REVIEW

# A Comprehensive Review of Small Interfering RNAs (siRNAs): Mechanism, Therapeutic Targets, and Delivery Strategies for Cancer Therapy

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**Abstract:** Small interfering RNA (siRNA) delivery by nanocarriers has been identified as a promising strategy in the study and treatment of cancer. Short nucleotide sequences are synthesized exogenously to create siRNA, which triggers RNA interference (RNAi) in cells and silences target gene expression in a sequence-specific way. As a nucleic acid-based medicine that has gained popularity recently, siRNA exhibits novel potential for the treatment of cancer. However, there are still many obstacles to overcome before clinical siRNA delivery devices can be developed. In this review, we discuss prospective targets for siRNA drug design, explain siRNA drug properties and benefits, and give an overview of the current clinical siRNA therapeutics for the treatment of cancer. Additionally, we introduce the siRNA chemical modifications and delivery systems that are clinically sophisticated and classify bioresponsive materials for siRNA release in a methodical manner. This review will serve as a reference for researchers in developing more precise and efficient targeted delivery systems, promoting ongoing advances in clinical applications.

**Keywords:** small interfering RNA, cancer, chemical modifications, delivery systems, bioresponsive materials

## Introduction

Cancer, also called malignancy, is a group of diseases caused by the rapid multiplication and spread of malignant cells.[1,2] According to statistics from the National Cancer Center, more than 100 types of cancer have been identified.[3] The ability of malignant tumor cells to metastasize via the blood-lymphatic system and subsequent proliferation to invade numerous tissues and organs throughout the body complicates the treatment of ordinary surgical resection, which is the main reason for the high mortality rate in cancer.[4–6]

Traditional cancer therapeutics, including surgical resection, radiotherapy, and chemotherapy, are widely employed.[7,8] Surgical resection utilizes advanced surgical instruments and imaging techniques to accurately remove non-metastatic tumor tissue.[9,10] However, it struggles to eliminate metastatic tumor cells. Radiotherapy, often used as an adjunctive treatment prior to surgery, effectively eradicates tumor cells using high-energy radiation such as X or γ, with the aid of CT imaging techniques.[11] Chemotherapy primarily combats tumors by impeding cell proliferation through the use of drugs. Nonetheless, it has notable side effects on normal proliferating cells and may induce drug resistance.[12,13] To address the limitations of conventional therapies, various novel cancer treatments have been developed, such as tumor immunotherapy.[14–17] Monoclonal antibody-based therapies targeting immune cells and pericyte therapy have proven to be highly effective approaches.[17,18] Immune checkpoint blockade (ICB) therapies have made remarkable strides in cancer treatment, notably in melanoma[19,20] and non-small cell lung cancer.[21] Relay cell therapy involving the transfusion of chimeric antigen receptor T cells (CAR-T) has demonstrated efficacy in treating a range of chemotherapy-resistant

**Graphical Abstract**



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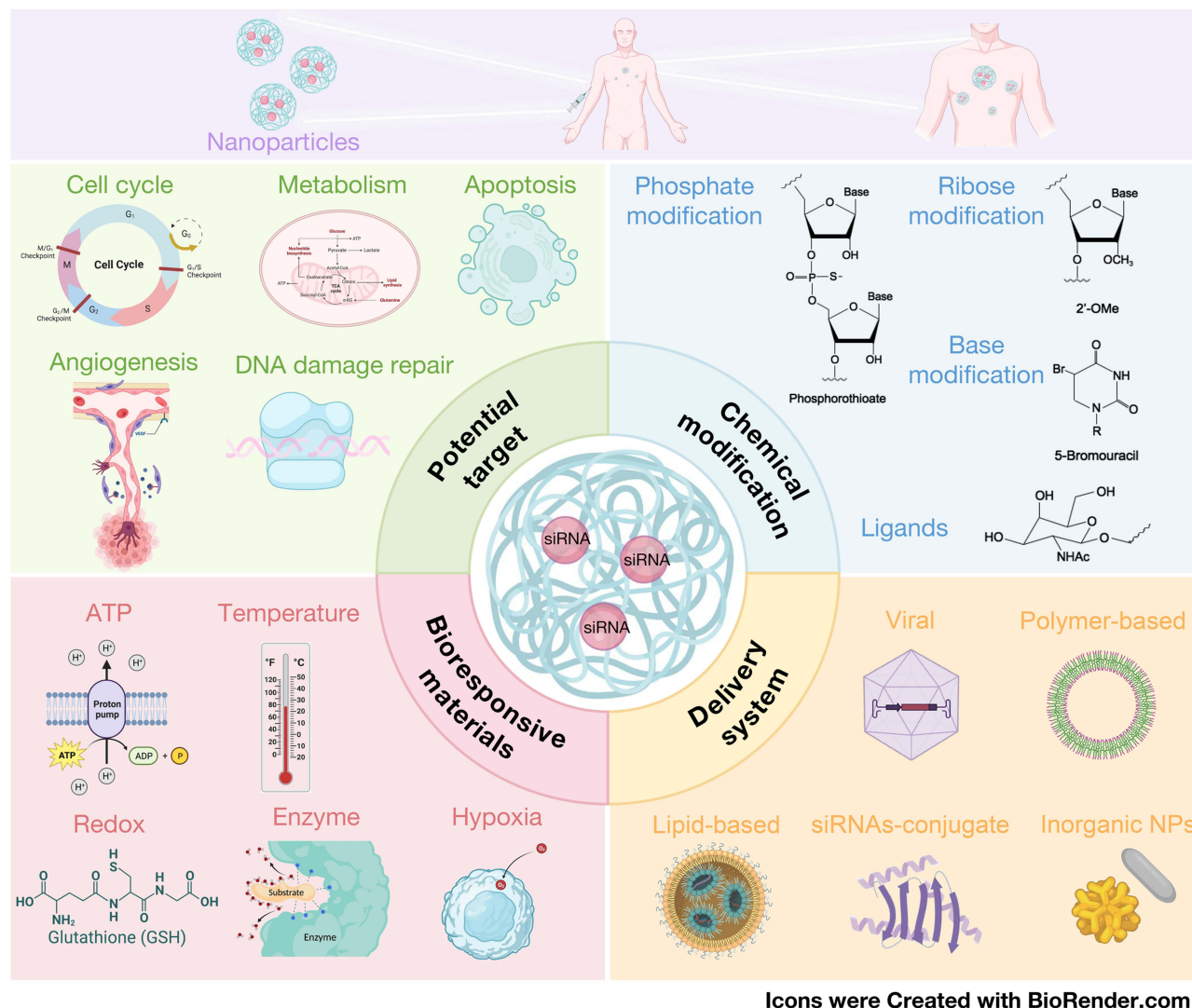
leukemias. However, activated CAR-T cells can undergo division and proliferation, triggering a cytokine storm and leading to severe side effects, including fatalities.[22–25]

The introduction of precision medicine in 2015 marked a significant milestone in the field of healthcare, facilitated by advancements in genome sequencing and bioinformatics technology.[26,27] This approach involves sequencing and analyzing the genomes of cancer patients to identify causative genes, thereby enabling the development of personalized cancer treatments for individuals. This personalized approach maximizes cancer treatment efficacy while minimizing toxic side effects.[28,29] A promising avenue within precision medicine is the use of small interfering RNA (siRNA), a nucleic acid-based drug that holds potential for disease treatment by selectively silencing disease-related genes through sequence-specific binding.[30–32] Notably, nucleic acid synthesis technology has enabled the precise, rapid, and cost-effective production of siRNA, with lower costs compared to small molecule and antibody drugs.[33,34] In addition, the design of siRNA sequences allows for the targeting of previously considered undruggable genetic loci, overcoming a major challenge in personalized therapy.[35] As a result, the siRNA modality has garnered significant attention due to its shorter research and development timeline, broader therapeutic scope, and versatility. In 2018, US Food and Drug

Administration (FDA) approved the first siRNA therapeutic, ONPATTRO™ (Patisiran) (Alnylam Pharmaceuticals, Inc.), for the treatment of transthyretin-mediated amyloidosis.[36,37] Currently, siRNA-based drugs are being utilized in the treatment of various diseases, including viral infections,[38,39] genetic disorders,[40–42] cardiovascular disease,[43,44] and cancer.[45–47] To date, four siRNA therapeutics have been approved by FDA, and more than 20 siRNA therapeutics have entered clinical trials[48] (Table 1).

Despite the potential of siRNAs in drug development, there are several extracellular and intracellular barriers that limit their application in vivo,[49,50] especially in systemic delivery. The extracellular barriers include degradation of naked siRNAs by endogenous nucleases in serum,[51] rapid renal clearance due to their small size and molecular weight,[52] activation of the innate immune system triggered by siRNAs through Toll-like receptors (TLR)-dependent or TLR-independent pathways,[53,54] plasma protein sequestration and entrapment by the reticuloendothelial system (RES),[55–57] and membrane impermeability due to their negative charge and high hydrophilicity.[58] The intracellular barriers mainly include endosomal entrapment[59,60] and off-target effects.[61] To overcome these barriers, chemical modifications and/or delivery systems are essential to enhance siRNA bioavailability in target regions and therapeutic efficacy. Various chemical modifications have been extensively investigated and successfully employed to enhance the properties of siRNAs, including stability improvement, reduction of off-target effects, and mitigation of immunogenicity.[62–64] These modifications can be categorized based on the specific sites of modification within the siRNA duplex. Examples include backbone modification,[65] ribose modification,[66,67] base modification,[68,69] and terminal modification.[70] When designing siRNAs, it is crucial to consider the modification type, position, and their impact on the charge of siRNA to ensure optimal gene silencing efficiency without compromising its effectiveness.[71] Efficient delivery systems play a crucial role in delivering siRNAs to specific locations and facilitating cellular uptake, thereby enhancing their in vivo bioavailability. A diverse range of delivery strategies have been developed and widely utilized for siRNAs transportation, such as viral vectors,[72–74] polymer-based delivery systems,[75–77] lipid-based delivery systems,[78,79] and conjugate delivery systems.[80,81] Moreover, to exert the gene silencing function, siRNAs must be dissociated from the delivery vector and exist freely in the cytoplasm.[82] Various bioresponsive materials have been employed to control siRNAs release under different conditions.[83,84]

Cancer cells possess a multitude of altered features compared to normal cells, offering numerous molecular targets for cancer therapy. Theoretically, siRNAs are capable of silencing all disease-related genes, including those associated with cancer. Therefore, siRNAs hold great promise for cancer treatment. This review aims to provide an overview of the mechanism of siRNA based on RNA interference (RNAi), highlight the advantages of siRNA in drug development, and explore potential targets for cancer therapy using siRNA therapeutics. Additionally, we summarize various chemical modifications, delivery systems, and bioresponsive materials utilized to enhance the properties of siRNA, improve gene silencing efficiency, and facilitate clinical application. Furthermore, we discuss ongoing clinical studies, potential challenges, and future prospect of siRNA therapeutics in cancer therapy.

