Cas technology, various methods of gene editing have been established to treat diseases [52,53]. CRISPR means clustered regularly interspaced short palindromic repeats, with the ability of binding to DNA specifically. CRISPR associated proteins (Cas) are responsible for DNA cleavage and the formation of double strain breaks (DSBs), initiating mechanism of DSB repair and realizing gene editing. Compared to other genome-editing tools such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR/Cas system exhibits higher accuracy, lower off-target effect, lower cytotoxicity and flexible designability. Among the several types, CRISPR/Cas9 represents a most common and simple system, which relies on CRISPR RNA (crRNA) to target and trans-activating crRNA (tracrRNA) to form complex with Cas9. This system was used in chimeric antigen-receptor T (CAR-T) cells and enabled improving anti-cancer effects without any adverse reaction [54]. Other types of gene editing system have been applied in tumor-bearing mice, for example, CRISPR/Cas12a knocked out genes encoding miR-21 [55] and CRISPR/Cas13a silenced target genes and caused collateral damage effect [56]. Even so, there is still a long way for gene editing to be used in human.

3.1.8. Peptide nucleic acids

Peptide nucleic acids (PNA) are synthetic analogs of DNA/RNA with 2-([2-aminoethyl] amino) acetic acid backbone. They can bind to DNA/RNA complementarily, allowing for antigene and antisense properties by inhibition of translation and transcription. PNA have been used in diagnosis and treatment of several diseases, such as cancer, and viral infections. Alessandro Bertucci et al. modified polyarginine-PNA conjugate targeting miR221 on the surface of mesoporous silica nanoparticles while keeping the anti-miR activity of PNA. Previous studies have shown that down-regulation of miR221 sensitize glioma cells temozolomide (TMZ). In this work, comparative analysis showed that the combination of TMZ and PNA nanosystems resulted in greater sensitivity of glioma cells to TMZ and enhanced cell apoptosis [57]. This kind of artificial analogs of DNA/RNA exhibits superior stability and biosafety, but there is still some concern about poor cellular uptake induced by PNA. The pharmacokinetic limitations of PNA can benefit from drug delivery strategies, and PNA may constitute a potential tool for modulating brain tumors [58–60].

3.2. Nucleic acid drugs approved by the FDA/EMA and in clinical trials

Over the past few decades, nucleic acid drugs have flourished and are drawing more attention. Drugs approved by the FDA or EMA are listed in Table 2. The ASO drugs are the majority, mostly due to their superior biosafety and easy design. Most of them aim at the treatment of virus infection and metabolic disorder. Tumor treatment options, however, have been less developed, especially for brain tumors. Several aspects account for this obstacle. First, there are no ideal targets for nucleic acids to achieve an effective therapy. Because of the variation between human genes and mutation sites, it is hard to develop a universal therapeutic platform for a single kind of nucleic acids. In addition, bare nucleic acids have severe immunogenicity and low stability properties in the presence

Table 2
Summary of nucleic acid-based drugs approved by the FDA/EMA and in clinical trials.

Name	Type	Route of administration	Conditions	Target	Phase	Status	NCT number
Fomivirsen (Vitravene)	ASO	IVI	CMV; Retinitis; HIV Infections	CMV UL123	NA	Completed	NCT00002187
					II	Completed	NCT00002356
					NA	Completed	NCT00002355
Mipomersen (Kynamro)	ASO	SC	AS	Apo B	II	Completed	NCT00002156
					III	Completed	NCT01598948
Eteplirsen (Exondys 51)	ASO	IV	DMD	DMD exon 51	III	Completed	NCT02255552
					II	Completed	NCT02420379
					II	Completed	NCT03218995
Nusinersen (Spinraza)	ASO	IT	SMA	SMN2 exon 7	II	Completed	NCT02286947
					III	Completed	NCT02292537
					II	Completed	NCT01839656
					I	Completed	NCT01494701
Inotersen (Tegsedi)	ASO	SC	ATTR	TTR		Approved for marketing	NCT03400098
Volanesorsen (Waylivra)	ASO	SC	FCS; HLP Type 1	Apo C	III	Completed	NCT02658175
Golodirsen (Vyondy 53)	ASO	IV	DMD	DMD exon 53	III	Enrolling by invitation	NCT03532542
Viltolarsen (Viltepso)	ASO	IV	DMD	–	–	Approved for marketing	NCT04337112
Casimersen (AMONDYS 45)	ASO	IV	DMD	DMD exon 45	III	Enrolling by invitation	NCT03532542
					II	Active, not recruiting	NCT04179409
Patisiran (Onpattro)	siRNA	IV	ATTR	TTR	–	Approved for marketing	NCT02939820
Givosiran (Givlaari)	siRNA	SC	AHP	ALAS1	–	Approved for marketing	NCT04056481
Lumasiran (OXLUMO)	siRNA	SC	EHC	HAO1	–	Approved for marketing	NCT04125472
Leqvio (Inclisiran)	siRNA	SC	EHC	PCSK9	–	Not yet recruiting	NCT05118230
Pegaptanib (Macugen)	Aptamer	IVI	DME; DR	VEGF-165	III	Completed	NCT01189461

