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REVIEW

Bioengineered Nanomaterials for siRNA Therapy of Chemoresistant Cancers

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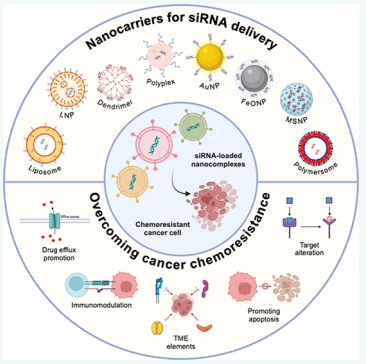
ABSTRACT: **Chemoresistance remains a long-standing challenge after cancer treatment. Over the last two decades, RNA interference (RNAi) has emerged as a gene therapy modality to sensitize cancer cells to chemotherapy. However, the use of RNAi, specifically small-interfering RNA (siRNA), is hindered by biological barriers that limit its intracellular delivery. Nanoparticles can overcome these barriers by protecting siRNA in physiological environments and facilitating its delivery to cancer cells. In this review, we discuss the development of nanomaterials for siRNA delivery in cancer therapy, current challenges, and future perspectives for their implementation to overcome cancer chemoresistance.**



KEYWORDS: *chemoresistance, cancer, siRNA, nanotechnology, gene therapy, nanoparticles, drug delivery, chemotherapy*

1. INTRODUCTION

Drug resistance remains a significant challenge in cancer treatments.[1] Resistance mechanisms often arise from either early innate adaptations or acquired resistance following prolonged treatment exposure. Innate adaptive responses occur rapidly and can lead to transient clinical responses, undermining the efficacy of initial therapies. In contrast, acquired resistance typically involves the activation of alternative pathways or the restoration of primary oncogenic mechanisms due to changes in signaling pathways or epigenetic modifications over time.[2] These adaptations enable cancer cells to survive and proliferate despite therapeutic interventions. Extensive research over the past decade has allowed the identification of resistance mechanisms, leading to the identification of promising targets for drug development.[3] Therefore, developing strategies to target these resistance components and eradicate cancer drug resistance remains a critical focus in modern cancer research.

Advances in gene therapy have made it possible to treat many life-threatening conditions with precision and individualized approaches. For instance, gene therapy has gained prominence in cancer treatment due to significant progress in genetic research and sequencing, which has informed the development of recent drug candidates.[3] RNA interference (RNAi) therapies, particularly those utilizing small interfering RNAs (siRNAs), offer promising opportunities to silence key genes involved in chemoresistance. siRNAs can selectively degrade messenger RNA (mRNA) transcripts, effectively reducing the expression of proteins that contribute to drug resistance. However, the clinical application of RNAi is limited by several challenges, including poor pharmacokinetic properties, rapid degradation by serum endonucleases, and off-target toxicity.[4,5] These limitations have hindered the translation of RNAi therapies from the laboratory to clinical practice.

Nanotechnology can be employed to engineer systems that can serve as delivery platforms for siRNA, addressing the challenges of nucleic acid delivery. Nanoscale drug delivery systems have proven to be effective and versatile carriers of siRNA for passive and active targeting, which is critical for cancer treatment, as it allows for specific siRNA delivery to tumor cells to minimize systemic off-target associated effects.[6−8] Additionally, these nanoparticles can be engineered to respond to specific stimuli or conditions within the tumor microenvironment, enabling controlled and sustained release of siRNA.[8] This approach not only improves the stability and bioavailability of siRNA but can also enhance its intended therapeutic effect. In this review, we examine the advantages and limitations of siRNA therapy in treating chemoresistant

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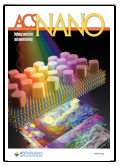

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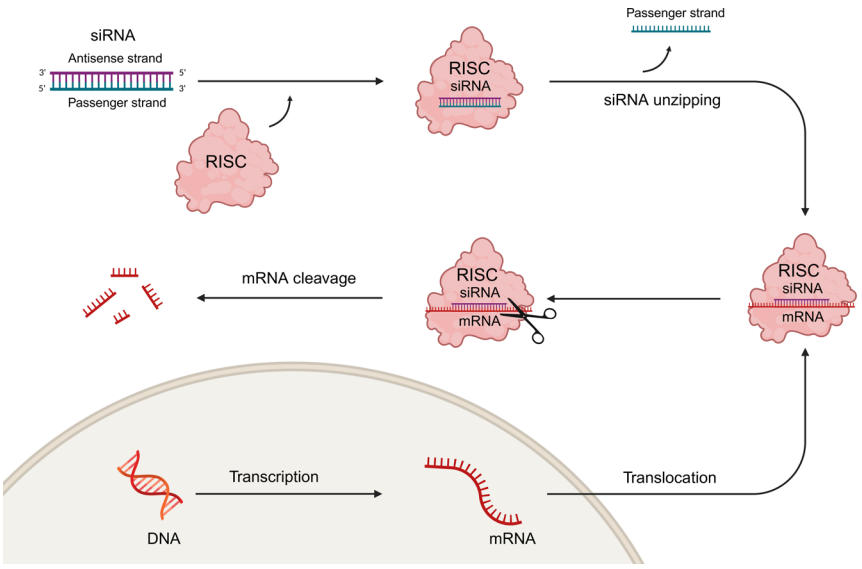

Figure 1. Schematic representation of siRNA-based RNAi gene therapy. First, small interfering RNA (siRNA) enters the RNA-induced silencing complex (RISC). The double-stranded siRNA is then unzipped, and the passenger strand is cleaved. The antisense strand directs RISC to the target mRNA, resulting in its degradation. Created with BioRender.com.

cancers and overview the potential of engineered nanoparticles as effective delivery platforms to overcome these challenges. By integrating RNAi technology with advances in nanotechnology, more effective and personalized treatments for overcoming cancer drug resistance can be developed, with the potential to improve patient outcomes. Multiple literature reviews have highlighted the importance of engineering nanomaterials as siRNA delivery platforms for cancer therapy and overcoming chemoresistance.[9−15] This review addresses the benefits and limitations, along with recent advancements in the engineering of various types of nanoparticles for siRNA delivery, while also offering an in-depth and updated discussion on how this technology aligns with the prevalent mechanisms of cancer chemoresistance.

2. OPPORTUNITIES AND CHALLENGES FOR SIRNA IN CANCER THERAPY

More than two decades after the initial discovery of RNAi by Fire et al., RNAi-based therapeutic strategies now hold significant potential for constructing innovative and targeted cancer medications.[16] RNAi is a conserved biological process exploiting double strands of RNA, e.g., siRNA and microRNA (miRNA), for sequence-specific suppression of any disease-associated gene expression.[17] siRNAs have distinct structural characteristics that include 5′-phosphate and 3′-hydroxyl termini, plus two 3′-overhanging ribonucleotides on each strand of the duplex.[18] To begin the RNAi process, the endoribonuclease Dicer splits siRNAs and dissociates the guide and passenger strands inside the RNA-induced silencing complex (RISC). The guide strand then connects to the target mRNA, accelerating its destruction by argonaute2 (AGO2) (Figure 1).[19] While both miRNA and siRNA can be used to suppress genes, a single miRNA may simultaneously affect the expression of several other genes. On the other hand, since siRNAs must identify the intricate spatial configuration of particular proteins, they usually yield more effective and selective gene silencing. Because of that, siRNAs offer more specificity than other targeted therapies such as small molecule inhibitors and monoclonal antibodies, given that they accomplish precise Watson−Crick base pairing with the target mRNA.[4] As a result, siRNAs offer unique benefits for creating tailored cancer treatments.

In cancer therapy research, siRNAs bring exciting opportunities to overcome chemoresistance as they can pinpoint any particular gene by selecting the appropriate nucleotide sequence along the targeted mRNA. Countless studies, e.g., the investigation by Lou et al. that identified multiple synthetic lethal interactions with Ras oncogene, have exploited siRNAs for genome-wide RNAi screening to discover possible targets in cancer-related signaling.[20−23] In addition, multiple siRNA-based tactics, e.g., suppressing tumor growth by inducing apoptosis and arresting the cell cycle progression in studies by MacDiarmid et al. and Ptasznik et al., have been devised to address drug resistance in cancer models.[24−27] RNAi therapies based on diverse siRNA molecules have been successful in targeting several signaling pathways implicated in cell proliferation and survival.[28−30] siRNAs can also suppress the expression of diverse multidrug resistance (MDR) genes to facilitate the accumulation of anticancer medications at the tumor site.[31] Therefore, siRNAs hold considerable promise to combat drug resistance in a range of cancer types.

Despite the high potential of siRNAs for cancer therapy, fundamental concerns such as poor pharmacokinetic characteristics, instability in the biological milieu, off-target toxicity, and immunogenicity have hindered their practical applicability.[17,32] Naked siRNAs are compromised by multiple clearance mechanisms after being intravenously administered. For instance, RNase enzymes have been demonstrated to degrade circulating siRNAs.[33] In addition, Kupffer cells of the reticuloendothelial system have been shown to remove siRNAs from the bloodstream.[34] The remaining siRNAs are almost entirely flushed out of the body via liver metabolism and kidney-mediated excretion.[34,35] siRNAs also have a limited half-life, which significantly impedes their therapeutic efficacy. The large molecular weight, high water solubility, and polyanionic character of siRNAs make it challenging for them to traverse the lipid bilayer of the plasma membrane

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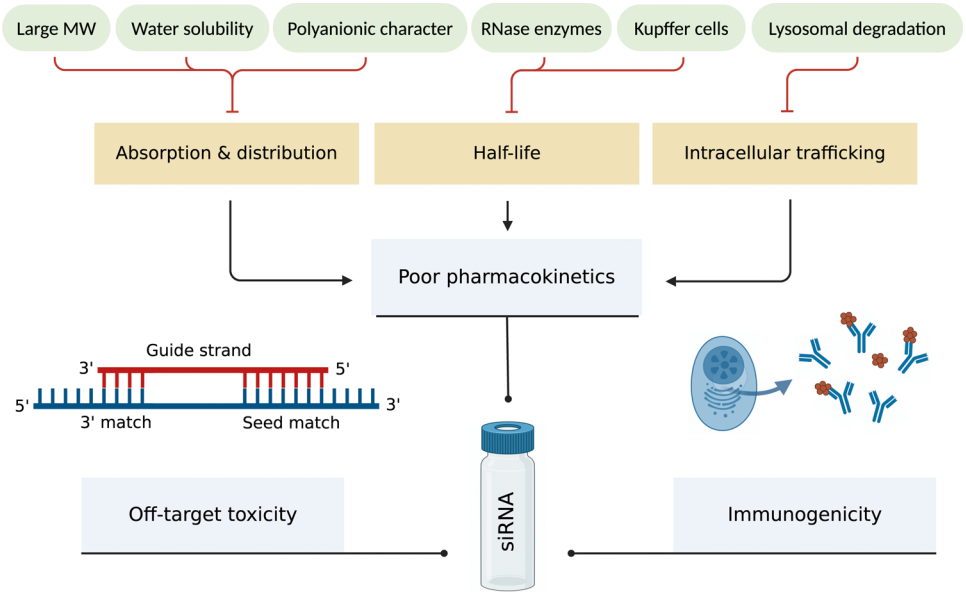

Figure 2. Shortcomings of small interfering RNA (siRNA)-based RNA interference (RNAi) therapy. Poor pharmacokinetics, off-target effects, and immunogenicity are the main drawbacks of the *in vivo* application of siRNAs for cancer therapy. MW, molecular weight. Created with BioRender.com.

independently.[36] Further, lysosomal degradation at the end of the endocytic pathway limits the intracellular accumulation of siRNAs.[37] For all these reasons, it is challenging for siRNA to reach target tissue and maintain an effective concentration inside cells (Figure 2).

Given their propensity to induce off-target reactions and immunogenicity, the safety profile of naked siRNAs is relatively unsatisfactory. Even though siRNAs are intended to silence a particular gene, they occasionally generate miRNA-like off-target toxic effects, which has raised safety concerns. siRNAs may yield miRNA-like silencing mechanisms at the three prime untranslated regions (3′ UTRs) of undesired transcripts, triggering their sequence-dependent control by seed region complementarity.[38] Additionally, the appearance of immunological responses is not surprising since the human immune system recognizes siRNAs as foreign pathogens. RNA-binding Toll-like receptors (e.g., TLR3, TLR7, and TLR8) recognize siRNAs, setting off the innate immune response by enabling the overproduction of cytokines and interferons. This results in the loss of siRNA efficacy and the induction of immunologic reactions.[39] The mentioned drawbacks (Figure 2) drastically hamper the therapeutic utility of siRNAs and their translation into the clinic.

