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Review Article

Advances in nano-delivery systems for overcoming the challenges of applying siRNA drugs in tumor therapy

Xiaoxin Yan[#], *Jinhu Liu*[#], *Jie Liu, Suyun Wei, Ruixin Li, Weiwei Mu, Na Zhang*[*], *Yongjun Liu*[*]

Department of Pharmaceutics, Shandong Key Laboratory of Targeted Drug Delivery and Advanced Pharmaceutics, NMPA Key Laboratory for Technology Research and Evaluation of Drug Products, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Cheeloo College of Medicine, Shandong University, Jinan 250012, China

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ABSTRACT

Tumors pose a serious threat to human life and health. In recent years, gene therapy against tumors has garnered considerable attention. Small interfering RNAs (siRNAs), an important class of nucleic acid drugs, can silence the mRNAs of tumor-associated genes with high specificity through RNA interference (RNAi), inhibiting tumor-related signaling pathways or protein expression and thereby exerting anti-tumor effects. However, anti-tumor siRNA drugs are currently in the clinical research stage, and none of these drugs have been approved for marketing, mainly because of the challenges in terms of safety, efficacy and targeted delivery. Nano-delivery systems can enhance siRNA stability and improve siRNA pharmacokinetics and biodistribution, while increasing their uptake by target cells to achieve precise delivery and controlled release, thereby serving as a promising solution to overcome the challenges of siRNA drug application. This review summarizes the existing research on the nano-delivery systems currently available to help siRNAs achieve organ-targeted delivery and enhance the anti-tumor efficacy of siRNAs, discussing the characteristics of siRNA action and the unique advantages of different types of nano-delivery systems. The aim was to provide novel ideas for the design and optimization of siRNA-based drug delivery and the development of novel anti-tumor formulations to promote the clinical translation and application of siRNA drugs.

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[*] Corresponding authors.
E-mail addresses: zhangnancy9@sdu.edu.cn (N. Zhang), liuyongjun@sdu.edu.cn (Y. Liu).
[#] These authors contributed equally.
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1. Introduction

Tumors are currently considered the leading cause of threat to human life and health [1–3]. Gene therapy against tumors has garnered considerable attention and involves the delivery of exogenous nucleic acid-based agents to replace/repair the defective genes or regulate gene expression to modulate the processes involved in tumorigenesis and progression [4,5]. Among the different kinds of gene therapy methods, small interfering RNA (siRNA) is particularly relevant and involves a type of double-stranded RNA (dsRNA) that is 20–23 base pairs in length and operates through the RNA interference (RNAi) mechanism. Specifically, with the enzymatic mediation of the RNA-induced silencing complex (RISC), siRNAs bind to the complementary target mRNAs to induce gene silencing, thereby suppressing tumor-related signaling pathways and protein expression with high specificity [6,7].

Compared to traditional small-molecule drugs, siRNAs have the following advantages. First, they effectively regulate the non-druggable targets. The numerous key biomolecules that drive tumorigenesis and tumor formation are difficult to develop into small-molecule drugs because of the following: (1) Structural constraints: their targets lack well-defined deep binding pockets or have flat interfaces (e.g., KRAS and HRAS; transcription factors MYC and p53); (2) similar structures among protein family members lead to poor selectivity and significant off-target toxicity. (e.g., phosphatases PTPs and SHP2); (3) protein-protein interaction (PPI) interfaces are wide, flat or charged (such as the Bcl-2 family and NF-κB); (4) intrinsically disordered proteins (IDPs) lack stable conformations for ligand binding (e.g., Tau and α-synuclein); (5) competitive inhibition is not feasible because the substrate has extremely strong affinity (e.g., the KRAS-GTP complex) [8,9]. The siRNA technology bypasses the protein level and directly binds to the mRNAs that encode and express these proteins, inducing their degradation or inhibiting translation, thereby regulating gene expression at the most basic level to achieve intervention in these non-druggable targets [10–12]. Second, siRNAs achieve targeting through nucleic acid sequence complementarity and base pairing, which drastically reduces the need for structural precision in molecular skeleton modifications or steric hindrance optimization, and this offers distinct advantages in regard to manufacturing and drug development [13–15]. Moreover, advances in human tumor genomics have enabled the systematic decoding and annotation of tumor-associated genes, expanding the potential applications of siRNAs in tumor therapy [16,17].

Despite the significant advantages of siRNA in anti-tumor treatment, its application in tumor therapy faces numerous challenges. First, as an exogenous molecule, siRNA possesses inherent immunogenicity. This property can trigger immune responses, leading to excessive cytokine release and, in severe cases, inflammatory reactions that cause organ failure or damage [18–20]. Second, siRNA drugs may induce toxicity-related issues, such as hybridization-dependent toxicity (caused by excessive binding to target genes and off-target hybridization with non-target genes [21,22], as well as carrier excipient-related toxicity during storage and administration. Furthermore, siRNA's short *in vivo* half-life combined with poor cell membrane permeability limits the ability of free siRNA to achieve efficient lysosomal escape and organ-targeted delivery [23,24]. To address these limitations, research strategies have focused on siRNA structural modifications and advanced drug delivery systems. Notably, nanoparticle-based delivery platforms developed during pharmaceutical research offer distinct advantages. These include precisely controllable physicochemical properties, mitigation of adverse effects, enhanced *in vivo* biodistribution and tissue metabolism, precise targeting mechanisms (both active and passive), and stimuli-responsive release (e.g., pH-, light- or temperature-sensitive systems) [25–28], making them promising candidates for delivery. Current nano-delivery systems primarily encompass lipid nanoparticles (LNP), polymeric carriers, and inorganic nanoparticles. Cell-derived, viral, and bacterial nano-drug delivery systems have also been extensively investigated. Based on the mechanism of siRNA action and the current landscape of anti-tumor siRNA drug development, this review systematically analyzes the challenges in anti-tumor siRNA therapeutics. It classifies nano-delivery systems and discusses their research progress and future directions to advance organ-targeted siRNA delivery and enhance anti-tumor efficacy.

2. Mechanisms of siRNA drug action and challenges in clinical application

2.1. *Development of siRNA drugs*

In 1998, Fire and Mello discovered and proposed the RNAi phenomenon in *Caenorhabditis elegans* [29], pioneering the RNAi-based therapeutic strategies. In 1999, Florence Wianny and colleagues demonstrated that introducing dsRNA in mice could suppress protein expression specifically, confirming the applicability of RNAi in mammals [30]. Tuschl and collaborators subsequently demonstrated that the dsRNA mediating the RNAi process *in vivo* was 21–23 nucleotides in length and named it siRNA [31,32]. The remaining core components of RNAi, Dicer enzymes and Argonaute-2 (Ago2) proteins, were discovered in 2001 and 2004, respectively [7,33]. Since then, siRNA drugs have undergone a surge in research and development. However, the first-generation siRNA drugs were withdrawn from clinical development because of safety and efficacy issues. The challenges were addressed by the researchers through siRNA chemical modifications and targeted drug delivery systems to achieve improved therapeutic outcomes. After over 20 years of research, the US Food and Drug Administration (FDA) approved the first siRNA product, patisiran, in 2018 for use in the treatment of hereditary transthyretin (TTA)-mediated amyloidosis polyneuropathy, employing LNPs as the delivery system [34]. Six additional siRNA drugs have since been approved (listed in Table 1). While siRNA drugs show great potential in disease treatment, those that target tumors remain in preclinical and clinical trials and have not yet received approval.

Table 1 – Seven marketed siRNA drugs.

Drugs	Company	Indication	Target site	Target organ	administration	Delivery system	Time to market
Onpattro® (Patisiran)	AlnyIam	hATTR	TTR	Liver	IV	LNP	2018
Givlaari® (Givosiran)	AlnyIam	AHP	ALAS1	Liver	SC	GalNAc	2019
Oxlumo® (Lumasiran)	AlnyIam	PH1	HAO1	Liver	SC	GalNAc	2020
Leqvio® (Inclisiran)	Novartis/AlnyIam	LDL-C	PCSK9	Liver	SC	GalNAc	2020
Amvuttra® (Vutrisiran)	AlnyIam	ATTR-PN	TTR	Liver	SC	GalNAc	2022
Rivfloza® (Nedosiran)	Novo Nordisk	PH1	LDHA	Liver	SC	GalNAc	2023
Qfitlia® (Fitusiran)	Sanofi/AlnyIam	Hemophilia A/B	SERPINC1	Liver	SC	GalNAc	2025

IV: intravenous injection; SV: subcutaneous injection

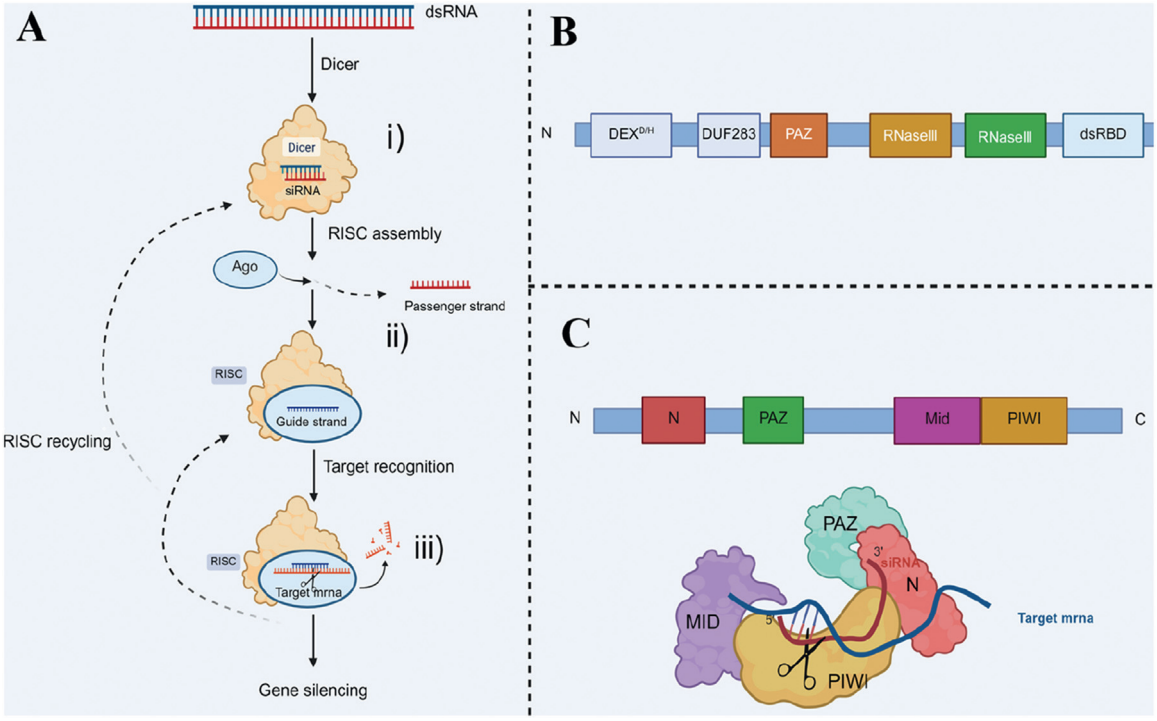


Fig. 1 – (A) Molecular mechanism and structural components of RNAi: (i) Processing of long dsRNA precursors into 21–23 bp siRNA duplexes by Dicer; (ii) Passenger strand cleavage by Ago, yielding a guide strand-loaded RISC; (iii) Target mRNA recognition and silencing through guide strand-mRNA complementarity; (B) Structural composition of Dicer; (C) Structural composition of Ago. Created in BioRender.

2.2. *Mechanism of siRNA drug action*

In the RNAi mechanism, three critical components are recognized as fundamental elements: the Dicer enzyme family, Ago proteins, and dsRNA-derived small RNAs (about 21–23 base pairs in length). These three components collectively constitute the RISC, which is responsible for executing RNAi-mediated gene silencing [35,36].

The RNAi process consists of three steps (Fig. 1A). First, double-stranded siRNAs are generated. Long dsRNAs transcribed from the genome are processed by Dicer, a cytoplasmic RNase III enzyme, into 21–23-base pair (bp) siRNA duplexes. Next, single-stranded siRNAs are generated. The Ago protein binds to the double-stranded siRNA, removing the passenger strand of the siRNA duplex and retaining the guide strand, yielding the single-stranded RNA (ssRNA). Dicer, Ago, and ssRNA form a functional RISC. In this process, the passenger strand and guide strand selection is determined by the relative thermodynamic stability of the duplex termini; the strand with its 5' end at the less thermodynamically stable terminus is preferentially incorporated into the guide strand [37]. The final step involves RISC scanning the transcriptome to identify the target mRNAs complementary to the guide strand, directing its endonuclease component to cleave the homologous sequences, thereby inducing gene silencing [6]. Dicer, which is the initiator of RNAi, contains a DEXD/H ATPase domain, a DUF283 domain, a PAZ domain, two tandem RNase III domains, and a dsRNA-binding domain (dsRBD) (Fig. 1B). The PAZ and RNase III domains are critical for the precise excision of the siRNA duplexes from the dsRNA termini. Ago, which is the core mediator of RNAi silencing, comprises four structural domains (Fig. 1C): an N-terminal domain, a Mid

(middle) domain, a PAZ domain, and a PIWI domain. Among these domains, the degradation of the target RNA is induced by the PIWI domain of the Ago protein [38].

2.3. *Challenges in clinical application of siRNA drugs*

To date, all siRNA-based anti-tumor drugs remain in the preclinical and clinical research stages, and none has been approved for clinical, primarily owing to the following challenges in siRNA drug development: (1) Immunogenicity: siRNA molecules trigger immune responses in the human body; (2) Toxicity issues, including hybridization-dependent toxicity caused by siRNA-gene mismatches and excipient-related toxicity; (3) Poor *in vivo* stability, characterized by rapid degradation by the ribonucleases or renal clearance, which results in a short half-life; (4) Delivery limitations: siRNAs generally lack an intrinsic targeting specificity for specific organs or tissues. Owing to its high molecular weight and hydrophilic properties, siRNA cannot passively penetrate cell membranes, and after cellular uptake, siRNAs are typically trapped within the lysosomes of cells, which hinders their therapeutic functioning. This section of the report integrates the clinical applications of siRNA drugs to systematically analyze the challenges associated with the utilization of siRNAs, hoping to provide a theoretical basis for improving the safety and effectiveness of siRNA drugs.

2.3.1. *Innate immunogenicity of siRNA*

In order to prevent pathogen invasion, the immune system can recognize foreign molecules through different intracellular and extracellular pathogen-associated molecular pattern (PAMP) receptors named PRRs. RNA recognition is primarily achieved through Toll-like receptor (TLR) signaling, with structural features such as single/double-stranded conformations, length variation and sequence motifs critically influencing TLR activation [39–41]. In mammalian systems, TLR3 recognizes the dsRNA and requires at least 40 bp of dsRNA for activation. As the length of dsRNA increases, the activation efficiency of TLR3 may improve, and longer dsRNA strands can facilitate the formation of tetramers or even larger polymeric complexes of TLR3, thereby increasing signal transduction and activation efficiency. TLR7, TLR8 and TLR13 detect the ssRNA fragments with distinct sequence preferences: TLR7 activation requires a ssRNA ligand that binds at its first site with only a single guanosine nucleotide and then at its second site with a ssRNA trimer containing a uridine dinucleotide (UU motif). TLR8 exhibits ligand binding similar to that of TLR7 but preferentially binds uridine at the first site and a UG dinucleotide at the second site. In contrast to TLR7/8, TLR13 shows strict sequence specificity for a defined 13-nt ssRNA motif (5'-CGGAAAGACC-3'). TLR9 recognizes the unmethylated CpG motifs in single-stranded DNA [42,43] (Fig. 2). TLRs activate downstream pathways through the myeloid differentiation factor 88 (MyD88), triggering nuclear factor-κB (NF-κB) activation and inducing various pro-inflammatory cytokines (IL-6, IL-8, IL-12 and TNF) or type I interferon responses, which collectively drive immune activation [44,45]. TLR expression levels vary across cell types; for example, upon TLR7 or TLR9 stimulation, plasmacytoid dendritic cells (pDCs) produce type I and type III interferons (IFNs), whereas the other cell types primarily generate NF-κB-dependent cytokines [46]. This unique TLR recognition mechanism underlies siRNA immunogenicity, which is one of the main barriers limiting the clinical translation of first-generation siRNA therapeutics [47].