Abbreviations: IVI, intravitreal injection; IV, intravenous injection; IT, intrathecal injection; SC, subcutaneous injection; NA, not applicable; CMV, Cytomegalovirus; AS, Atherosclerosis; Apo, apolipoprotein; DMD, Duchenne Muscular Dystrophy; SMA, Spinal Muscular Atrophy; SMN, survival motor neuron protein; FCS, Familial hyperchylomicronemia; HLP, Hyperlipoproteinemia; TTR, Transthyretin; ATTR, Hereditary transthyretin amyloidosis; AHP, Acute Hepatic Porphyria; ALAS1, aminolevulinate synthase 1; HAO1, Hydroxyacid oxidase 1; EHC, Primary Hyperoxaluria; PCSK9, Proprotein Convertase Subtilisin/Kexin Type 9; DME, Diabetic Macular Edema; DR, Diabetic Retinopathy.

of nucleases and low accumulation at the tumor site due to the biological barrier such as BBB/BBTB. Therefore, the development of carriers is crucial to the progression in drugs for brain tumor therapy. Currently, more studies have been conducted in this field and we believe these nucleic acid-based drugs may be utilized from bench to bedside soon.

4. Delivery of nucleic acids for brain tumors

Because of the existence of RNase and nuclease in the serum, it is usually difficult for exogenous nucleic acids to stay active, which leads to unsatisfactory therapeutic effect, a short half-life, and large dosage of medication. Therefore, appropriate administration routes and carriers need to be selected to achieve safe and efficient brain delivery of nucleic acids and a long period of blood circulation. Here, we summarize the advantages and disadvantages of different routes of administration in Table 3 and list some scientific research progress or drugs under clinical trials to elaborate.

4.1. Local delivery

Both the BBB and BBTB pose great challenges for the direct targeting of brain tumor tissue. Local delivery enables the bypassing of the barriers completely, allowing the high concentration aggregation of drugs at the tumor site in the brain. Additionally, local delivery methods avoid the long systemic blood circulation, reduce the degradation of nucleic acid drugs, and off-target effects.

4.1.1. Intrathecal injection

Intrathecal injection is the main clinical strategy used for local delivery to the brain, which inject contents directly into the subarachnoid cavity through lumbar puncture. The drugs will then diffuse in the CSF and reach an effective blood concentration quickly [72]. The efficiency of intrathecal injection to the brain has been proven by Seija Lehnardt et al., *via* the administration of miRNA let-7 to CSF. It was observed that Toll-like receptor 7 (TLR7) was activated to induce neurodegeneration, while mice lacking TLR7 were resistant to this effect. This strategy allows lower effective dose, greatly prolonging the half-life *in vivo* [61].

4.1.2. Nose-to-brain delivery

Nose-to-brain delivery methods bypass the BBB due to the constant replacement of the olfactory receptor neurons, which leads to an area of communication between structures. Several materials have been developed with the purpose of taking advantage of this characteristic to increase transportation of drugs to the tumor tissue [62–64,73]. One of such materials is chitosan nanoparticles loaded with siRNA targeting Galectin-1 (Gal-1) as shown by Van Woensel et al. The aim of these was to reduce Gal-1 in the tumor microenvironment. In this study it was found that the intranasal delivery led to a great alteration in the tumor tissue, having changed the Immune and inflammatory characteristics of the microenvironment, as well as leading to the normalization of the tumor vasculature [62,74].

In addition, for this purpose, some plant-derived strategies have been proposed and are being developed. For example, Zhuang et al. designed grapefruit-derived nanovectors (GNVs) and GNV-coated polyethylenimine (pGNVs) to deliver miR17 to brain tumor tissue in mice. Folic acid (FA) was also modified to enhance the targeting ability. The authors found that mice treated with FA-pGNV/miR17 showed delayed tumor growth [75]. This not only represents a significant step in this type of delivery method's progression but demonstrates the potential behind natural products being used for this type of application. Furthermore, plant-derived miRNAs have recently shown potential as treatment strategies for several conditions [76] including, for example, colon and breast cancer [77,78]. This opens the door for the use of these strategies in cancer treatment and the possibility for their use in brain cancer.