# siRNA and Potential Targets for Cancer Therapy
## Mechanism of RNA Interference (RNAi)

RNAi is an important defense mechanism for eukaryotic cells, capable of degrading exogenously invading genetic material such as viruses.[111] The mechanism of RNAi involves two main types: microRNAs (miRNAs) and small interfering RNAs (siRNAs), which are crucial for gene regulation. miRNAs are transcribed from endogenous genes, and their primary transcripts (pri-miRNA) are processed in the cell nucleus by Drosha and DGCR8 to form precursor miRNA (pre-miRNA). After being transported to the cytoplasm by Exportin 5, pre-miRNA is further processed by an enzyme called Dicer to remove the hairpin structure. The processed pre-miRNA is then loaded into the RNA-induced silencing complex (RISC) that comprises the Argonaute 1–4 (Ago1–Ago4) protein and separates from the complementary RNA strand. The miRNA-RISC complex binds to the 3' untranslated region (UTR) of the target mRNA in an imperfectly complementary manner, ultimately leading to mRNA degradation or inhibition of mRNA translation.[112] Conversely, siRNA-mediated RNAi initiates with the introduction of double-stranded RNA (dsRNA) into the cell. This dsRNA can originate from diverse sources, including exogenous genetic material such as viruses or synthetic siRNAs. Upon entry, the dsRNA is recognized and processed by Dicer, which cleaves the dsRNA

**Table 1** siRNA-Based Cancer Therapeutics in Clinical and Preclinical Trials

| Therapeutics | Target | Carrier/Delivery Route | Disease/Clinical Outcome | Sponsor/Collaborators | Clinical Trial Status | Ref. |
|---|---|---|---|---|---|---|
| **Liver Cancer** | | | | | | |
| TKM-080301 | PLK1 | SNALP/IV | Hepatocellular carcinoma | Arbutus Biopharma Corporation | NCT02191878 (Phase I/II) | [85] |
| DCR-MYC | MYC | LNP/IV | Hepatocellular carcinoma | Dicerna Pharmaceuticals, Inc | NCT02314052 (Phase I/II) | [86] |
| **Colorectal Cancer** | | | | | | |
| APN401 | CBLB | Naked siRNA | Colorectal cancer | Wake Forest University Health Sciences, National Cancer Institute (NCI) | NCT03087591, (Phase I) | [87] |
| TKM-080301 | PLK1 | SNALP | Colorectal cancer with hepatic metastases | National Cancer Institute (NCI), National Institutes of Health Clinical Center (CC) | NCT01437007 (Phase I) | [88] |
| NBF-006 | GSTP | LNP/IV | Colorectal cancer | Nitto BioPharma, Inc | NCT03819387 (Phase I) | [89] |
| **Pancreatic Cancer** | | | | | | |
| siG12D LODER | KRAS G12D | LODER Polymer/EUS biopsy needle | Pancreatic cancer | Silenseed Ltd. | NCT01676259, NCT01188785 (Phase I/II) | [90,91] |
| Exosomes with KRAS G12D siRNA | KRAS G12D | iExosomes/IV | Metastatic pancreatic adenocarcinoma, Pancreatic ductal adenocarcinoma | M.D. Anderson Cancer Center | NCT03608631 (Phase I) | [92,93] |
| Atu027 | PKN3 | Cationic lipoplex/IV | Pancreatic ductal | Silence Therapeutics GmbH, Granzer Regulatory Consulting & Services | NCT01808638 (Phase I/II) | [94] |
| DCR-MYC | MYC | LNP/IV | Pancreatic neuroendocrine tumors | Dicerna Pharmaceuticals, Inc | NCT02110563 (Phase I) | [86] |
| APN401 | E3 ubiquitin | IV | Pancreatic cancer | Wake Forest University Health Sciences, National Cancer Institute (NCI) | NCT02166255 (Phase I) | [87] |
| NBF-006 | GSTP | LNP/IV | Colorectal cancer | Nitto BioPharma, Inc | NCT03819387 (Phase I) | [89] |
| TKM-080301 | PLK1 | SNALP | Pancreas cancer with hepatic metastases | National Cancer Institute (NCI), National Institutes of Health Clinical Center (CC) | NCT01437007 (Phase I) | [88] |
| **Renal Cell Cancer** | | | | | | |
| APN401 | E3 ubiquitin | IV | Renal cell cancer | Wake Forest University Health Sciences, National Cancer Institute (NCI) | NCT02166255 (Phase I) | [87] |
| ARO-HIF-2 | HIF-2$\alpha$ | TRiM (RGD-siRNA conjugate) | Clear cell renal cell carcinoma | Arrowhead Pharmaceuticals | NCT04169711 (Phase I) | [95] |
| **Prostate Cancer** | | | | | | |
| PNT2258 (ss DNA)* | BCL2 | Lipid (non-targeted) | Prostate cancer | Sierra Oncology, Inc | NCT01191775 (Phase I) | [96] |
| si-PT-LODER | HSP90 | Polymeric matrix (LODER polymer) | Prostate cancer | Silenseed Ltd. | Preclinical | [30] |

| **Neuroendocrine/Brain Tumors** | | | | | | |
|---|---|---|---|---|---|---|
| TKM-080301 | PLK1 | SNALP | Neuroendocrine tumors | Arbutus Biopharma Corporation | NCT01262235 (Phase I/II) | [97] |
| NU-0129 | Bcl2L12 | Gold NP/IV | Gliosarcoma, Recurrent glioblastoma | Northwestern University, National Cancer Institute (NCI) | NCT03020017 (Early Phase I) | [98,99] |
| **Melanoma** | | | | | | |
| PNT2258 (single-stranded DNA)* | BCL2 | Lipid (non-targeted) | Melanoma | Sierra Oncology, Inc | NCT01191775 (Phase I) | [96] |
| NCT00672542 | LMP2, LMP7, MECL1 | NC | Metastatic melanoma | Scott Pruitt, Duke University | NCT00672542 (Phase I) | [100] |
| **Gastric Cancer** | | | | | | |
| TKM-080301 | PLK1 | SNALP | Gastric cancer with hepatic metastases | National Cancer Institute (NCI), National Institutes of Health Clinical Center (CC) | NCT01437007 (Phase I) | [88] |
| **Leukemia** | | | | | | |
| SPC2996 | Bcl-2 | IV | Chronic lymphocytic leukemia | Santaris Pharma A/S | NCT00285103 (Phase I/II) | [101] |
| **Myeloma** | | | | | | |
| SNS01-T | eIF5A$^{K50R}$ plasmid eIF5A | Polyethylenimine (non-targeted) | Multiple myeloma | Senesco Technologies, Inc. | NCT01435720 (Phase I/II) | [102] |
| DCR-MYC | MYC | LNP/IV | Multiple myeloma | Dicerna Pharmaceuticals, Inc | NCT02110563 (Phase I) | [86] |
| **Non-Small Cell Lung Cancer** | | | | | | |
| NBF-006 | GSTP | LNP/IV | Colorectal cancer | Nitto BioPharma, Inc | NCT03819387 (Phase I) | [89] |
| **Breast Cancer** | | | | | | |
| TKM-080301 | PLK1 | SNALP | Breast cancer with hepatic metastases | National Cancer Institute (NCI), National Institutes of Health Clinical Center (CC) | NCT01437007 (Phase I) | [88] |

(*Continued*)

**Table 1** (Continued).

| Therapeutics | Target | Carrier/Delivery Route | Disease/Clinical Outcome | Sponsor/Collaborators | Clinical Trial Status | Ref. |
|---|---|---|---|---|---|---|
| **Lymphoma** | | | | | | |
| CpG-STAT3 siRNA (CAS3/SS3) | STAT3 | CpG oligodeoxynucleotide | B-cell non-Hodgkin lymphoma | City of Hope Medical Center, National Cancer Institute (NCI) | NCT04995536 (Phase I) | [103] |
| PNT2258 (single-stranded DNA)* | BCL2 | Lipid (non-targeted) | Lymphoma, Non-Hodgkin's lymphoma, Diffuse large B-cell lymphoma | Sierra Oncology, Inc | NCT01191775 (Phase I) NCT01733238, NCT02226965 (Phase II) | [96,104] |
| SNS01-T | eIF5A$^{K50R}$ plasmid eIF5A | Polyethylenimine (non-targeted) | Mantle cell lymphoma in relapse, Diffuse large B-cell lymphoma in relapse | Senesco Technologies, Inc. | NCT01435720 (Phase I/II) | [102] |
| DCR-MYC | MYC | LNP/IV | Non-Hodgkin's lymphoma | Dicerna Pharmaceuticals, Inc | NCT02110563 (Phase I) | [86] |
| **Prostatic Cancer** | | | | | | |
| SXL01 | AR | SC | Prostatic cancer, Castration-resistant | Institut Claudius Regaud | NCT02866916 (Phase I) | [30] |
| **Ovarian Cancer** | | | | | | |
| TKM-080301 | PLK1 | SNALP | Ovarian cancer with hepatic metastases | National Cancer Institute (NCI), National Institutes of Health Clinical Center (CC) | NCT01437007 (Phase I) | [88] |
| **Solid Tumors** | | | | | | |
| ALN-VSP02 | KSP, VEGF | SNALP/IV | Solid tumors | Alnylam Pharmaceuticals | NCT00882180, NCT01158079 (Phase I) | [105] |
| CALAA-01 | RRM2 | AD-PEG-Au nanoparticles/IV | Solid tumors | Calando Pharmaceuticals | NCT00689065 (Phase I) | [106] |
| Atu027 | PKN3 | Naked siRNA/IV | Advanced solid tumors, | Silence Therapeutics GmbH, Granzer Regulatory Consulting & Services | NCT00938574 (Phase I) | [107] |
| DCR-MYC | MYC | LNP/IV | Solid tumors | Dicerna Pharmaceuticals, Inc | NCT02110563 (Phase I) | [86] |
| **Advanced Cancers** | | | | | | |
| siRNA-EphA2-DOPC | EphA2 | DOPC LNP/IV | Advanced malignant solid neoplasm | M.D. Anderson Cancer Center, Gateway for Cancer Research, Institutional Funding for Federally Supported Clinical Trials (IFSCT), National Cancer Institute (NCI) | NCT01591356 (Phase I) | [108–110] |

**Note**: *PNT2258 do not contain siRNA.
**Abbreviations**: LNP, lipid nanoparticles; IV, intravenous injection; EUS biopsy Needle, endoscopic ultrasound biopsy Needle; N/A, not available; NC, no carrier; SC, subcutaneous injection; EphA2, EPH receptor A2 (ephrin type-A receptor 2); DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; BCL2L12, B cell lymphoma 2-like protein 12; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; Cbl-b, casitas-B-lineage lymphoma protein-b; HIF-2α, hypoxia-inducible factor-2α; ssDNA, single-stranded DNA.
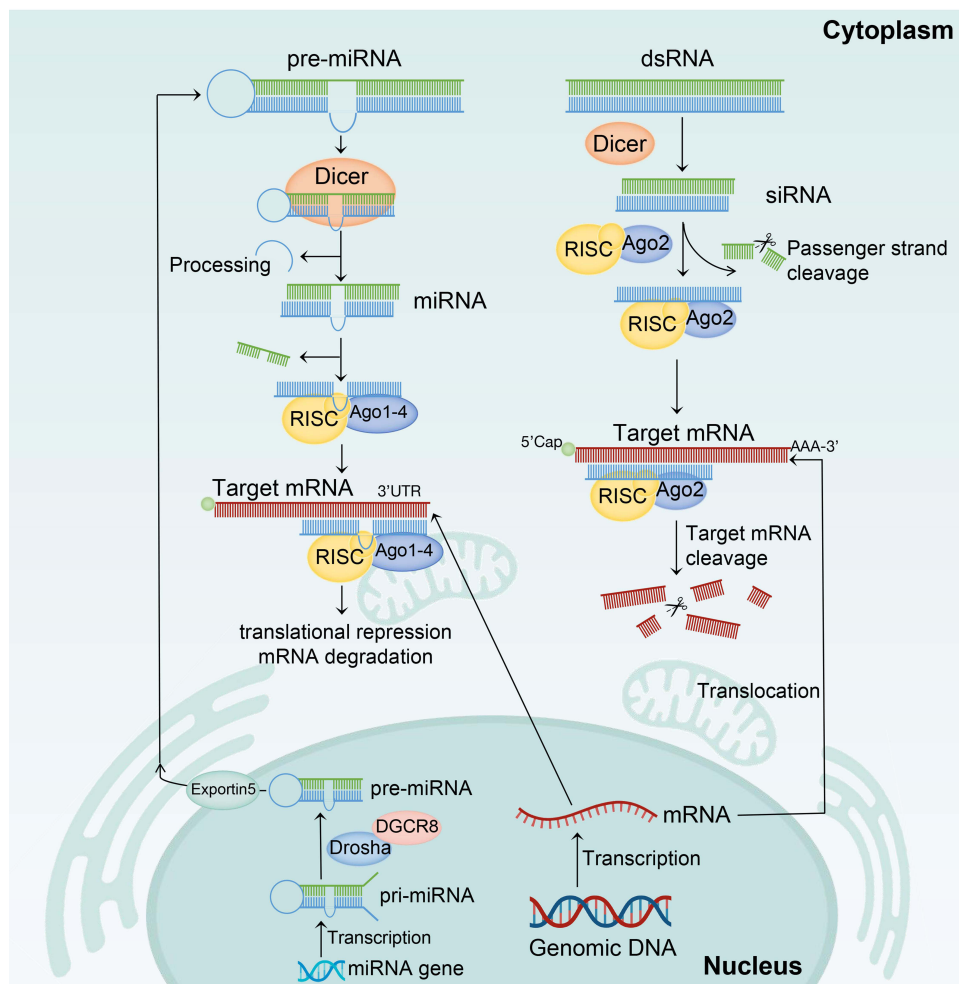
**Figure 1** Mechanism of microRNA (miRNA) and small interfering RNA (siRNA) action. miRNA: The miRNA gene is transcribed into pri-mRNA in the cell nucleus and then processed by Drosha and DGCR8 to form pre-miRNA. The pre-miRNA is transported to the cytoplasm by Exportin 5, where it undergoes further processing by Dicer to remove the stem-loop structure, forming mature miRNA. The miRNA is then loaded into the RISC that comprises Argonaute 1–4 (Ago1-Ago4), and the passenger strand is discarded. The miRNA-RISC pairs with the target mRNA in an imperfectly complementary manner, leading to mRNA degradation or translational repression. siRNA: The precursor of siRNA, such as double-stranded RNA (dsRNA), is first recognized and processed by Dicer. Dicer cleaves the precursor molecules into small siRNA fragments, typically 21–23 nucleotides in length. These siRNA fragments are then incorporated into the RNA-induced silencing complex (RISC). Once formed, the siRNA-RISC complex binds to a specific target mRNA molecule through base pairing between the siRNA and the mRNA sequence. The siRNA-RISC complex, specifically the Argonaute-2 endonuclease (Ago2) within RISC, then induces cleavage of the target mRNA at a specific site. This cleavage prevents the target mRNA from being translated into protein, ultimately leading to a reduction in target protein expression. The icons (cell, mitochondria, endoplasmic reticulum, miRNA gene, genomic DNA, and mRNA) were Created with BioRender.com.