2.3.2. *siRNA hybridization dependent toxicity*

To date, only a limited number of liver-targeted siRNA drugs have been approved. These treat specific disorders including human hereditary transthyretin amyloidosis (hATTR), primary hyperoxaluria (PH), acute hepatic porphyria (AHP), heterozygous familial hypercholesterolemia (HeFH), atherosclerotic cardiovascular disease (ASCVD) and hemophilia. Beyond siRNA's inherent immunogenicity, hybridization-dependent toxicity represents another critical barrier to clinical translation [48]. This toxicity arises from two primary mechanisms: siRNA interactions with non-target genes (off-target effects) or excessive hybridization with target genes (on-target side effects) (Fig. 3). These adverse effects primarily occur during downstream steps of RISC loading, including passenger strand cleavage and aberrant target gene recognition.

2.3.2.1. *Off-target effects of siRNA*

siRNAs may exhibit non-specific effects during their action, including unintended binding to non-target genes and the subsequent downregulation of their expression. This phenomenon is termed off-target effects (OTEs) and arises from two primary mechanisms: (1) Seed region complementarity: Saturation of the siRNA-mediated RNAi pathways can aberrantly upregulate miRNA-like activity, which is mediated by the complementarity between the 5'-end (seed region) of the siRNA antisense strand and the 3'-untranslated region (3'-UTR) of transcripts [49–51]. Preclinical studies have demonstrated that the hepatotoxicity observed with high-dose RNAi therapies in animal models is attributed primarily to OTEs [52]. The number of Watson-Crick base pairs directly modulates this complementarity, and increasing the seed region match length induces a logarithmic additive reduction in the off-target mRNA expression [53]. In order to mitigate this effect, strategies such as designing siRNAs with minimal seed region complementarity to 3'-UTRs or introducing chemical modifications (e.g., 2′-OMe substitutions in the sugar moiety) are adopted [54–56]. Furthermore, to reduce hepatotoxicity during clinical use, siRNA) drugs are typically administered as low-frequency, high-dose injections, and liver function and coagulation parameters are monitored. For example, inclisiran (which targets PCSK9) initiates treatment with two 284 mg subcutaneous injections (Month 0 and Month 3), followed by injections every 6 months, which maintains LDL-C suppression efficacy while preventing cumulative liver enzyme elevation [57]. Similarly, fitusiran for hemophilia treatment begins with a dose of 50 mg, administered every 2 months, accompanied by the monitoring of liver function and antithrombin (AT) levels, with subsequent doses adjusted based on the AT activity levels [58,59]. (2) RISC complex mispairing: Misfunction occurs when passenger strands (rather than guide strands) are erroneously incorporated into the RISC complexes, which suppresses the genes complementary to the passenger

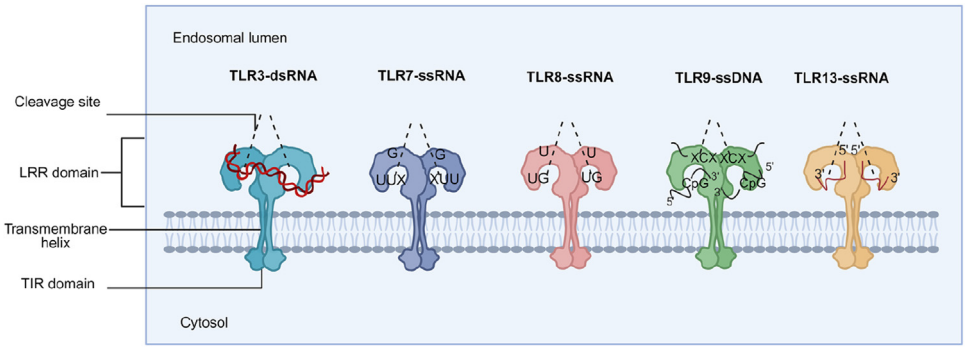


Fig. 2 – TLR receptors recognize different nucleic acid molecules: TLR3: Recognizes dsRNA. TLR9: Binds single-stranded DNA with unmethylated CpG motifs. TLR7: Requires a guanosine at the primary binding site and a UUX trinucleotide sequence at the secondary site. TLR8: Prefers uridine at the primary site and a UG dimer at the secondary site. TLR13: Targets a specific 13-nucleotide ssRNA sequence (5'-XCGGAAAGACCXX-3'). Created in BioRender.

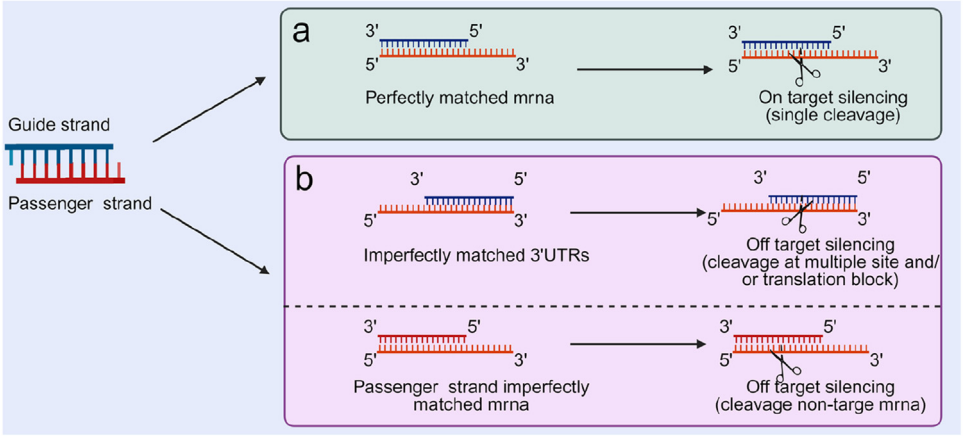


Fig. 3 – Comparison of the mechanism of on-target and OTEs of siRNA. (a) On-target effects: Guide strand binds to target mRNA, mediating gene silencing. Excessive target cleavage disrupts cellular functions; Organ toxicity occurs when mRNA of related genes is cleaved in cells of non-target tissues and organs (b) OTEs: Seed region (5' end of guide strand) complementarity to 3'-UTR induces unintended mRNA cleavage; Passenger strand incorporation into RISC complex causes downregulation of partially complementary genes. Created in BioRender.

strand, thereby inducing adverse effects. This risk can be mitigated through asymmetric siRNA designs incorporating chemical modifications, such as 5-nitroindole substitutions, and these specifically impair the passenger strand-RISC assembly [60,61].

2.3.2.2. *On-target side effects of siRNA*

The OTEs of siRNAs include excessive downregulation of the target gene or the expression of the target gene in non-target cells, which can lead to adverse effects and cause several detrimental outcomes. First, when a siRNA excessively inhibits the expression of a target gene, it can drastically reduce the synthesis of the encoded protein. For example, fitusiran, a siRNA drug that targets AT, promotes hemostasis in hemophilia and induces thrombosis and disseminated intravascular coagulation in non-diseased animals at high doses [62,63]. Second, abnormal compensatory feedback regulation is a prominent issue. A precise feedback regulatory network exists within cells. When the expression of target genes is inhibited, cells maintain homeostasis by upregulating or downregulating the expression of other related genes [64]. For example, the siRNA-mediated silencing of cell cycle regulators may dysregulate proliferation, differentiation, or apoptosis, potentially driving oncogenesis or tissue atrophy [65]. Third, siRNAs exert gene silencing effects on non-target cells, causing dysfunction in these cells, thereby leading to side effects. A prominent case is that of MRX34, a liposomal miR-34a mimic employed in anticancer therapy, which has been shown to accumulate not only in cancer cells but also in immune cells, triggering pro-inflammatory signaling activation, resulting in severe cytokine release syndrome (CRS) and liver injury [66].

2.3.3. *Potential toxicity of excipients*

Toxicity concerns in siRNA therapeutics may also arise due to excipient-related degradation, as pharmaceutical adjuvants (e.g., cationic lipids or polymer-based carriers) can undergo hydrolysis or oxidation during storage or *in vivo* circulation, generating toxic degradation products, which cause toxicities often in a dose dependent manner, making it critical to

establish mechanisms that balance therapeutic efficacy and toxicity for siRNA drug applications [67–69]. Notably, certain cationic liposomes and inorganic nanoparticles, which are commonly used in preclinical studies, induce inflammatory responses and programmed cell death through TLR activation or mitochondrial dysfunction [70]. Patisiran was the first globally approved siRNA drug for hATTR, and novel non-toxic nanocarriers are being explored even today based on its original LNP delivery system [71,72]. Additionally, the choice of administration route critically impacts the siRNA drug safety profiles. For example, an intravenous delivery of nanoformulations may provoke infusion-related adverse events, including the hypersensitivity reactions observed during patisiran therapy, which necessitate rigorous hemodynamic monitoring and prophylactic administration of corticosteroids and antihistamines to mitigate risks [73,74]. In contrast, the clinically approved siRNA drugs such as inclisiran (for HeFH) and lumasiran (for PH) are administered through a subcutaneous abdominal injection, and this localized delivery strategy enhances therapeutic specificity by minimizing systemic immune activation, reducing off-target gene silencing in non-diseased tissues, and alleviating the renal metabolic burden [75,76].

2.3.4. *Challenges of siRNA drug delivery*

Following intravenous administration, nanomedicines targeting solid tumors must successfully navigate the five-stage cascade: circulation in the bloodstream, accumulation at tumor sites, penetration into tumor tissue, internalization by target cells, and intracellular release of therapeutic payloads. siRNA encounters significant barriers at each stage of this process.

2.3.4.1. *Pharmacokinetic characteristics*

First, a naked siRNA is unstable and highly susceptible to degradation by the ubiquitous nucleases in the bloodstream and extracellular matrix (ECM), resulting in extremely low bioavailability and a short half-life of just a few minutes [24,77,78]. Second, tissue distribution remains a significant obstacle, limiting the clinical application of siRNA drugs. Non-specially designed siRNA molecules are taken up by the liver, and this is mainly attributed to the strong endocytosis ability of liver cells and the abundant endothelial fenestra structure in the liver, which renders the liver a major “trap” for siRNA molecules [79]. Moreover, the high molecular weight (~12–14 kDa) and the polyanionic nature of siRNAs lead to electrostatic repulsion with negatively charged cell membranes, hindering their cellular uptake [12].

2.3.4.2. *Biological barrier hinders the penetration of siRNA*

Effective siRNA delivery to non-hepatic tissues, such as the central nervous system (CNS), heart, lungs and bones, remains a formidable challenge, particularly for organs with specialized protective barriers. The blood-brain barrier (BBB), for example, exemplifies this difficulty: BBB is composed of tightly connected endothelial cells, a basement membrane, pericytes and astrocytic end-feet, and it imposes stringent physical restrictions (tight junction pore size <1 nm) and biochemical barriers (efflux pumps such as P-gp, metabolic enzymes) that severely restrict substance entry into the brain. Owing to their high molecular weight ($\approx$13 kDa) and strong hydrophilicity, siRNAs cannot passively diffuse across the BBB. Furthermore, owing to their lack of receptor-specific pathways (such as TFR1, LDL, GLUT1 and CAM1) unique to endothelial cells, siRNAs are susceptible to clearance by efflux pumps, resulting in their extremely low brain delivery efficiency [80–82].

Although the tumor vasculature exhibits pathological hyperpermeability (the basis of the EPR effect), its highly tortuous architecture and disordered hemodynamics, characterized by regional blood stasis or high-velocity flow, result in heterogeneous nanocarrier distribution. Furthermore, vascular endothelial growth factor (VEGF)-driven hyperproliferation exacerbates the vascular abnormalities, thereby impairing effective siRNA carrier retention and extravasation at target sites [83,84].

The tumor stromal barrier constitutes another formidable obstacle to drug delivery. This barrier is primarily composed of ECM components, which are oversecreted by cancer-associated fibroblasts (CAFs), and includes densely packed collagen fibers (e.g., types I and IV), fibronectin networks, and hyaluronic acid macromolecules. Concurrently, signaling molecules such as transforming growth factor-β (TGF-β) drive continuous ECM remodeling and fibrosis, which further increases matrix rigidity, physically impeding siRNA penetration [85,86].

2.3.4.3. *Lysosomal escape*

Following endocytosis, the siRNA becomes encapsulated within early endosomes (pH 6.5), which subsequently mature into late endosomes (pH 4.5). The abundant proteolytic enzymes within these compartments degrade siRNA, with successful escape occurring primarily during the transition from early to late endosomes (pH 6.0–5.0). Current strategies for nanocarrier-mediated endolysosomal escape rely primarily on three core mechanisms: the proton sponge effect (e.g., PEI buffers pH changes through amine protonation, inducing chloride influx, which causes osmotic swelling and endosomal rupture); membrane fusion (e.g., ionizable lipid-based LNPs undergo protonation at acidic pH, driving lipid phase transitions, which facilitate the fusion of endosomal membranes to form pores); photochemical internalization (e.g., porphyrin photosensitizers generate singlet oxygen $^1O_2$ under light irradiation, oxidatively damaging the endosomal membranes to achieve spatiotemporally controlled release).

Although LNPs, polymer nanoparticles, liposomes and other carriers leverage their inherent properties to promote endosomal escape, the overall efficiency remains low (under 1%). By contrast, free siRNA exhibits extremely low endosomal escape efficiency (below 0.01%) [87,88]. Consequently, achieving efficient lysosomal escape represents a critical challenge for successful siRNA therapy.

3. Modification of the siRNA structure

To mitigate these limitations, a primary approach entails structural modification of siRNA. This strategy primarily

encompasses two methodologies: chemical modification of the siRNA molecule itself or conjugation via GalNAc technology.

3.1. *Chemical structure modification of siRNA*

Based on the modification site, these chemical alterations are categorized as targeting the phosphodiester backbone, sugar moiety or nucleobases.

3.1.1. *Ribose ring modifications*

Ribose ring modifications represent the most extensively utilized and mature strategy in siRNA chemical engineering. This approach encompasses substituent modifications, such as 2'-position modifications (e.g., 2'-OMe, 2'-F, 2'-MOE [55,89]), which increase nuclease resistance, improve the binding affinity of the siRNA target mRNA, and reduce immunostimulation. Additionally, 5'-morpholino (5'-Mo) modifications block the passenger strand 5'-phosphorylation, mitigate OTEs, and enhance RNAi efficacy. Clinically validated modifications for HeFH treatment include inclisiran (ALN-PCSsc), which incorporates phosphorothioate (PS), 2'-OMe, 2'-F and 2'-deoxy modifications [90–93]. Similarly, givosiran, which is indicated for AHP in adults, features PS, 2'-OMe and 2'-F modifications within its sugar moiety, thereby conferring enhanced nuclease resistance, improved stability and lowered immunogenicity. Further, conformationally restricted modifications, such as unlocked nucleic acids (UNAs), locked nucleic acids (LNAs), glycol nucleic acids (GNAs), (S)-constrained ethyl-bridged nucleic acids ((S)-cEt-BNAs), and tricyclo-DNA (tcDNA), along with their combined derivative [52,94], facilitate guide strand loading into the RISC complex while reducing OTEs.