Accordingly, the most significant concerns in the development of siRNA-based therapies are to reduce adverse effects, extend circulation time, and improve efficient tissue-specific delivery of siRNAs with sufficient penetration and concentration to the intended region. Different viral delivery platforms have been created to address the shortcomings of naked siRNAs and improve the efficiency of RNAi treatment in living organisms.[40] Although viral vectors like adeno-associated viruses have high transfection efficiency, concerns about insertional mutagenesis and immunogenicity limit their use in siRNA therapy.[41] Current breakthroughs in nanotechnology and the advent of advanced customized delivery systems bring opportunities to reassess the efficacy of siRNAs in cancer therapy, particularly resistant genotypes. These nanoparticle-based delivery systems increase the biological stability of siRNAs, transport them to the specific region of interest, and minimize off-target effects.[42] In the next section, we will discuss the capabilities of nanoparticle-based delivery systems for siRNA delivery.

3. ADVANTAGES OF NANOPARTICLES FOR SIRNA DELIVERY

Nanotechnology holds promise in circumventing the hurdles associated with conventional drug delivery, notably those related to biodistribution and intracellular trafficking.[43] Nanoparticles exploiting passive and active targeting offer possibilities for improved cancer diagnosis and therapy specificity.[44−47] The passive targeting properties of nanoparticles augment the therapeutic efficacy of the encapsulated siRNAs by enhancing their stability and solubility, extending their circulation periods, and enabling their transport across membranes. Applying active targeting-based modifications further yields nanoparticles with tissue-specific drug delivery properties and reduced off-target effects.[47−49]

3.1. Passive Targeting. Nanoparticles can exploit tumor vascular anomalies to extravasate into tumor tissues preferentially. Moreover, solid tumors have insufficient lymphatic drainage, which causes nanoparticle accumulation to increase. These describe the basic principles for the passive targeting of nanoparticles, termed the enhanced permeability and retention (EPR) effect.[50] Thus, encapsulation of siRNAs into nanoparticles protects them from RNase breakdown in circulation, reduces their early renal clearance, extends their half-life, and passively guides them into tumor tissue owing to the EPR effect (Figure 3).[42] These advantages have been shown in multiple studies, including the investigation by Meng et al. demonstrating the ability of mesoporous silica nanoparticles (MSNPs) to provide efficient and protected delivery of doxorubicin-P-glycoprotein (P-gp) siRNA conjugates to the tumor site to overcome drug resistance in breast cancer *in vivo*.[51] Another example is the study by Wang et al. that designed gold nanorod-siRNA nanoplexes for targeted silencing of Bcl-2 associated athanogene domain 3 (BAG3)

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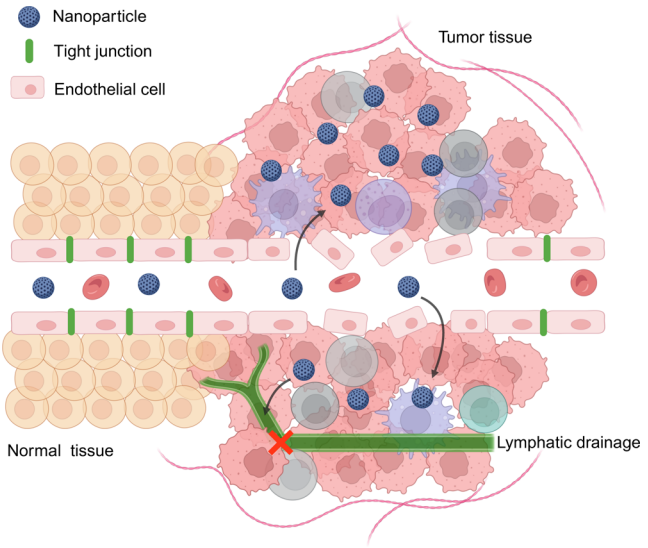

Figure 3. Schematic illustration of the enhanced permeability and retention (EPR) effect. Nanoparticles selectively extravasate into tumor tissue by exploiting vascular abnormalities through gaps between adjacent endothelial cells without tight junctions. Furthermore, solid tumors have inadequate lymphatic drainage, which leads to a gradual increase in nanoparticle accumulation. Created with BioRender.com.

and promoting apoptosis in human oral squamous cell carcinoma cell cal-27 xenograft nude mice.[52] As a different approach to improving the transport of nanoparticles from blood vessels to the tumor, Wang et al. proposed breaking through the tumor vascular basement membrane with hyperthermia. The basement membrane is a mechanical barrier, entrapping nanoparticles in the subendothelial space. It was found that inflammation caused by local hyperthermia drew platelets to the nanoparticles, subsequently attracting neutrophils to these spaces. Moving neutrophils across the basement membrane proceeded to surpass the barrier, enabling the release of nanoparticles and allowing them to penetrate deeper into tumors.[53]

Passive targeting involves fine-tuning the physicochemical properties of nanoparticles, e.g., size, shape, stiffness, and surface traits, to establish an optimal interaction with the biological environment.[54] For example, the surface properties of nanoparticles impact their passive targeting ability. In the systemic circulation, plasma proteins with negative charge adhere to the surface of cationic nanoparticles (i.e., the opsonization process) and form aggregates that can be identified by innate immune system elements (e.g., complement proteins), enabling the mononuclear phagocyte system (MPS) cells, especially Kupffer cells and macrophages, to recognize and remove them from circulation.[55] Complement attachment and identification by MPS may be prevented if the surface is neutral or slightly negatively charged. With this in mind, a stealth coating of nanoparticles with biocompatible polymers, particularly polyethylene glycol (PEG), is often used. Such polymers enable differences in the surface potential and hydrophobicity of the nanoparticles, reducing their phagocytosis, extending their systemic circulation, and improving their biodistribution.[56] Nevertheless, while essential for the stealth qualities of nanoparticles, the PEG coating may also impede cellular uptake.[57] Further, there is a growing concern regarding the potential of PEG immunogenicity since high anti-PEG antibody titers have been reported in several clinical studies.[58,59] As an alternative to stealth coatings, nanoparticles may have CD47 molecules, a well-known 'do not eat me' signal, functionalized to their surface to evade phagocytosis.[60] Consequently, surface-engineered nanoparticles hold great potential for evading the immune system and natural clearance mechanisms and transporting their encapsulated siRNAs to the tumor sites.

However, findings about the EPR phenomenon in murine xenograft tumors with extensive vasculature may not be accurately reflected most solid human tumors, given variables such as small relative tumor size, heterogeneous tumor perfusion, and dissimilar tumor microenvironment (TME).[61−63] This may hinder the translation of nanotechnology for human application.[64] However, several strategies have been proposed to strengthen the EPR effect via permeability-enhancing elements (e.g., TNF-α, angiotensin-II, and sonoporation) in low-EPR tumors or bypass the EPR effect (e.g., regional delivery and vasculature targeting) for tumors in isolated organs (e.g., brain and bone).[65,66]

3.2. Active Targeting. Active targeting consists of functionalizing the surface of nanoparticles with ligands that bind to receptors or other motifs that are highly expressed by tumor cells, tumor-blood vessels, and other TME elements.[67] It was developed as a complementary technique to passive targeting for boosting delivery efficacy by increasing targeting accuracy and improving retention at the tumor region.[68,69] Various active ligands, particularly antibodies, oligonucleotides (e.g., aptamers), and peptides (e.g., RGDs), are often attached to the surface of nanoparticles to strengthen their affinity for certain cells, resulting in tailored siRNA delivery (Figure 4).[70−72] Ligand-mediated recognition of cancer cells improves nanoparticle cellular uptake through endocytosis.[73] In

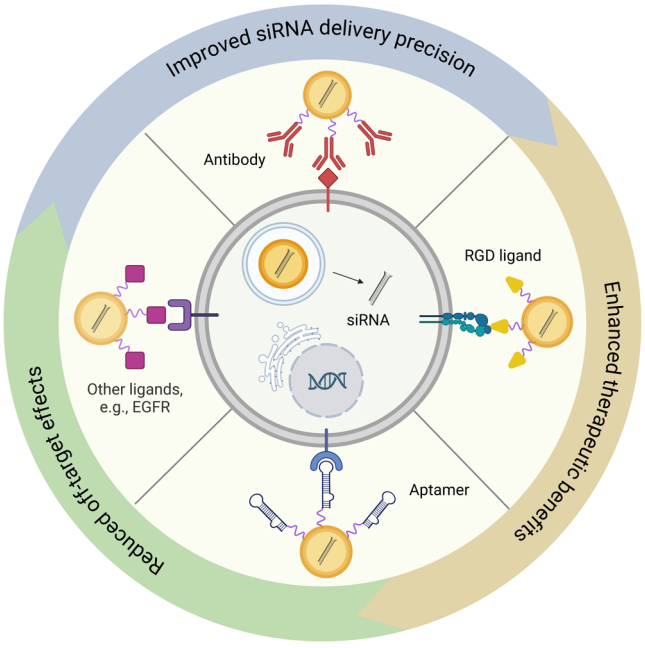

Figure 4. Nanoparticle active targeting approaches. Small interfering RNA (siRNA) delivery is achieved by attaching ligands to the surface of nanoparticles to increase their delivery to tumor cells. Common active ligands include antibodies, oligonucleotides (e.g., aptamers), and peptides (e.g., RGD). EGFR, epidermal growth factor receptor. Created with BioRender.com.

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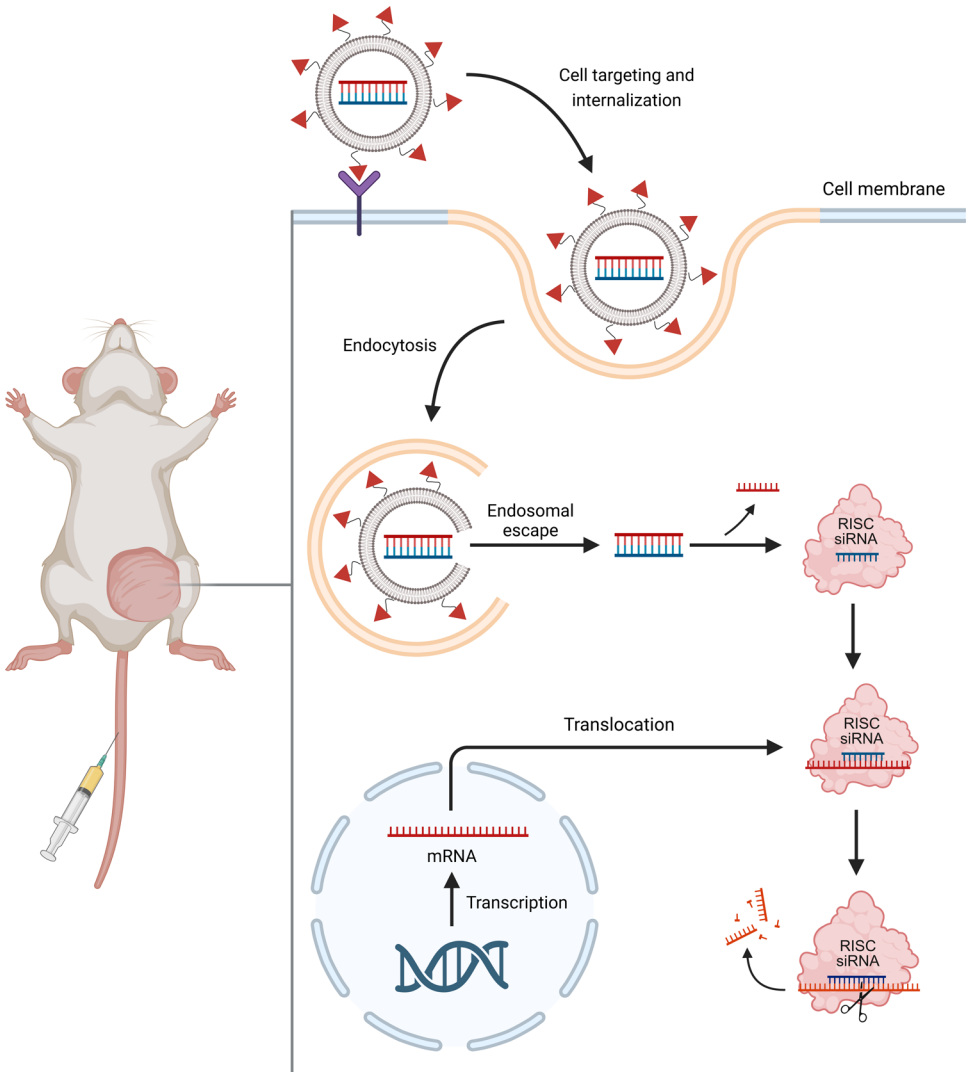