4.1.3. Intramuscular injection

Intramuscular injection methods have been widely used for the administration of nucleic acids, specifically with the development of the mRNA vaccines for SARS-CoV-2 [65]. However, in a study by Ma et al., it has also shown promising results in what concerns to the treatment of glioblastoma. A single administration of an adeno-associated viral (AAV) vector expressing angiostatin, effectively suppressed the growth of human glioma in the brain of nude mice. Additionally, 40% of the animals treated with this vector survived the entirety of the experimental duration, in opposition to 100% of the tumor-bearing mice in the control group that died before the end of the experimental period. Furthermore, the high levels of angiostatin, resulting from the injection of the vector, were still detectable >250 days after a single injection. Thus, the method presented in this study arises as a promising gene therapy option with efficient and sustained product delivery, leading to a suppression of glioma growth in the brain of the mice [66].

4.1.4. Intratumoral implantation

This type of drug delivery methods guarantees the bypassing of the BBB as well as high concentrations of drug at the target site (Fig. 3). Furthermore, it can be performed at the same time as the resection surgery, allowing for immediate initiation of the treatment. These methods present a set of problems such as the difficulty in drug replenishing and the necessity for invasive implantation procedures which could damage the surrounding brain structures. The penetration into the tumor tissues and the development of drug resistance mechanisms also presents possible complications [67]. Carmustine wafers, also known as Gliadel®, are currently the only implantable devices that have been approved by the FDA for local treatment of brain tumors. These wafers are implanted following tumor resection surgery and consist of

Table 3
Summary of local delivery and systemic delivery.

Routes of administration		Pros	Cons	Examples
Local delivery	Intrathecal injection	Avoid the obstacle of BBB; Avoid the degradation caused by nuclease	Slow distribution in the cerebrospinal fluid (CSF); Treat with higher trauma and agony	[61]
	Nose-to-Brain delivery	Non-invasive and efficient	Limited drug weight and surface properties; Mucociliary clearance and unclear mechanism	[62–64]
	Intramuscular injection	Faster efficacy	Muscle pain and spasm	[65,66]
	Intratumoral implantantion	Bypassing the BBB; High concentrations of drug at the target site; Implantation at the same time as the resection surgery	Difficulty in drug replenishing; Invasive implantation procedures; Risk of damage to brain structures; Penetration into the tumor tissues and possible development of drug resistance	[67]
Systemic delivery	Oral delivery	Ease for multiple administrations; Convenience for the patient	Need to overcome the enzymatic barrier of nucleases in the intestine, the mucus barrier and the plasma membrane; Need to overcome the BBB	[68,69]
	Intravenous injection	Long blood circulation; Least invasive	Easy to degradation caused by nuclease; Hard to cross the BBB	[70]
	Subcutaneous injection	Faster efficacy	Limited dosage; Repeated administration	[71]