3.1.2. *phosphodiester backbone modification*

Chemical modifications of the phosphodiester backbone primarily involve substituting the non-bridging oxygen atoms. The key substitutions include PS, which enhances nuclease resistance and extends the circulation half-life, phosphorodithioate (PS_2), which promotes guide strand loading into the RISC complex and reduces the OTEs, and methylphosphonate (MP) or methoxypropylphosphonate (MOP) modifications, which mitigate siRNA hepatotoxicity. Peptide nucleic acid (PNA) incorporation increases hydrophobicity and confers nuclease resistance [95–97]. Notably, the 5'-phosphate group of the guide strand (antisense strand) is essential for RISC complex loading, and to prevent enzymatic degradation by phosphatases, stabilizing modifications such as 5'-(E)-vinylphosphonate (5'-(E)-VP) and 5'-MP are routinely employed [98–101].

3.1.3. *Base modification*

Base modifications primarily consist of three forms: purine modifications, pyrimidine modifications and base substitutions. Representative purine modifications include N6-methyladenosine (m6A) [102], while pyrimidine modifications encompass 5-methylcytidine (m^5C), 3-methyluridine (m^3U), 5-methyluridine (m^5U), pseudouridine, 2-thiouridine (s^2U) and 5-propargyluridine (5-pU) [103–105]. Substitution modifications incorporate structures such as 2,4-difluorotoluyl ribonucleoside (rF). Collectively, these modifications bolster siRNA stability, minimize immunogenicity, and alleviate OTEs [106,107].

3.2. *GalNAc-siRNA*

GalNAc (N-acetylgalactosamine) serves as a targeting ligand for the asialoglycoprotein receptor (ASGPR) on hepatocytes. Covalently conjugated to the 3'-terminus of the siRNA sense strand via trivalent linkage, GalNAc-siRNA conjugates mediate liver-specific delivery (>90% hepatic accumulation versus <5% for unmodified siRNA) [87,108]. Of the seven clinically approved siRNA therapeutics, all except patisiran employ GalNAc conjugation with subcutaneous administration (Table 1). Compared to unconjugated siRNA, GalNAc-siRNA not only enables targeted delivery but also enhances nuclease resistance, thereby improving stability. As an endogenous sugar moiety, GalNAc confers high biocompatibility while mitigating siRNA immunogenicity. Furthermore, the subcutaneous route of administration reduces required dosing versus intravenous delivery, lowering both treatment costs and off-target toxicity risks [109,110]. Notably, GalNAc technology extends its utility to hepatic delivery of diverse oligonucleotide classes, underscoring its versatile therapeutic applicability [111].

4. Types of siRNA nano-delivery systems

Beyond structural modification, nano-delivery systems represent a key alternative strategy for siRNA delivery. Patisiran, the first FDA-approved siRNA therapeutic for TTR amyloidosis, exemplifies this approach by incorporating structural modifications within LNP carriers. This dual strategy not only enhances siRNA stability but also enables liver-targeted delivery, improves cellular uptake and achieves significant therapeutic efficacy [71]. Critically, nano-delivery systems enhance siRNA stability, facilitate active/passive targeting, promote cellular internalization and mediate lysosomal escape [112,113]. This review focuses on siRNA nano-delivery platforms, detailing advances in: (i) lipid-based carriers (liposomes, LNPs); (ii) polymer-based systems (chitosan, dendrimers, protein nanoparticles); (iii) inorganic vectors (iron oxide, gold, carbon nanotubes); (iv) biomimetic carriers (cell membranes, extracellular vesicles, living cells); and (v) other platforms including viral/bacterial vectors (Fig. 4, Table 2).

4.1. *Lipid-based nano-delivery systems*

Lipid-based nanocarriers represent the predominant platform for nucleic acid delivery. Structurally, they comprise two principal categories: LNPs and liposomes. Cationic or ionizable lipids within these systems electrostatically complex with negatively charged siRNA, subsequently co-assembling with phospholipids to form stable nanoparticles. These architectures demonstrate favorable biocompatibility while achieving exceptional nucleic acid encapsulation efficiency (>90%).

Table 2 – Nanocarriers for siRNA delivery in tumor treatment.

Carrier type	Carrier name and loaded drug	Animal model	Featured significance	Reference
LNP	MG-LNP (anti-PU.1 siRNA)	LPS-induced model of systemic inflammation and the inducible CK-p25 model of neurodegeneration and neuroinflammation	i. Optimized formulation (ionizable lipid cKK-E12: phospholipid ratio) enables natural targeting of activated microglia, enhancing delivery efficiency by 24%–35% under inflammatory conditions (LPS stimulation *in vitro*). ii. Intracisternal injection (i.c.st.): Directly bypasses the BBB.	[124]
	RBP131 (anti-HBV siRNA)	HBV transgenic mouse models	i. Liver targeting: Utilizes the liver endothelial fenestrations for passive hepatocyte targeting, independently of the ApoE/LDLR pathway. ii. Endosomal escape: Features the ionizable lipid LC8 (pKa ≈6.21), optimizing pH-responsive endosomal escape. iii. For the treatment of Hepatitis B.	[125]
	DSPE-PNMVA24(siGFP)	Zebrafish BALB/c mice (Focusing on siRNA Delivery Technology Optimization)	i. Passive membrane targeting: Hydrophobic DSPE anchors the lipid bilayer to target cell membranes passively. ii. pH-responsive release: LNPs incorporate pH-sensitive lipid CSL3, undergoing conformational changes in acidic endosomes to promote siRNA release. iii. 65% GFP silencing in A549/GFP cells (*vs* 40% with PEGylated particles).	[127]
Liposome	Plofsome (siMDK/CRISPR-Cas9 RNP)	Orthotopic brain tumor models in nude mice	i. Elevated H_2O_2 in the TEM triggers oxidative hydrolysis of the HPBA moiety, releasing the "4-arm PEG-oDP" conjugate. ii. Post-cleavage lipid particles regain fusion capability, enabling membrane fusion-mediated cytosolic delivery iii. Overcomes temozolomide (TMZ) resistance in GBM	[131]
Polymer carriers	cRGD-BCP (siKRAS)	PANC-1 tumor-bearing nude mice	i. cRGD peptide targeting: Binds $\alpha v\beta 3/\alpha v\beta 5$ integrins (overexpressed on tumor cells/neovasculature). ii. GSH-responsive release: Elevated intracellular GSH in tumor cells cleaves disulfide bonds in the shell-forming P(TMC-DTC) complex, releasing siKRS. iii. Endosomal escape facilitation: PEI inner core promotes endosomal escape via the proton sponge effect. iv. Overcomes KRAS G12D resistance, extending survival time in mice.	[138]
	G-CMssOA/D&P (DOX/PD-L1 siRNA)	Orthotopic 4T1-bearing breast cancer tumor model	i. DOX/siRNA co-loading: Electrostatic complexation forms DOX-siRNA nanoparticles (D&P). ii. EGFR-targeted encapsulation: G-CMssOA encapsulates D&P via nanoprecipitation into the hydrophobic core. iii. GSH/pH dual-responsive release: Elevated tumor GSH cleaves OAss disulfide bonds (releasing D&P), while acidic pH (5.5) protonates CMCS micelles (triggering swelling & accelerated release). iv. Efficacy: Achieves highly effective breast cancer therapy.	[139]

(*continued on next page*)

Table 2 (*continued*)

Carrier type	Carrier name and loaded drug	Animal model	Featured significance	Reference
	FAPi-IPMs (siHSP47/siHMGB1)	C57BL/6 male mice with hepatic fibrosis	i. Ionizable oligomers (IOs): Tertiary amine headgroups protonate under acidic conditions, electrostatically adsorbing siRNA. ii. FAPi peptide targets fibroblast activation protein (FAP) receptors on activated HSCs. iii. Endolysosomal escape: Tertiary amines (pKa ≈6.0) undergo protonation in the acidic endolysosomal environment (pH 5.0). iv. siHSP47 suppresses collagen hypersecretion and siHMGB1 modulates inflammation, cooperatively mitigating liver fibrosis.	[140]
Inorganic carriers	PDA@siBRAF/CaP-SA (siBRAF)	B16F10 tumor-bearing BALB/c mice	i. Core-shell nanoparticle design: siRNA coordinates with Ca^{2+} via phosphate diester groups and nucleobases, forming a CaP-siRNA shell encapsulating a PDA photothermal core. ii. pH-responsive payload release: Acidic TME (pH 6.5) trigger CaP degradation, releasing Ca^{2+} (inducing hydrogel formation) and siBRAF. iii. Photothermal activation: NIR irradiation induces rapid heating of PDA, accelerating CaP dissociation and burst siRNA release. iv. Intratumoral injection enables efficient melanoma suppression.	[148]
	DOX-EZH2si-VMR@CM (DOX/ EZH2 siRNA)	BALB/c nude mice bearing 4T1 tumors	i. CM-coating-mediated active homing to the TEM; Vortex magnetic Fe_3O_4 nanorods (VMNs) leverage hyperferric tumor conditions for passive tumor enrichment. ii. PEI on the VMR surface undergoes protonation, achieving lysosomal escape.	[149]
	10B/siPD-L1 nanoparticles	BALB/c mice bearing 4T1 tumors	i. siRNA covalently conjugates to ^{10}B-polymer PBA groups; π-π/diSulfide stabilization. ii. cRGD targets tumor $\alpha v\beta 3$. iii. ATP/GSH dual-responsive release: Elevated tumor ATP competitively binds PBA groups, dissociating siRNA; High intracellular GSH reduces disulfide bonds, disrupting nanostructure. iv. Therapeutic mechanisms: 10B neutron capture generates α particles and lithium nuclei for selective tumor cell eradication; siPD-L1 silences PD-L1, reversing T-cell immunosuppression to enhance antitumor immunity.	[150]
Cellular component-based carriers	BSPC@HM NCs (Sav1 siRNA)	Rat and porcine models of myocardial IR injury	i. Core assembly: siRNA electrostatically complexes with cationic helical polypeptides (P-Ben), forming the inner core (BSPC). ii. HM targeting: Platelet membrane (PM) targets microthrombi in I/R injury zones; Macrophage membrane (RM) targets to inflammatory sites. iii. Acidic microenvironment of injured regions (pH 6.8) triggers charge reversal in the PC layer to positive polarity, resulting in HM shedding and core exposure.	[156]

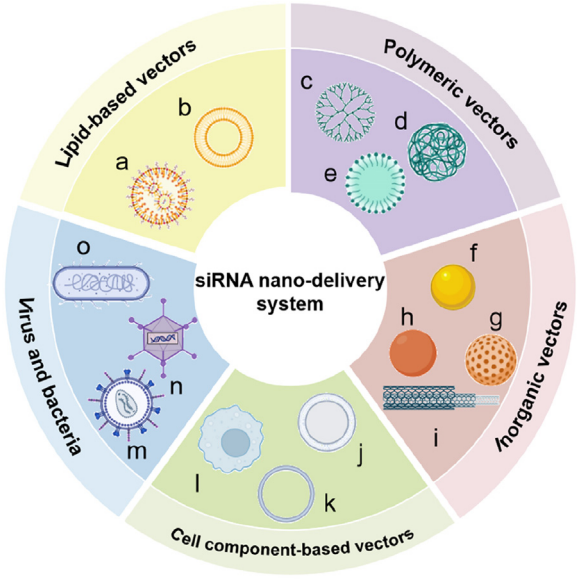


Fig. 4 – Different types of siRNA nano-delivery systems: a-LNP, b-Liposome, c-Dendrimer, d-Polymer, e-Emulsion, f-Gold NP, g-Silica NP, h-TiO_2/Iron NP, and i-Carbon nanotubes, j-Cell membrane, k-EVs, l-Cells, m-Lentiviral, n-Adenovirus, o-Viruses. Created in BioRender. https://BioRender.com/qgs84hm

4.1.1. *LNPs*

LNPs are lipid vesicles with a homogeneous lipid core, capable of encapsulating both hydrophilic and hydrophobic drugs, and represent one of the most advantageous non-viral delivery systems [114–117]. After endocytosis, in acidic endosomes, cationic ionized lipids combine with anionic endosomal membranes, leading to the destruction of endosomal membranes and the escape of siRNA drugs from endosomal membranes [118,119]. However, current LNP formulations exhibit limited endosomal escape efficiency, necessitating development of novel ionizable lipids. Clinically approved LNPs (e.g., in patisiran) feature a four-component architecture: (1) Ionizable lipids (e.g., DLin-MC3-DMA) mediate nucleic acid encapsulation and endosomal escape; (2) Helper lipids (e.g., distearoylphosphatidylcholine, DSPC) enhance structural stability while modulating membrane fusion and targeting; (3) Cholesterol regulates membrane fluidity and stabilizes bilayer architecture; (4) PEGylated lipids (e.g., PEG2000-DMG) prolong circulation half-life and enable surface functionalization [120–123].

To optimize LNP biodistribution and siRNA delivery efficacy, researchers engineered key LNP components. As the critical functional element, ionizable lipids have become a major research focus. Langer et al. systematically optimized ionizable lipid structures, phospholipid selection and lipid-to-mRNA ratios to develop seven LNP formulations. Screening identified MG-LNP (molar composition: ionizable lipid cKK-E12/cholesterol/phospholipid/PEG-lipid = 35:46.5:16:2.5; cKK-E12:RNA = 10:1) as the optimal candidate (Fig. 5A). This formulation demonstrated superior *in vivo* delivery efficiency versus the commercial reagent Invivofectamine® 3.0, achieving 92% ± 2% transcriptional silencing of PU.1 at 500 ng/mL siRNA within 12 h, confirming exceptional delivery performance [124]. Huang et al. developed an ionizable lipid-like material (lipidoid) designated LC8, synthesized from alkyl acrylate, alkyl acrylamide and amino molecular precursors. When formulated with auxiliary components, LC8 formed ionizable lipidoid nanoparticles (RBP131, Fig. 5B) exhibiting >90% siRNA encapsulation efficiency. For hepatic delivery, LC8 demonstrated efficacy comparable to Onpattro®'s benchmark lipid DLin-MC3-DMA. Strikingly, formulation RB-HBV008 significantly suppressed hepatitis B virus expression relative to entecavir [125]. Beyond ionizable lipids, PEGylation represents the predominant surface modification strategy for prolonging systemic circulation by minimizing immune recognition and reducing macrophage clearance [126]. Berger et al. synthesized poly(N-methyl-N-vinylacetamide) (PNMVA, Fig. 5C), which inhibits protein corona formation while exhibiting stealth properties comparable to DSPE-PEG. DSPE-PNMVA conjugates demonstrated favorable safety profiles with reduced hepatic accumulation, establishing a novel lipid platform for siRNA delivery [127]. Current research focuses on developing advanced LNP components, with promising next-generation lipids anticipated for enhanced siRNA therapeutics.

4.1.2. *Liposomes*

Liposomes are closed vesicles formed by single or multiple concentric phospholipid bilayers enclosing an aqueous interior. This unique structure allows simultaneous encapsulation of both hydrophilic compounds within the aqueous core and lipophilic substances incorporated within the phospholipid layers. The phospholipid bilayer structure can fuse with cell membranes, enabling efficient intracellular delivery of siRNA and enhancing delivery efficiency [128–130]. Structurally, liposomes consist of phospholipids (natural or synthetic) and cholesterol. Natural phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), are frequently blended with synthetic analogs—including DOPC, DOPE, DSPC and DPPC—to construct charge-neutral vesicles. Cationic lipids like DOTAP, though prone to inducing cytotoxicity, facilitate nucleic acid encapsulation via electrostatic interactions. Cholesterol, a critical modulator of bilayer rigidity and colloidal stability, optimizes membrane fluidity and prevents premature cargo leakage [118].