Figure 5. Nanoparticle-based small interfering RNA (siRNA) therapy. Steps include: (i) targeting tumors based on the interaction between targeting ligands on the surface of nanoparticles and receptors overexpressed on tumor cells, (ii) endocytosis-mediated nanoparticle internalization, (iii) siRNA endosomal escape, and (iv) siRNA-induced gene silencing. In the cytosol, the released siRNA enters the RNA-induced silencing complex (RISC), the double-stranded siRNA unwinds, and the passenger strand is cleaved. The antisense strand then directs RISC to the target mRNA, leading to mRNA degradation. Created with BioRender.com.

addition, ligands may interact with their targets in an agonistic/antagonistic pattern in favor of cancer treatment.[74]

Increasing nanoparticle delivery to tumors with antibodies is an emerging strategy that leverages the promise of antibody-conjugated drugs and nanotechnology for cancer therapies.[75] The strong affinity of tumor antigen−antibody binding can be leveraged to increase nanoparticle drug delivery to the tumor site.[76] Yang et al. demonstrated substantial tumor targeting and uptake of epidermal growth factor receptor (EGFR) antibody-functionalized iron oxide nanoparticles (FeONPs) after systemic administration in an orthotopic pancreatic cancer model.[77] Antibody fragments, e.g., antigen-binding fragments (Fab), may likewise be used to improve tumor absorption and diffusion rates.[78] For example, Okamoto et al. decorated lipid nanoparticles (LNPs) with a Fab′ antibody against heparin-binding epidermal growth factor (EGF)-like growth factor (αHB-EGF) for targeted delivery of polo-like kinase 1 (Plk1) siRNA to breast cancer cells overexpressing HB-EGF on their surface.[79] As alternatives to antibodies, aptamers are short fragments of single-stranded DNA or RNA molecules with a tridimensional architecture that firmly bind to cognate targets on tumor cells. Kim et al. demonstrated that functionalizing doxorubicin-loaded gold nanoparticles (AuNPs) with prostate-specific membrane antigen (PSMA) aptamers confers considerable targeting capabilities and significantly improves their therapeutic effects in prostate cancer cells.[80] Notably, aptamers are preferable agents to target neoplasms due to their potentially reduced immunogenic responses, versatile chemical modification, and simplicity of manufacturing compared to antibodies.[81]

Substantial efforts have been made to devise delivery methods based on functionalizing nanoparticles with RGD peptides that recognize integrin receptors expressed by tumor cells and vasculature.[82−84] The expression of integrins is increased on the surface of malignant cells since integrins mediate adhesion to extracellular matrix (ECM) and activate intracellular signaling pathways that control cytoskeletal architecture, angiogenesis, and survival.[85] Therefore, targeting integrins with RGD peptides offers both active tumor targeting and potential therapeutic effects. Jiang et al.

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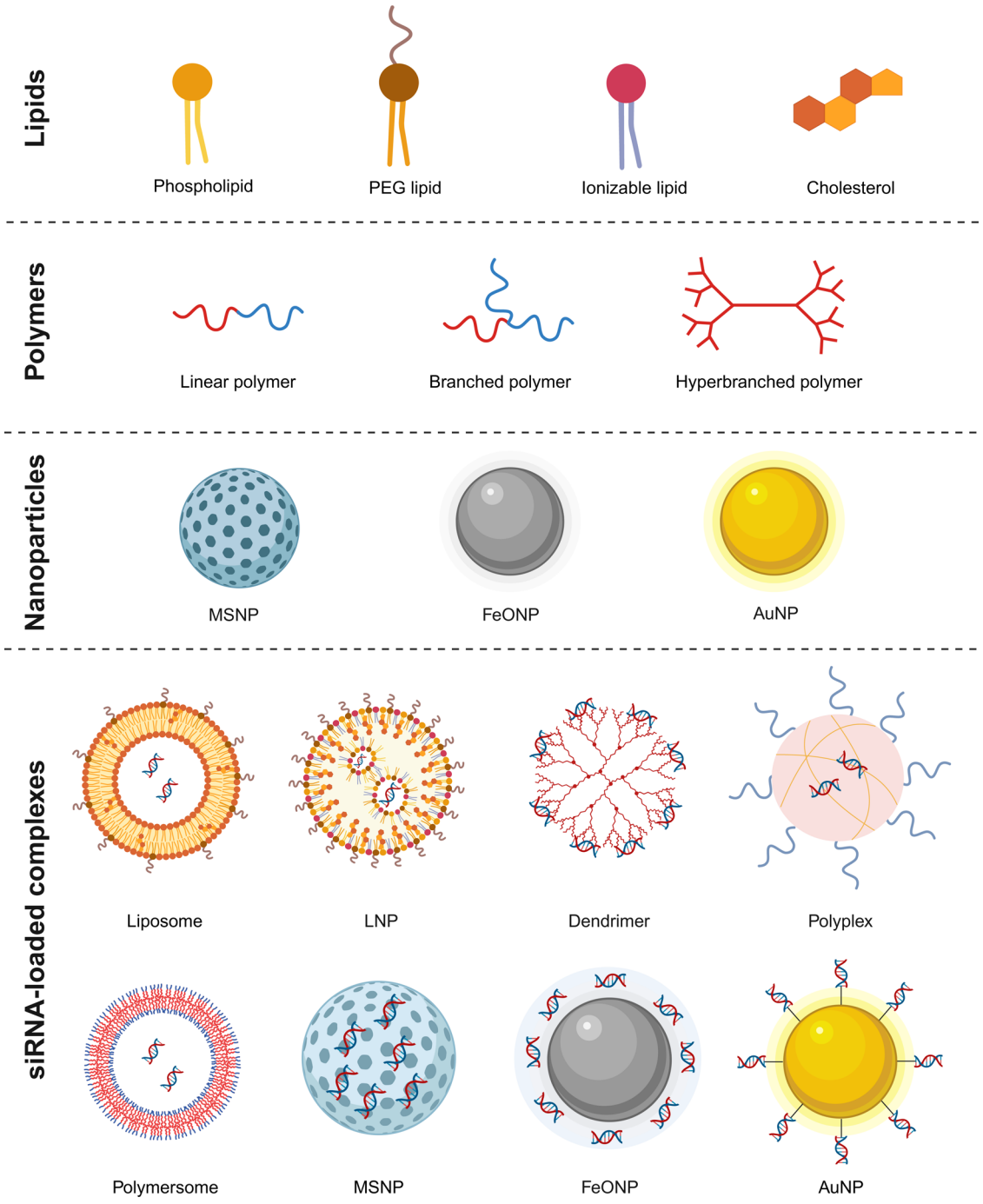

Figure 6. Materials used for engineering nanoparticles for small interfering RNA (siRNA) delivery. PEG, polyethylene glycol; MSNP, mesoporous silica nanoparticle; FeONP, iron oxide nanoparticle; AuNP, gold nanoparticle; LNP, lipid nanoparticle. Created with BioRender.com.

demonstrated the ability of RGD-functionalized liposomes in the targeted delivery of P-gp siRNA and suppressing tumor growth compared to nonmodified liposomes in a murine model of drug-resistant breast cancer.[86] Schiffelers et al. developed RGD-functionalized PEGylated polyethylenimine (PEI) nanoparticles to deliver vascular endothelial growth factor receptor 2 (VEGFR2) siRNA, which conferred selective tumor uptake of siRNA given the RGD-mediated recognition of tumor neovasculature expressing integrins and suppressed tumor angiogenesis and growth in N2a tumor-bearing mice.[87] A review article by Danhier et al. highlights the importance of RGD-based nanoparticles in targeted cancer therapy.[88] Additional ligands have also been the focus of research, particularly small molecules (e.g., folate) and transferrin, due to their high affinity for associated receptors upregulated on the surface of tumor cells.[89,90] Jones et al. fabricated folate-targeted PEI-poly(ε-caprolactone) (PCL)-PEG triblock copolymers for tailored delivery of TLR4 siRNA and paclitaxel to SKOV-3 ovarian cancer cells, resensitizing them to paclitaxel treatment.[91] Yhee et al. designed tumor-targeting transferrin nanoparticles for rapid tumor cell-specific uptake of

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Table 1. Common Physicochemical Characterization Methods for Nanoparticles[a]

Technique	Description	Measured parameters	Advantages/disadvantages	Refs
TEM	Generates images of nanoparticles at extremely high magnification using a focused beam of electrons.	Size (>1 nm and <1 μm), size distribution, and shape (2D projection)	- Provides sub-nm resolution of nanoparticle morphology. - Provides information on the internal structure of nanoparticles. - Nanoparticles must be electron transparent and resist high beam energy and vacuum. - Expensive.	96,97
SEM	Scans the surface of nanoparticles with secondary electrons emitted upon their interaction with the electron beam.	Size (>2−3 nm and <10 μm), size distribution, and shape (2D projection)	- Provides single-particle resolution. - Reduces the likelihood of beam-mediated damage to the nanoparticles. - Nonconductive nanoparticles require conductive coatings. - Restricted penetration depth.	98,99
AFM	Generates images of nanoparticles based on the interplay between a cantilever that scans a surface and the forces generated by the sample.	Size (<1 nm in vertical resolution or ≈10−20 nm in lateral resolution), size distribution, and shape (3D imaging)	- Suitable for both dry and wet specimens. - Nanoparticles must be immobilized on a surface. - Samples are typically deposited on a hard surface. - Time-consuming measurements.	100,101
DLS	Determines nanoparticle size by detecting the light scattered from the interaction of light with nanoparticles.	Hydrodynamic diameter (>5 nm and <10 μm) and size distribution	- Suitable for nanoparticles in suspension. - Compatible with diverse solvents. - Determines the behavior of nanoparticles in solution. - Requires minimal sample volume. - Lack of information on nanoparticle shape. - Not suitable for highly polydisperse particle specimens.	102,103
MS	Characterizes the composition of nanoparticles following digestion and dissolution using ionization techniques.	Size (>0.1 nm and <50 nm), size distribution, and composition	- Suitable for dry nanoparticles and nanoparticles in solution. - Ionization may affect the stability of nanoparticles.	104−106
ELS	Converts the measured electrophoretic mobility of nanoparticles into zeta potential.	Zeta potential	- Suitable for nanoparticles in suspension. - Minimal sample preparation. - Fast analysis. - Usually combined with DLS.	107,108
XRD	Uses X-ray diffraction and analyzes the FWHM of the Bragg reflections.	Crystallite size (≈1−100 nm)	- Provide data on crystal structure. - Does not characterize nanoparticle size.	109,110

[a]Abbreviations: TEM, transmission electron microscopy; SEM, scanning electron microscopy; AFM, atomic force microscopy; DLS, dynamic light scattering; MS, mass spectrometry; ELS, electrophoretic light scattering; XRD, X-ray diffraction; FWHM, full width at half-maximum.

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Table 2. Common Pharmacokinetic and Safety Characterization Methods for Nanoparticles[a]

Type of characterization	Measured parameters	Standard protocols	Refs
Analytical	Cargo encapsulation and release	**Fluorescence spectrometry**	111−113
		Measures changes in the fluorescent drug/probe optical features as a result of its interaction with nanoparticles.	
		Colorimetric assay	114
		Uses reagents that induce a measurable color shift in the presence of the unloaded or released drug.	
		HPLC	115
		Measures the amount of drug loaded into or released out of nanoparticles with considerable precision.	
Cellular	Biological stability	**Protein corona analysis**	116
		Investigates the impact of protein corona formed around nanoparticles on their stability and interaction with biological elements.	
		Incubation with biological fluids	117,118
		Evaluates protein corona formation, nanoparticle behavior, and cellular interactions in a biological fluid exposure model.	
	Cellular toxicity	**Fluorescence microscopy**	119,120
		An imaging technique that observes viability, morphology, and cytoskeletal architecture of cells following exposure to nanoparticles.	
		MTT colorimetric assay	121,122
		Determines viable cell number based on the cleavage of MTT by mitochondrial enzymes to the blue formazan product.	
	Cellular uptake	**Flow cytometry**	123,124
		Measures the scattering and fluorescence of cells in stream, thereby enabling the reliable quantification of nanoparticle uptake.	
		Confocal microscopy	125,126
		A highly sensitive imaging technique with optical sectioning capability that enables the evaluation of nanoparticle uptake by cells.	
Animal	Biodistribution	**Fluorescence imaging**	127
		A noninvasive imaging method that relies on the detection of fluorescently labeled nanoparticles with a defined wavelength of light, particularly in the second near-infrared window, enabling imaging in animals.	
		Luminescence imaging	128
		A low-cost and noninvasive imaging technique that uses nanoparticle-generated luminescence to determine nanoparticle biodistribution within the body.	
		MRI	129
		A noninvasive imaging technique that determines the biodistribution of magnetic nanoparticles acting as contrast agents.	