into smaller fragments, typically around 20 nucleotides in length. These fragments are then loaded onto a protein complex known as the RISC. Within the RISC, one of the strands of the dsRNA, referred to as the guide strand, is selected to direct the complex to its target mRNA. The guide strand of the RISC binds to the complementary sequence on the target mRNA through base pairing. This interaction leads to the recruitment and activation of proteins, such as Argonaute 2 (Ago2), that facilitate the cleavage or degradation of the target mRNA. Consequently, the production of the corresponding protein is prevented or significantly reduced. RNAi serves as a powerful tool in research and exhibits potential therapeutic applications (Figure 1).[113,114]

## Advantages of siRNAs for Cancer Therapy

RNAi encompasses three types of dsRNAs capable of inducing gene silencing: short hairpin RNA (shRNA), endogenous small RNA (miRNA), and siRNA. Among these, synthetic siRNAs are the most suitable for use as drugs due to their unique advantages over chemotherapeutic drugs and other anti-cancer agents, owing to the special RNAi

mechanism.[30,114–116] Four key points highlight the superiority of siRNA drugs: the specificity in silencing. siRNAs of 20 nucleotides in length can recognize any target gene with high specificity and minimal off-target effects due to the base complement pairing recognition mechanism. Second, the exceptional safety profile. siRNAs exert their post-transcriptional gene silencing effects exclusively in the cytoplasm, preventing nuclear entry and genome integration, thus minimizing the risk of host gene mutations. Third, the remarkable efficiency. Even with few fragments, siRNAs can induce significant gene silencing effects in cells. Fourth, the unlimited number of potential targets. Advances in molecular biology and whole genome sequencing have facilitated the establishment of large human genomic databases, cDNA libraries, and disease-causing gene databases. By simply designing siRNAs based on the mRNA sequence of the target gene, it becomes possible to obtain an siRNA capable of effectively silencing any disease-causing gene.[30,116]

## Potential Targets for siRNAs Drugs for Cancer Therapy

Cancer cells exhibit substantial differences from normal tissue cells. Hanahan et al have comprehensively summarized the fourteen most important characteristics of cancer together with the corresponding therapy strategies.[1,117,118] These traits include sustained proliferative signaling, loss of inhibition of proliferation, replicative immortalization, migration and metastasis, induction of angiogenesis, resistance to apoptosis, genomic susceptibility to mutation, evasion of immune attack, induction of cancer-promoting inflammation, derangement of energy metabolism, unlocked phenotypic plasticity, non-mutational epigenetic reprogramming, aging cells, and polymorphic microbiomes. While these altered traits contribute to cancer development and progression, they also offer a wealth of molecular targets for cancer therapy.

The targeted siRNA silencing of specific cancer pathogenesis and relevant target genes holds promise for interventions in cancer therapy. Figure 2 illustrates potential therapeutic targets for siRNA drugs, including: 1) cell cycle-related signaling pathways involving cyclins, cyclin-dependent kinases (CDKs), polo-like kinases (Plks), and mitosis-related molecules;[119,120] 2) cell proliferation-related signaling pathways involving protein kinase N3 (PKN3), ephrin type-A receptor 2 (EphA2), AKT1/2/3, and other molecules that promote cell proliferation;[121–124] 3) cancer metastasis-related signaling pathways, such as hepatocyte growth factor receptor (HGFR), transforming growth factor-β (TGFβ), chemokine receptor (CCR2, CXCR2) and molecules facilitating HCC metastasis;[125,126] 4) cancer angiogenesis-related signaling pathways involving vascular endothelial growth factors (VEGFs), vascular endothelial growth factor receptors (VEGFRs), matrix metalloproteinase 9 (MMP9), focal adhesion kinase (FAK), and other molecules that promote cancer angiogenesis;[127] 5) cancer cell anti-apoptotic proteins including Survivin, Bcl2, Bcl-xL, which inhibit cancer cell apoptosis;[128] 6) DNA damage repair-related molecules such as methyltransferase (MGMT), and poly(adenosine diphosphate-ribose) polymerase (PARP), which inhibit programmed cancer cell death;[129] 7) molecules associated with cancer immune escape, such as programmed cell death protein 1 (PD1) and its ligand (PDL1), cytotoxic T lymphocyte-associated protein 4 (CTLA4) and other immune test site molecules;[15,130] 8) genes related to cancer metabolism, such as glucose transporters (GLUTs), lactate dehydrogenase A (LDH-A), hypoxia-inducible factors (HIFs), glutaminases, fatty acid synthases;[131] 9) oncogenes, including c-myb, c-Myc, and RAS genes, which are closely associated with cancer development and tumor progression;[132,133] 10) In addition, there are cancer tyrosine kinase-related signaling pathways, such as epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor (IGF-1R), Ras family proteins, BCR-ABL tyrosine kinases,[134] and cancer cell multidrug resistance genes (MDRs).[135,136]

In cancer therapy, the abundance of potential targets necessitates careful selection based on the following principles: 1) Preferential expression in tumor cells, while being minimal, absent, or present at negligible levels in normal cells; 2) Key involvement in promoting tumorigenesis; 3) Preferably a broad-spectrum gene applicable to multiple tumor models. These principles include the Therapeutic Index and the Therapeutic Window, which together define the safety and efficacy of a treatment regimen.[137,138] Differential expression of target genes in tumor cells and normal cells reduces damage to healthy tissue; The critical role of target genes in tumorigenesis increases the likelihood of successful treatment; In addition, the broad-spectrum nature of these genes facilitates the dissemination of therapeutic solutions and offers greater market potential. By considering these three factors, the selection of a safe and effective therapeutic target with broad applicability becomes possible.
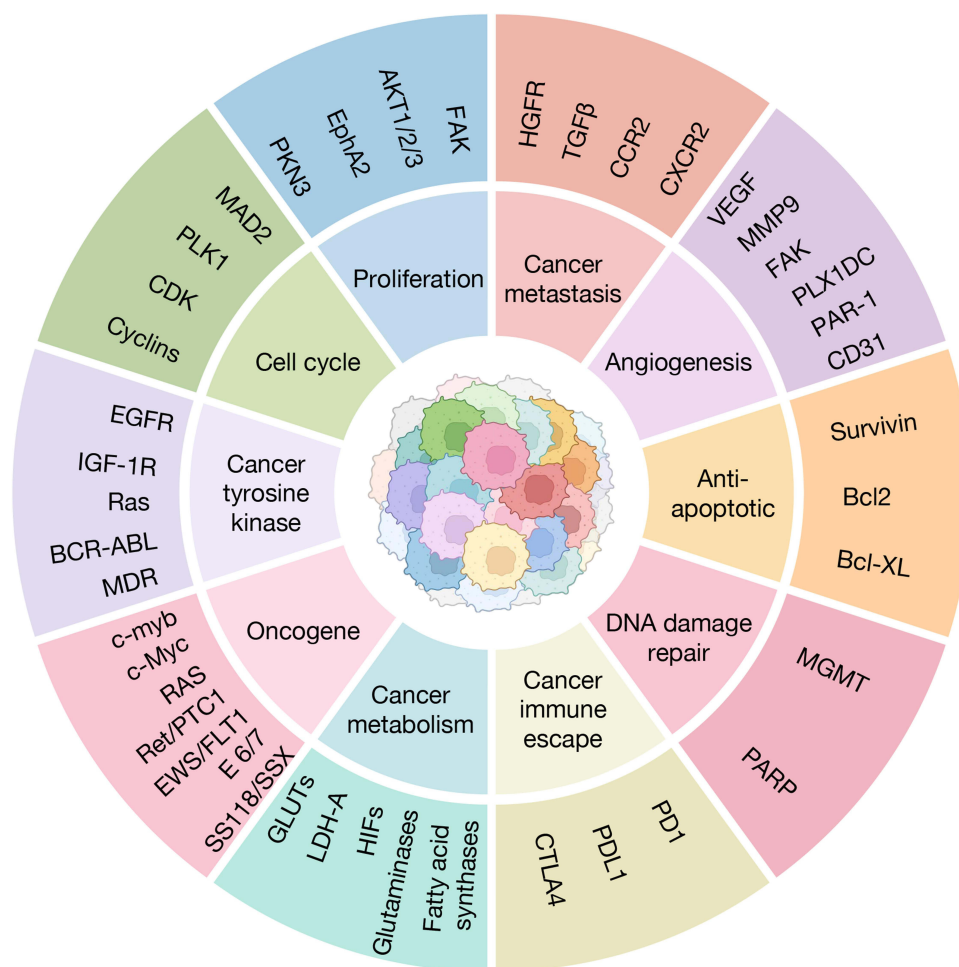
**Figure 2** The targeting pathways of siRNA in cancer treatment. The inner circle illustrates the diverse pathways involved in cancer treatment, encompassing: cell cycle regulation, proliferation, cancer metastasis, angiogenesis, anti-apoptotic processes, DNA damage repair, cancer immune escape, cancer metabolism, oncogene, and cancer tyrosine kinase. The outer circle represents the specific siRNA interference targets corresponding to these pathways, which have been explored in preclinical research. The tumor cells in the center were Created with BioRender.com.

## Challenges in siRNA Drugs Development

Although siRNA offers significant advantages in cancer therapy, it is important to acknowledge the multitude of cancer-related genes that can be targeted using this approach. Nevertheless, the utilization of siRNA drugs in clinical cancer therapy encounters several obstacles that need to be overcome.

The first challenge lies in the inherent instability of siRNAs under physiological conditions, making them susceptible to rapid clearance. As shown in Figure 3, the physiological milieu harbors a multitude of nucleases, leading to the degradation of siRNAs upon entry into the bloodstream.[139] Furthermore, phagocytes are abundant in tissues and organs such as blood, liver, and spleen, particularly in the mononuclear phagocytic system (MPS)/reticuloendothelial system (RES), including mononuclear macrophages, phagocytic cells, and neutrophils, which engulf siRNAs entering the body.[57] Moreover, significant amounts of siRNA are efficiently eliminated during their circulation to the kidneys by glomerular filtration.[140] The unmodified siRNAs have a short half-life in blood, lasting only a few minutes to one hour.[139] Even if siRNA successfully reaches the target cells, it initially encounters the endosomes and lysosomes, which have an acidic environment with a pH range of 5–6 and an even lower pH of 4.5 within the lysosomes. These compartments contain a number of nucleases capable of rapidly degrading siRNAs.[141]