By engineering specialized liposomal architectures, intracellular entry pathways can be modulated to achieve precise drug release within tumor microenvironment (TME). Yu et al. developed a polymer-locked fusogenic liposome (Plofosome) enabling BBB penetration. This system was fabricated via anchoring 4-arm PEG-oDPs onto Angiopep-2 (Ang)-modified fusogenic liposomes (Fig. 5D). Critically, 4-arm PEG-oDPs dissociate post-BBB penetration upon exposure to tumor-associated ROS (H_2O_2), triggering target cell membrane fusion and simultaneous siRNA/CRISPR-Cas9 RNP release. In the absence of H_2O_2, however, liposomes remain sequestered within endolysosomal compartments through endocytosis – enabling spatiotemporally controlled activation exclusively in target cells [131]. Cationic lipids within liposomes electrostatically complex with negatively

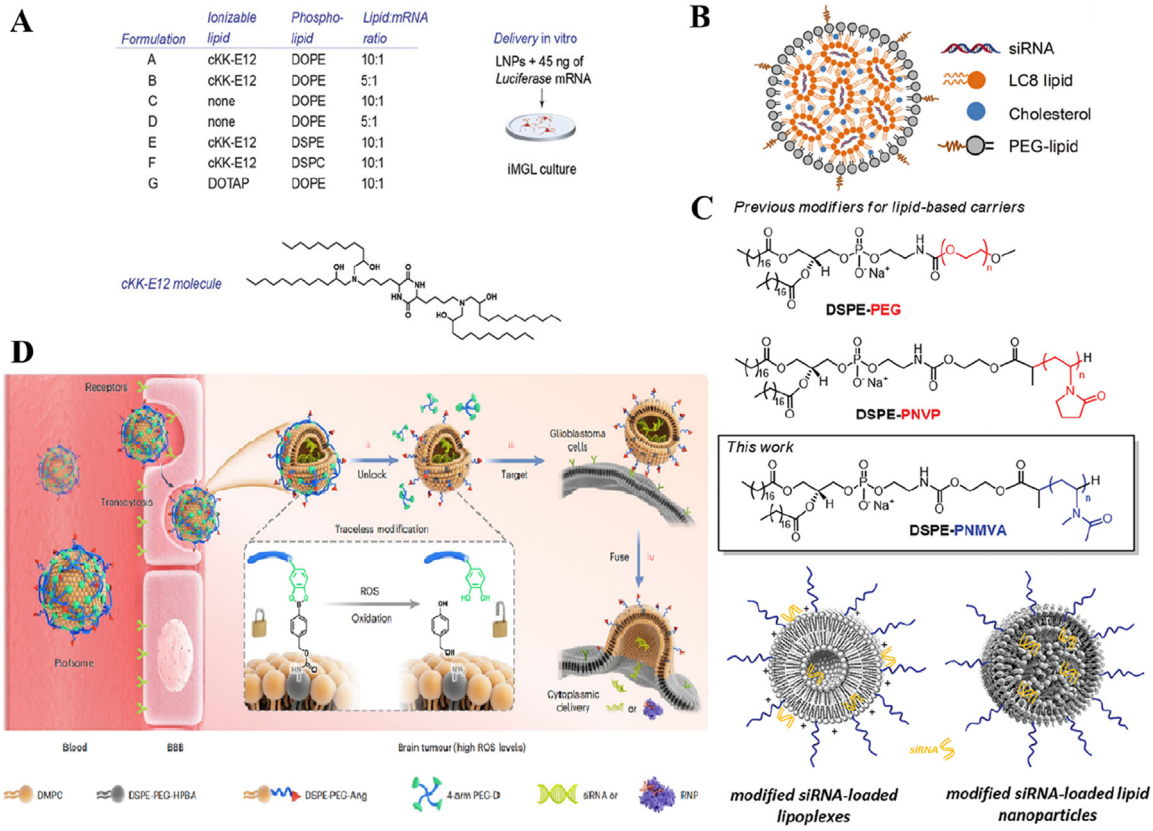


Fig. 5 – (A) LNP formulation variations focused on ionizable lipid and phospholipid composition, as well as lipid:mRNA ratio. Below: Structure of lead ionizable lipid, cKK-E12. Adapted with permission [124] copyright 2023 John Wiley and Snos. (B) Schematic illustration of RBP131/siRNA nanoparticle and the compositions. Adapted with permission [125] copyright 2022 Springer Nature. (C) Schematic illustration of the strategy for the PNMVA surface modification of siRNA-loaded lipoplexes and LNPs. Adapted with permission [127], copyright 2023. John Wiley and Snos. (D) Plofsomes for GBM-targeted siRNA delivery and CRISPR–Cas gene editing. Adapted with permission [131] copyright 2024 Springer Nature.

charged siRNAs, enhancing encapsulation efficiency while boosting cellular uptake and therapeutic efficacy [132]. For instance, Li et al. engineered TPGS-coated cationic liposomes (Bcl-2 siRNA/Dox-TPGS-LPs) co-encapsulating Bcl-2 siRNA and doxorubicin (Dox). Compared to free siRNA, this system elevated intracellular siRNA concentrations 12-fold and achieved 94% silencing efficiency at 100 nM Bcl-2 siRNA [133].

4.2. *Polymer-based nano-delivery systems*

Cationic polymers represent a cornerstone of non-viral RNA delivery systems, broadly categorized as natural or synthetic based on origin. Natural variants include polysaccharides (e.g., chitosan, hyaluronic acid), proteins (e.g., gelatin), and cell-penetrating peptides (e.g., TAT). Synthetic counterparts encompass polyethyleneimine (PEI), poly(lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA) and dendrimers such as PAMAM [134–136]. Polymer nanoparticles mediate siRNA encapsulation through electrostatic complexation (cationic polymers) or hydrophobic interactions (amphiphilic block copolymers).

Polymer nanoparticles achieve targeted delivery through surface functionalization with ligands such as RGD, NGR, GE11, HER2-targeting peptides and transferrin receptor-binding peptides [137]. Huang et al. developed a bioreactive chimeric polymer conjugated with cRGD peptide (cRGD-BCP), comprising PEG-P(TMC-DTC)-PEI and cRGD-PEG-P(TMC-DTC) (Fig. 6A). This system mediated efficient siKRAS delivery to PANC-1 pancreatic cancer cells, achieving near-complete tumor growth inhibition at 3 mg/kg in murine pancreatic cancer models with significant antitumor efficacy [138]. Stimuli-responsive polymers enable TME-specific siRNA release (e.g., pH-, enzyme- or temperature-triggered), enhancing both safety and therapeutic performance. Researchers engineered GE11-functionalized dual-reactive carboxymethyl chitosan micelles (Fig. 6B) that deliver pH/GSH-responsive co-release of PD-L1 siRNA and DOX. This approach enhanced apoptosis in 4-T1 breast cancer cells while increasing intratumoral infiltration of $CD4^+$/$CD8^+$ T lymphocytes [139]. Zhou et al. designed pH-responsive polymeric micelles (FAPi-IPM) via self-assembly of oligomers (IOLA4, IOLA8, IOA2), PLA-PEG and FAPi-PEG-PLA targeting peptide (Fig. 6C). These micelles specifically target hepatic stellate cells (HSCs), where lysosomal protonation (pH 5.0) of tertiary amine groups induces membrane disruption, releasing siHSP47 and siHMGB1 for hepatic fibrosis therapy

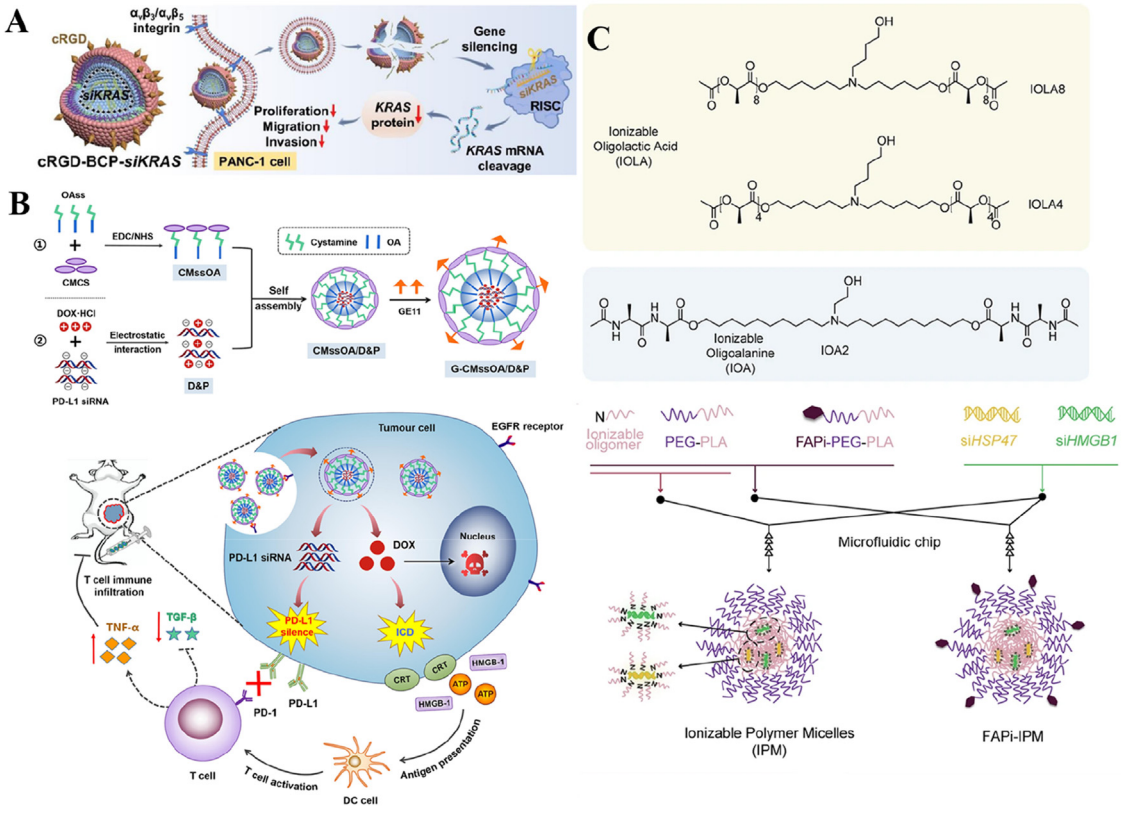


Fig 6 – (A) cRGD-installed bioresponsive chimeric polymersomes (cRGD-BCP) for highly efficient siKRAS delivery to PANC-1 tumor. Adapted with permission [138] copyright 2023 Elsevier. (B) G-CMssOA/D&P accumulates in tumor tissues, releasing DOX and PD-L1 siRNA under low pH and high glutathione (GSH) conditions. Adapted with permission [139] copyright 2023 Elsevier. (C) Three IOs with PEG-PLA were prepared by microfluidic chip to obtain IPM. siHSP47 and siHMGB1 were delivered to treat hepatic fibrosis by targeting activated HSCs with the addition of FAPi-PEG-PLA in the formulation. Adapted with permission [140] copyright 2025 Springer Nature.

[140]. Polymer-based drug delivery has made remarkable progress. With the continuous development of materials science and biotechnology, more polymeric materials with excellent performance are expected to be developed for drug delivery.

4.3. *Inorganic materials-based nano-delivery systems*

Inorganic nanomaterials, including calcium phosphate (CaP), iron oxide (Fe_3O_4), gold (Au), silica (SiO_2), carbon nanotubes (CNTs) and emerging boron-based nanostructures, have gained prominence as versatile RNA delivery platforms. These systems demonstrate favorable biocompatibility and transfection efficiency in preclinical models [141–143]. Their exceptional payload capacity derives from high surface-to-volume ratios and chemically tunable surfaces, permitting RNA loading via electrostatic adsorption, covalent conjugation, or physical encapsulation within cavities; the dominant loading mechanism depends on specific material properties. Compared to alternative nano-delivery systems, inorganic nanomaterials (e.g., gold nanorods, CNTs) exhibit unique theranostic capabilities including photothermal conversion, superparamagnetic behavior, and ROS generation. These attributes facilitate synergistic integration of immunotherapy with photothermal therapy (PTT) and radiotherapy for advanced tumor treatment [144–147].

The pH responsiveness of inorganic nanocarriers synergized with photothermal effects enables precise spatiotemporal control of siRNA release within TEM. Lu et al. engineered polydopamine (PDA) nanoparticles as photothermal cores, subsequently constructing CaP shells via biomineralization (PDA@CaP) to encapsulate siBRAF (PDA@siBRAF/CaP, Fig. 7A). Following intratumoral injection within alginate gel, tumor acidity dissolved the CaP shell, releasing Ca^{2+} (which gelled alginate to enhance penetration) and siBRAF, while PDA-mediated photothermal activation further modulated siRNA release. This strategy synergized photothermal ablation, Ca^{2+} overload, and BRAF silencing in melanoma models [148]. Magnetic inorganic nanocarriers achieve targeted delivery and enhanced tissue penetration through external field manipulation. Lu et al. designed vortex-engineered Fe_3O_4 nanorods (Fig. 7B) for co-delivery of DOX and EZH2 siRNA (DOX-EZH2si-VMR@CM). Fluorescence co-localization analysis demonstrated significantly enhanced nuclear translocation of DOX compared to free drug