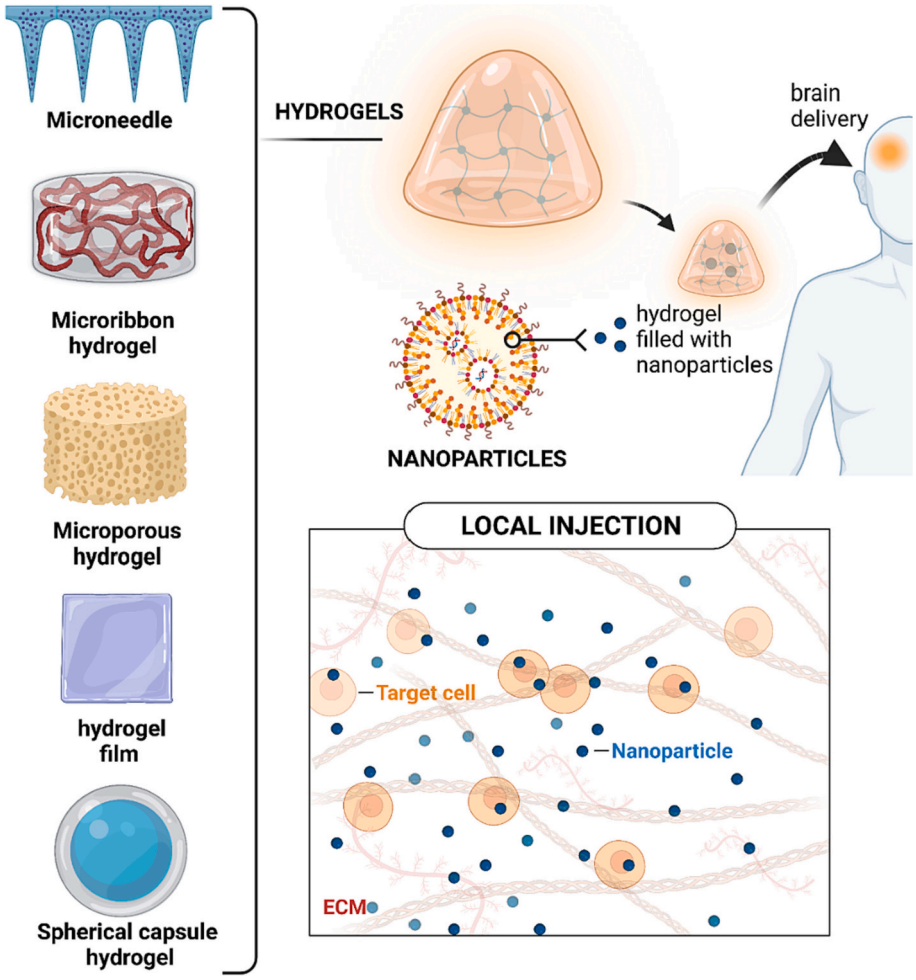

Fig. 3. Localized drug delivery approach. Intratumoral implantation of functional hydrogel-based systems loaded with nanoparticles for localized delivery in brain tumor.

an alkylating agent that leads to DNA and RNA crosslinking in dividing cells and binds and modifies glutathione reductase [79,80].

Several other methods have been in development for the use in intratumoral drug delivery consisting of microreservoirs [81], drug-eluting beads [82], microelectromechanical systems [83], titanium wires [84], convection-enhanced delivery (CED) [85], hydrogels [86], nanofiber discs [87] and several types of nanoparticles [67,80]. Guerrero-Cázares et al. developed nanoparticles of poly(β-amino ester)s (PBAEs) which are biodegradable and loaded with plasmid DNA. Particles were injected in GBM mouse models, transfecting tumor cells selectively. Furthermore, these systems showed a diffusion comparable to that of other nanosystems. Thus, PBAE nanoparticles emerge as a promising system for local and targeted delivery of nucleic acids to brain tumors [88]. Shatsberg et al. used polymeric nanogels for intratumor delivery of miR-34a, known for tumor suppressing activity in GBM. These nanogels led to a great level of down regulation of the target genes. Furthermore, it significantly inhibited tumor growth in human U-87 MG GBM-bearing SCID mice [89].

4.1.5. *Alternative routes to overcome the BBB*

Additionally, in response to the difficulty in the delivery of materials through the BBB, some methods of BBB disruption have been developed. Such methods include the use of ultrasounds for the creation of fenestrations in the BBB. Yutong Guo et al. combined microbubble-enhanced focused ultrasound (MB-FUS) and cationic lipid-polymer hybrid nanoparticles (LPHs:siRNA) to overcome the BBB. Data showed an improvement of >10-fold of siRNA delivery into the brain tumor microenvironment [90]. This disruption of the BBB's structure facilitates the targeting of the tumor tissue by different therapeutic options, particularly for those rely on systemic delivery [67,91]. Notably, this method must be finely controlled to minimize the long-term damage on the BBB.

4.2. *Systemic delivery*

Though multiple routes of administration have been discovered to facilitate the therapeutic efficiency of nucleic-acid drugs, the systemic administration has been the most widely used in the clinic. Different from local delivery, systemic delivery allows drugs to pass through all organs, thus ensuring their arrival at tumor lesions. Systemic delivery usually includes oral delivery, intravenous injection, subcutaneous injection, *etc.*, which requires a stable formation of nucleic acid drugs (See Fig. 4.). Oral delivery methods are vastly used in the administration of compounds. In the specific case of cancer treatments, these are used to administer chemotherapeutic agents, for example [68]. However, the use of this type of method to deliver nucleic acids presents a set of challenges such as the need to overcome the enzymatic barrier of nucleases, the mucus barrier, and the plasma membrane of the intestine. These make oral availability of nucleic acids very low and gives rise to the need for means of protection [69,92]. An example of such protective measures is the use of nanoparticle encapsulation. More specifically, chitosan nanoparticles have arisen as promising vectors for oral delivery due to their stability, ability to encapsulate nucleic acids, functionalization possibility and their ability to cross the intestinal wall [92,93].