[a]Abbreviations: HPLC, high-performance liquid chromatography; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; MRI, magnetic resonance imaging.

red-fluorescent-protein (RFP) siRNA with negligible cytotoxicity in RFP overexpressing melanoma cells.[92]

Nanoparticles with targeting moieties still face delivery challenges. After internalization through endocytosis pathways, nanoparticles must enable the endosomal escape of encapsulated siRNAs and facilitate their cytosolic delivery. In the acidic medium of endosomes, protonation of surface moieties of nanoparticles can induce the proton sponge effect, leading to the inflow of water and ions, followed by osmotic swelling and rupture of endosomes and the cytosolic release of siRNAs.[93] Nanoparticle PEG coatings may hinder endosomal escape, and thus pH-sensitive linkers have been employed to facilitate the cleavage of PEG chains and expose the positive surface charge of nanoparticles in the endosomes.[94] Several other targeting strategies have also been explored, including the development of stimuli (e.g., light, temperature, magnetic field) or condition (e.g., acidic pH or reductive status)-responsive nanoparticle-based delivery platforms.[65] Thus, precision engineering of nanoparticles is crucial to attain desired cell- and organ-targeting effects.

4. CLASSES OF NANOPARTICLES FOR SIRNA DELIVERY

Several classes of nanoparticles have been designed and evaluated for drug delivery. Based on their tunability, biocompatibility, and safety, the following nanoparticles can be utilized for nucleic acid cancer therapies (Figures 5 and 6). The physicochemical properties of nanoparticles (e.g., shape, size, and zeta potential) have a substantial impact on their biological interactions; thus, precise characterization of nanoparticles is essential.[95] Table 1 summarizes the characterization methods for determining the physicochemical properties of nanoparticles. Further, Table 2 highlights common methods for examining the pharmacokinetic and safety profiles of nanoparticles.

4.1. Lipid-Based Nanoparticles. Lipid-based nanoparticles are commonly used in drug delivery systems, given their excellent biocompatibility and biological stability, as well as their affinity for biological membranes, such as plasma and endosomal membranes, enabling their cellular uptake and endosomal escape of their encapsulated cargos. The formulation process of these nanoparticles is simple, precise, and tunable, allowing the synthesis of nanoparticles with desired properties (e.g., surface potential and chemistry and particle size). Moreover, lipid-based nanoparticles have both hydrophilic and hydrophobic regions, allowing them to encapsulate therapeutics with diverse structures and physicochemical characteristics.[130] Lipid-based nanoparticles are now being evaluated and used in clinical settings to treat solid tumors.[131]

4.1.1. Liposomes. Liposomes are the most prevalent type of lipid-based nanoparticles, constructed from phospholipid

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Table 3. Features and Examples of Lipid-Based Nanoparticles for siRNA Delivery[a]

Type	Features	Examples			Notes	Refs
		Lipid	Targeting ligand	siRNA		
Cationic liposomes	- Noncovalent encapsulation of siRNAs	DOTAP	Angiopep-2, tLyP-1	VEGF siRNA	- PEG coating reduces hemolytic potential and immunogenicity of cationic lipids and prolongs their circulation but reduces their cellular uptake.	135−137
	- High siRNA loading efficiency	DOPE	cRGD	RRM1 siRNA	- Targeting ligands reduce off-target liposome uptake and improve on-target uptake.	
	- High cellular uptake - Nonspecific interactions - Immunologic reactions - Rapid clearance	DOTMA, DOPE	RGD	MYCN siRNA		
Anionic liposomes	- Noncovalent encapsulation of siRNAs	DOPG, DOPE	Cationic K16 peptide	BACE1 siRNA	- PEG coating and cationic peptides help improve cellular uptake.	142,143
	- Low siRNA loading efficiency				- Targeting ligands enhance uptake by target cells.	
	- Low toxicity and immunogenicity - Low cellular uptake	POPC, POPG	Fibronectin-targeted peptide	CypA siRNA	- Complexation of siRNA with protamine enhances loading efficiency.	
LNPs	- Noncovalent encapsulation of siRNAs - Gains positive charge in acidic environments	DLin-MC3-DMA	Hyaluronan	Plk1 siRNA	- LMW PEG lipids improve circulation stability and half-life and lessen nonspecific protein adsorption.	145,151−153,156
	- High siRNA encapsulation in low pH - Lack of protein adsorption-induced clearance	BAMPA-O16B		CD47 and PD-L1 siRNAs	- Optimal PEG lipid percentage is critical for obtaining the optimal particle size.	
	- Biocompatibility and lack of hemolytic and immunologic reactions	iBL0104	c(GRGDSPKC)	Plk1 siRNA	- Targeting ligands enhance uptake by target cells.	
	- Efficient endosomal escape				- Helper lipids improve siRNA entrapment, formulation stability, and membrane fusion.	

[a]Abbreviations: siRNA, small interfering RNA; DOTAP, 1,2-dioleoyl-3-trimethylammonium propane; Angiopep-2, low-density lipoprotein receptor-related protein receptor; tLyP-1, neuropilin-1 receptor; VEGF, vascular endothelial growth factor receptor; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; RPM1, ribonucleotide reductase subunit M1; DOTMA, N-[1- (2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride; MYCN, v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homologue; LMW, low molecular weight; PEG, polyethylene glycol; BACE1, beta-site amyloid precursor protein cleaving enzyme 1; DOPG, 1,2-dioleoyl-*sn*-glycero-3-PG; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylglycerol; CypA, cyclophilin A; Plk1, polo-like kinase 1; PD-L1, programmed death-ligand 1.

building blocks self-assembled into unilamellar and multilamellar vesicles due to their amphiphilic nature. Their stability and transport efficiency depend on lipid content, surface charge, and particle size.[132,133] Numerous studies have used film hydration, reverse phase evaporation, and solvent injection preparation procedures to achieve acceptable drug encapsulation and generate uniformly sized liposomes.[134] Liposomes composed of cationic lipids, e.g., 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), and zwitterionic lipids, e.g., 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), have been used for siRNA delivery due to their interaction with the negative backbone of siRNAs. For example, Yang et al. prepared active targeted DOTAP-based cationic liposomes for the delivery of VEGF siRNA and docetaxel to gliomas and demonstrated a synergistic tumor suppression effect *in vivo*.[135] Khatri et al. generated cRGD-decorated DOPE-containing liposomes for targeted delivery of ribonucleotide reductase catalytic subunit M1 (RRM1) siRNA to lung cancer cells *in vitro* and *in vivo*.[136] Tagalakis et al. employed RDG-decorated and MYCN oncogene siRNA-loaded DOTMA/DOPE liposomes to treat neuroblastoma *in vivo* and showed considerable and targeted siRNA transfection of tumor cells.[137]

Of note, the surface charge of liposomes may cause nonspecific interactions as well as immunologic reactions and facilitate their clearance through the MPS system. A protective PEG coating can mask the surface charge, thereby extending the circulation time and reducing the immunogenicity of liposomes. However, it may discourage the electrostatic interactions essential for cellular absorption and interfere with the endosomal escape of liposomes by blocking their fusion with the lipid membrane.[138,139] This issue can be minimized by stabilizing cationic liposomes with anionic glycosaminoglycans (e.g., hyaluronic acid) or even PEGylated hyaluronic acid, which improves serum stability and cellular absorption compared to PEGylated liposomes.[140] Another critical factor in developing liposomes is particle size, which can determine the extent of MPS-mediated liposome dissociation. Liposomes with a diameter of less than 100 nm can result in reduced phagocyte uptake.[141] Given the toxicity of quaternary ammonium in cationic lipids, anionic liposomes have also been designed for siRNA delivery. For example, Tagalakis et al. employed cationic K16 peptide-decorated anionic liposomes to encapsulate beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) siRNA and facilitate its delivery to the brain. Surface modification of anionic liposomes with the K16 motif improved cellular uptake.[142] In another investigation, Xu et al. conjugated Cyclophilin A (CypA) siRNA with cationic protamine and encapsulated the complex into the core of targeted PEGylated anionic liposomes against the extra-domain B (EDB) of

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ACS Nano 2024, 18, 34425−34463

fibronectin that is overexpressed on glioma cells for down-regulation of the tumor-promoting CypA gene in glioma tumors.[143]

4.1.2. Lipid Nanoparticles. Lipid nanoparticles (LNPs), including solid LNPs (SLPs) and nanostructured lipid carriers (NLCs), are one of the most clinically relevant delivery platforms (Table 3).[144] These nanoparticles are primarily composed of ionizable and cationic lipids, helper lipids, and PEG lipids.[145−147] Ionizable lipids possess ionizable head groups that maintain a neutral charge at physiological pH but can become positively charged through protonation in acidic environments. There are multiple advantages associated with incorporating these lipids into LNPs, e.g., enhancing siRNA encapsulation efficiency by incorporating low pH buffers during preparation, preventing nuclease-mediated siRNA breakdown in the circulation, improving biocompatibility by avoiding interactions with blood and immune cells, and enabling endosomal escape.[148−150] A few examples of LNPs for siRNA delivery to tumor cells include works done by Cohen et al.and Liu et al., by using DLin-MC3-DMA and BAMPA-O16B ionizable lipids to construct LNPs for delivery of Plk1 siRNA to drug-resistant glioma tumors and CD47 and programmed death-ligand 1 (PD-L1) siRNAs for glioma immunotherapy, respectively.[151,152] In addition, Guo et al. generated RGD-decorated ionizable LNPs for the targeted delivery of Plk1 siRNA to liver tumors *in vivo*.[153] Of note, developing next-generation ionizable lipids by tailoring their head groups and hydrophobic tails to various nucleic acids is currently an ongoing and promising approach.[154,155]

Low-molecular weight (LMW) PEG lipids consisting of a hydrophilic PEG polymer anchored to a lipid moiety (e.g., DMG or DSPE) were shown to improve circulation stability and half-life, lessen nonspecific protein adsorption, and conserve the on-target delivery of nanoparticles.[156,157] Helper lipids like cholesterol and phospholipids are also involved in enhancing siRNA encapsulation, formulation stability, and membrane fusion.[145] LNPs extravasate into tumor tissue and enter cells by endocytosis, where the positive charge of ionizable lipids facilitates the endosomal escape and cytosolic release of siRNAs through destabilizing interactions with the negatively charged endosomal lipid membranes.[146,150] The overall lipid content of these nanoparticles can also be fine tuned, for instance adjusting the PEG lipid percentage of LNPs can lead to optimal particle sizes for siRNA delivery.[158] Efficient, single-step, and scalable rapid-mixing methods (e.g., T-mixing and microfluidic mixing) are used for fabricating homogeneous LNPs of desired size.[159]Notably, LNPs have made significant strides in clinical applications; for instance, Patisiran (Onpattro), an LNP-based siRNA drug, was approved in 2018 for hereditary polyneuropathy, and LNP-mRNA vaccines have successfully been employed in clinical settings to combat the coronavirus disease 2019 (COVID-19).