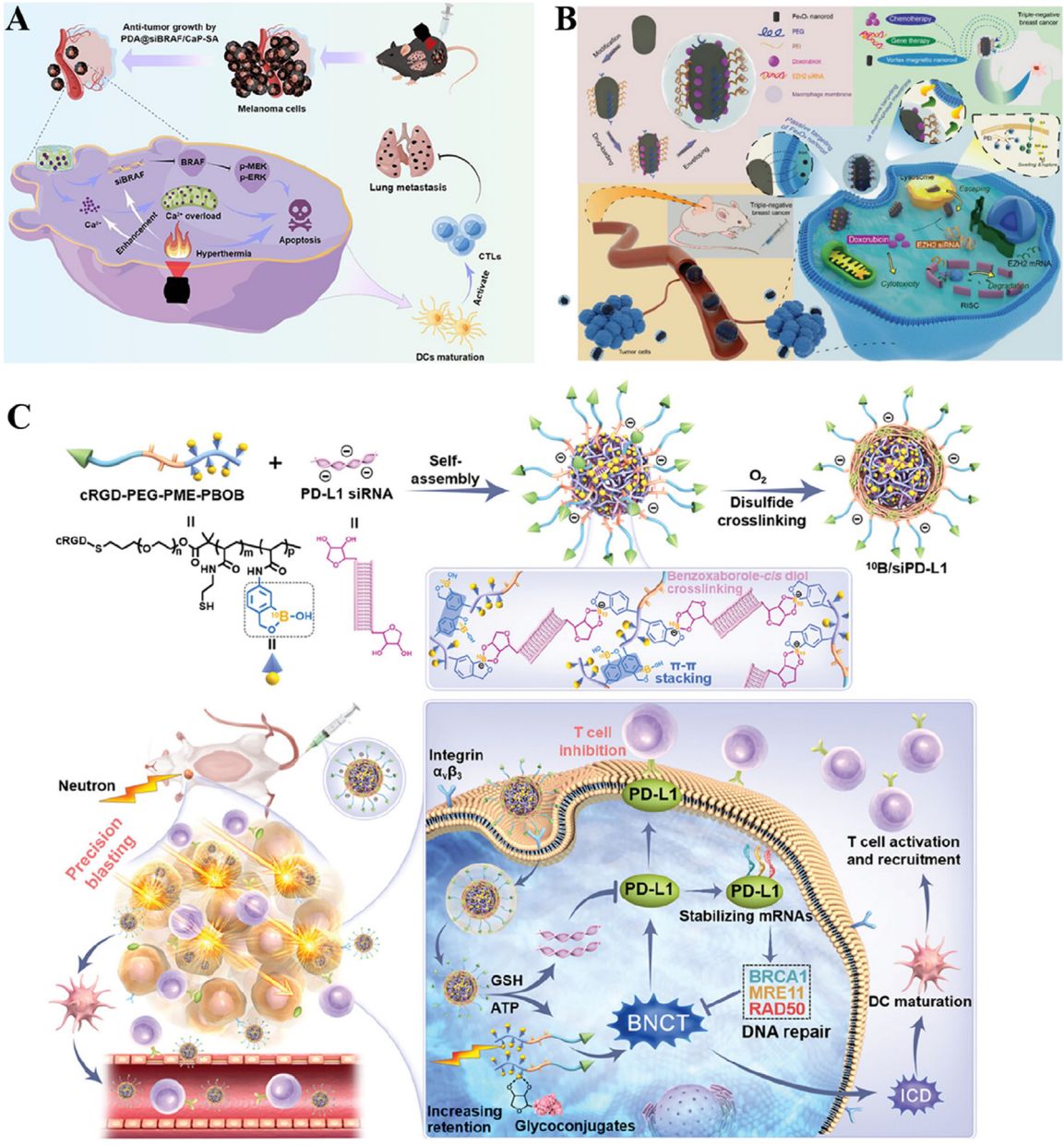


Fig. 7 – (A) Injectable hydrogel platform based on sodium alginate (SA) with multifunctions of PTT and Ca^{2+}-overload-induced cell apoptosis. Adapted with permission [148] copyright 2023 American chemical Society. (B) Schematic of synthesis and mechanism of novel nanodrug targeting triple-negative breast cancer (TNBC). Adapted with permission [149] copyright 2023 John Wiley and Sons. (C) Schematic illustration of the preparation of 10B/siPD-L1 nanoparticles and their application in combined BNCT-immunotherapy. Adapted with permission [150] copyright 2025 John Wiley and Sons.

administration [149]. Boron neutron capture therapy (BNCT) leverages tumor-localized ^{10}B agents combined with neutron irradiation for precise tumor eradication with minimal healthy tissue damage. Deng et al. developed cRGD-functionalized nanoparticles (^{10}B/siPD-L1) co-delivering >95% enriched ^{10}B and PD-L1 siRNA (Fig. 7C). Tumor-specific ATP/GSH levels triggered simultaneous payload release: siRNA suppressed PD-L1 expression while downregulating DNA repair proteins, concurrent with ^{10}B-enabled neutron capture. This dual mechanism concurrently blocked immune checkpoints, inhibited DNA damage repair, and induced immunogenic cell death (ICD) [150].

4.4. *Cellular component-based nano-delivery systems*

Biomimetic nano-delivery systems leveraging cellular components exhibit superior immune evasion capabilities [151]. Cell membrane-coated nanoparticles exemplify this approach, where reconstituted membrane components (proteins and glycans) confer the surface properties

and biological functions of native cells. This biomimetic camouflage enables evasion of immune recognition and clearance, significantly prolonging systemic circulation. Furthermore, by harnessing the tumor-homing capacity of immune cells and inherent homotypic/heterotypic adhesion mechanisms, these systems overcome biological barriers to achieve tumor-targeted payload release [152–154]. Over the past decade, such biomimetic strategies have been widely investigated in drug delivery systems, particularly through the utilization of cell membranes, extracellular vesicles (EVs), and live-cell vectors for siRNA transport [155].

Nanocarriers fabricated through co-extrusion of cellular membranes with synthetic materials exhibit dual biomimetic properties: immune evasion and precise targeting. Zhou et al. engineered nanocomposites (NCs) featuring reversibly camouflaged platelet-macrophage hybrid membranes (HM) (Fig. 8). These biomimetic BSPC@HM NCs comprise a cationic nanocore, a charge-reversible poly(L-lysine)-cis-aconitate (PC) intermediate layer, and an outer HM shell. Within the inflammatory microenvironment of myocardial ischemia-reperfusion (I/R) injury, PC undergoes charge reversal to release cationic nanoparticles containing siSav1 (BS NCs). BSPC@HM NCs demonstrated extended circulation ($T_1/_2$ = 27.9 h in rats; 4-fold longer than BSPC NCs [7.2 h]) with reduced hepatic accumulation and optimized pharmacokinetics. *In vitro*, 95% gene silencing efficiency was achieved at pH 6.8, while *in vivo* treatment reduced myocardial fibrosis and infarct size to ~15%—significantly below control values. This study validates the therapeutic potential of membrane-engineered delivery systems [156]. To address inefficient siRNA delivery caused by endosomal trapping, Yan et al. engineered exosomes with elevated membrane cholesterol (30% Chol-MEs). These modifications enhanced membrane fusion—achieving 57.8% fusion efficiency within 2 h—and quadrupled cellular uptake in HCT116 cells versus 5% Chol-MEs. Critically, 30% Chol-MEs bypassed endosomal pathways, enabling direct cytoplasmic PLK1 siRNA delivery. This resulted in 84.1% PLK1 mRNA knockdown (5.3-fold greater than controls) and near-complete tumor suppression in colon cancer models, demonstrating a novel strategy to overcome endosomal escape barriers [157]. Additionally, researchers harnessed neutrophils' inherent inflammatory tropism to develop siTNFα-loaded cationic liposome-neutrophil complexes (siTNFα/TP/NEs) for rheumatoid arthritis targeting. *In vivo* studies confirmed exceptional targeting specificity and therapeutic efficacy, establishing these complexes as promising cell-based therapeutics [158].

4.5. Others

Beyond conventional nano-delivery platforms, viral- and bacterial-based systems—such as engineered adenoviruses or attenuated Salmonella vectors—achieve high transfection efficiencies with sustained transgene expression profiles. However, their clinical translation faces significant challenges including intrinsic immunogenicity (e.g., neutralizing antibody production) and biosafety concerns (e.g., insertional mutagenesis or systemic infections), requiring rigorous risk-benefit evaluation in therapeutic development [159–161].

Viral vectors—including lentivirus, adenovirus, and adeno-associated virus (AAV)—constitute established platforms for siRNA delivery, despite limited current research application. Notably, the increasing clinical approval of viral vector therapies over the past decade underscores their potential for optimized siRNA delivery [162–164]. For instance, Zolgensma®, an AAV9-based gene therapy, mediates delivery of the human SMN1 gene to ameliorate spinal muscular atrophy by restoring SMN protein expression [165]. Similarly, Kymriah^{TM} (the first FDA-approved lentiviral therapy, 2017) achieves durable remission in pediatric relapsed B-cell acute lymphoblastic leukemia through chimeric antigen receptor T-cell engineering [166]. Recently, Qiu et al. employed lentivirus-mediated RNAi to explore circCSNK1G3-specific shRNA in glioma models, revealing that circCSNK1G3 overexpression induces miR-181d upregulation while depleting CBX7 expression [167].

Bacteria exhibit potent invasive capacity and intrinsic tissue tropism, enabling precise targeting of pathological sites such as tumors or inflammatory loci. These microorganisms function as self-replicating bioreactors capable of sustained therapeutic production and release. While their preparation offers relative simplicity and cost-efficiency, concerns regarding induced immunogenicity persist. Current research primarily employs attenuated bacterial vectors for siRNA delivery [168,169]. Researchers genetically engineered *Escherichia coli* Nissle 1917 (EcN) to produce abundant outer membrane vesicles (OMVs), incorporating synthetic genetic circuits to enhance RNA synthesis and encapsulation. In proof-of-concept studies delivering PD-L1 siRNA, intravenous administration in 4T1 subcutaneous tumor models achieved twofold reduction in PD-L1 expression, with single-dose treatment yielding 49.4% tumor growth inhibition [170]. Tang et al. developed urease-powered OMV nanorobots through noncovalent surface conjugation of urease enzymes. These catalyze urea decomposition to propel nanorobots, which—when loaded with siRNA—penetrated deeply into tissue cores inaccessible to conventional therapeutics, significantly enhancing delivery depth and coverage [171]. Genetic modification of bacteria remains relatively facile. This approach enables straightforward integration of genes encoding therapeutic proteins or drug molecules, permitting efficient expression and secretion of pharmaceutical compounds. Such capabilities expand the therapeutic repertoire while facilitating development of personalized treatment strategies [172,173].

4.6. Safety of nano-delivery systems

In the development of nano-delivery systems, carrier safety represents a critical consideration for clinical translation. Safety profiles vary across different types of nanocarriers due to their distinct material properties and design characteristics.

Lipid-based nanocarriers generally demonstrate favorable safety profiles owing to their structural mimicry of biological membranes. Primary safety concerns relate to potential cytotoxicity from cationic lipids (e.g., DOTAP) and PEGylation-induced immunogenicity. The advent of ionizable lipids (including COVID-19 mRNA vaccine

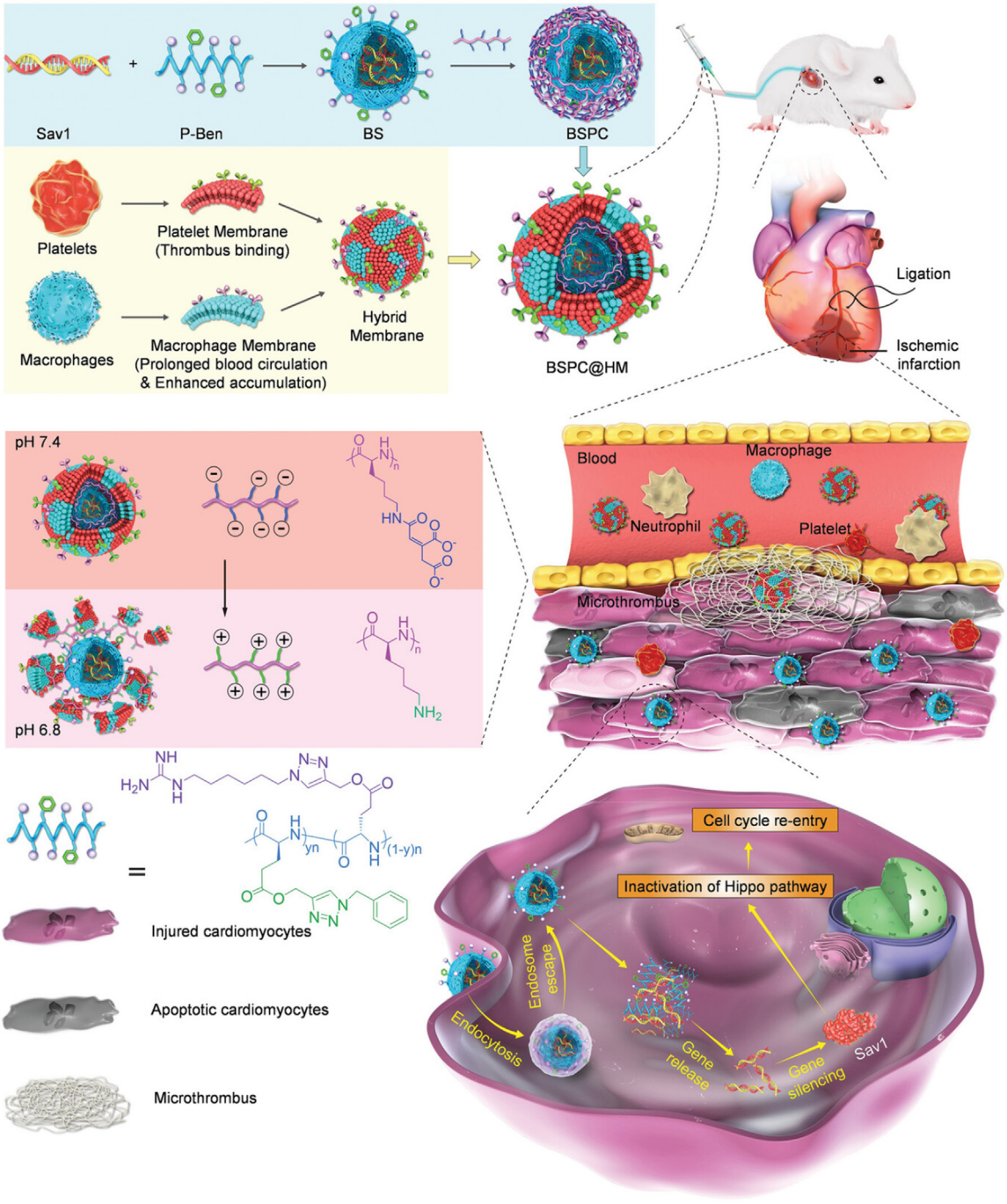


Fig 8 – Schematic illustration of the biomimetic BSPC@HM NCs reversibly camouflaged with HM for the myocardial siSav1 delivery and IR injury management. Adapted with permission [156] copyright 2023 John Wiley and Sons.

components ALC-0315 and SM-102) alongside optimized natural lipid formulations (e.g., DSPC-cholesterol systems) has substantially mitigated these risks. This safety advancement is clinically validated by patisiran—an LNP-based therapeutic—which exhibits manageable toxicity profiles during extended (>1 year) clinical use [174,175]. Polymer-based nanocarriers demonstrate safety profiles comparable to lipid systems, though vigilance remains warranted for potential PEG-induced immunogenicity and cationic polymer cytotoxicity. Notably, comparative sunitinib-loading studies reveal both lipid and polymeric carriers exhibit excellent corneal permeability/retention with no observed toxicity or irritation in *ex vivo* models [176,177]. In contrast, inorganic nanoparticles present heightened safety concerns due to persistent bioaccumulation and limited degradation pathways. Conventionally engineered inorganic particles >5 nm (e.g., gold, hafnium oxide) display extended retention (>100 d) within the mononuclear phagocyte system

(liver/spleen), potentially inducing chronic inflammation and organ damage. Quantum dots containing heavy metals (e.g., cadmium selenide) may leach toxic ions (Cd^{2+}) through non-biodegradation, causing nephrotoxicity [178,179]. Mitigation strategies employing ultrasmall designs (<5 nm, e.g., Ag_2S-NPs) combined with surface modifications (PEG/citrate) or biodegradable matrices show promise for enhanced renal clearance [180]. Cell-derived carriers like EVs offer inherent biocompatibility advantages due to endogenous composition. Repeated administration studies (10 doses/30 d) demonstrate significantly reduced inflammatory cell infiltration and tissue damage for EV-based systems versus PEGylated liposomes [174,181]. Nevertheless, precision targeting challenges persist for cell-based therapies (e.g., CAR T-cells) in complex microenvironments like solid tumors, where off-target toxicity remains a critical safety consideration [182]. Microorganism-derived nanocarriers (bacterial/viral vectors) retain inherent immunogenicity despite attenuated pathogenicity through virulence reduction. PAMPs in these systems may trigger potent inflammatory responses or immune activation, potentially causing adverse events like cytokine storms. Furthermore, genetic mutations could induce virulence reversion, while insertional mutagenesis may cause genomic instability. These collective safety challenges necessitate rigorous assessment for clinical translation [183,184].