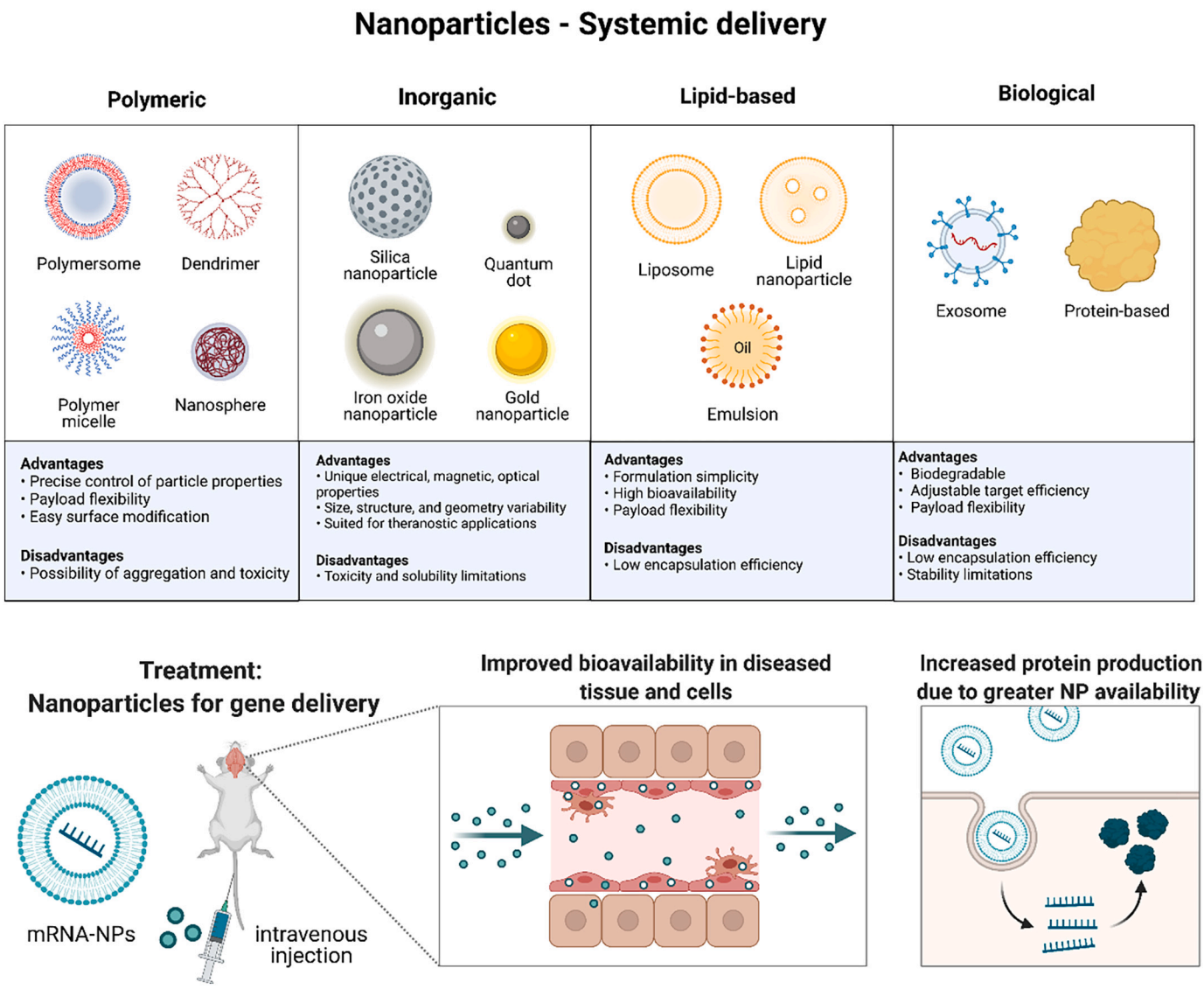

Fig. 4. Hybrid nanomaterials in systemic delivery of nucleic acid. Examples of designed nanocarrier systems (polymeric, inorganic, lipid-based or biological) for systemic delivery of nucleic acid with their advantages and disadvantages.