4.2. Polymeric Nanoparticles. Polymeric nanoparticles are promising materials for siRNA delivery due to their simplicity of fabrication, broad structural customization and functionalization, transfection efficacy, and exceptional biocompatibility.[160] Diverse natural, e.g., cyclodextrin and chitosan, and synthetic, e.g., polylactic-*co*-glycolic acid (PLGA) and PEI, polymers have been employed in nanocarriers for cancer gene delivery.[7] These polymers can be synthesized using well-defined techniques, including the Michael addition reaction, reversible addition−fragmentation chain transfer polymerization (RAFT), radical polymerization, atom transfer radical polymerization (ATRP), and ring-opening polymerization.[161] The intrinsic characteristics of polymers exert a pronounced influence on the performance of the produced nanoparticles. Cationic polymers electrostatically adsorb siRNAs to generate polymeric nanoparticles. PEI, a cationic polymer with an abundance of primary, secondary, and tertiary amines, possesses a high concentration of positive charges. This allows PEI to facilitate the efficient encapsulation of siRNAs and their escape from endosomes.[162] For example, Cubillos-Ruiz et al. fabricated PEI-based PD-L1 siRNA nanocomplexes to reprogram tumor-associated dendritic cells and elicit antitumor immunity.[163] Nevertheless, the abundance of positive charges expedites the elimination of the PEI-based vectors within the bloodstream, thereby constraining their systemic administration. As previously noted, incorporation of a PEG coating may address the problem. In this regard, Yang et al. synthesized CD44-targeted nanoparticles composed of hyaluronic acid-PEI/hyaluronic acid-PEG and successfully used them for MDR1 siRNA transfection of chemoresistant ovarian cancer cells.[164] The abundance of positive charges may also impede the release of siRNAs, a challenge that has been addressed through the introduction of disulfide bonds in PEI-based polymeric nanoparticles, which are degraded under the intracellular reductive conditions to release the siRNA.[165]

Poly dimethylaminoethyl methacrylate (PDMAEMA), manufactured by free radical polymerization of the DMAEMA monomer, is another cationic polymer with tertiary amines possessing great capabilities in loading nucleic acids and escaping endosomes.[166] Zhu et al. utilized PDMAEMA−PCL−PDMAEMA triblock copolymers by the RAFT method that formed positively charged nanomicelles in water. Micelles were complexed with green fluorescent protein (GFP) siRNA and showed efficient gene knockdown efficiency in breast cancer cells.[167] Of note, PEI and PDMAEMA can be toxic given that they are non-degradable; thus, degradable cationic polymers have been designed. However, PEI cytotoxicity can be diminished by preparing fluorinated PEI, enabling efficient gene transfection at lower N/P ratios.[168] Poly-β-amino ester (PBAE), a cationic polymer, has ester bonds within its structure, which renders it susceptible to hydrolysis by esterases *in vivo*, resulting in less toxicity.[169] Tang et al. demonstrated the potential of pH-responsive PBAE nanoparticles delivering siRNAs targeting Snail and Twist transcription factors and paclitaxel in repressing breast cancer growth and metastasis.[170] Polyamino acids are another type of degradable cationic polymer generated by linking amino acids with amide bonds; thus, proteases can break them down *in vivo*.[171] Anionic polyamino acids such as biodegradable PLGA with low toxicity have also been incorporated for siRNA delivery.[172] For example, in order to overcome resistance in lung cancer cells, Su et al. created PLGA nanoparticles and coated them with cationic PEI polymer. This allowed the signal transducer and activator of transcription-3 (STAT3) siRNA and paclitaxel to be encapsulated and delivered to lung cancer cells *in vitro*.[173]

Chitosan, a natural, biocompatible, and degradable polymer, has been frequently employed in developing polymeric nanoparticles for siRNA delivery. The presence of primary amines in the repeating unit of chitosan gives it a positive charge, playing a crucial role in the effective loading of siRNAs as well as endosomal escape.[174] Wei et al. developed an oral delivery platform using N-((2-hydroxy-3-trimethylammo-

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Table 4. Features and Examples of Different Types of Polymeric Nanoparticles for siRNA Delivery[a]

Type	Features	Structure	Targeting ligand	siRNA	Notes	Refs
Polymersomes	- Noncovalent encapsulation of siRNAs	(PEG-P(TMC-DTC)-PEI) asymmetric triblock copolymer	cNGQ	Plk1 siRNA	- Encapsulated siRNAs into aqueous core.	183
	- Amphiphilic and biodegradable nature				- Conferred extended circulation periods, tumor accumulation, and cell internalization.	
	- Colloidal stability	PEG and polyGMA hydrophilic terminal blocks and the ionizable poly-ImHeMA internal block	Folate	Hsp90 siRNA	- Interacted electrostatically with siRNAs.	185
	- Tunable membrane permeability				- pH-responsive nature: membrane-disrupting capabilities at endosomal pH.	
	- Lower biocompatibility but higher stability compared to liposomes				- Compatibility with blood cells at plasma and cytosolic pH levels.	
	- High chemical versatility given the block copolymers				- Incorporating PEG with block copolymers conferred prolonged circulation.	
Dendrimers	- Noncovalent electrostatic adsorption of siRNAs	Generation 4 PAMAM	Arginine-rich motif	Hsp27 siRNA	- Stable dendriplexes with siRNAs were formed due to positive surface charge.	190
	- Monodisperse and radially symmetric structure				- Arginine residue improved delivery efficiency.	
					- Highly branched architecture.	
	- High cost and low final yield of production	Generation 5 PAMAM	RGD	GFP siRNA	- Higher PAMAM generations are more compact and have higher surface charge density.	191
	- Low solubility of higher generations				- Exhibited superior loading capacity compared to naïve PAMAM.	
	- Considerable siRNA loading capacity	Cationic and surface-neutral PAMAM	LHRL	Bcl-2 siRNA	- Complexed with the siRNA via internal cationic charges.	192
					- Conferred free tertiary amines for the proton sponge effect.	
					- Exhibited great targeting specificity and low toxicity.	
		PAMAM-histidine-PEG	Triptorelin	Cy3-tagged siRNA	- Conjugation with PEG enhanced the biocompatibility of dendrimers and improved their stability in circulation.	194
Polyplexes	- Electrostatic interaction with siRNAs	PAMAM−PEG−PLL	-	Bcl2-siRNA	- Transfection efficiency may be enhanced by using smaller particles.	199
	- Efficient loading of siRNAs				- More cationic polymer relative to siRNA allowed for the effective loading of nucleic acids.	
	- PEGylation enhanced the stability of polyplexes.				- Polymer chains sterically prevent siRNA degradation by nucleolytic enzymes	
	- Possible toxicity with PEI					
	- High solubility					
	- Tunable size and composition	PEG−PEI-Ce6	-	RRM2 siRNA	- Produced ROS under irradiation, which disassembled the complex and facilitated siRNA cellular entry and endosomal escape.	200

[a]Abbreviations: PEG, polyethylene glycol; P(TMC-DTC), poly(trimethylene carbonate-co-dithiolane trimethylene carbonate); PEI, polyethylenimine; Plk1, polo-like kinase 1; siRNA, small interfering RNA; polyGMA, polyglycerol methacrylate; poly-ImHeMA, poly imidazole-hexyl methacrylate; HSP90, heat shock protein 90; PAMAM, poly amidoamine; GFP, green fluorescent protein; LHRH, luteinizing hormone release hormone; PLL, poly L-lysine; RRM2, ribonucleotide reductase subunit M2; ROS, reactive oxygen species.

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nium) propyl) chitosan chloride nanoparticles with high telomerase reverse transcriptase siRNA encapsulation efficiency given their positive charge and porous structure. The platform enabled the codelivery of siRNA and paclitaxel, causing synergic tumor suppressive effects in a subcutaneous syngeneic transplantable Lewis lung carcinoma model.[175] Bastaki et al. synthesized a nanoplatform by conjugating hyaluronate and trans-activator of transcription (TAT, a cell-penetrating peptide) with trimethyl/thiolated chitosan, demonstrating efficient siRNA encapsulation, serum stability, significant uptake by cancer cells, and low toxicity. The nanoparticles enabled the regulated release of STAT3 and PD-L1 siRNAs and suppressed tumor growth in breast and melanoma cancers *in vitro* and *in vivo*. Trimethyl chitosan, a partial quaternized derivative of chitosan, exhibits improved water solubility and cell penetration, while thiolated chitosan possesses enhanced mucoadhesive properties and structural stability.[176] Other polysaccharide-based nanosystems have also been used for siRNA delivery to cancer cells. Hyaluronic acid, an anionicbiopolymer, possesses attributes such as biodegradability, nontoxicity, and nonimmunogenicity, making it an optimal carrier polymer for applications in systemic drug delivery.[177] Genesh et al. generated CD44-targeted hyaluronic acid−PEI−PEG nanoparticles for tailored downregulation of Plk1 in sensitive and resistant lung cancer cells.[178] Modifications with cationic polyamines like PEI help improve the encapsulation of negatively charged siRNAs.

Generally, polymeric nanoparticles can be classified into two groups: nanocapsules and nanospheres, which are further subdivided into subclasses, including polymersomes, polyplexes, and dendrimers (Table 4).[179]

4.2.1. Polymersomes. Polymersomes are self-assembled amphiphilic, biodegradable, and/or stimulus-sensitive block copolymers. They have drawn interest as nanocarriers due to their adjustable supramolecular structure, colloidal stability, and tunable membrane permeability.[180,181] They are able to encapsulate siRNAs in their aqueous core due to the hydrophilic nature of block copolymers. Increased volume of the inner aqueous core and higher nucleic acid encapsulation have been shown with polymersomes with asymmetrical membranes produced from ABC triblock copolymers.[182] Zou et al. designed chimaeric polymersomes self-assembled from biodegradable PEG-poly(trimethylene carbonate-*co*-dithiolane trimethylene carbonate)-PEI (PEG-P(TMC-DTC)-PEI) asymmetric triblock copolymer and cNGQ-PEG-P(TMC-DTC) diblock copolymer. The polymersomes encapsulated Plk1 siRNA within their aqueous lumen and shielded it from degradation. These polymersomes displayed extended circulation times, high tumor accumulation and selectivity, high internalization via cNGQ-mediated recognition of $\alpha 3\beta 1$ integrin receptors overexpressing on A549 lung cancer cells, and rapid release of Plk1 siRNA within the cytoplasm. These collective characteristics led to successfully treating nude mice harboring orthotopic A549 human lung tumors.[183] Using polymersomes with ionizable membranes, which interact electrostatically with siRNAs, is another approach to enhance encapsulation efficiency.[184] Gallon et al. devised folate-targeted, pH-responsive, and heat shock protein (Hsp)90 siRNA-loaded polymersomes using PEG and polyglycerol methacrylate (polyGMA) hydrophilic terminal blocks and the ionizable poly imidazole-hexyl methacrylate (poly-ImHeMA) internal block. The pH-responsive nature of polymersomes conferred membrane-disrupting capabilities at endosomal pH while maintaining compatibility with blood cells at plasma and cytosolic pH levels. In cultured human cervical carcinoma cells, the polymersomes induced efficient silencing of Hsp90.[185] It is also worth noting that incorporating PEG with block copolymers during the fabrication process yields polymersomes with a prolonged blood circulation.[180] Film rehydration, solvent exchange, direct dissolution of polymeric materials, and probe sonication are frequently used methods for polymersome manufacturing.[181]

4.2.2. Dendrimers. Dendrimers are monodisperse and radially symmetric molecules with a highly branched architecture synthesized through divergent or convergent methods, in which synthesis begins from the interior (i.e., the dendrimer core) or from the exterior (i.e., the molecular structure that eventually becomes the dendrimer arm), respectively. Dendrimers commonly comprise three parts: internal core, repeating units, and surface functionalization modifications. The chemical composition and synthesis of repetitive units determine the generation of dendrimers and control their size, charge density, and globular structure.[186] Given the existence of protonatable amine groups, dendrimers such as poly amidoamine (PAMAM) exhibit positive surface charge, enabling the formation of a strong complex with siRNAs.[187] Notably, higher-generation dendrimers are more compact, raising the surface density of amine groups to establish a more robust interaction with negative siRNAs.[188] Multiple reactive surface groups also permit the incorporation of additional therapeutic molecules into the nanocarrier or functionalization of their surface with targeted ligands, making dendrimers promising gene delivery platforms.[189] Liu et al. fabricated the cell-penetrating arginine-rich motif-terminated triethanolamine core generation 4 PAMAM dendrimers that formed stable dendriplexes with Hsp27 siRNA. The positive surface potential of dendrimers enabled effective siRNA electrostatic adsorption and condensation, while the tertiary amines within their interior allowed for intracellular siRNA release. Further, these dendrimers displayed robust gene silencing and anticancer effects in prostate cancer models compared to their nonarginine-bearing dendrimer equivalent.[190] Another investigation by Waite et al. demonstrated the superior capacity of RGD-modified generation 5 PAMAM dendrimer in delivering GFP siRNA to U87 cells compared to naïve PAMAM, underscoring the importance of active targeting.[191] To attenuate the possible toxicity associated with the positive charge of dendrimers, Patil et al. synthesized luteinizing hormone-releasing hormone (LHRL)-decorated, internally cationic, and surface-neutral PAMAM dendrimers for tailored delivery of Bcl-2 siRNA. The presence of internal cationic charges facilitated the formation of complexes as well as the protection of siRNA molecules. Further, the lower degree of quaternization conferred free tertiary amines for the proton sponge effect. These nanoparticles showed cancer cell-specific targeting with very low toxicity even at high concentrations.[192] Moreover, conjugation with hydrophobic polymers (e.g., PEG) may enhance the biocompatibility of dendrimers and improve their stability in circulation.[193] Tambe et al. introduced triptorelin-targeted PAMAM-histidine-PEG dendritic nanoconstructs to deliver Cy3-tagged siRNA to luteinizing hormone release hormone (LHRH)-overexpressing breast cancer cells. These nanoconstructs were nontoxic, serum stable, and delivered the siRNA to cancer cells with high specificity.[194]

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Table 5. Features and Examples of Inorganic Nanoparticles for siRNA Delivery[a]