5. Organ-targeting study of siRNA nano-delivery systems

The design of organ-specific siRNA delivery systems has become a pivotal focus in nucleic acid therapeutics, aiming to optimize gene silencing efficacy while mitigating systemic OTEs. Current progress is most advanced in liver (where GalNAc-conjugated siRNAs exploit asialoglycoprotein receptor-mediated uptake), and significant advances have also been achieved in targeting other organs [185].

5.1. *Liver targeting*

Liver targeting remains the most clinically advanced application for siRNA therapeutics, with all seven FDA-approved siRNA drugs exhibiting hepatic specificity. While patisiran employs LNP delivery, the other six utilize covalent GalNAc ligand conjugation targeting ASGPRs on hepatocytes (Table 1). Recent advances now enable precision targeting of hepatic subpopulations. For instance, Han et al. developed a novel lipid formulation (AA-T3A-C12, Fig. 9A) for LNP-mediated HSP47 siRNA delivery to liver-resident fibroblasts. This system achieved ~65% silencing of heat shock protein 47 (HSP47)—predominantly expressed by activated fibroblasts—demonstrating twofold higher potency than FDA-approved MC3-based formulations. The optimized delivery significantly inhibited collagen production and attenuated fibrotic progression [186]. Mannose-mediated macrophage targeting exploits specific receptor interactions. Zhou et al. designed mLNP-siHMGB1 nanoparticles surface-functionalized with DSPE-PEG2000-Man ligands (Fig. 9B) to selectively silence HMGB1 expression in hepatic macrophages, providing a therapeutic strategy for non-alcoholic fatty liver disease (NASH) [187].

5.2. *Eye targeting*

Local ocular siRNA delivery offers simplified administration compared to systemic approaches by bypassing circulatory challenges via topical/intraocular injection, minimizing off-target toxicity risks [188]. Guo et al. developed PACD—a ternary copolymer integrating PEG, siRNA-binding domains, and pH-responsive modules (C7A-DBA copolymer)—for intravitreal delivery (Fig. 10A). This system penetrated the blood-retinal barrier (BRB) through transcytosis, internalizing into retinal cells to achieve 65% fibroblast-specific HSP47 silencing with superior endosomal escape versus Lipo2000. These findings elucidate critical design principles for non-viral ocular delivery systems, emphasizing pH-responsive cargo release and BRB-penetrating capabilities [189]. The eye's accessibility makes localized administration preferable for ophthalmic therapy, providing precise treatment, rapid onset, enhanced safety, and improved patient comfort. Topical siRNA instillation represents a particularly advantageous strategy [190]. Recently, Baran-Rachwalska et al. developed ProSilic® (Fig. 10B), a silicon-based platform for non-invasive topical ocular siRNA delivery. While exhibiting lower transfection efficiency than Lipofectamine™ RNAiMAX in human corneal epithelial cells (HCE-S) *in vitro*, ProSilic® demonstrated superior biosafety: commercial formulations caused 50% membrane damage, whereas ProSilic® maintained integrity in >86% of cells [191].

Ocular drug delivery faces bioavailability challenges due to physiological barriers including the blink reflex, tear clearance, and corneal tight junctions. To overcome these limitations, employing a liposome-gel biphasic system for siRNA delivery (e.g., chitosan/polyvinyl alcohol-based nanofiber gels) presents a viable strategy. This approach prolongs drug residence time while enhancing tissue permeability. Furthermore, leveraging pH- or light-responsive nanomaterials enables development of sustained-release formulations, thereby reducing administration frequency and improving patient compliance.

5.3. *Brain targeting*

Brain-targeted siRNA delivery confronts significant challenges including the BBB, enzymatic degradation, limited tissue penetration, and inefficient cellular uptake [192,193]. Nanocarrier systems provide essential platforms for overcoming these barriers, with notable progress achieved through ligand-mediated targeting strategies. For instance, the fusogenic Plofsomes (as mentioned in Section 3.1.2) incorporate DSPE-PEG-Ang, where Ang targets the LRP-1 on BBB endothelial cells and glioblastoma (GBM) (Fig. 5D). Due to the locking effect of the 4-arm PEG-oDP, the fusogenic Plofsomes pass through the BBB endothelial cells via the endocytosis pathway. Then, under the high expression of H_2O_2 in the GBM tissue, the 4-arm PEG-oDP is cleaved and released, and the fusogenic Plofsomes deliver the siRNA to the cytoplasm of GMB cells through the membrane fusion mechanism [131]. Additionally, Tang et al. developed a C-

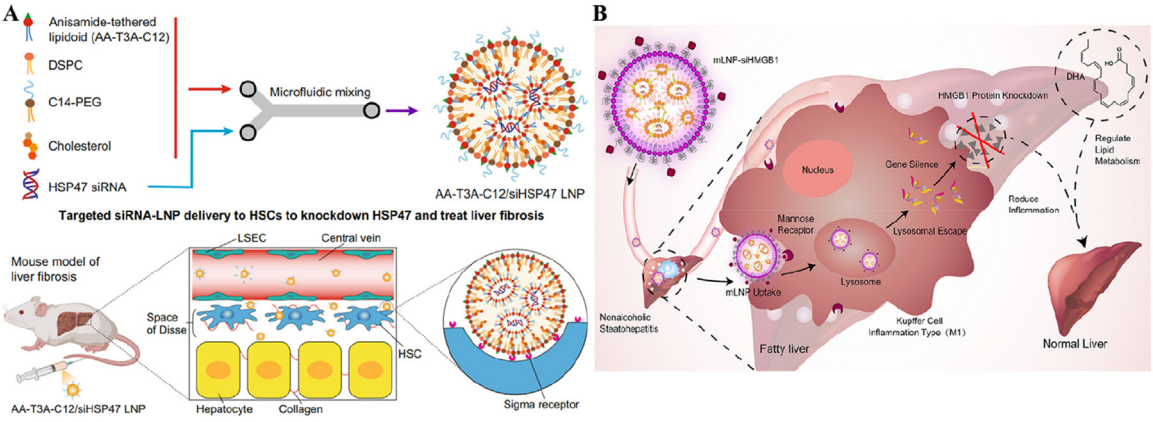


Fig. 9 – (A) Preparation and application of ligand-tethered lipidoid nanoparticles for targeted siRNA delivery to HSCs to treat liver fibrosis. Adapted with permission [186] copyright 2023 Springer Nature; (B) a mannose-modified HMGB1-siRNA loaded stable nucleic acid lipid particle delivery system (mLNP-siHMGB1) was designed for targeting liver macrophages with mannose receptor mediation, thereby silencing HMGB1 protein expression and treating NASH. Adapted with permission [187] copyright 2022 Elsevier.

DP7-ACP-T7-modified DAT-LNP/siRNA system (Fig. 10C). Here, DP7 (a cell-penetrating peptide) mediates both delivery and immune activation, while T7 targets transferrin receptors overexpressed on glioma cells, and triggers endocytosis mediated by the caveolin pathway and the clathrin pathway. This carrier successfully delivered siSlit2 across the BBB and brain-tumor barrier [194]. Another study employed NP-CTX-R10 for siRNA delivery. Chlorotoxin (CTX) binds matrix metalloproteinase-2 (MMP-2) overexpressed on glioma surfaces, enabling tumor targeting. Polyarginine (R10) is a transmembrane peptide that causes endosomal membrane rupture, thereby promoting siRNA escape from endosomes [195].

The BBB constitutes the principal obstacle for intravenous drug delivery in neurological disorder treatment. Beyond developing nanocarriers for receptor-mediated transcytosis across the BBB, alternative administration routes—including non-invasive intranasal delivery or invasive approaches (intrathecal/parenchymal injection)—may be strategically employed. Furthermore, to prevent microglial clearance of nanocarriers, surface modification using "stealth" polymers (e.g., PEG) or anti-phagocytic peptides (e.g., TAT derivatives) can be employed. Alternatively, exosome-encapsulated siRNA nanoparticles may be utilized, capitalizing on endogenous CD47 "don't-eat-me" signals displayed on exosomal membranes to inhibit microglial phagocytosis.

5.4. *Bone targeting*

Bone tissue presents significant delivery challenges due to low vascularity and a dense mineralized matrix that impedes nanoparticle penetration, resulting in inefficient accumulation at skeletal sites. Current strategies predominantly employ targeting peptide modifications to enhance siRNA delivery within bone microenvironments. Cui et al. developed BT-Exo-siShn3, an exosomal system derived from human iPSC-differentiated mesenchymal stem cells (MSCs) and functionalized with the bone-targeting peptide DSPE-PEG-Mal-Cys-SDSSD. Both *in vitro* and *in vivo* studies demonstrated specific osteoblast uptake, targeted bone accumulation, and gene silencing efficacy comparable to Lipofectamine 3000 in osteoblasts, effectively inhibiting osteoclastogenesis [196]. Separately, researchers designed $\alpha v\beta 3$ integrin-targeting cRGD-modified nanomicelles for siM13 delivery (Fig. 10D). Intra-articular injection enhanced siM13 uptake in injured chondrocytes, suppressing MMP13 expression by 72% and attenuating cartilage degradation in osteoarthritis models [197]. Beyond peptides, bone targeting can be achieved through antibody-functionalized nanocarriers leveraging antigen-antibody specificity. Bedingfield et al. created mAbCII-SirNPs: Col2-targeting antibody-coated polymeric nanoparticles delivering siMMP13. These achieved 90% *in vivo* gene silencing and reversed arthritic pathology by sustaining drug retention in joint tissues through antibody-mediated binding [198].

Although hydroxyapatite (HA), a major component of the bone microenvironment, is frequently utilized as a target for directed delivery, conventional drugs or nanocarriers exhibit weak affinity for HA. This limitation causes nonspecific adsorption across the bone matrix, drastically reducing delivery efficiency to target cells (e.g., osteoclasts or bone tumor cells). To address these delivery challenges, engineering carrier materials coupled with bisphosphonates (BPs) is suggested to promote HA binding, thereby enhancing nanoparticle retention and accumulation on bone surfaces.

5.5. *Targeting Spleen, Kidney and Lung*

Current application of siRNA for splenic delivery has shown promising advancements, particularly driven by LNP technology. The emergence of Selective Organ Targeting Lipid Nanoparticles (SORT LNPs) have enabled improved spleen-, kidney- and lung-targeted siRNA delivery [199,200]. Vaidya et al. engineered SORT LNPs (Fig. 11A) that established distinct organ tropism: 18PA-15 LNPs exhibited ~50% splenic accumulation with 57% silencing of the spleen-specific CD31 gene, while DODAP-20 LNPs achieved >80% FVII gene silencing in the liver ($EC_{5n} \approx 0.17$ mg/kg). DOTAP-50 LNPs

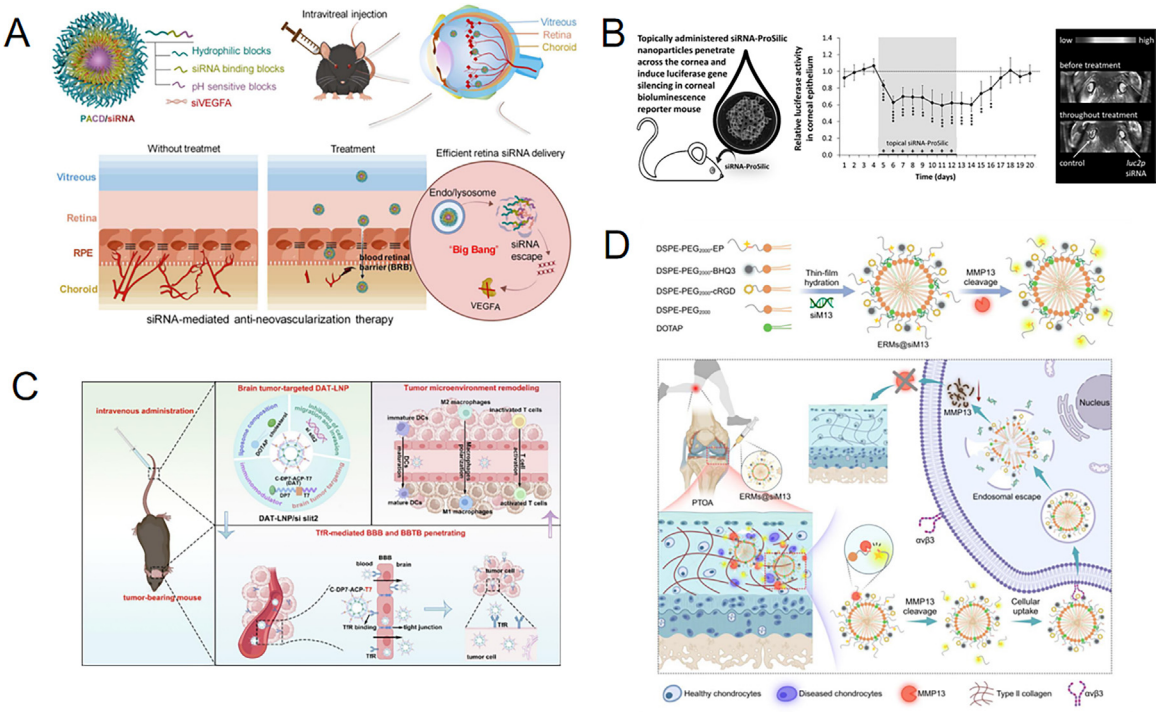


Fig. 10 – (A) Schematic illustration of PACD polymeric micelles enabling efficient siRNA delivery in the retina, abrogating hypoxia-induced angiogenesis. Adapted with permission [189] copyright 2024 Elsevier; (B) Non-viral hybrid silicon–LNP system (ProSilic®), composed of biodegradable silicon nanoparticles (SiNPs), lipids and amino acids, for siRNA complexation and corneal delivery via non-invasive topical administration. Adapted with permission [191] copyright 2020 Elsevier; (C) Cholesterol-DP7-ACP-T7 (C-DP7-ACP-T7)-modified DOTAP liposome (DAT-LNP)/siRNA delivery system addressing three challenges in glioma therapy: enhancing intravenous delivery across BBB and blood-brain tumor barrier (BBTB); enabling brain tumor-targeted nucleic acid delivery [194] copyright 2024 Elsevier; (D) Proposed action mechanism of ERMs@siM13 in the early-stage posttraumatic osteoarthritis (PTOA) mouse model. Adapted with permission [197] copyright 2024 Elsevier.

demonstrated ~60% lung accumulation, though with higher EC_{5n} values than other formulations [201]. Notably, SORT LNPs facilitated prolonged gene editing (>660 d) in lung stem cells *in vivo* [202]. Parallel work by Li et al. developed zwitterionic polymer LNPs (ZP-LNPs) through polycarboxybetaine (PCB)-functionalized 1,2-dimyristoylglycerol (DMG) assembly into DMG-PCB_n (Fig. 11B). Screening identified B4-3@siRNA and B5-1@siRNA with >85% siRNA encapsulation efficiency, enabling liver- and lung-targeted delivery, respectively. *In vivo* fluorescence imaging revealed 3-fold higher hepatic siRNA accumulation (B4-3@Cy5-siRNA vs. P-LNP@Cy5-siRNA) and 15-fold greater pulmonary accumulation (B5-1@Cy5-siRNA). Therapeutic delivery of siTNF-α achieved 68% TNF-α reduction in liver inflammation models and 72% reduction in lung inflammation models, validating ZP-LNPs' potential for organ-specific disease modulation [203].