Moreover, these have been extensively explored for the treatment of brain tumors as described elsewhere in this paper. In addition, a recent study showed that acerola exosome-like nanovesicles (AELNs) delivered nucleic acids systemically through oral administration [94]. Results showed the presence of signal in the brain, following oral administration of an AELN/miRNA mixture in mice. Once more, this shows that natural products are promising candidates in the development of tumor treatment strategies.

Intravenous injection is the most efficient, simple, and common route of systemic administration. Injected intravenously, drugs can flow with blood throughout the body and reach the tumor site. However, when naked nucleic acids are exposed to nuclease in the serum, they are rapidly degraded and inactivated. On the other hand, without any modification, only a little amount will accumulate passively through the enhanced permeability and retention (EPR) effect [70], while the rest will be directly excreted by the kidney or affect normal cells, known as the off-target effect. Therefore, the systemic route requires more strict targeting ability. For brain tumors, the additional challenge is how to penetrate the BBB. Several carriers and ligands or peptides have been designed to deliver the therapeutic nucleic acids and target to the brain.

4.3. *Vehicles for systemic delivery*

An ideal carrier for nucleic acid delivery to brain should possess special characteristics such as biocompatibility, stability, and efficient cellular uptake. The diffusion across the BBB mainly depends on the lipophilicity, surface charge, relative molecular mass, and the concentration gradient of the drug on both sides of the BBB. The various carriers commonly used to deliver nucleic acid drugs through different mechanisms of traversing the BBB and the recent advances in the field are further explored below.

4.3.1. *Viral carriers*

It is now believed that virus can traverse the BBB through different mechanisms, such as direct cytokinesis, Trojan horse and disruption of tight junctions. As a result, several methods have been developed to deliver therapeutic nucleic acids based on viral particles [66,95–97]. Adeno-associated viral (AAV), which may transduce neurons, astrocytes, oligodendrocytes, and endothelial cells through direct transcytosis, have been established for *in vivo* gene therapy in clinic. In 2012, Glybera®, the first gene therapy product based on the AAV method, was approved by EMA, demonstrating the ideal safety and great application potential of AAV vectors. In 2017, Yescarta® [98], retroviral vector-

based drug for large B cell lymphoma, was approved by the FDA. The neurotropic JC polyomavirus (JCPyV) may be effective to traverse the BBB through trafficking into immune cells. Recently, JCPyV virus-like particles (VLPs) has been developed to deliver suicide genes that can convert non-toxic substrate into lethal compounds. Data showed a prominent inhibition of brain tumor growth and significantly prolonged survival of mice [99]. Oncolytic virotherapy is designed to selectively infect tumor cells and exhibit antitumor effects on genetically mediated cytotoxic immunotherapies while retaining the surrounding normal organs [100]. Their replication ability overcomes the low transduction efficiency of non-replicating virus; however, this approach remains difficult to apply due to the immune responses and potential danger.

4.3.2. Polymeric carriers

Polymeric nanocarriers are spherical structures synthesized by natural or synthetic materials, including polymersomes, dendrimers, micelles and polymeric nanospheres [101–103], which emerge as the promising carriers with precisely controlled characteristics and easy surface modification. By improving the pharmacokinetic properties, polymeric carriers can prolong the duration of nucleic acid drugs in the circulation, alter the distribution *in vivo*, increase their feasibility of crossing the BBB, and achieve delayed drug release. Branched polyethylenimine (PEI) of high molecular weight presents satisfying complexation efficacy with nucleic acids through the high density of cationic charge but higher toxicity, while liner PEI of small molecular weight has better biosafety. Therefore, Michael et al. generated a series of liner PEIs and modified with tyrosine modifications which could improve the complexation efficacy of siRNAs and transfection efficacy in cancer cells. It has been confirmed that the tyrosine-modified PEIs/siRNA complex possesses a higher capacity for siRNA delivery and improved gene knockdown effect [104]. Similarly, chitosan (CS) is a liner polymer with good biodegradability and special physical characteristics that CS only dissolves in acidic environment and is insoluble at normal pH value [105]. Stimuli-responsive chitosan has been designed based on the characteristics and methods such as alkylation, acylation, amidation and other modifications have been employed to improve the solubility in normal cellular conditions [105–107]. Polyamidoamine (PAMAM) is a cationic polymeric dendrimer with three-dimension structure and abundant peripheral groups, leading to a high cargo payload and on-demand tuning property. Recently, Aleksandra's group designed an amphiphilic dendrimer (AD) composed of two hydrophobic chains and one hydrophilic PAMAM dendron for nucleic acid delivery to microglia. Data showed stable nanoparticles of AD and siRNA have been formed to prevent from degradation and mediate cellular uptake by microglia, inducing effective gene silence and corresponding biological effect. Notably, AD nanocarrier mediated siRNA delivery didn't cause inflammatory reaction and unexpected off-target effect [108].