The second challenge concerns the cellular uptake of free siRNAs. Due to its hydrophilic nature and negative charge, siRNA encounters obstacles when attempting to cross the negatively charged hydrophobic cell membranes and enter cancer cells, resulting in charge repulsion and impermeability.[57] The third hurdle involves the escape of siRNAs from
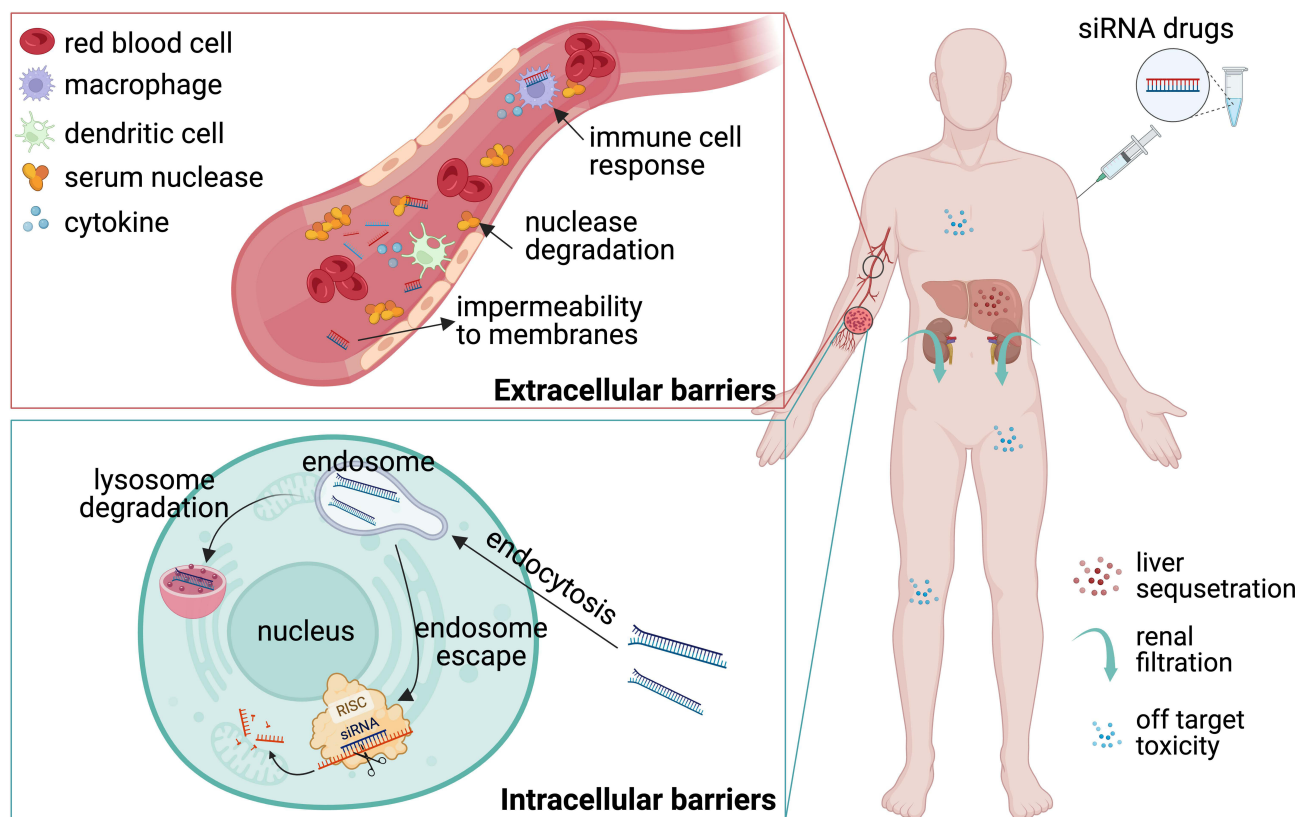
**Figure 3** Barriers to the delivery of siRNA in vivo. After administration, siRNAs can encounter several obstacles. Sequestration and excretion: siRNAs may be sequestered or excreted by organs like the liver and kidneys, limiting their distribution and reducing their effectiveness in reaching target tissues. Extracellular barriers: nucleases present in the bloodstream can degrade siRNA molecules, rendering them ineffective before they can reach their intended targets; macrophages are capable of recognizing and engulfing foreign particles, including siRNAs, hamper their delivery to target cells and reduce their availability; siRNA is a negatively charged molecule, and it faces significant challenges in crossing biological membranes. Intracellular barriers: once internalized by target cells, siRNAs may encounter challenges in escaping the endosomal-lysosomal pathway, potentially hindering their ability to reach the desired intracellular target mRNA and diminishing their efficacy; off-target effect led to unwanted toxicities. Created with BioRender.com.

endosomes. The assembly of siRNA into the RISC complex for gene silencing can only occur in the cytoplasm. However, siRNA faces limitations in freely penetrating biological membranes, requiring strategies to achieve efficient endosomal escape and release into the cytoplasm, creating a barrier to siRNA delivery.[57,141] The fourth obstacle involves the immunogenicity of siRNAs. Toll-like receptors (TLRs), which are pattern recognition receptors expressed on immune cells, can recognize pathogen-associated molecular patterns, including CpG DNA and viral dsRNA. TLR3, TLR7, and TLR8 specifically recognize nucleotide sequences in siRNAs, such as the UG dinucleotide and 5'-UGU-3' motifs. Upon injection into the body, siRNAs activate the innate immune system, resulting in the production of substantial cytokines.[54,142]

The fifth challenge focuses on the off-target effects of siRNAs. Although siRNAs, with a length of 20 nucleotides, have a high specificity in terms of base-complementary pairing, cells contain numerous long-stranded mRNAs and miRNAs. Incomplete complementary pairing of siRNAs can lead to the degradation of unintended mRNAs and miRNAs, causing non-specific gene regulatory effects. Furthermore, cells express various miRNAs to regulate gene expression, and these miRNAs can have sequences identical to the target mRNAs. Consequently, siRNAs not only degrade target mRNAs but also miRNAs, leading to unpredictable changes in gene expression.[143]

As previously mentioned, siRNA holds immense potential in cancer therapy but is accompanied by various challenges. Therefore, it is crucial to identify safe and efficient delivery strategies that can fully exploit the benefits of siRNA drugs in cancer therapy. In essence, siRNA delivery systems must fulfill the following requirements: 1) ensuring the serum stability of siRNA; 2) facilitating siRNA immune evasion; 3) attenuation of siRNA interactions with plasma proteins and phagocytes; 4) prevention of renal clearance; 5) enhancing the ability of siRNA to cross the vasculature and

reach cancerous tissues; 6) facilitating cellular uptake of siRNA; 7) promoting siRNA escape from endosomes; 8) high biocompatibility and non-toxicity. Only when these conditions are met can efficient delivery of siRNA drugs be achieved.[114] In the following section, we present a comprehensive overview of siRNA delivery strategies.

## Delivery Strategies for siRNA Drugs

siRNA, when used as a therapeutic drug, faces challenges such as a short half-life, poor serum stability, and susceptibility to nuclease degradation. Furthermore, its negative charge hinders easy entry into tumor cells through the cell membrane and is prone to degradation by intracellular lysosomes. Consequently, the direct use of siRNAs as therapeutic drugs to treat diseases is challenging. To overcome these limitations, chemical modifications and nano-delivery systems have emerged as common strategies to facilitate the therapeutic application of siRNA drugs.

## Chemically Modified siRNAs

While chemical modification does not serve as a delivery vehicle for siRNA, it does play a critical role in enhancing the inherent properties of siRNAs. Significant improvements in serum stability, immune evasion capability, and the assembly of siRNAs into RISC can be achieved through the use of appropriate chemical modifications.[57,144] Therefore, it is imperative to discuss the impact of chemical modifications on siRNA delivery efficiency before introducing siRNA delivery vectors.

In general, siRNAs offer multiple sites that can be chemically modified, including the terminus, backbone, ribose moieties, and bases.[30] Several commonly used methods for siRNA modification are shown in Figure 4. Experimental evidence has demonstrated that phosphorothioate (PS) modification enhances nuclease resistance, pharmacokinetic properties, and serum stability of the modified material. However, excessive PS modification can lead to serious toxic effects.[145] Furthermore, 2'-fluoro (2'-F) or 2'-O-methyl (2'-OMe) modifications of siRNA ribose have been widely used in commercial siRNAs. These modifications improve serum stability, extend half-life, enhance RNAi ability, and reduce
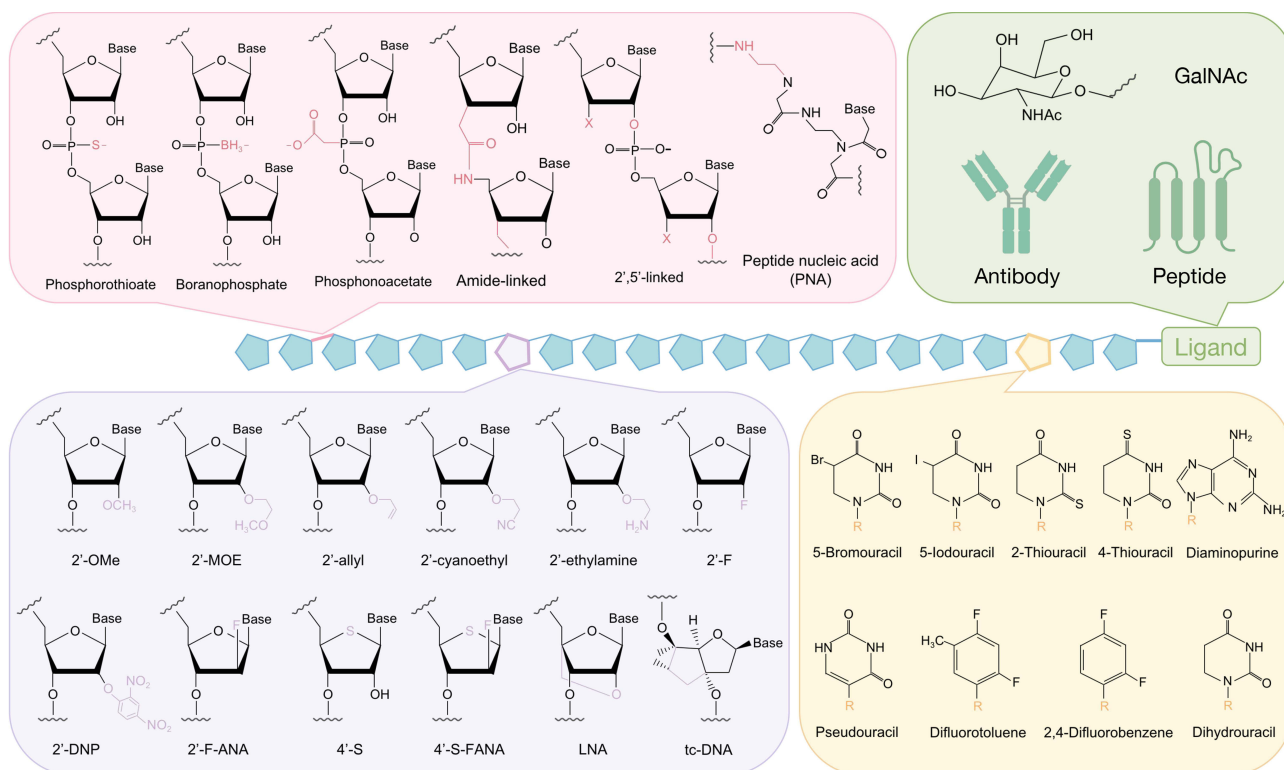


**Figure 4** Chemical modifications commonly used in siRNA therapeutics. According to the structure of the RNA nucleotide, it can be classified into phosphate (pink), ribose (purple), and base modifications (yellow). Besides, Ligands (green, such as GalNAc, antibodies, and peptides) enable specific cell types delivery.

off-target effects.[144] Other siRNA modification methods include the incorporation of locking nucleic acids (LNAs) at the 2' and 4' positions of methylene-linked ribose, as well as the replacement of phosphodiester nucleotides with phospho-nothioate at the 3' end of the RNA backbone.[114] Additionally, modification of siRNA with the small molecule 2.4-dinitrophenol (DNP) not only reduces nuclease degradation but also facilitates cellular uptake by aiding cell membrane crossing.[146]

While numerous modification methods exist to enhance the stability and other properties of siRNAs, it is critical to strike a balance between the gene-silencing effect and safety when using chemical modifications. It should be noted that certain chemical modifications can affect the effectiveness of siRNAs. For instance, a boranophosphonate modification within the siRNA antisense strand may increase nuclease resistance but reduce the effectiveness of RNAi.[147] As far as safety is concerned, the nucleotides generated by the degradation of regular siRNAs are indistinguishable from those naturally occurring in vivo. However, many chemical modifiers utilized are not native to the human body. Consequently, a critical evaluation of the safety of substances generated by the metabolism of these chemically modified siRNAs in humans is essential.