Despite progress with SORT LNPs, certain organ-specific challenges persist. Spleen targeting struggles to achieve cellular specificity within its heterogeneous immune architecture, often leading to unintended immune activation and silencing in target subsets like marginal zone B cells or red pulp macrophages. Kidney delivery faces dual challenges: glomerular size exclusion limits nanoparticle access to podocytes and mesangial cells, while efficient receptor-mediated efflux (e.g., via megalin-cubilin in proximal tubules) reduces siRNA retention. Lung targeting contends with rapid mucociliary clearance, alveolar macrophage sequestration, and poor penetration through pathological mucus/surfactant layers, limiting epithelial bioavailability. To address these, multicomponent nanoparticles with cell-selective ligands (e.g., CD169 for splenic macrophages) could enhance specificity. Kidney-targeted systems may employ sub-6 nm carriers with near-neutral charge to evade filtration, coupled with competitors of tubular efflux receptors. Lung delivery optimization requires mucus-penetrating designs (e.g., dense PEGylation) combined with macrophage-evasion tactics (CD47 mimetics) and stimuli-responsive release triggered by organ-specific cues (renal proteases, pulmonary redox gradients).

6. Advances in nano-delivery systems to enhance the therapeutic effect of anti-tumor siRNA drugs

6.1. *Direct inhibition of tumor proliferation and metastasis*

Tumor metastasis constitutes a defining hallmark of malignancy, severely complicating clinical management

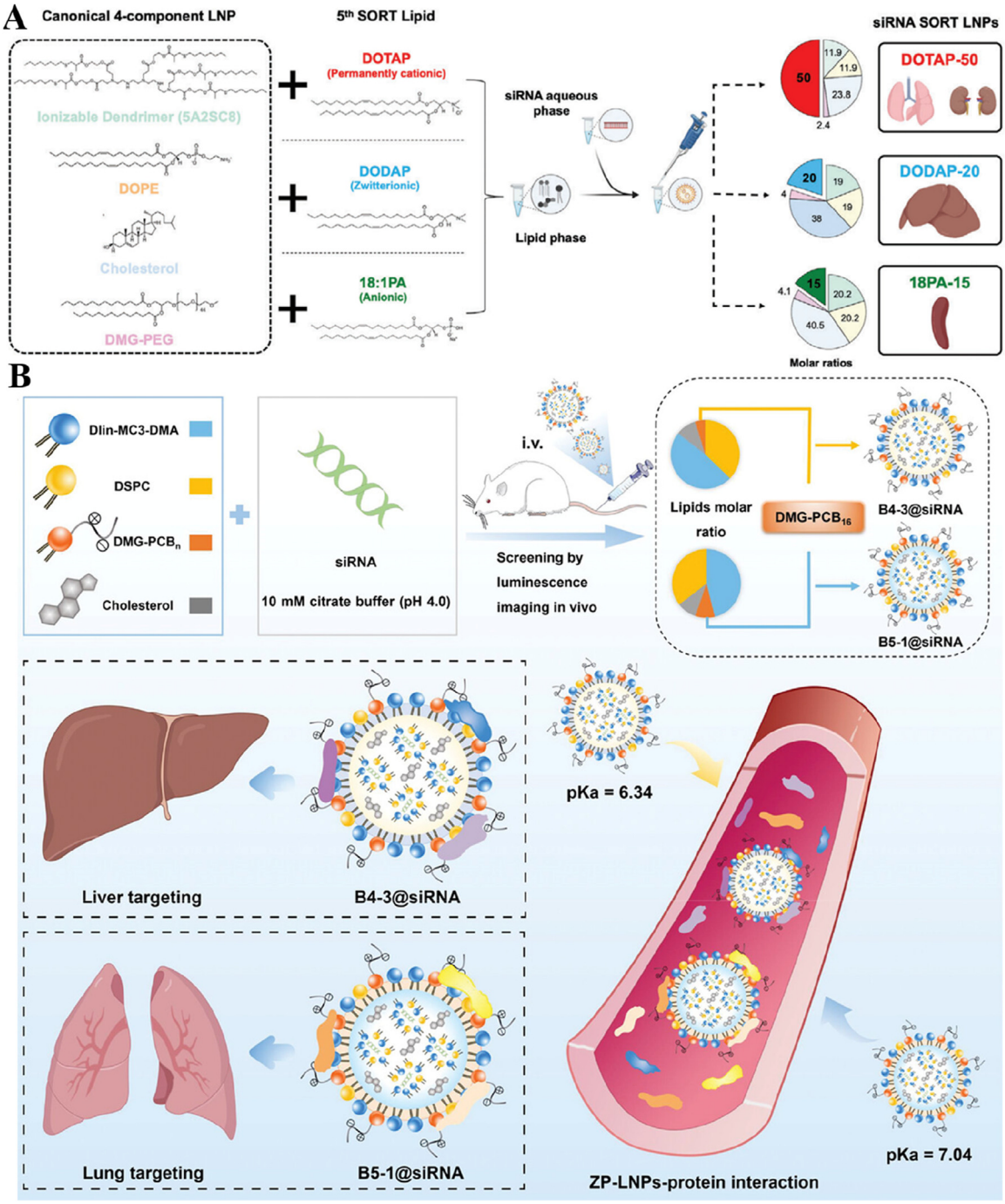


Fig. 11 – (A) Formulation scheme for siRNA SORT LNPs consisting of five lipids. LNPs containing unique SORT lipids delivered functional siRNA to lungs and kidneys (DOTAP-50), liver (DODAP-20), and spleen (18PA-15) in mice. Adapted with permission [201] copyright 2024 John Wiley and Sons; (B) Schematic illustration of the construction and delivery of ZP-LNPs: Synthesis and screening of ZP-LNPs. B4-3@siRNA and B5-1@siRNA target the liver and lung, respectively. Adapted with permission [203] copyright 2025 John Wiley and Sons.

and correlating with higher mortality rates [204–206]. To address this challenge, current research prioritizes developing strategies that simultaneously suppress primary tumor proliferation and metastatic dissemination through pathway-specific interventions. Emerging approaches employ siRNA therapeutics targeting pro-metastatic signaling pathways (e.g., MMP-2, VEGF or EMT-related genes) to disrupt metastatic niches, demonstrating significant preclinical efficacy [207–209].

In advanced non-small cell lung cancer (NSCLC), overexpression of lysophosphatidylcholine acyltransferase 1 (LPCAT1) drives brain metastasis (BM). Jiang et al. engineered

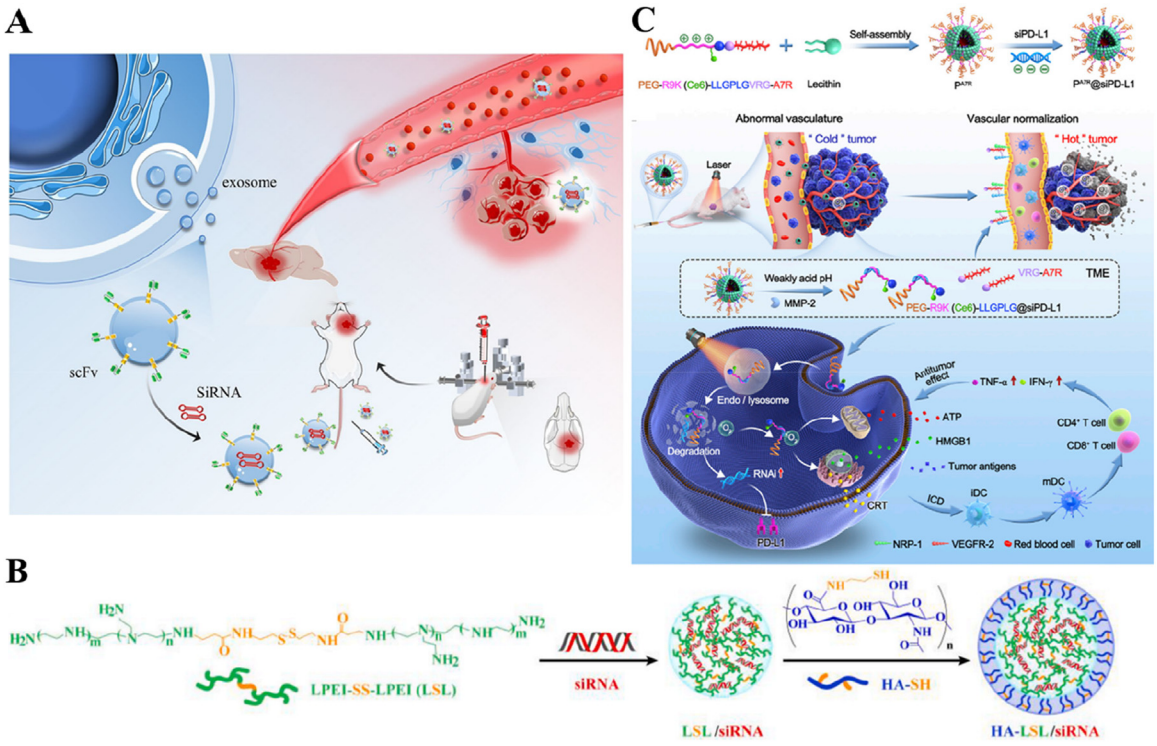


Fig. 12 – (A) Schematic illustration of exo^{scFv} construction, mouse model of lung cancer BM and RNAi treatment. Adapted with permission [210] copyright 2024 Springer Nature; (B) Formation of a dual-targeting nanosystem (HA-LSL/siTGF-β) with HAase and GSH dual-bioresponsive function. Adapted with permission [209] copyright 2023 Elsevier; (C) Illustration of MMP-2-responsive, peptide-assembled micelleplexes for enhanced photoimmunotherapy: composition and self-assembly process of P^{A7R}@siPD-L1, and the antitumor mechanism of P^{A7R}@siPD-L1-mediated photoimmunotherapy. Adapted with permission [211] copyright 2025 Elsevier.

anti-EGFR single-chain variable fragment (scFv)-modified exosomes (exo^{scFv}) (Fig. 12A), which cross the BBB following intravenous administration to deliver LPCAT1 siRNA to metastatic NSCLC lesions. *In vivo* experiments demonstrated a 4.51-fold reduction in bioluminescence intensity of brain metastases compared to the control group at Day 19 ($P < 0.01$). TUNEL assays revealed elevated apoptosis, while Ki67-positive cells decreased by 61.5%, confirming dual suppression of tumor proliferation and metastasis [210]. Yang's group developed an HA-LSL/siTGF-β nanoplatform that releases siTGF-β in response to hyaluronidase and GSH (Fig. 12B). Combined with PD-L1 blockade, this system achieved 79.1% ± 3.2% tumor growth inhibition in orthotopic 4T1/NIH 3T3 tumors by Day 16, extended median survival to 55 d (vs. 35 d in controls), reduced lung metastatic nodules by 82%, and suppressed EMT while enhancing immune-mediated clearance of circulating tumor cells [209]. Similarly, MMP-2-responsive P^{A7R}@siPD-L1 nanomicelles targeting tumor vasculature via A7R peptide-mediated vascular normalization (Fig. 12C) synergized with Ce6 photodynamic therapy to reduce lung metastasis volume by 81%, silence PD-L1 by 72%, elevate ROS levels 3.2-fold, and induce durable antitumor immunity [211]. Collectively, these studies advance metastatic NSCLC treatment through integrated targeting of oncogenic signaling, immune modulation, and vascular remodeling.

6.2. *Inhibition of angiogenesis*

Tumor growth and metastasis are critically dependent on neovascularization, which supplies oxygen and nutrients while facilitating metastatic dissemination [212,213]. Consequently, siRNA-mediated inhibition of tumor angiogenesis has emerged as a prominent anticancer strategy. Targeting angiogenic regulators like VEGF suppresses formation and compromises maintenance of tumor-associated vasculature. In a representative study, Li et al. engineered a dual-functional siRNA complex (TriRGD-siRNA-F) featuring 5'-terminal trimeric cyclic RGD peptides targeting $\alpha v\beta 3$ integrin receptors on neovascular endothelium, while its 3'-terminal trifluoropropyl groups enable direct cytoplasmic delivery via a non-endocytic mechanism. This design bypasses lysosomal degradation while enabling dual silencing of VEGF-A/VEGFR2 genes, significantly inhibiting angiogenesis and inflammatory responses in choroidal neovascularization (CNV) models (Fig. 13A) [207]. Complementary work by Li et al. developed RGD-modified chitosan nanocarriers co-delivering VEGF siRNA and phenethyl isothiocyanate (PEITC), leveraging RGD's affinity for integrin α on tumor vasculature. *In vitro* analyses demonstrated 87.3% inhibition of CD31-DAB-positive areas, 92.9% VEGF suppression, and 90.1% HIF-1α downregulation. In BALB/c nude mouse xenografts, this system achieved

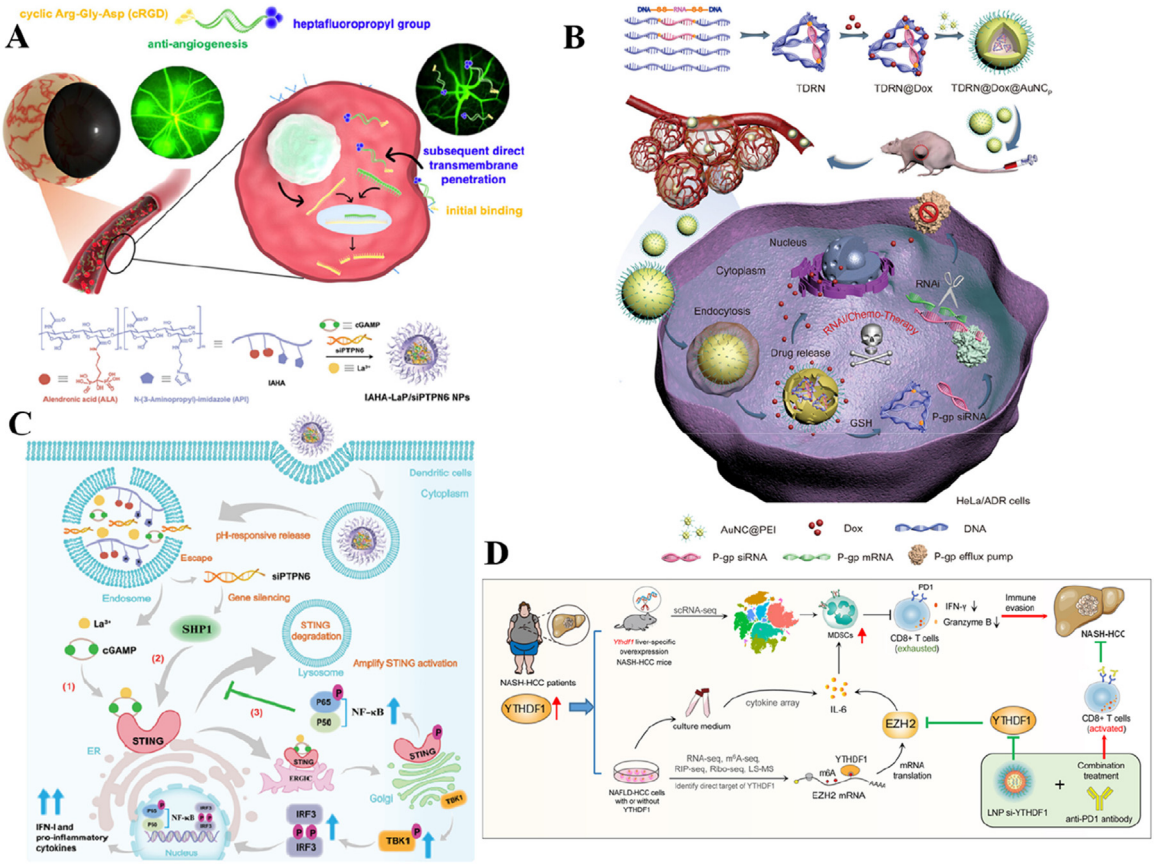


Fig. 13 – (A) Schematic illustration of intracellular delivery of the siRNA conjugate (TriRGD-siRNA-F) for anti-angiogenic therapy targeting CNV. Adapted with permission [207] copyright 2025 American chemical Society; (B) Schematic illustration of programmable tetrahedral DNA-RNA nanocages woven with stimuli-responsive siRNA (TDRN@Dox@AuNCp) for synergistic RNAi/chemotherapy of multidrug-resistant tumors. Adapted with permission [217] copyright 2024 John Wiley and Sons; (C) IAHA-LaP/siPTPN6 NPs amplify STING activation in DCs through three mechanisms [225] copyright 2025 John Wiley and Son; (D) Targeting YTHDF1 using LNP-encapsulated siRNA significantly enhanced the efficacy of anti-PD-1 blockade in a mouse model of NASH-HCC. Adapted with permission [228] copyright 2023 Elsevier.