4.3.3. Lipid-based carriers

Similar lipid composition confers the ability of liposome and other lipid nanoparticles (LNPs) to cross the BBB, thus attracting a wide attention on their biomedical applications. Numerous studies have attempted to apply lipid-based carriers to load nucleic acids [109–111], with the cationic liposomes being the most common carriers. Xiyang Sun et al. loaded paclitaxel (PTX) and siRNA of survivin, an inhibitor of apoptosis highly expressed in GBM, into cationic liposome to realize chemo-gene therapy. QPCR analysis showed a significant down-regulation of survivin mRNA levels in U251 cells treated with liposome-encapsulated siRNA compared to that with survivin siRNA alone. To overcome the off-target effect of nucleic acid drugs in systemic delivery, the modification of target ligand is a common method. Thus, A15 and angiopep-2 were linked on the liposome (DP-CLPs-PTX-survivin siRNA) to track $CD133^+$ cancer cells and target low-density lipoprotein receptor-related protein (LRP), respectively. Finally, DP-CLPs-PTX-survivin siRNA has been proved efficient to induce apoptosis inside the glioma, especially $CD133^+$ glioma cells [112]. Another method to achieve targeting and reduce toxicity is the utility of tumor microenvironment such as pH [113,114], redox condition [115–117], oxygen condition and others. Hongmei Liu's group designed an ionizable liposome with enhanced endocytosis under hypoxic condition and at low pH values. In tumor environment, there would grow tertiary amines and aminoimidazole with high cationic charge on liposomes, which enhances the uptake by tumor cells. While in normal organs, the low density of positive charges mediates an inefficient endocytosis. It has been confirmed that ionizable liposome injected from the tail vein can selectively deliver PLK1 siRNA to brain tumors and hence inhibit tumor growth [118].

4.3.4. Inorganic carriers

The specific size and relative molecular weight enable nanoparticles at nanoscale to diffuse across the BBB, resulting in a large number of studies based on inorganic nanoparticles. With the continuous development of nanotechnology, inorganic nanomaterials have been designed with unique physicochemical properties and variable size and structure, providing attractive opportunities for nucleic acid delivery. Porous silicon nanoparticles (pSiNPs) possess high porosity and specific surface area, along with high cargo load efficiency and quick degradation into non-toxic products. Therefore, pSiNPs can be prepared to deliver nucleic acids and coated with PEI, CS or other polyplex to realize controlled release [119,120]. Carbon-based nanoparticles in different morphology are also employed due to easy preparation and prominent biosafety, like cationic carbon dots [121] and carbon nanotubes [122]. For better stability and bioavailability, they always need to be integrated with functional molecules to deliver nucleic acids systemically and target to brain tumor selectively. Metal-based nanoparticles have drawn extensive attention due to their multifunction, such as gold nanoparticle with light absorbance bands in the near-infrared region and capacity of photothermal conversion. Lester et al. anchored one strand of dsDNA on the spherical gold nanoparticle and facilitated ssDNA delivery through photothermal effect. With the irradiation of continuous-wave lasers, the local temperature would be raised above the melting temperature (T_m) of the DNA duplex, realizing controlled release of therapeutic nucleic acids [123]. The structure of gold nanoparticles decorated with high density of nucleic acid is known as spherical nucleic acid (SNA). Though with the advantages of multifunction and efficient delivery, it is still essential to monitor the long-term toxicity due to their accumulation in normal tissue. Wrapping in polyethylene glycol (PEG) is a general and useful strategy to avoid this obstacle [124], blocking the biomolecules away from nanoparticles and improving the biosafety.