Type	Features	Structure	Targeting ligand	siRNA	Notes	Refs
AuNPs	- Attach to siRNAs by thiol-gold interaction or surface adsorption - Tunable physicochemical properties - Simple synthesis - Biocompatibility	Positively charged gold nanocluster	-	NGF siRNA	- Improved siRNA stability, circulation lifespan, and tumor accumulation.	211
	- Multiple forms (spherical, nanocluster, nanorods) - Enable higher cellular uptake compared to nontargeted nanoparticles	Gold nanorods	Octreotide	ASCL1 siRNA	- Attachment to the siRNAs via a cationic polymer polyarginine.	210
FeONPs	- Attach to siRNAs by noncovalent electrostatic interaction or covalent modification - Biocompatibility - Thermal/magnetic properties - Small size - Theranostic application	Disulfide−PEG−PEI-FeONPs	Folic acid	PD-L1	- Minimal cytotoxicity. - PEI enhanced siRNA loading capacity. - PEG modification enhanced stability and reduced cytotoxicity - Functioned as a contrast agent for cancer MRI and a carrier for siRNA delivery.	216
	- Possible oxidative toxicity - Enable siRNA delivery and noninvasive imaging of tumor response	MPAPs-dextran-FeONPs	EPPT1,	Plk1 siRNA	- EPPT1 enhanced tumor specificity, while MPAPs improved cellular uptake and endosomal escape.	215
MSNPs	- Noncovalent or covalent encapsulation or surface adsorption of siRNAs - Large surface area - Substantial pore volume - Flexible pore size - Versatile surface chemistry	PEI-phosphonate-MSNPs	-	P-gp siRNA	- The phosphonate group allowed electrostatic doxorubicin binding and PEI coating. - PEI coating enabled interaction with the siRNA.	220
	- Biocompatibility - Low cytotoxicity.	KALA-PEI-MSNPs	-	VEGF siRNA	- KALA, a fusogenic peptide, enabled efficient endosomal escape.	221

[a]Abbreviations: AuNPs, gold nanoparticles; NGF, nerve growth factor; siRNA, small interfering RNA; ASCL1, chaete-scute complex-like 1; FeONPs, iron oxide nanoparticles; PEG, polyethylene glycol; PEI, polyethylenimine; PD-L1, programmed death- ligand 1; MRI, magnetic resonance imaging; MPAPs, myristoylated polyarginine peptides; Plk1, polo-like kinase 1; MSNPs, mesoporous silica nanoparticles; P-gp, P-glycoprotein; VEGF, vascular endothelial growth factor receptor.

4.2.3. Polyplexes. Polyplexes are formed spontaneously by the electrostatic condensation of nucleic acids with cationic polymers such as chitosan, PEI, and poly L-lysine (PLL). This is critical for gene transfer since transfection efficacy may be enhanced by using smaller particles, especially *in vivo*.[187] To effectively condense nucleic acids into smaller nanoparticles with positive surface charge, incorporating more cationic polymer relative to oligonucleotide is common practice.[195] Polymer chains sterically block nucleolytic enzymes, protecting entrapped nucleic acids from degradation.[187] Further, the inclusion of covalent cross-linkers into the particle core or incorporating hydrophobic components such as alkyl groups may encourage particle formation via hydrophobic aggregation and increase the packing stability of polyplexes.[196,197] Similar to dendrimers, PEGylation enhances the stability of polyplexes in the biological milieu.[198] Patil et al. designed a PAMAM−PEG−PLL triblock nanocarrier for Bcl2- siRNA delivery to cancer cells. PLL works as a penetration enhancer and yields primary amines to build polyplexes with siRNAs via electrostatic interactions. The tertiary amine groups of the PAMAM dendrimer can act as a proton sponge, allowing siRNA to be transported from the endosome to the cytoplasm. Incorporating the PEG linker connecting PLL to PAMAM dendrimers not only enhanced the siRNA protection against plasma nucleases but also decreased the cytotoxicity of PLL and the overall triblock nanocarrier. These nanocarriers were efficiently internalized by cancer cells and successfully suppressed the expression of the Bcl-2 gene.[199] As another example, Zhang et al. developed ROS-responsive polyplexes containing the PEGylated PEI, ROS-cleavable linker, photosensitizer Ce6, and ribonucleotide reductase subunit M2 (RRM2) siRNA. Under irradiation, the polyplex produced ROS, which disassembled the complex, destabilized the cell membrane, and facilitated siRNA cellular entry and endosomal escape. The polyplex reduced tumor growth in a patient-derived xenograft mouse model of hepatocellular carcinoma.[200]

4.3. Inorganic Nanoparticles. Inorganic nanoparticles can be designed with specific size, shape, and surface properties well-suited for *in vivo* gene delivery. Accordingly, several studies have looked at how physicochemical properties and surface chemistry affect their targeting capacity and interaction with the biological environment in various models (Table 5).[201] Low immunogenicity, biocompatibility, and high encapsulation efficiency make inorganic nanomaterials, particularly gold, silica, and iron oxide nanoparticles, attractive candidates for siRNA delivery.[202−204]

4.3.1. Gold Nanoparticles (AuNPs). AuNPs have highly tunable physicochemical characteristics and straightforward synthesis methods. These nanoparticles can be readily decorated with siRNAs via dynamic covalent attachment or surface adsorption. The gold−thiol interaction, for instance, has been used to conjugate siRNAs to the naked or polymer-modified surface of AuNPs.[205] The elevated glutathione levels in the cytoplasm of target cells provide a reducing environment that may break this dynamic bond and facilitate siRNA release.[206] Numerous investigations have also employed electrostatic adsorption for conjugating siRNAs to positively

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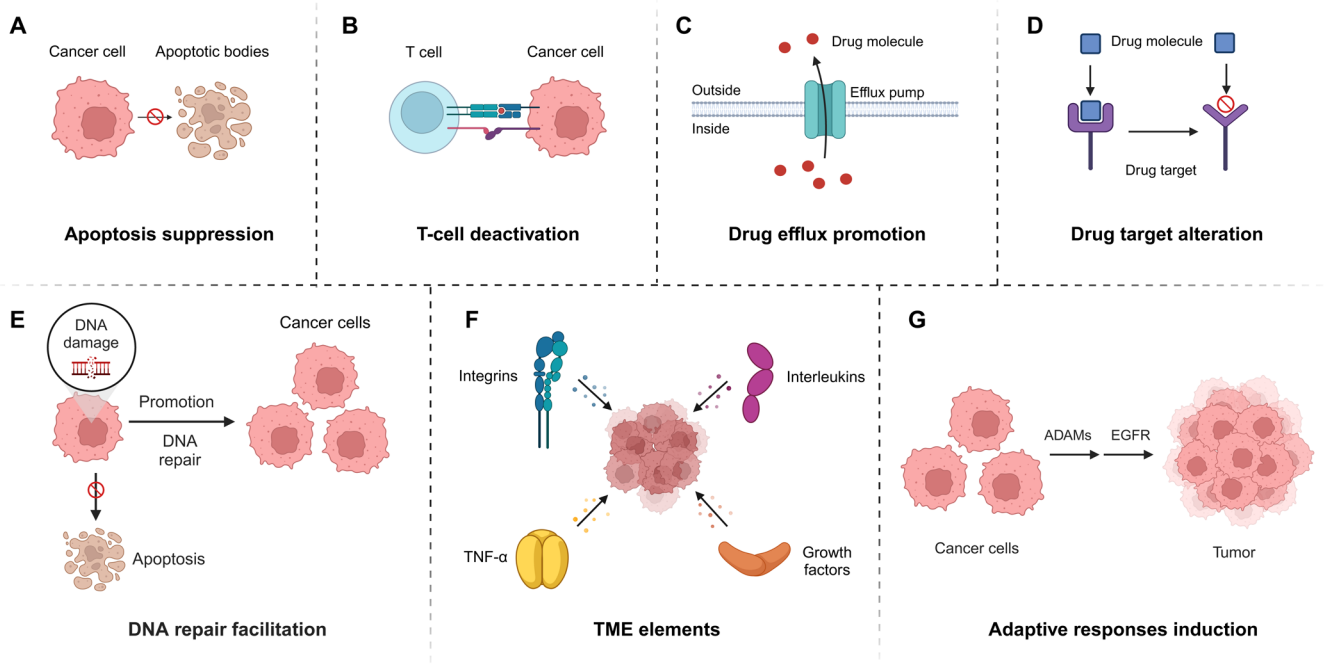

Figure 7. Diverse mechanisms of cancer chemoresistance. (A) Pro-survival mechanisms and impediment of apoptosis pathways. (B) Induction of an immunosuppressive tumor microenvironment (TME) by T cell exhaustion. (C) Promotion of drug efflux capacity in tumor cells by upregulating ATP-binding cassette (ABC) transporters. (D) Alteration of chemotherapy drug targets. (E) Prevention of tumor cell death mediated by chemotherapy drug-induced DNA insults through manipulating elements involved in DNA repair. (F) Upregulation of TME elements (e.g., integrins, interleukins, tumor necrosis alpha (TNF-α), or growth factors) involved in apoptosis suppression and tumor cell survival. (G) Triggering adaptive responses by disintegrin and metalloproteinases (ADAMs) that promote tumor cell survival signaling including epidermal growth factor receptor (EGFR) signaling. Created with BioRender.com.

charged AuNPs.[207] Further, the surface of AuNPs has been frequently modified with various polycations and targeting ligands, which allow nanoparticles to interact with specific receptors on the surface of target cells.[208] Achieving efficacious targeting of nanoparticles requires careful consideration of ligand characteristics, concentration, and affinity.[209] Xiao et al. developed octreotide functionalized gold nanorods for targeted codelivery of achaete-scute complex-like 1 (ASCL1) siRNA and doxorubicin to neuroendocrine cancer cells overexpressing somatostatin receptors. A pH-sensitive hydrazone linkage was used to affix doxorubicin to the nanoparticles. The cationic polymer polyarginine was also attached to the nanoparticles to facilitate complexation with siRNA. Cellular uptake, gene silencing, and antiproliferative effects increased in human carcinoid cells by the engineered nanocomplex compared to nontargeted AuNPs.[210] Another example is the work by Lei et al. in synthesizing positively charged small AuNPs from the one-step reduction of Au3+ in the presence of glutathione (GSH) and oligoarginine that adsorbed the nerve growth factor (NGF) siRNA electrostatically. AuNPs enhanced the serum stability of the siRNA, improved its circulation lifespan, and improved its cellular uptake and tumor accumulation, resulting in effective gene silencing and tumor suppression in pancreatic cancer models.[211]

4.3.2. Iron Oxide Nanoparticles (FeONPs). Superparamagnetic FeONPs are biocompatible and relatively safe magnetic nanoparticles with diverse theranostic applications.[212] Engineered FeONPs have shown several advantages as siRNA delivery platforms given their intrinsic targeting capabilities or in thermal/magnetic-driven settings. Many of these delivery systems are designed based on positively charged engineered FeONPs that adsorb anionic siRNAs. FeONPs coated with cationic lipid-like materials have shown promising *in vivo* siRNA delivery under magnetically guided targeting.[213] Notably, the coating layer has to be carefully designed since it not only enhances the siRNA loading capacity but also makes it possible for FeONPs to traverse biological barriers *in vivo* efficiently and reach their intended target site.[214] Mahajan et al. developed multifunctional probes comprising dextran-coated FeONPs functionalized by the tumor-selective peptide EPPT1, increasing tumor specificity, and the ligand myristoylated polyarginine peptides (MPAPs), enhancing cellular uptake by electrostatic interaction and mediating the endosomal escape of siRNAs. These nanoparticles facilitated targeted Plk1 siRNA delivery to pancreatic cancer cells, resulting in efficient gene silencing, tumor suppression, and apoptosis induction *in vitro* and *in vivo*.[215] In another example, Luo et al. generated folic acid- and disulfide-PEG-conjugated PEI complexed with FeONPs as a delivery platform for PD-L1 siRNA, which resulted in the targeted PD-L1 knockdown in gastric cancer cells. Cationic PEI on the surface of nanoparticles enabled the effective adsorption and condensation of siRNAs. The PEG modification reduced cytotoxicity and promoted the stability of the PEI-siRNA complex before cell internalization. The breakdown of disulfide bonds under the intracellular reductive conditions then enabled the release of PEI-siRNA in the cytoplasm.[216]

4.3.3. Silica Nanoparticles. Biocompatible MSNPs are potential candidates for cancer gene delivery due to their large surface area, substantial pore volume, flexible pore size, and versatile surface chemistry. The primary method for

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incorporating siRNAs into MSNPs is electrostatic adsorption into the pores and on the surface of the nanoparticles.[217] MSNPs must be engineered with an appropriate architecture to enhance their *in vivo* performance and eventual translation to the clinic.[218] To achieve optimal siRNA loading and release rate, pore size and surface modifications must be carefully considered. Pores with a smaller diameter (2.5−5 nm) may facilitate controlled release, whereas larger pores may accommodate more siRNAs but release them more rapidly.[204] Furthermore, to improve siRNA loading, protein adsorption, and release rates, the anionic nature of MSNPs is often modified through surface covalent binding of cationic macromolecules.[219] Meng et al. developed MSNPs functionalized with a phosphonate group that permits electrostatic doxorubicin binding to the porous interior and enables coating with PEI, allowing MSNPs to deliver P-gp siRNA. These nanoparticles were able to codeliver doxorubicin and P-gp siRNA to the cytoplasm and nucleus of KB-V1 drug-resistant cancer cells to accomplish cell killing in an additive or synergistic fashion.[220] Li et al. introduced PEI and fusogenic peptide (KALA)-functionalized and VEGF siRNA-encapsulated magnetic MSNPs to eradicate lung cancer cells. With a siRNA protective effect and minimal cytotoxicity, the fabricated delivery system efficiently entered cells, evaded the endolysosomes, released the loaded siRNA into the cytoplasm, and inhibited neovascularization in lung tumors *in vivo*.[221]

5. OVERCOMING CANCER CHEMORESISTANCE BY NANOPARTICLE-BASED SIRNA DELIVERY

The current landscape of cancer therapy is challenged by drug resistance (Figure 7), which negatively impacts patient outcomes.[1,2] As previously discussed, siRNAs have shown great promise in modulating the genetic factors behind chemoresistance, but their clinical application has been hampered by issues such as poor pharmacokinetics and off-target toxicity. To address these limitations, smart nanoparticle-based delivery platforms with targeting capabilities have been developed, offering advantages to enhancethe efficacy of siRNA therapies. In this section, we discuss recent advances in the engineering of nanoparticles for siRNA delivery, to overcome cancer chemoresistance through diverse strategies (Table 6, Figure 8).