## siRNA Delivery Systems

In addition to chemical modification, researchers have developed various types of delivery systems to facilitate the transport of siRNAs into target tissues and cells. Delivery systems can be broadly divided into viral and non-viral vectors. Viral vectors are known for their efficient nucleic acid delivery (Figure 5A); however, they possess certain limitations,[148,149] such as: 1) the ability of some viruses to only deliver nucleic acids to dividing cells; 2) limited nucleic acid loading capacity (≤ 5 kb); 3) potential immunogenicity and toxicity; and 4) the risk of insertional mutagenesis. Given the extensive existing reviews on viral vectors, this article focuses on the systematic description of non-viral siRNA vectors (Figure 5B–E).
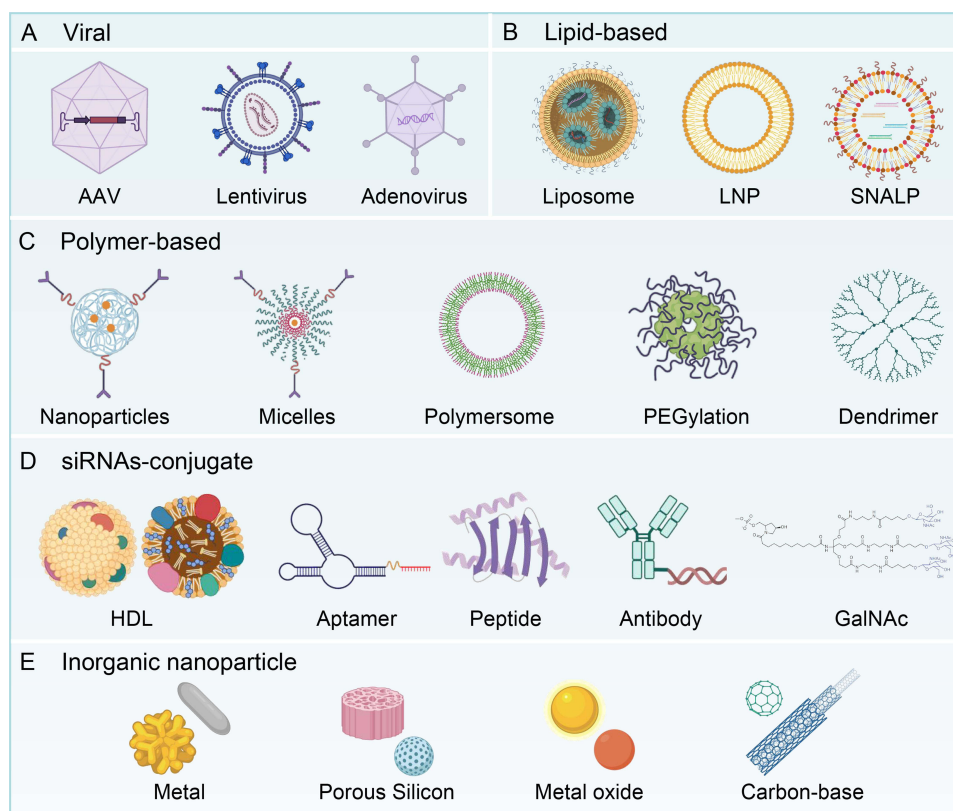


**Figure 5** Delivery systems for delivering siRNAs to target tissues and cells can be classified into (**A**) viral and (**B–E**) non-viral vectors. Structure of the triantennary GalNAc used in several drug candidates from Alnylam Pharmaceuticals. The icons were Created with BioRender.com.

## Lipid-Based siRNA Delivery Systems

Lipid-based siRNA delivery vectors include various types, including anionic liposomes, neutral liposomes, cationic liposomes, stable nucleic acid-lipid particles (SNALPs), and lipidoid nanoparticles (Figure 5B). Among these, cationic liposomes such as the commercial Lipofectamine have gained significant importance in DNA and RNA transfection, including siRNA transfection. In particular, Lipofectamine RNAiMAX has shown remarkable efficacy in achieving high transfection efficiencies at the cellular level. The mechanism underlying the use of cationic liposomes for cell transfection involves electrostatic interaction between positively charged lipids and negatively charged cell membranes, facilitating the phagocytosis of siRNA by cells.[150]

In terms of biocompatibility and pharmacokinetics, anionic or neutral liposomes generally outperform cationic liposomes due to the negatively charged nature of biological membranes. A remarkable neutral lipid, 1.2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC), has been utilized to improve the encapsulation efficiency of siRNA. In 2005, Landen et al developed a neutral liposome (siRNA-EphA2-DOPC) containing siRNA targeting the oncoprotein EphA2 using DOPC. Notably, within 48 hours after administration of this siRNA-EphA2-DOPC liposome to an in-situ mouse model of ovarian cancer, a significant downregulation of EphA2 expression was observed.[109] Currently, siRNA-EphA2-DOPC is undergoing Phase I clinical trials at the M.D. Anderson Cancer Center (MDA). Recently, Sahu et al have developed a nanocarrier system composed of DOPC that can effectively stabilize mTOR siRNA and successfully deliver it (NL-mTOR siRNA). In vitro and in vivo experiments have demonstrated that NL-mTOR-siRNA can inhibit the growth and invasion of breast cancer and restore tumor morphology. Furthermore, the neutral liposomes enhance the accumulation of siRNA in breast cancer tissue, facilitate its distribution within the tumor, and enhance its anti-tumor potential.[151] Ramos-Gonzalez et al employed a novel neutral DOPC liposome-siRNA system (L-siRNA) respiratory delivery strategy that effectively silenced WT1 in vitro B16F10 melanoma cells and in vivo lung metastatic melanoma mouse models, inhibiting cell proliferation, significantly reducing tumor weight, and delaying animal mortality.[152]

Although cationic liposomes may not exhibit the same level of biocompatibility as other liposome types and are prone to clearance by the mononuclear phagocyte system, their notable advantage lies in their high cellular uptake capacity. Therefore, cationic liposomes currently remain the preferred choice. Cationic lipids, such as dioleoyl-phosphatidylethanolamine and 1,2-dioleoyl-3-trimethyl propane (DOTAP), can be formulated into cationic liposomes that efficiently transport negatively charged siRNAs through electrostatic interactions.[153] Sorensen et al successfully delivered TNF-α siRNA via DOTAP liposomes and effectively inhibited bacterial lipopolysaccharide (LPS)-induced mortality in a sepsis mouse model.[154] Additionally, Balgobind et al developed a cationic lipid carrier system consisting of cholesteryl cytofectins, 3β-N-(N',N'-dimethylaminopropyl)-carbamoyl cholesterol (Chol-T) or N, N-dimethylaminopropylaminylsuccinylcholesterylformylhydrazide (MS09), and neutral helper lipid dioleoylphosphatidylethanolamine (DOPE). The liposomes demonstrated effective transfection and gene silencing in cells overexpressing *HER2/neu*.[155] Jarallah et al designed an efficient siRNA nanoliposome carrier using Genzyme Lipid 67 (GL67), DC-Chol, and DOPE lipids. Physicochemical characterization results showed that it exhibited a favorable impact on the metabolic activity and uptake rate of A549 cells.[156] It is important to note that although liposomes are designed to possess a positive charge to facilitate cellular uptake, this positive charge also accelerates their clearance by mononuclear phagocytes. Therefore, strict control of the charge of cationic liposomes is required, typically achieved by maintaining an appropriate complex phosphorus ratio (N/P) of around 2 to 3.[157]

PEGylation of liposomes is an important method for enhancing drug delivery efficiency. By coating the liposome surfaces with a layer of polyethylene glycol (PEG), the particle size can be reduced, preventing aggregation and fusing during storage. In addition, PEGylation decreases clearance by the mononuclear phagocyte system and prolongs the blood half-life of liposomes.[158] However, liposome PEGylation also has some disadvantages. For example, the spatial site-blocking effect and negative electrical properties of PEG can hinder cellular uptake, decrease fusion of liposomes with endosomal membranes, and inhibit endosome escape.[158] These challenges can be addressed by rationally designing the PEG density and length on the liposome surface or by using pH-sensitive chemically bonded PEGs.[159] While the optimal PEG density and length are still under investigation, pH-sensitive chemical bond-optimized PEG modification has demonstrated effectiveness.[160] For instance, liposomes linked to PEG via oxime bonds remain stable at pH 7.4 in

a neutral environment but exhibit accelerated siRNA release and improved gene silencing at pH 5.5 in an acidic environment. Additionally, HEMA-histidine-methacrylic acid (HEMA)-modified PEGylated liposomes exhibit stability under neutral conditions. In contrast, the 1.2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) liposome nucleus is positively charged, and upon entering the endosome, the imidazole and methacrylic acid residues become protonated in the acidic environment, converting the negatively charged PEG to a positive charge. This dissociation exposes the positively charged liposome nucleus, promoting fusion with the endosome and facilitating endosome escape.[161]

SNALPs (Stable Nucleic Acid-Lipid Particles) represent the most well-known lipid-based siRNA delivery vectors. Notably, the FDA-approved siRNA drug for Ebola treatment is delivered by SNALPs, a class of lipid nanoparticles encapsulating siRNA with a particle size of approximately 120 nm. SNALPs have a lipid bilayer structure consisting of a mixture of cationic lipids and pro-fusion lipids on the outer layer, while siRNA is encapsulated within the inner layer, with the surface of the lipid bilayer structure modified with PEGs.[162] The presence of PEGs on the surface allows SNALPs to achieve prolonged circulation and passive enrichment at the tumor sites through the enhanced permeability and retention (EPR) effect. Once SNLAPs reach the tumor site, they can be rapidly taken up by tumor cells, facilitating efficient siRNA delivery. Alnylam Pharmaceuticals made a significant breakthrough by encapsulating two siRNAs (VEGF siRNA and KSP siRNA) simultaneously in SNALPs, naming the formulation ALN-VSP02. In April 2009, ALN-VSP02 entered a phase I clinical trial for advanced solid tumors, with an initial cohort of 28 patients showing good tolerance and antitumor activity following the administration of the six highest doses (1.25 mg/kg). Notably, one patient with endometrial cancer achieved complete remission.[163] Tekmira Pharmaceuticals has also developed SNALP-Plk1 siRNA (TKM080301), which is currently undergoing phase I and Phase II clinical trials for various solid tumors and lymphomas.[164] Furthermore, Abdel-Bar et al developed a stable SNALP formulation capable of co-delivering Dox and siCD47, synergistically enhancing tumor immunogenic cell death (ICD). The optimized SNALPs exhibited an encapsulation efficiency of over 65% for siRNA and Dox and improved serum stability. The combination of siCD47 and Dox-loaded SNALPs demonstrated potent anti-tumor activity in a tumor challenge model.[165]

Lipid nanoparticles (LNPs) are siRNA delivery vehicles composed of lipids, cholesterol, and PEGylated lipids with a structure like SNALPs, which also have a lipid bilayer structure with PEGylated surfaces and siRNAs encapsulated in the core.[163] Akinc et al developed a rapid method for chemical synthesis of lipid-like libraries and performed a screen to identify the most effective lipid-like nanoparticles for siRNA delivery.[166] One of the most promising lipid-like nanoparticle components was 98N12-5, with which siRNAs encapsulated with ApoB or FVII factors could achieve 75–90% gene silencing in non-human primate hepatocytes.

## Polymer-Based siRNA Delivery Systems

Polymer-based delivery vehicles, also known as polymeric nanoparticles, are a class of solid biodegradable colloidal nanoparticles commonly used for drug delivery and are mainly classified into water-soluble polycationic nanoparticles and polymeric nanoparticles[167] (Figure 5C).

Various water-soluble polycationic nanoparticles are available, including those derived from cyclodextrin polymers (CDP) and polyethyleneimine (PEI). Among these, CDP nanoparticles have shown promise for clinical delivery of siRNA drugs.[77] A notable example is the CDP-based siRNA drug CALLA-01, developed by Calando Pharmaceuticals. CALLA-01 consists of four components: CDP, adamantane-PEG (AD-PEG), adamantane-PEG-transferrin (AD-PEG-Tf), and siRNA. These components self-assemble into nanoparticles, with the positively charged CDP and siRNA forming the particle core.[168] AD-PEG incorporation creates a PEG shell on the particle surface, which increases particle stability and reduces clearance by the mononuclear phagocyte system. In addition, AD-PEG-Tf is coupled to transferrin, which specifically binds to the highly expressed transferrin receptor CD71 in tumor cells, thus enabling targeted drug delivery.[169] In a study conducted by Wu et al, a polymer-siRNA nanoparticle (PEG-PEI/siRNA) was synthesized and evaluated as a non-viral carrier for siRNA targeting CD44v6 in gastric cancer cells. This nanoparticle exhibited a relatively high gene transfection efficiency and low cytotoxicity.[170]

The main polymer nanoparticles used in this area are derived from polycaprolactone (PCL), poly (lactic acid) (PLA) and poly (lactic acid glycolic acid) (PLGA).[167] A notable study has reported a self-assembled micellar nanoparticle (MNP) called micelle plex, formed by the self-assembly of poly(ethylene glycol)-b-polycaprolactone)-b-poly(2-(2-oxo-

1,3,2-dioxapospholoyloxy)ethyl methacrylate) (mPEG-b-PCL-b-PPEEA) triblock polymer.[171] The micelle plex consists of a hydrophobic core formed by the PCL block, the cationic hydrophilic phosphate PPEEA responsible for siRNA binding, and an outer PEG layer that stabilizes the particle. By delivering GFP siRNA, micelle plex effectively downregulates GFP expression in HEK293/GFP cells by 40%-70%. In addition, micelle plex has demonstrated the successful delivery of various siRNAs targeting tumor-associated genes, such as AC siRNA, HIF1 siRNA, and CDK4 siRNA, resulting in significant inhibition of tumor growth in various tumor models.[172,173] Recently, Ma et al employed positively charged polydimethylaminoethyl methacrylate (PDMAEMA) and poly [oligo (ethylene glycol) methyl ether methacrylate] (POEGMA) to produce miktoarm star polymer nanoparticles. These star nanoparticles, when complexed with siRNAs targeting βIII-tubulin and Plk1, demonstrated efficient cellular uptake and endosomal escape. Upon nebulization, the star-siRNA nanoparticles inhibited lung tumor growth by accumulating in the lung and silencing the expression of βIII-tubulin and Plk1.[174]