4.3.5. Biological carriers

The utility of biomaterials as carriers to deliver nucleic acids emerges as a strategy with excellent biosafety. More importantly, most biological carriers can penetrate the BBB through receptor-mediated transcytosis or adsorption-mediated transcytosis, which are strong and effective pathways. Exosomes function as natural carriers in organisms, responsible for the transport of biomolecules between cells and thus applying for the therapeutic nucleic acid delivery [125]. Gyeungyun et al. obtained a glioblastoma-targeting exosome (T7-exo) by preparing plasmids encoding the Lamp2b and T7 peptide and then transfecting into 293 T cells. T7 is a peptide binding to transferrin receptor (TfR) which is highly expressed on endothelial cells of cerebral capillaries and glioblastoma cells, leading to a successful BBB penetration and tumor targeting. With electroporation of antisense miRNA oligonucleotides against miR-21, a significant knockdown of miR-21 levels and death of tumor cells have been detected *in vivo* and *in vitro* [126]. Engineering exosomes from various organisms have been widely used to construct delivery platform, which presents universal biocompatibility but relatively low loading efficiency [127]. Hence other biomolecules are explored to deliver nucleic acids. Ferritin is an important iron storage protein with spherical structure and internal cavity, binding to TfR1 and maintaining iron homeostasis. Based on its excellent physical and

chemical properties, Yan and Fan's group applied heavy chain apoferritin (HFn) as ferritin drug carriers (FDC) for tumor drug delivery [128]. Thanks to the ability of binding to TfR1 and penetrating the BBB, HFn provides a potential strategy for nucleic acid delivery and brain tumor therapy. Other proteins like human serum albumin (HAS) and apolipoprotein E3-reconstituted high-density lipoprotein (ApoE-rHDL) are also employed as drug carriers in the treatment of glioblastoma [129–131]. Due to their low immunogenicity and easy degradation, proteins as carriers have promising applications in nucleic acid delivery.

5. Conclusion and future perspectives

As an endogenous molecule, nucleic acids used to treat brain tumor have great advantages and potential prospects. Firstly, therapeutic nucleic acids, including ASO, siRNA, miRNA, DNA, CRISPR/Cas system, present excellent biosafety and biocompatibility. Secondly, they function at the gene or protein level and change the pathogenesis, probably showing a small effective dosage. Thirdly, the designable structure and sequence make it possible to apply nucleic acids in the treatment of different disease and realize precision medicine. Moreover, simple synthesis process leads to a low commercial cost, indicating great potential in clinical transformation. Tremendous types of nucleic acid drugs have been prepared and approved to treat multiple diseases but few are used for brain tumor therapy. The challenge lies in the instability in the presence of nuclease, obstacle of BBB/BBTB and the off-target effect. Therefore, it is essential to choose the appropriate administration route and develop effective drug carriers.

Different materials have been used to deliver nucleic acids systemically. Viral carriers are the first to be applied due to their simple structures and ability of crossing the BBB, and already develop a mature technique to construct delivery platform. However, limited loading of nucleic acids reduces the therapeutic effects and potential toxicity and immune reaction caused by viruses cannot be overlooked. Polymeric carriers have been developed to improve the side effect, including PEI, CS, PAMAM, *etc.* The cationic polymer can be integrated with nucleic acids by electrostatic adsorption and prevents the degradation by nuclease, leading to an enhanced stability. Similarly, lipid-based carriers such as liposomes are promising alternatives approved by the FDA. They possess a potential ability to overcome the biological barriers but low specificity. To realize controlled release of nucleic acids, several types of liposomes responsive to tumor microenvironment have been designed [132]. Inorganic nanocarriers are developed in recent years as their multifunction and high loading capacity, which makes the nucleic acid-based therapy efficient. Thanks to the unique physical and chemical properties, inorganic nanoparticles contribute to the theranostic applications and facilitate the controlled release. However, there are still some concerns about the long half-life and potential toxicity. Biological carriers like exosomes, ferritin and other proteins have been confirmed to have a superior biosafety, successful BBB penetrating and targeting delivery of nucleic acids to brain tumor. Multiple ligands and strategies are also used to overcome the biological barrier, such as targeting peptide, membrane encapsulation and receptor-mediated delivery to glioma [133–136].

Although there remain numerous obstacles, it is undeniable that nucleic acids have proposed an efficient platform and great clinical potential for brain tumor therapy. Due to differences in genomes, many nucleic acid drugs need specific designs to cater for individuals, which is an irreplaceable advantage and facilitate the precision medicine. We expect to see more nucleic acid drugs being applied in a wider aspect.

CRediT authorship contribution statement

Zixia Zhang: Writing – review & editing. **João Conniot:** Writing – review & editing. **Joana Amorim:** Writing – review & editing. **Yiliang Jin:** Writing – review & editing. **Rajendra Prasad:** Writing – review & editing. **Xiyun Yan:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing. **Kelong Fan:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing. **João Conde:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

J.Conde is a co-founder and shareholder of TargTex S.A. The remaining 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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