Further, Table 7 presents Supporting Information about the preclinical usage of nanoparticles. The current progress in clinical studies of siRNA delivery for cancer therapy is also highlighted in Table 8.

5.1.1. Overcoming Drug Efflux. Drug efflux by the overexpression/overactivity of many known ATP-binding cassette (ABC) transporters is one of the most investigated cancer drug resistance mechanisms.[222] MDR1, also called P-gp or ABCB1, was the initial transporter recognized to grant intrinsic or acquired chemoresistance through the active dumping of cytotoxic chemotherapies from the intracellular environment.[222,223] Further, the overexpression of efflux proteins has been demonstrated in cancer stem cells that intrinsically resist chemotherapeutics.[224,225] Several studies have leveraged MDR1 siRNA-incorporated nanoparticles to address cancer chemoresistance. Heidari et al. generated aminated MSNPs with a large pore size of 5 nm for MDR1 siRNA delivery. Given their positive surface charge and porous structure, the nanoparticles efficiently adsorbed siRNAs on their surface and within large pores. However, while the presence of large pores enables the adsorption of substantial quantities of siRNAs, it also permits RNase diffusion, leading to siRNA degradation. Moreover, siRNAs on the nanoparticle surface are susceptible to degradation by endonucleases. To address this issue, nanoparticles were coated with chitosan through electrostatic interactions with siRNAs to protect them from enzymatic degradation. The chitosan coating was subsequently PEGylated to enhance nanoparticle water solubility, prevent their detection by the reticuloendothelial system, and extend their circulation time. This modification also facilitated surface functionalization with TAT cell-penetrating peptide and folate targeting ligand. The fabricated nanosystem demonstrated optimal cellular uptake in folate receptor-rich HeLa-RDB malignant cells. Further, due to the chitosan and TAT-mediated sponge effect and endosomal escape, the internalized siRNAs degraded MDR1 mRNA, generating a chemo-sensitized phenotype of MDR cancer cells.[25] Of note, adding a dimeric TAT, which has shown a greater propensity to evade endosomes, may augment the efficacy of such delivery systems.[226] In addition to the direct targeting of MDR1, silencing Rack1 and Src which participate in tuning the P-gp function may also be an innovative approach to overcome MDR1-mediated cancer chemoresistance.[227]

Another strategy to provide efficient MDR-1 siRNA delivery is PEI modification of nanocarriers. Generally, the high positive charge density of PEI facilitates effective condensation of siRNA through electrostatic interaction.[228] Further, PEI can buffer the low endosomal pH and disrupt endosomes, facilitating efficient endosomal escape.[221] However, notable nonspecific toxicity due to interrupting cellular and mitochondrial membrane stability and stimulation of intracellular apoptosis has hindered the clinical incorporation of high molecular weight PEI.[229] LMW PEIs exhibit manageable toxicity levels but demonstrate lower efficacy as transfection agents. Interestingly, LMW-branched PEI (bPEI) offers a beneficial balance by reducing toxicity without compromising its effectiveness as a transfection agent. Additionally, bPEI possesses favorable attributes of hydrophilic polymers, such as flexible chains and a hydrophilic quality, leading to decreased opsonization.[230] Mendes et al. employed an LMW-bPEI-phospholipid conjugate to engineer the surface of liposomes.[231] Covalently attaching lipids to bPEI improved its interaction with cell membranes and resultant transfection efficacy.[232] These nanoparticles were used to simultaneously deliver paclitaxel and MDR1 siRNA to drug-resistant ovarian cancer cells *in vitro* and *in vivo*. The nanocarrier provided high encapsulation efficiency, exhibited long-term stability in ion- and protein-rich environments, and were biocompatible. The LMW bPEI-modified liposomes also demonstrated greater cellular uptake and deeper penetration into tumor spheroids than PEGylated liposomes. Further, intracellular delivery of siRNA and paclitaxel sensitized tumor cells to chemotherapy and reduced tumor growth in xenograft animal models.[231] These findings highlight the potential of LMW bPEI for improving the properties of nanoparticles as siRNA carriers.

Since the discovery of MDR1, many other ABC transporters have been studied; breast cancer resistance protein (BCRP; also called ABCG2) and MDR-associated protein 1 (MRP1, also called ABCC1) are prominent transporters linked to chemoresistance in a wide variety of malignancies, including breast, colon, prostate, and lung cancers.[222] To address doxorubicin resistance mediated by MRP1 in prostate cancer cells, Yang et al. constructed a photothermally enabled

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Table 6. siRNA Nanomaterials to Overcome Cancer Chemoresistance[a]

Type of resistance	Mechanism of resistance/model	Nanoparticle-based systems				Refs
		Nanoparticle	Targeting ligand	Payload (s)	Outcome	
Drug efflux	MDR1 overexpression/cervical cancer (*in vitro*)	PEG-chitosan-coated NH_2-MSNPs	TAT peptide and folate	MDR1 siRNA	Efficiently targeted cancer cells and generated a chemosensitized phenotype.	25
	MDR1 overexpression/ovarian cancer (*in vitro* and *in vivo*)	LMW-bPEI -modified liposomes	-	Paclitaxel/MDR1 siRNA	Effectively delivered payloads to tumor cells and sensitized them to paclitaxel.	231
	MRP1 overexpression/prostate cancer (*in vitro* and *in vivo*)	mPEG-coated gold nanorods	AS1411 aptamer	Doxorubicin/ MRP1 siRNA	Selective delivery to tumor cells, released payloads in response to NIR, and improved doxorubicin effect.	233
DNA repair	Rev3L-mediated DNA repair/ovarian cancer (*in vitro* and *in vivo*)	Fusogenic lipid-coated MSNPs	iRGD	Rev3L siRNA	Selective delivery to tumor cells, silenced Rev3L, and improved sensitivity to cisplatin.	241
	MGMT-mediated DNA repair/GBM (*in vitro* and *in vivo*)	Chitosan−PEG−PEI-coated FeONPs	Chlorotoxin	MGMT siRNA	Delivery to brain, targeted GBM cells, and sensitized them to temozolomide-induced apoptosis via silencing MGMT.	244
	Rac1-facilitated DNA repair/breast cancer (*in vitro* and *in vivo*)	pH-responsive mPEG-*b*-PDPA polymeric nanoparticles	-	Cisplatin prodrug/ Rac1 siRNA	Delivered payloads to cancer cells where cisplatin prodrug was converted to the active drug, and its effect was enhanced by Rac1 silencing.	251
Apoptosis inhibition	Upregulation of ant-iapoptotic proteins/malignant melanoma (*in vitro* and *in vivo*)	pH-responsive kojic acid−based cationic liposomes	-	Paclitaxel/Bcl-2 siRNA	Efficient delivery of payloads to malignant cells and tailored knockdown of Bcl-2 improved sensitivity to paclitaxel.	259
	Upregulation of anti-apoptotic proteins/breast cancer (*in vitro* and *in vivo*)	PLR−PEG-modified MSNPs	AS1411 aptamer	Doxorubicin/Bcl-2 or Bcl-xL siRNA	Caused a 40-fold drop in doxorubicin IC50 through targeted delivery of payloads and silencing anti-apoptotic proteins.	260
	Upregulation of anti-apoptotic proteins/breast cancer (*in vitro* and *in vivo*)	PPEMMRA polymeric nanoparticles	-	Paclitaxel/Bcl-xL and Mcl-1 siRNAs	Enabled the rapid release of siRNAs followed by the slow release of paclitaxel within 72h, enhancing the sensitivity of cells to paclitaxel-induced apoptosis.	261
Adaptive response induction	Activation of EGFR signaling/NSCLC (*in vitro*)	PEGylated NLCs	LHRH	Paclitaxel/EGFR siRNA	Increased sensitivity of malignant cells to paclitaxel via targeted delivery of payloads and effective inhibition of EGFR signaling.	274
	AXL RTK overexpression/NSCLC (*in vitro*)	Porous gelatin nanoparticles	EGFR antibody (cetuximab)	AXL siRNA	Targeted knockdown of AXL in cancer cells diminished EMT and apoptosis resistance, rendering them vulnerable to EGFR TKIs.	285
	Midkine growth factor overexpression/hepatocellular carcinoma (*in vitro* and *in vivo*)	Ultrasmall LNPs	SP94 peptide	Sorafenib/midkine siRNA	Efficiently transported payloads to cancer cells and eradicated resistance to sorafenib via specific repression of midkine.	290
Drug target alteration	BCR-ABL1 oncogenic kinase mutation/CML (*in vitro*)	PEI-lipid cationic lipopolymer	-	BCR-ABL1 siRNA	Selectively lowered BCR-ABL1 transcripts in malignant cells and improved their therapeutic response to imatinib.	301
	EML4-ALK fusion gene mutation/NSCLC (*in vitro* and *in vivo*)	Hollow gold nanoshells	RGD	Doxorubicin/ALK siRNA	Accumulated in the tumor region during laser irradiation, selectively delivered payloads to cancer cells, and produced considerable tumor suppression.	298
	Androgen receptor signaling amplification/CRPC (*in vitro* and *in vivo*)	PEI−PLGA polymeric micelles	-	Docetaxel/AR siRNA	Selectively silenced AR signaling in cancer cells and improved the anticancer impact of docetaxel.	305
TME elements alteration	β3-integrin overexpression/breast cancer (*in vitro* and *in vivo*)	ECO cationic lipid-based nanoparticles	RGD	β3-integrin siRNA	Enabled targeted delivery of siRNA to malignant cells and repressed tumor metastasis and relapse.	308
	β1-integrin overexpression/advanced colorectal cancer (*in vitro*)	DDAB-mPEG−PCL nanoparticles	-	Regorafenib/ β1-integrin siRNA	Preferentially delivered payloads to tumor cells, thereby reducing the β1-integrin gene expression, leaving cells more sensitive to regorafenib.	309
	IL17RB-induced tumor growth and spread/breast cancer (*in vitro*)	Carboxymethyl dextran-containing cationic chitosan nanoparticles	-	Doxorubicin/ IL17RB siRNA	Strengthened the cytotoxicity of doxorubicin toward cancer cells by selectively delivering payloads and silencing IL17RB.	312
Immune suppression	PD-L1 upregulation/breast cancer (*in vitro* and *in vivo*)	Redox-responsive trimethyl chitosan-based nanoparticles	T7 peptide	Doxorubicin/PD-L1 siRNA	Augmented the therapeutic impact of doxorubicin by targeted delivery of payloads to cancer cells and specific PD-L1 silencing.	323
	PD-L1 upregulation/colorectal cancer and esophageal squamous cell carcinoma (*in vitro* and *in vivo*)	PEGylated calcium phosphate core-lipids shell nanoparticle	GE11 peptide	FOLFOX regimen/PD-L1 siRNA	Provided a superior long-term antitumor effect given the targeted delivery mechanism as well as efficient PD-L1 downregulation.	324
	Oncogenic STAT3 induction/melanoma (*in vitro* and *in vivo*)	Liposome-protamine-hyaluronic acid nanoparticles	-	STAT3 siRNA	Conferred targeted STAT3 silencing that improved the antitumor effect of gemcitabine-loaded lipid-coated calcium phosphate nanoparticles.	327