PLA and PLGA are FDA-approved polymeric materials for medical use. Yang et al developed a PLA-based cationic lipid-assisted polymeric nanoparticle (CLAN) with double emulsified nanoparticles encapsulated with siRNA, named CLAN$_{siRNA}$, prepared by block polymer PEG-PLA and cationic lipid N,N-bis (2-hydroxyethyl)-N-methyl-N-(2-cholesteryloxycarbonyl aminoethyl) ammonium bromide (BHEM-Chol).[175] The particle size of CLAN$_{siRNA}$ was around 170 to 200 nm, and the incorporation of the cationic lipid BHEM-Chol significantly increased the encapsulation rate to over 90%. In a mouse model of MDA-MB-435s breast carcinoma, injection of CLAN$_{siRNA}$ encapsulated with Plk1 siRNA effectively inhibited tumor growth.[175] In addition, Ghareghomi et al developed PLGA-based nanoparticles (NPs) functionalized with folic acid and loaded with magnetic nanoparticles for imaging. These NPs were co-loaded with Wtmn and hTERT siRNA to achieve efficient cytotoxicity and growth suppression in SKOV-3 cancer cells by targeting hTERT/telomerase activity, providing a potential combinatorial therapy for ovarian cancer.[176]

Dendrimers, a type of nanocarrier with a star-shaped or branched structure, are utilized for drug conjugation in tumor diagnosis and treatment and are extensively researched as potential nucleic acid carriers.[177–181] Various dendrimers, such as poly(L-lysine) dendrimers (PLL), carbosilane dendrimers, polyethyleneimine (PEI) dendrimers, and poly-(amidoamine) dendrimers (PAMAM), have been employed for siRNA delivery. Recent studies have demonstrated that these dendrimers effectively transport siRNA into cells and contribute to cancer treatment. Gorzkiewicz et al revealed that a novel PLL dendrimer, containing lysine and arginine or histidine residues, is capable of efficiently transferring siRNA into cells.[182] Additionally, Olmo et al confirmed that Schiff-based carbosilane copper (II) metallodendrimers can deliver pro-apoptotic siRNAs Mcl-1 and Bcl-2 into human breast cancer cells (MCF-7), enhancing the anti-cancer effect of siRNA.[183] Furthermore, Pan et al discovered that generation 4 PAMAM (G4 PAMAM), conjugated with polyethylene glycol (PEG)-modified 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (PEG2K-DOPE), can deliver MDR-1 siRNA and the drug DOX to MDR cancer cells through the EPR effect, reducing the P-gp and drug resistance of MDR cancer cells.[184] Chen et al synthesized a bola-amphiphilic PAMAM dendrimer, demonstrating its potential for delivering siRNA drugs to tumors and cancer cells and treating various cancer models.[185]

## siRNA-Conjugate Delivery Systems

siRNA-conjugate delivery systems involve the direct coupling of siRNA to a delivery material, resulting in a single-component drug delivery system with specific characteristics. The initial approach to siRNA conjugation involved coupling siRNA to a lipophilic molecule like cholesterol. However, various coupling systems have been developed. These include the coupling of siRNA to polymers, peptides, antibodies, nucleic acid aptamers, or small molecules[35,186] (Figure 5D).

Lipophile-siRNA conjugates, in which cholesterol is coupled to the 3' end of the siRNA sense strand via a pyrrolidone bond, not only increase the transfection efficiency of siRNA in vitro but also enhance the pharmacokinetics of siRNA in vivo.[187] In 2007, Wolfrum et al investigated the use of high-density lipoprotein (HDL) to further optimize cholesterol-siRNA conjugates, resulting in an 8- to 15-fold increase in siRNA gene silencing effect in vivo.[188] Recently, Masimov et al developed chitosan nanoparticles conjugated with HDL to shield siRNAs from enzymatic degradation. These nanoparticles possess the capability to target siRNAs in liver cancer cells expressing SR-B1 receptors.[189] Moreover, antibody-siRNA-coupled delivery systems, leveraging the specificity of antibodies, enable targeted delivery

of siRNA. For instance, by coupling a targeting antibody against the transferrin receptor CD71 to siRNA via biotin-strand affinity action, siRNA can cross the blood-brain barrier and inhibit the expression of reporter genes in intracranially transplanted rat brain tumor cells.[190] Wang et al reported novel photoresponsive antibody-siRNA conjugates (PARCs), consisting of anti-programmed death-ligand 1 antibody (α-PD-L1) and siRNA targeting intracellular PD-L1 mRNA, linked by a photo-cleavable linker. This complex enables tumor-specific light-induced siRNA delivery and photo-activated immunogene therapy.[191] Similarly, nucleic acid aptamer-siRNA-coupled delivery systems, utilizing the specificity of nucleic acid aptamers for protein binding, serve as targeted siRNA delivery systems. For example, the coupling of nucleic acid aptamers targeting prostate-specific membrane antigen (PSMA) to siRNA through biotin-strand affinity action successfully increases siRNA transfection efficiency in PSMA-highly expressed cells.[192] Besides, Yang et al developed a multifunctional RNA nanoparticle, EGFRapt-3WJ-Alexa647-si$KRAS^{G12C}$, capable of delivering siRNA targeting $KRAS^{G12C}$ to non-small cell lung cancer (NSCLC) cells. The EGFR-targeting RNA aptamer (EGFRapt) further enhances specificity for the tumor.[193]

Cell-penetrating peptides (CPPs) are used as siRNA coupling agents to facilitate the crossing of cell or endosomal membranes, thereby improving siRNA transfection efficiency or endosomal escape. An example of a CPP is the TAT transcription activator protein derived from the HIV-1 virus. TAT can be coupled to the 3' end of the siRNA antisense strand using sulfosuccinimidyl-4-(p-maleimidophenyl) butyrate and other heterobifunctional bridge bonds. This coupling strategy significantly improves the intracellular delivery efficiency of siRNA.[194] Yang et al proposed the use of graphene nanoparticle-loaded siRNA to silence Rictor molecules, which are essential for the mammalian rapamycin (mTOR) complex 2 (mTORC2) complex. They modified a CPP to treat breast cancer, leading to enhanced gene delivery to tumor cells.[195] In a study by Cai et al, a CPP-modified metal-organic framework nanoplatform named PEG-CPP33 @ORI@survivin siRNA@ZIF-90 (PEG-CPP33@NPs) was designed for targeted co-delivery of oridonin (ORI), a naturally occurring antitumor active component, and survivin siRNA in vivo.[196] However, it is important to note that CPP-siRNA couplers may pose potential side effects due to the membrane-penetrating ability and immunogenicity of CPP.[197] Additionally, Ma et al constructed a nanobubble modified with CPP-conjugated DOX and CPP-conjugated c-myc siRNA, along with asparagine-glycine-arginine peptide (NGR) modification. When combined with ultrasound, the CPP- and NGR-modified formulation led to enhanced drug accumulation in tumors and demonstrated potent anti-tumor effects.[198]

In addition to the aforementioned siRNA-coupled delivery systems, one of the most promising clinical applications is the N-acetylgalactosamine-siRNA (GalNAc-siRNA)-coupled delivery system. This system exhibits a high affinity for the asialoglycoprotein receptor (ASGPR) present on the hepatocyte surface, enabling hepatic targeting of siRNA.[199] Khan et al developed a safe, effective, and biocompatible formulation called GalNAc@PEG@siRNA-PLGA, which encapsulates survivin siRNA in GalNAc-modified polyethylene glycolized PLGA nanocouples (NCs). They evaluated the synergistic antitumor effects of this formulation for targeted delivery to mice with hepatocellular carcinoma.[200] Li et al established a GalNAc-modified nanocarrier formulation containing 5'-FU and siRNA targeting the VEGFs. This formulation exhibited significant anti-metastatic activity against C5WN1-HCC cells and effectively suppressed tumorigenic and pulmonary metastases with a tumor suppression rate of 96%.[201] Additionally, Alnylam Pharmaceuticals has developed a trivalent GalNAc-siRNA delivery system to enhance liver-targeting capabilities. Three siRNA drugs, namely ALN-TTRsc, ALNPCS, and ALN-AT3, have been prepared by coupling trivalent GalNAc with TTR siRNA, PCSK9 siRNA, or AT siRNA, respectively. These drugs have entered clinical trials for the treatment of thyrotropin amyloidosis, hypercholesterolemia, and hemophilia.[114] Recently, Porter et al investigated the potential of GalNAc-conjugated siRNA, known as SLN124, for treating beta-thalassemia and myeloproliferative neoplasms. SLN124 exhibits liver-targeting properties and can effectively silence the *TMPRSS6* gene, resulting in increased endogenous hepatic phospholipid synthesis. The initial phase I clinical trial in human subjects assessed the safety and tolerability of escalating single doses of SLN124 (1.0, 3.0, and 4.5 mg/kg) in healthy volunteers. The study revealed dose-dependent impacts on iron metabolism and red blood cell markers, with sustained effects lasting up to 56 days following a single dose.[202]

# Inorganic Nanoparticle siRNA Delivery Systems

Inorganic materials offer the opportunity to develop nanocarriers with controllable size and morphology. These materials possess unique properties that contribute to the carriers' favorable characteristics, including excellent biocompatibility, non-immunogenicity, non-toxicity, ease of scale-up, and straightforward surface functionalization (Figure 5E).

Gold nanoparticles (AuNPs) have garnered significant attention as inorganic nanocarriers due to their favorable physicochemical properties. Yi et al successfully obtained sub-50 nm modified glucose nanoparticles (Glu-NPs) through a two-step bottom-up self-assembly process involving unimer polyion complexes (uPICs) and AuNPs. These Glu-NPs show the potential for systemic delivery of siRNAs to cancer stem-like cells (CSCs)-rich breast cancer models. The study shows that Glu-NPs specifically recognize CSCs in MDA-MB-231 breast cancer spheroid culture models and enhance antitumor effects in in situ breast tumor models.[203] Baghani et al developed a delivery system using trimethyl-chitosan (TMC)-coated AuNPs as nanocarriers capable of transporting EGFR-siRNA into breast cancer cells. These nanoparticles were efficiently taken up by breast cancer cells and exhibited a significant gene silencing effect on target genes, providing a potential therapeutic strategy for breast cancer treatment.[204] However, it is important to note that gold nanoparticle-based carriers also have limitations, including low encapsulation efficiency, poor storage stability, and slow in vivo escape.

Mesoporous silica nanoparticles (MSNPs) have been widely employed for nucleic acid delivery due to their robust loading capacity and biodegradability. In a study conducted by Mora-Raimundo et al, MSNPs were utilized to deliver SOST siRNA and osteostatin, resulting in increased expression of frontal osteogenesis-related genes, improved bone microarchitecture, and alleviated osteoporosis symptoms.[205] Kumar et al developed a nanocarrier based on MSNPs by modifying them with poly-l-arginine (PLR) and PEG to endow siRNA binding ability and enhance biocompatibility. They then conjugated the nanocarrier with AS1411 aptamer for cancer cell targeting. The multifunctional nanocarrier demonstrated excellent target specificity, and the co-delivery of DOX and siRNA against BCL-2/BCL-xL showed strong potential in overcoming drug resistance in triple-negative breast cancer cells.[206] Alternatively, superparamagnetic iron oxide nanoparticles (SPIONs), such as $Fe_2O_3$ or $Fe_3O_4$, exhibit superparamagnetic magnetism at specific sizes and can serve as siRNA carriers for targeted delivery to specific tissues or tumors in the presence of an external magnetic field. To facilitate electrostatic interactions with anionic siRNAs, surface-engineered cationic compound modifications are required for SPIONs. For instance, PEI- or PEG-coated iron oxide nanoparticles ranging from 20 to 100 nm have demonstrated superior siRNA delivery activity.[207–211] In a recent study, Chung et al introduced a delivery system based on SPIONs to deliver siRNA targeting the O6-methylguanine-DNA methyltransferase (MGMT) gene, aiming to enhance the sensitivity of glioma cells to the alkylating drug temozolomide. The SPIONs were coated with polymer and conjugated with the targeting ligand chlorotoxin and cell-penetrating polyarginine ($R_{10}$), thereby enhancing the transfection efficiency of siRNA.[212]