81.2% tumor growth inhibition after 21 d, significantly suppressing vascular proliferation and tumor progression [214]. Collectively, inhibiting tumor neovascularization disrupts both metabolic support and metastatic routes, establishing RNAi-based vascular targeting as a promising therapeutic paradigm in oncology.

6.3. *Reversal of tumor drug resistance*

Despite advances in anticancer drug development that have enhanced therapeutic efficacy, tumor drug resistance persists as a critical clinical challenge [215,216]. Nano-delivery systems delivering therapeutic siRNA to silence resistance-associated genes have established a pivotal strategy to counteract tumor resistance.

EZH2, a histone methyltransferase, promotes tumor progression by catalyzing H3K27 trimethylation (H3K27me3) to silence tumor suppressor genes and facilitating epithelial-mesenchymal transition (EMT), thereby accelerating metastasis and chemoresistance. To overcome DOX resistance in TNBC, Lu et al. developed the vortex magnetic nanorod system DOX-EZH2si-VMR@CM (Fig. 7B) for co-delivering DOX and siEZH2. This approach blocked EZH2-mediated epigenetic drug resistance pathways, reducing DOX IC_{50} from 0.6798 μg/ml (monotherapy) to 0.1952 μg/ml (combination)—representing a 3.5-fold enhancement in potency [149]. Regarding extrinsic resistance mechanisms, the efflux pump P-glycoprotein (P-gp) critically contributes to multidrug resistance. Researchers engineered stimulus-responsive DNA-RNA tetrahedral nanocages (TDRN; Fig. 13B) co-delivering DOX and P-gp-targeting siRNA (TDRN@Dox@AuNCp). This system achieved 91.0% tumor growth inhibition with 47.4% regression in HeLa/ADR xenografts after five intravenous doses, effectively countering multidrug resistance [217]. Researchers have developed an ATP-responsive nanocarrier based on phenylboronic acid-modified ε-poly-L-lysine (ε-PLL-PBA). The phenylboronic acid (PBA) groups form reversible covalent bonds with cis-diols on siRNA ribose moieties. Within tumor cells (intracellular ATP: 3–5 mM), ATP competitively dissociates these bonds to enable precise siRNA release. This system achieved co-delivery of siP-gp (targeting P-gp efflux pumps) and siBcl-2 (targeting

anti-apoptotic Bcl-2), successfully reversing DOX resistance in breast cancer models [218]. Similarly, ATP-responsive LNPs functionalized with PBA- designated LNP-P/siMITF - have been developed for siMITF delivery, successfully suppressing tumor growth and metastasis in melanoma [219]. Collectively, engineered nano-delivery systems enable precise siRNA targeting of tumor resistance mechanisms, significantly enhancing anti-tumor efficacy and representing a promising approach for developing novel therapeutic strategies.

6.4. *Enhancement of anti-tumor activity of immune cells*

The complex TME contains immunosuppressive cells and cytokines that impair effector T cell function and antigen-presenting cell (APC) activity [220–222]. APCs primarily include B cells, dendritic cells (DCs), and macrophages. These cells capture tumor antigens, process them into peptide-MHC complexes for presentation to T cells, and activate T cells via CD80/CD86 costimulatory molecules. Enhancing APC-mediated antigen uptake and cross-presentation via siRNA-based strategies can amplify antitumor immune responses [223]. For instance, Xu et al. developed a cationic liposome (Lipofectamine 3000)-encapsulated nano-adjuvant carrying RCT-synthesized RNA scaffolds co-loaded with siCD47 and siSIRPα. This system achieves dual CD47-SIRPα checkpoint blockade *in vivo* by overriding "don't eat me" signals, In *in vitro* cell experiments, macrophage (BMDM) phagocytic uptake increased to 20.3% (compared to 14.6% in the control group), while in the B16F10 melanoma model, RIG-I/MDA5 activation induced IFNβ production, leading to increased MHC-I and MHC-II expression on tumor-associated macrophages (TAMs) and DCs, with a concurrent 2.4-fold increase in $CD8^+$ T cell infiltration into tumors [224]. Similarly, Wang et al. developed a nanodrug (Fig. 13C) designed to triple-amplify STING signaling in DCs. This system integrates three components: siPTPN6, cGAMP (a STING agonist), and lanthanum phosphate (LaP) nanoparticles, which were co-precipitated and encapsulated within an IAHA polymer shell—a modified hyaluronic acid grafted with N-(3-aminopropyl)-imidazole (API) and alendronate. LaP-siRNA/cGAMP promotes $CD11c^+$ DC maturation through a triple mechanism: (1) La^{3+} boosts cGAMP-mediated STING phosphorylation, activating the TBK1-IRF3-NF-κB axis. (2) siRNA silences SHP-1 protein, enhancing K63-linked ubiquitination of STING; (3) Activated NF-κB blocks STING lysosomal degradation. This synergistic action significantly increases secretion of proinflammatory cytokines: IFNβ (2.4-fold), TNFα (1.4-fold), and IL-6 (1.5-fold) [225].

Tumor cells often evade immune surveillance by suppressing CTL activity through immune checkpoint pathways . Current RNAi therapies restoring cytotoxic T lymphocyte (CTL) function primarily deliver siRNA to directly block the PD-1/PD-L1 immune checkpoint [226,227]. Other strategies target complementary immunosuppressive pathways, creating synergy with PD-1 inhibitors. YTHDF1, an RNA reader protein recognizing N6-methyladenosine (m6A) modifications, is significantly over expressed in tumor tissues of NASH-HCC patients and mouse models. Wang et al. demonstrated that it recruits MDSCs and suppresses $CD8^+$ T cells by activating the EZH2-IL-6 signaling axis. The team delivered chemically modified siYTHDF1 to liver tumor cells via LNPs, effectively blocking EZH2-IL-6 signaling. Tumor cell IL-6 secretion and MDSC ($CD11b^+Gr\text{-}1^+$) numbers were significantly reduced, while the proportion of Granzyme B^+/IFN-γ^+ $CD8^+$ T cells increased (Fig. 13D) [228].

7. Combined application of siRNA

7.1. *Combined application with other gene therapies*

Beyond RNAi therapeutics, both DNA-based (e.g., plasmid DNA, viral vectors) and mRNA-based platforms demonstrate significant therapeutic potential by delivering nucleic acid sequences that encode therapeutic genes. This enables endogenous production of functional proteins to either tag tumor cells for enhanced immune recognition or directly augment activation, homing, and cytotoxic capabilities of immune cells such as T cells and NK cells [229–231]. Co-delivering RNAi therapeutics like siRNA with DNA- or mRNA-based therapies provides a multi-targeted strategy to simultaneously engage tumor cells and their microenvironment across multiple dimensions, thereby enhancing anti-tumor efficacy. Alex G. Hamilton et al. utilized LNPs to co-deliver CAR mRNA and PD-1 siRNA, achieving dual T cell engineering. This approach simultaneously enhanced tumor-targeting specificity through CAR expression while reversing microenvironmental immunosuppression via PD-1 knockdown, synergistically improving CAR-T efficacy with ensured safety for solid tumors [232]. Separately, researchers demonstrated profound HBV suppression and immune control in animal models through co-delivery of siHBV (targeting viral replication) and mIL-2 mRNA (activating immunity), presenting a promising strategy for functional cure of hepatitis B [233].

7.2. *Combined application with chemotherapy*

Chemotherapeutic agents remain cornerstone cancer treatments. Beyond overcoming resistance, co-delivering siRNA with chemotherapeutics offers two key advantages: (1) TME Remodeling: siRNA targeting immunosuppressive (TGF-β, PD-L1, CSF-1R) or angiogenic factors (VEGF) reverses immunosuppression, enhances chemosensitivity, improves drug penetration, and creates synergistic microenvironments [227,234]. (2) Pharmacokinetic optimization: Nanocarriers improve therapeutic profiles—extending half-lives, enhancing tumor accumulation via EPR/active targeting, promoting cellular uptake, and reducing systemic toxicity [235,236]. Chen et al. developed an amphiphilic polymer-based nanocarrier (PMBOP-CP) for the co-delivery of the chemotherapeutic prodrug FuOXP (a 5-fluorouracil-oxaliplatin conjugate) and Xkr8-targeting siRNA (siXkr8), a phospholipid scramblase. This delivery system significantly enhanced pharmacokinetic parameters: the half-life of FuOXP was extended 16-fold, while tumor platinum concentration reached 10.9 times that of the free drug. Crucially, siXkr8 blocked chemotherapy-induced phosphatidylserine (PS) externalization, thereby disrupting the "don't eat me" signal to macrophages. This

intervention reversed immunosuppression within the TME, increasing the M1/M2 macrophage ratio [237].

7.3. *Combined application of RNAi with photothermal and radiotherapy*

The synergistic integration of siRNA with PTT or radiotherapy addresses fundamental limitations of conventional physical treatments through distinct yet complementary mechanisms. At the molecular level, siRNA targeting HSP90/HSP70 silences the HSF1-SIRT1 regulatory axis. This significantly reduces tumor thermal tolerance thresholds, enabling irreversible damage at 42–45 °C while enhancing photothermal conversion efficiency [238]; Furthermore, siRNA precisely inhibits key factors within the DNA damage repair pathway, prolonging the retention of DNA damage markers and markedly enhancing tumor cell radiosensitivity [239,240]. Immunologically, photothermal/radiation-induced ICD releases tumor antigens, while siRNA suppression of anti-apoptotic genes (Bcl-2/Bcl-xL) further enhances calreticulin (CRT) and HMGB1 exposure to boost DC antigen presentation—simultaneously, inhibition of immunosuppressive pathways (PD-L1/TGF-β/IDO) reverses Treg expansion and M2 macrophage polarization, promoting $CD8^+$ T-cell infiltration and synergistically enhancing antitumor immunity [150,241,242]. At the delivery level, specialized carriers (e.g., gold nanoshell/hafnia composites) leverage photothermal and radiation-responsive properties for spatiotemporally controlled siRNA release. Near-infrared (NIR) laser-triggered localized hyperthermia enhances the EPR effect, promoting tumor-targeted accumulation. Concurrent X-ray activation enables precise siRNA release from the carrier. Real-time monitoring of treatment response is facilitated via photoacoustic and MRI imaging. This establishes an integrated therapeutic framework extending from local tumor eradication to systemic immune surveillance [243–247].

8. *Ex vivo* applications of siRNA

Beyond its established role in *ex vivo* gene silencing, current *ex vitro* siRNA applications now span multiple disciplines, encompassing several key areas: (1) For Organ Transplantation and Preservation: Targeted siRNA delivery against inflammatory factors inhibits endothelial cell activation, mitigating post-transplant immune rejection, while silencing ischemia-reperfusion injury-related genes extends organ survival [248–251]. (2) For molecular mechanism research at the cellular level: siRNA technology facilitates precise silencing of specific genes. It is utilized in *ex vitro* cellular systems to investigate gene function (e.g., observing resulting phenotypic and functional alterations in cell growth, differentiation, and apoptosis), dissect signaling pathways (e.g., silencing critical pathway regulators like those in the Wnt/β-catenin or Notch pathways, combined with single-cell sequencing to decode downstream transcriptional consequences and regulatory mechanisms), and engineer cell models with targeted gene deficiencies. This provides an essential tool for elucidating molecular mechanisms and advancing drug development [232,252,253]. (3) For viral pathogenesis studies: Designing siRNAs targeting viral genes to silence critical viral factors in *ex vivo* systems constitutes a cornerstone approach for investigating viral replication cycles, host-pathogen interactions, and pathogenesis mechanisms. For instance, researchers delivered siHBV, which effectively silenced pgRNA and HBx RNA derived from cccDNA, thereby blocking viral transcription, inhibiting viral replication, and synergizing with other drugs for the treatment of chronic hepatitis B [254].

9. Summary and outlook

RNAi therapy has demonstrated significant clinical progress, evidenced by the approval of seven siRNA drugs for therapeutic use. However, no siRNA therapeutics have been approved for marketing against oncological indications. Delivering siRNA therapeutics to silence tumor-associated targets—such as those involved in tumor proliferation, metastasis, angiogenesis, and chemoresistance—offers a strategy for precise tumor cell eradication. Ensuring siRNA safety remains a critical priority in drug development, as safety concerns have halted numerous candidates during clinical trials. Furthermore, enhancing therapeutic efficacy and achieving tumor-specific delivery represent pivotal challenges. Given the historical trajectory of siRNA drug development and the persistent challenges encountered in its application, particularly concerning safety and targeted delivery, this review focuses on nano-delivery systems for antitumor siRNA drugs. It systematically examines how these systems enhance siRNA stability, safety, and targeting capabilities, and explores how combining them with immunotherapy and other modalities can improve antitumor efficacy. This review aims to provide insights to support the development and clinical translation of siRNA drugs.

Currently, siRNA drugs represent an active area of research in antitumor therapy. Nevertheless, realizing their industrialization and advancing more siRNA-based antitumor drugs through clinical trials to market approval will require concerted efforts across multiple domains. In vector development, the most notable progress for siRNA drugs has occurred in liver-targeted delivery, facilitated by specific hepatic cell receptors and enhanced targeting via GalNAc conjugation. However, delivering siRNA to organs such as the lung, kidney, and brain presents greater challenges due to complex anatomical structures and stringent size requirements for therapeutic agents. Developing vectors for these organs necessitates a comprehensive evaluation of safety, efficacy, quality, and manufacturing feasibility. Optimizing vector physicochemical properties to better protect siRNA and reduce immunogenicity is crucial. Incorporating targeting ligands or chemical moieties can improve tumor cell-specific accumulation and cellular uptake, thereby enhancing delivery efficiency. In preclinical toxicity assessment, current models exhibit limitations in accurately predicting human responses; approximately 30% of siRNA candidate drugs are discontinued in clinical trials due to unexpected toxicity. There is an urgent need to develop more predictive preclinical toxicity assessment models. This

requires integrating multiple factors, including physiological differences across species and *in vivo* drug metabolic pathways, to enable more precise safety evaluations and mitigate clinical trial risks.

Despite the numerous challenges in the clinical translation of siRNA-based antitumor drugs, we are confident they can be overcome. Progress in novel siRNA chemical modifications, improved conjugation methods, AI-assisted carrier design, and the discovery of organ-specific receptors holds significant promise. Such advancements could enable the development of more siRNA drugs with specific targeting capabilities for different organs, applicable not only to tumors but also to other diseases, thereby driving new breakthroughs in this field.

Conflicts of interest

The authors declare that there is no conflicts of interest.

CRediT authorship contribution statement

Xiaoxin Yan: Writing – original draft. **Jinhu Liu:** Writing – original draft. **Jie Liu:** Supervision. **Suyun Wei:** Investigation. **Ruixin Li:** Supervision. **Weiwei Mu:** Supervision. **Na Zhang:** Writing – review & editing. **Yongjun Liu:** Writing – review & editing, Validation, Supervision.

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