Calcium phosphate (CaP) nanoparticles have several advantages in terms of biocompatibility, ease of preparation, and high affinity for RNA. Furthermore, they exhibit rapid solubilization at low pH within early cellular lysosomes after endocytosis, leading to increased osmotic pressure and the subsequent release of encapsulated siRNA into the cytoplasm. This unique characteristic has been utilized to enhance the transfection efficiency of CaP nanoparticles. Huang et al demonstrated that lipid-coated CaP nanoparticles exhibit elevated $Ca2^+$ concentrations upon cellular internalization.[213,214] In addition, the incorporation of PEG-phospholipids and the targeting ligand anisamide into lipid-coated CaP nanoparticles resulted in a significant improvement in siRNA delivery compared to previous formulations (40-fold in vitro and 4-fold in vivo). Kara et al synthesized two arginine-modified CaP nanoparticles with different chemical and morphological characteristics to serve as targets for survivin and cyclin B1 silencing through specific siRNA vectors. Using A549 non-small-cell lung cancer cells, Kara et al demonstrated that CaP-Arg-siRNA-mediated inhibition of these genes led to a substantial reduction in cell growth and the induction of apoptosis.[215] Furthermore, Hsieh et al proposed a novel immunotherapy strategy for treating glioblastoma by developing a CaP-based delivery system that overcomes the blood-brain barrier (BBB). This system encapsulates the NO donor and siRNA targeting PD-L1 within CaP cores, coats them with a lipid bilayer, and modifies them with the CXCR4 antagonist peptide.[216]

Carbon nanotubes (CNTs), typically ranging from 1–2 nm in diameter and 50 nm to 1 cm in length, have been shown to effectively deliver siRNA when fabricated by functionalization methods. Siu et al non-covalently functionalized carbon nanotubes with lipopolymers (DSPEPEG) and PEl to facilitate siRNA delivery to target cells.[217] They observed successful hepatic GAPDH siRNA uptake and gene silencing in a mouse model. Zhao et al succeeded in self-assembling peptide lipids (PL) and sucrose laurate (SL) on CNTs, leading to the construction of bifunctional lipid-coated single- and multi-walled CNT delivery systems (SCNT-PS and MCNT-PS). After 21 days of treatment with SCNT-PS/siRNA, certain tumors regressed completely. Furthermore, SCNT-PS and MCNT-PS nanoparticles showed no significant toxic effects on cells at concentrations up to 60 µg/mL.[218] Besides, Wen et al introduced a delivery system based on CNTs using multiwalled carbon nanotubes (MWNTs) to co-deliver sorafenib and siRNA targeting EGFR. This co-delivery system demonstrated a positive anti-tumor effect on liver cancer in both in vitro and in vivo experiments.[219]

## Other siRNA Delivery Systems

In addition to the aforementioned siRNA delivery systems, there are several other potential systems that have not found wide application. Examples include exosomes, oligonucleotide nanoparticles (ONP), RNAi microsponges, and electro/photoporation.

Exosomes, 40–100 nm in size, are natural vesicles released from cellular multivesicular vesicles fused to the cell membrane. They serve as carriers of various types of RNA, including coding and non-coding RNAs, between cells.[220] Isolated exosomes can be used as delivery vehicles for siRNA.[221] Alvarez et al developed brain-targeted siRNA delivery vectors using exosomes derived from dendritic cells.[222] First, they expressed the rabies virus glycoprotein (Rabies glycoprotein) fused to the Lamp2b protein of dendritic cells. Subsequently, dendritic cell exosomes were isolated, and siRNAs were loaded into the exosomes through electrotransfer.[222] By administering exosome-siRNA via tail vein injection in mice, specific delivery of siRNA to neuronal cells was achieved, resulting in over 60% down-regulation of target gene expression in the mouse cortex, midbrain, and striatum.[222] Recently, Huang et al developed an exosome-based delivery system using exosomes derived from primary patient cells. The exosomes were loaded with siRNAs targeting CCDC80, a potential target involved in tumor metastasis and chemoresistance. The exo-siCCDC80 demonstrated ideal biocompatibility and biosafety. The delivery of exo-siCCDC80 showed excellent antitumor effects and increased chemotherapy sensitivity in multiple animal tumor models.[223] Since exosomes are vesicles secreted by cells themselves, they are completely non-toxic and non-immunogenic.[221]

Oligonucleotide nanoparticles (ONPs) are three-dimensional nanostructures formed by hybridization of complementary DNA fragments, offering structural programmability, spatial addressability, and biocompatibility. To enhance stability, Ponnuswamy et al employed oligo-lysine-PEG to wrap DNA nanostructures, thereby improving their resistance to low salt denaturation and nuclease degradation.[224] Nucleic acid nanostructures hold great promise as carriers for siRNA drugs. Various methods have been employed to load nucleic acid nanostructures with nucleic acid drugs. It has been reported that pre-designed functional nucleic acid sequences can be assembled into DNA dendrimers for siRNA delivery.[225] Additionally, DNA origami structures, such as DNA tetrahedral nanostructures developed by Lee et al, can serve as multivalent nanocarriers for siRNA drugs.[226] Lee et al developed tumor-targeted folate-ONP by modifying ONP with trivalent folic acid (folate). By administering folate-ONP loaded with luciferase siRNA via tail vein injection into tumor-bearing mice expressing luciferase, the expression of the luciferase gene in the tumor was downregulated by 60% without inducing a significant immune response.[226] Additionally, Xu et al developed octahedral DNA origami frameworks (OctDOFs) to shield siRNAs from RNase degradation and protein binding. This strategy effectively reduces connective tissue growth factor (CTGF) and heat shock protein 72 (HSP72), sensitizing cancer cells to chemo-photothermal therapy.[227] Furthermore, Ding et al developed a novel siRNA delivery system with excellent thermal stability and physiological stability against enzymatic degradation.[228] They achieved this by grafting DNA onto the linear polymer polycaprolactone (DNA-g-PCL), utilizing functional siRNA on a cross-linked strand, and forming spherical nanogels by nucleic acid hybridization.

RNAi microsponges consist entirely of cleavable RNA strands and are only processed by the cells' RNA machinery after cellular uptake. This conversion of stable hairpin RNA into siRNA provides inherent protection during delivery and translocation to the cytoplasm.[229] Lee et al hybridized linear single-stranded DNA, including luciferase-resistant siRNA,

with short DNA strands and sealed the cut in the circular DNA using T4 DNA ligase. Closing the rolling circle transcription (RCT) of the circular DNA generated multiple tandem repeats of the hairpin RNA structure, forming a spherical sponge-like structure. The incorporation of a single RNAi microsponge enables the delivery of over 500,000 copies of siRNA to the cell.[229] Roh et al proposed a novel method called Multi-RNAi-MS to produce multiple components of multimeric siRNA by simultaneous self-assembly and dense packing into composite sponge-like porous microstructures using RCT.[230]

The delivery of siRNA by electroporation involves the application of short electrical pulses to temporarily modify the permeability of tissue and cell membranes.[231] Yang et al developed the Rolling Microneedle Electrode Array (RoMEA), which combines closely spaced microneedle electrodes with a rolling structure. RoMEA utilizes parallel circular blades with micro-needle arrays as electrodes, applying synchronized electric fields while rolling to enable large-scale in vivo tissue electroporation on irregular living tissue surfaces. This innovative approach facilitates effective siRNA delivery and gene silencing.[232] Shokouhi et al introduced an electroactive nanoinjection (ENI) platform with vertically configured conductive nanotubes for rapid TRIOBP siRNA delivery into cells, effectively achieving gene silencing. This system is non-viral, reusable, and operates at low voltage.[233] Additionally, Wayteck et al proposed a nanoparticle-sensitized photoporation technique for transfecting siRNA into primary cytotoxic T lymphocytes (CTL). A photothermal effect is generated by irradiating gold nanoparticles (AuNPs) attached to the cell surface with laser light. This effect leads to a transient permeation of the cell membrane by causing the evaporation of water near the AuNPs and the formation of water vapor nanobubbles (VNBs). Rapid expansion and collapse of the VNBs generate high-pressure shock waves, resulting in local membrane damage and the formation of reversible pores in the cell membrane. These pores allow direct access for siRNA to the cytoplasm, thus enabling efficient transfection.[234] Xiong et al successfully down-regulated PD1 expression by embedding light-sensitive iron oxide nanoparticles (IONPs) into biocompatible electrospun nanofibers and using photothermal effects for cellular photopuncture. This photothermal nanofiber effectively delivered siRNA to adherent and suspended cells, such as embryonic stem cells, without affecting cell proliferation or phenotype.[235]

## Bioresponsive Materials

In the context of siRNA drugs, the stable and efficient delivery of siRNA to the target site is crucial for therapeutic effectiveness. It is necessary for siRNA to be effectively accumulated at the focal site within a nano-delivery system. However, in order to exert its gene silencing effect, the siRNA must be completely dissociated from the delivery vehicle and exist freely in the cytoplasm.[82] Therefore, drug release behavior also plays a crucial role in determining the therapeutic efficacy of siRNA. Smart bioresponsive materials offer a solution for controlled drug release by responding to specific biological signals, environmental factors, or pathological conditions within the organism. These materials can undergo physical, chemical, or biological changes triggered by factors such as pH, redox potential, enzymes, oxygen levels, temperature, or ATP levels.[83,84] These changes can lead to dissolution, contraction, dissociation, or degradation of the delivery system, as illustrated in Figure 6.

### pH-Responsive Biomaterials

pH-responsive biomaterials are collectively referred to as materials that can change in response to variations in pH levels in their environment. The pH responsiveness of these materials primarily arises from the protonation of ionizable groups or acid degradation of chemical bonds within the material, which leads to the dissolution, shrinkage, dissociation, and degradation of the material. Consequently, pH-responsive biomaterials offer the potential for targeted drug delivery at different levels and precise release at specific sites. Common pH-responsive materials include polymers synthesized from acrylic acid, methacrylic acid, maleic anhydride, and N,N-dimethylaminoethyl methacrylate.[241–245] In a recent study, Zhang et al developed a multifunctional siRNA delivery system (CHCE/siRNA nanoparticles) by self-assembly of histidine- and cholesterol-modified carboxymethyl chitosan with anti-EGFR antibodies. This system exhibited both tumor-targeting and pH-responsive capabilities, enabling effective treatment by overcoming biological barriers[236] (Figure 6A). Wang et al developed an ultra-pH-responsive peptide nanocarrier that can dynamically assemble in response to pH changes in the tumor microenvironment and intracellular lysosomal environment, thereby efficiently delivering siRNA into cancer cells. The carrier exhibits high transfection efficiency and targeting specificity, exerting high toxicity to cancer cells while showing low toxicity to normal cells.[246]
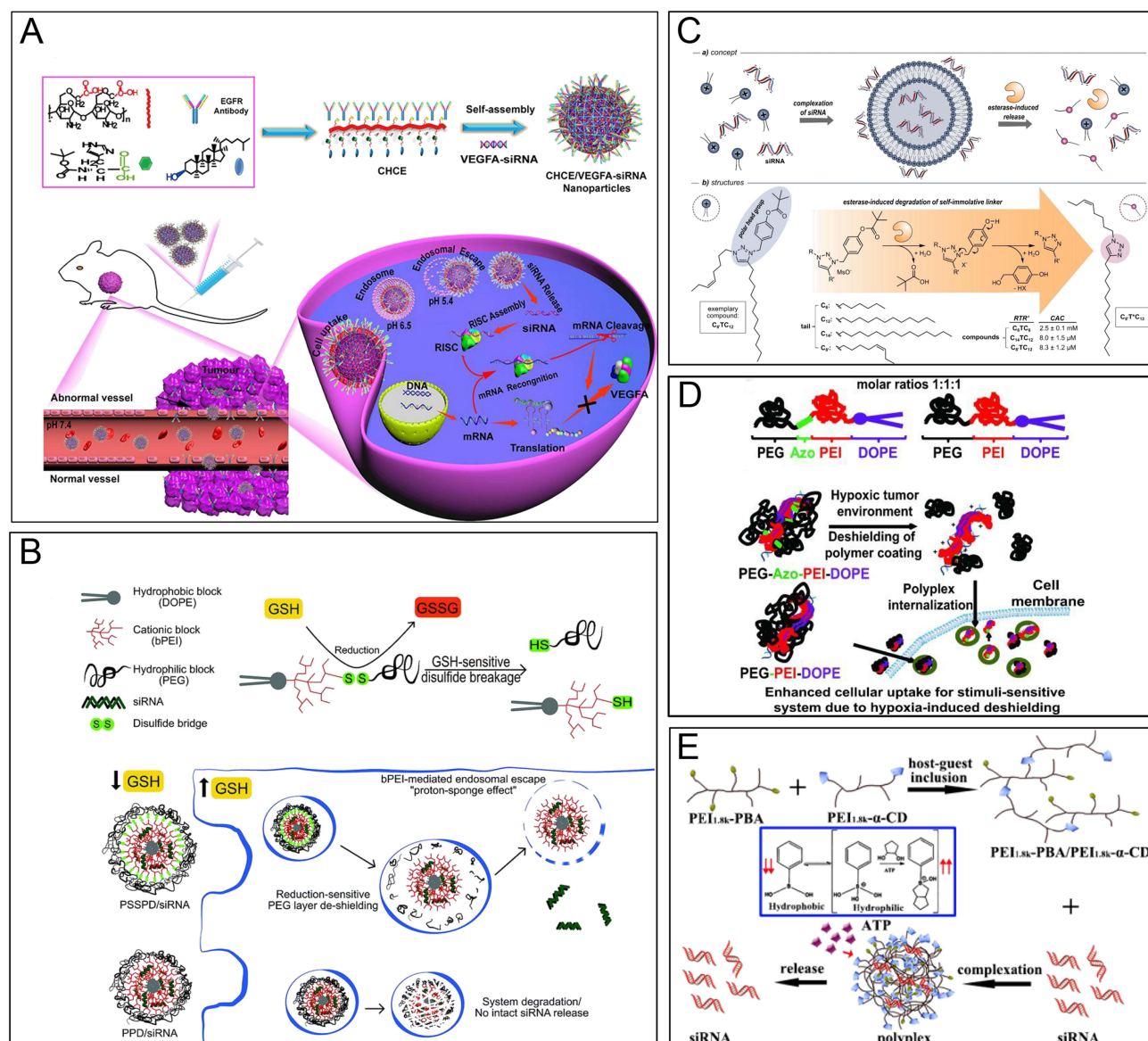
**Figure 6** Bioresponsive materials. (**A**) Schematic diagram of siRNA delivery systems with dual tumor-targeting and pH-responsive capabilities, which can break through biological barriers and penetrate deep into tumors to achieve better tumor therapeutic effects. International Journal of Nanomedicine 2022;17:953–967' used with permission from Zhang XY, Qin B, Wang M et al. Dual pH-responsive and tumor-targeted nanoparticle-mediated anti-angiogenesis siRNA delivery for tumor treatment. Int J Nanomedicine. 2022;17:953–967. Copyright 2022 Dove Medical Press Ltd.[236] (**B**) Schematic diagram of reduction-sensitive PEG-SS-PEI-PE and GSH-mediated PEG de-shielding, and proposed mechanisms for intracellular stimulus-sensitive siRNA delivery. Reproduced with permission from Mutlu Agardan NB, Sarisozen C, Torchilin VP. Redox-triggered intracellular siRNA delivery. Chem Commun (Camb). 2018;54(49):6368–6371.[237] Copyright 2018, The Royal Society of Chemistry. (**C**) Schematic diagram of triazolium amphiphiles capable of binding siRNA and releasing the nucleic acid payload via enzyme-responsive. Reproduced with permission from Hollstein S, Ali LMA, Coste M et al. A triazolium-anchored self-immolative linker enables self-assembly-driven siRNA binding and esterase-induced release. Chemistry. 2023;29(8):e202203311.[238] Creative Commons CC BY license. Copyright 2022, The Authors, published by Wiley-VCH GmbH. (**D**) Schematic diagram of hypoxia-induced siRNA uptake and silencing using a nanocarrier consisting of PEG2000, azobenzene, PEI (1.8 kDa), and DOPE units (named PAPD). Reproduced with permission from Perche F, Biswas S, Wang T, Zhu L, Torchilin VP. Hypoxia-targeted siRNA delivery. Angew Chem Int Ed Engl. 2014;53(13):3362–3366.[239] Copyright 2014, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (**E**) Schematic diagram of self-assembly of PEI1.8k-PBA/PEI1.8k-α-CD supramolecular polymer and release of siRNA through multimer disassembly under ATP stimulation. Reproduced with permission from Jiang C, Qi Z, Jia H et al. ATP-responsive low-molecular-weight polyethylenimine-based supramolecular assembly via host-guest interaction for gene delivery. Biomacromolecules. 2019;20(1):478–489.[240] Copyright 2019, American Chemical Society.

## Redox-Responsive Biomaterials

Based on the redox reaction, nanocarriers can regulate drug release under reducing or oxidizing conditions. Intracellular glutathione (GSH) has been observed to have a concentration ranging from 2–10 mM, significantly higher than extracellular levels. To exploit this disparity, researchers have proposed a novel redox-sensitive micellar nanopreparation named PEG-SS-PEI-PE (PSSPD). This preparation utilizes a triple conjugate of polyethylene glycol, polyethyleneimine,

and phosphatidylethanolamine. Upon exposure to increased GSH levels in tumor cells, the PEG shield is removed, facilitating the release of siRNA inside the cells[237] (Figure 6B). In a recent study, Li et al developed an innovative redox-responsive polyprodrug nanoplatform for targeted siRNA delivery and synergistic cancer therapy. The platform consists of a polyprodrug of 10-hydroxycamptothecin (polyHCPT) as the core, lipid-PEG as the shell, and surface modification with lactobionic acid (LA). Elevated GSH concentrations disrupt the disulfide bonds in polyHCPT, leading to the release of HCPT molecules and Bcl-2 siRNA (siBcl-2), thereby inducing apoptosis through HCPT and synergistically inhibiting tumor growth by silencing the anti-apoptotic gene via siBcl-2.[247] It has also been found that reactive oxygen species (ROS) are prevalent in conditions such as tumors, stroke, and atherosclerosis.[248–250] To exploit the redox potential differences (eg, glutathione) and ROS groups (eg, hydrogen peroxide, hydroxyl radicals) in diseased tissues and cells, researchers have developed various polymers based on disulfide bonds, thioethers, and selenides as nanocarriers for targeted siRNA release at the site of the lesion. These advances aim to enhance drug targeting and improve therapeutic efficacy.[247,251–256]

## Enzyme-Responsive Biomaterials

The utilization of enzyme-responsive materials for siRNA release represents an innovative strategy that enables precise control over siRNA release. This approach offers several advantages, including accurate release control, which helps prevent premature release and potential side effects in non-target tissues. This strategy shows promise by improving therapeutic efficacy and minimizing damage to healthy tissue. Hollstein et al performed a study on triazolium-based amphiphiles that demonstrated their ability to bind to siRNA and facilitate enzyme-responsive release of the nucleic acid payload. Upon the addition of esterases, the linker undergoes cascade degradation, preventing the formation of complexes with neutral triazole compounds and resulting in the release of negatively charged siRNA[238] (Figure 6C). Additionally, Shi et al designed a novel siRNA carrier called polyfluorinated polyarginine (PFC-PR), which is responsive to both oxidoreductases and enzymes. PFC-PR responds to the overexpression of tissue protease B and elevated levels of glutathione in cancer cells. This response enhances the release of siRNA in cancer cells, allowing for targeted delivery and effective cancer therapy.[257]

## Hypoxia-Responsive Biomaterials

In various human body lesions, such as tumors, myocardial ischemia, and vascular diseases, localized sites experience a depleted oxygen environment. To address this, prodrugs and molecular probes designed specifically for these conditions are extensively employed in disease diagnosis and treatment.[258,259] A commonly used oxygen-depleted group is 2-nitroimidazole (2-NI), which is hydrophobic under normal physiological conditions. However, in the depleted oxygen environment, 2-NI is reduced in the presence of nitroreductase to form the hydrophilic compound 2-aminoimidazole (2-AI), leading to nanoparticle depolymerization and subsequent drug release.[239,260] Another commonly used depleted oxygen-sensitive group is azobenzene, which Torchilin et al used as a linking group between PEI and PEG. In the absence of oxygen, the azobenzene bond breaks, leading to the detachment of PEG and the exposure of the positive charge of PEI. The alteration improves the cellular uptake of siRNA while reducing its potential toxic effects[239] (Figure 6D).

## Thermo-Responsive Biomaterials

Temperature serves as a convenient and effective trigger, as thermal stimulation can dilate blood vessels and facilitate drug release. Certain polymers possess temperature-sensitive properties, such as poly-N-isopropylacrylamide (NIPAM). These temperature-sensitive polymers exhibit a low critical solubility temperature (LCST). Below the LCST, hydrogen bonding occurs between the polymer and water molecules, resulting in a water-soluble swollen state. However, as the temperature rises, the hydrogen bonds break, causing the polymer to become insoluble and collapse. Exploiting this phase change behavior in drug delivery systems enables controlled drug release at 40°C.[261] In a study by Honda et al, a thermos-responsive copolymer was developed by combining siRNA with N-isopropylacrylamide (NIPAAm) and hydrophilic N,N-dimethylacrylamide (DMAA). This copolymer undergoes a coil-to-globule transition, allowing for temperature-controlled interaction between siRNA and intracellular proteins involved in gene silencing.[262]

## ATP-Responsive Biomaterials

Adenosine-triphosphate (ATP) serves as a molecular currency for intracellular energy delivery and is more abundant intracellularly than extracellularly. ATP can either compete for adsorption to drug binding sites to trigger drug release[263–266] or disrupt the structure of the carrier by inducing conformational changes.[267] One study reported ATP-responsive nanomicelles modified with phenylboronic acid (PBA) for the controlled release of siRNA. The findings revealed that under normal physiological conditions, siRNA can be encapsulated within the micelles through electrostatic interactions. The PBA component binds to the ribose of siRNA, stabilizing the micelles. However, when the micelles reach tumor sites with elevated ATP levels, ATP competitively binds to PBA, leading to the disruption of the micelle structure and subsequent release of siRNA.[265] In another study investigating ATP-triggered siRNA release, a supramolecular assembly was constructed using low molecular weight polyethyleneimine (LMW-PEI) through host-guest interactions between $PEI_{1.8k}$-α-CD and $PEI_{1.8k}$ -PBA. This assembly facilitated the cellular uptake of siRNA through ATP-dependent endocytosis[240] (Figure 6E).

## Summary and Outlook

The utilization of siRNA has proven to be highly effective in inhibiting the expression of cancer-related genes and reducing the proliferation of cancer cells. By targeting genes associated with cancer metastasis, signaling pathways, drug sensitivity, and apoptosis, siRNA can significantly decrease the malignancy of cancer and improve patient prognosis. In this review, we introduce the mechanism of RNAi and potential targets for siRNA drugs in cancer treatment and summarize the current clinical trials of siRNA therapeutics for cancer. Furthermore, recent advancements in various chemical modifications, delivery systems, and bioresponsive materials that facilitate the stability, safety, bioavailability, and controlled release of siRNAs, are discussed. The review provides a comprehensive overview of siRNA therapeutics and guidance for the investigation and clinical application of siRNA drugs.

siRNA holds promise for the future of disease treatment, particularly those caused by the dysregulation of specific genes. Molecular therapies utilizing siRNA have shown potential for addressing abnormal gene expression or mutations associated with various conditions, including cancer, viral infections, genetic disorders, and pain management. Despite the immense potential of siRNA therapy, the clinical utilization of siRNA-based drugs remains constrained. This can be attributed to the inherent challenges of siRNA itself and the delivery systems. An ideal siRNA delivery system should possess the following functions: 1) maintaining siRNA stability in circulation, 2) improving siRNA's pharmacokinetic characteristics, extending its half-life in the body, and enhancing its distribution in target tissues, 3) promoting cellular uptake of siRNA, 4) facilitating siRNA escape from endosomes, 5) being non-toxic or low in toxicity and non-immunogenic, and 6) exhibiting high transfection efficiency and achieving efficient in vivo delivery of siRNA across cell culture and animal models. The key challenge in achieving the aforementioned functions lies in overcoming physiological barriers within the body and successfully delivering the drugs to the site of the lesion. Advances in nanotechnology have contributed to the maturation of siRNA technology as scientists strive to overcome the safety and delivery obstacles associated with siRNA-based drugs.[268]

Currently, nanodrug delivery primarily relies on external forces, such as magnetic interactions and ultrasound, to overcome physiological barriers. However, the efficiency of this delivery method remains low.[269] In nature, organisms exhibit a diverse array of intricate and sophisticated dynamic systems. For example, multicellular organisms actively transport substances across cells by utilizing their own energy. Cellular scaffolding proteins employ microtubules as tracks to facilitate intracellular substance delivery. Bacterial rotary motor proteins enable flagella to move directionally, resembling propellers. Viral DNA packaging motor proteins assist in loading DNA during replication processes. All of these energy mechanisms are dependent on the biological energy ATP. Regrettably, these systems have not yet been directly applied to siRNA nanodrug delivery. Nevertheless, with the continuous development of new materials and formulation technologies, it is anticipated that siRNA will soon overcome the limitations of the delivery system and find extensive use in tumor treatment.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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