[a]Abbreviations: MDR1, multidrug resistance 1; PEG, polyethylene glycol; MSNPs, mesoporous silica nanoparticles; siRNA, small interfering RNA; LMW-bPEI, low molecular weight branched polyethylenimine; mPEG, methoxy PEG; MRP1, MDR-associated protein 1; NIR, near-infrared; MGMT, O^6-methylguanine-DNA methyltransferase; FeONPs, iron oxide nanoparticles; GBM,

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nanosystem consisting of methoxy PEG (mPEG)-coated gold nanorods. This system was functionalized with DNA and the AS1411 aptamer, and was subsequently loaded with doxorubicin and MRP1 siRNA.[233] Of note, mPEG, resulting from the addition of a methyl ether cap to PEG, prevents hydrogen bonding at the cap end, restricting nonspecific interactions with proteins and other PEG chains.[234] The nanoparticles showed tumor site trafficking and substantial uptake by cells via endocytosis owing to their active and passive targeting abilities. The gold nanorods also underwent photothermal conversion, which led to the dehybridization of DNA duplexes and the release of doxorubicin and MRP1 siRNA. The combined gene and chemotherapy had a synergistic therapeutic impact and repressed tumor growth *in vitro* and *in vivo*.[233] Such delivery systems enable the local release of payloads following systemic administration, allowing targeted drug development. Concerns regarding toxicity from high temperatures or power intensity have prompted discussions about laser irradiation; however, the 808 nm mild laser irradiation used in this work is not anticipated to cause any safety issues.[235]

Due to their heterogeneous nature, tumors may recruit more than one ABC transporter to induce a multidrug resistance phenotype. This has led to developing biocompatible pH-sensitive drug delivery platforms to overcome doxorubicin-mediated resistance in human breast cancer cells. These nanoparticles were based on carbonate apatite and showed effective delivery of MDR1 and BCRP siRNAs. Due to the presence of calcium ions, the carbonate apatite nanoparticles possessed a positive charge that allowed for surface adsorption of siRNAs through electrostatic interactions. The rapid dissolving rate of carbonate apatite nanoparticles in the acidic environment of endosomes allowed effective siRNA release into intracellular compartments. This resulted in a synergistic increase in the sensitivity of cancer cells to doxorubicin.[236,237] Implementing active targeting mechanisms, in addition to the pH sensitivity, may further improve the efficacy of this system. The presented findings emphasize the significance of enhancing cancer cell chemosensitivity by incorporating particular siRNAs to overcome drug efflux mechanisms.

5.1.2. Eradicating DNA Repair Capacity. Several chemotherapy drugs trigger death responses by direct or indirect DNA disruption, while malignant cells may employ multiple DNA repair mechanisms to survive. Accordingly, malignant cell responses against chemotherapeutics may be manipulated by several elements involved in DNA damage responses.[238] Most mammalian-encoded DNA polymerases are involved in DNA repair and damage tolerance. Accordingly, some of these enzymes might be viable targets for cancer therapeutic strategies.[239] For example, reversionless 3-like (Rev3L), the catalytic subunit of Pol ζ DNA polymerase, was shown to be crucial for bypassing most DNA-damaging mechanisms.[240] In a study by Kim et al., fusogenic lipid-coated MSNPs were synthesized and conjugated with the iRGD peptide for targeted siRNA delivery against Rev3L to impede DNA repair in cisplatin-resistant CAOV-3 human ovarian cancer cells. Generally, liposomes have a limited carrying capability for siRNAs and are susceptible to payload leakage. To tackle the problem, a calcium ion precipitation method was used, which allowed high siRNA loading capacity by stabilizing it and reducing its leakage. This method involved utilizing the calcium ions that

Table 6. continued

glioblastoma; PDPA, poly(2-(diisopropylamino) ethyl methacrylate; PLR, poly-L-arginine; EGFR, epidermal growth factor receptor; NSCLC, nonsmall cell lung cancer; NLCs, nanostructured lipid carriers; LHRH, luteinizing hormone release hormone; RTK, receptor tyrosine kinase; EMT, epithelial-mesenchymal transition; TKIs, tyrosine kinase inhibitors; LNPs, lipid nanoparticles; CML, chronic myeloid leukemia; EML4-ALK, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase; CRPC, castration-resistant prostate cancer; PLGA, polylactic-*co*-glycolic acid; AR, androgen receptor; TME, tumor microenvironment; ECO, (1-aminoethyl)iminobis[N-oleicylsteinyl-1-aminoethyl)propionamide]; DDAB, dimethyldidodecylammonium bromide; PCL, poly(ε-caprolactone); IL17RB, interleukin 17 receptor B; PD-L1, programmed death-ligand 1; STAT3, signal transducer and activator of transcription-3.

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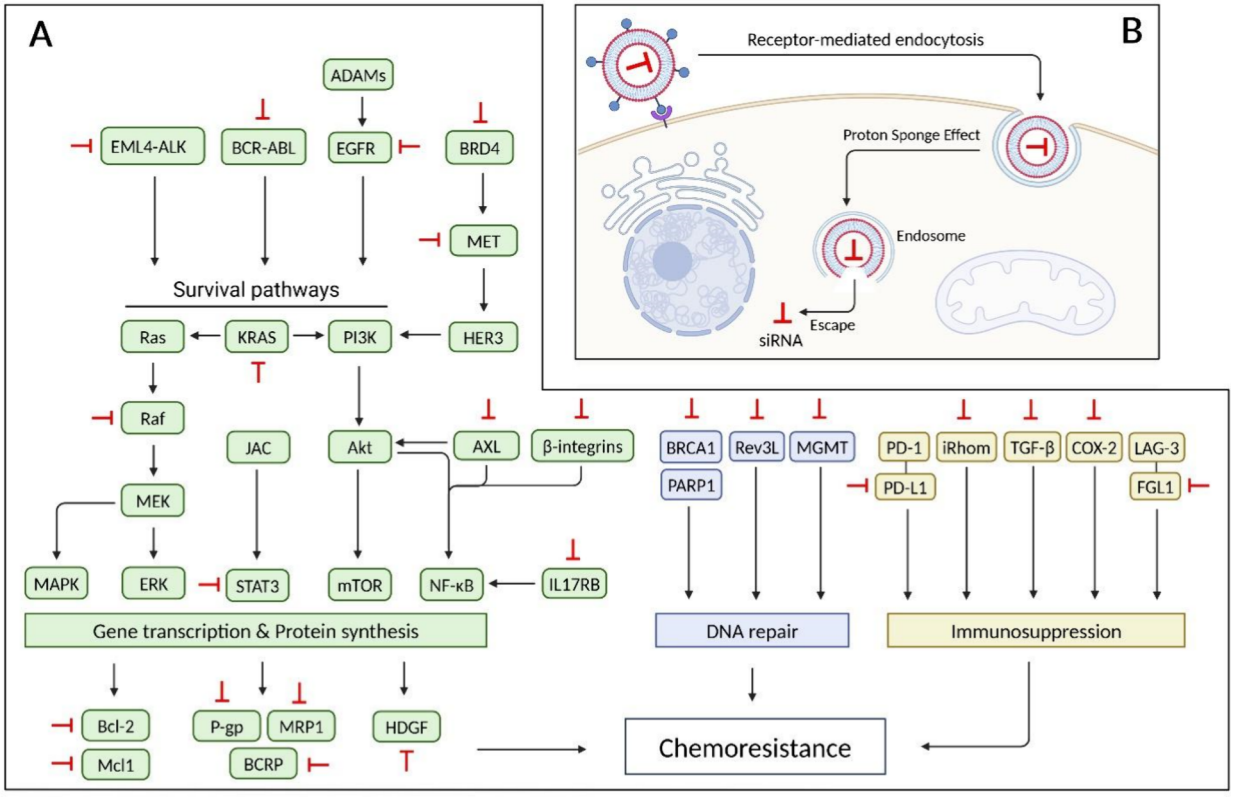

Figure 8. Schematic of (A) mechanisms of chemoresistance and (B) nanoparticles for siRNA delivery to silence drug resistance targets, particularly upstream and downstream factors involved in the promotion of cancer cell survival as well as factors involved in the repair of damaged genome and immunosuppression. ADAM, a disintegrin and metalloproteinases; EML4-ALK, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase; BCR-ABL, breakpoint cluster region-Abelson; EGFR; epidermal growth factor; BRD4, bromodomain-containing protein 4; MET, mesenchymal-epithelial transition factor; KRAS, Kirsten rat sarcoma viral oncogene; PI3K, phosphoinositide 3-kinase; HER3, human epidermal growth factor receptor; JAC, Janus kinase; STAT3, signal transducer and activator of transcription 3; MAPK, mitogen activated protein kinase; ERK, extracellular signal-regulated kinase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; P-gp, p glycoprotein; MRP1, multidrug resistance-associated protein 1; BCRP, breast cancer resistance protein; HDGF, hepatoma-derived growth factor; IL17RB, interleukin-17 receptor B; BRCA1, breast cancer type 1 susceptibility protein; PARP1, poly(ADP-ribose) polymerase 1; Rev3L, reversionless 3-like; MGMT, O6-methylguanine-DNA methyltransferase; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TGF-β, transforming growth factor-beta; COX-2, cyclooxygenase-2; LAG-3, lymphocyte-activation gene 3; FGL1, fibrinogen-like protein 1. Created with BioRender.com.

not only neutralize the negative charge of the silicon oxide surface of the nanoparticles and siRNAs but also form a precipitate with silicic acid produced from the partial dissolution of silicon throughout the loading process. This effectively sealed the nanostructure allowing entrapment of the siRNA, resulting in an efficient loading capacity with reduced leakage. Further, the fusogenic lipid coating, as well as the iRGD peptide, enabled the nanoparticles to recognize target cells, resulting in efficient intracellular siRNA delivery. It was demonstrated that fusogenic lipids must have a relatively low phase transition point close to room temperature to remain in a fluidic liquid crystal phase *in vivo*, the same phase to which endogenous extracellular vesicles attain during fusion. In addition, the positive charge of fusogenic lipids was shown to enhance absorption and fusion, given the electrostatic attraction between the anionic plasma membrane and the cationic lipids. Further, incorporating PEG into the nanoparticle formulation allowed for the dehydration of the space between the fusogenic lipid coating and the plasma membrane, promoting fusion efficiency. The fusogenic lipid coating disintegrates upon internalization, allowing the MSNPs to release the siRNA cargo into the cytosol. The nanosystem showed silencing of Rev3I, which led to improved anticancer responses of cisplatin in ovarian tumors *in vitro* and *in vivo*.[241]

Overcoming drug resistance is especially critical in cancers with limited therapeutic options, such as glioblastoma multiforme (GBM). The therapeutic efficacy of GBM conventional chemotherapy drug Temozolomide is limited by chemoresistance conferred by O^6-methylguanine-DNA methyltransferase (MGMT), which repairs Temozolomide-mediated DNA ruptures.[242] The chemical MGMT inhibitor O^6-benzylguanine (BG) has been administered systemically in clinical efforts to impede DNA repair and minimize MGMT activity. However, this approach has achieved limited success due to low bioavailability, poor barrier penetration, short serum half-life, and serious myelotoxicity when combined with Temozolomide.[242,243] In this regard, utilizing an MGMT siRNA nanocarrier may improve therapeutic effects without serious off-target toxicity. A platform built on FeONPs has been proposed to silence MGMT using siRNAs, sensitizing GBM cells to Temozolomide. FeONPs were coated with a chitosan-PEG (CP)-PEI copolymer and conjugated to MGMT siRNA and GBM targeting ligand chlorotoxin. Chlorotoxin enabled brain penetration and tailored tumor cell internalization via receptor-mediated transcytosis and

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Table 7. siRNA-Based Nanomedicines in Preclinical Cancer Research[a]

Nanocarrier	Nanosystem size/ charge	Payload(s)	Cancer	Main effect	Refs
Aptamer- functionalized shell−core nanoparticles	119.20 ± 1.34 nm/ −17.21 ± 0.43 mV	β-tubulin III, AR, and CXCR4 siRNAs (100 nM/kg) and paclitaxel (10 mg/kg)	Paclitaxel- resistant prostate cancer	Synergistic effect in reducing tumor growth in subcutaneous and orthotopic mouse cancer models.	335
CD44-targeted-hyaluronic acid−based nanoparticles	173.3 ± 13.7 nm/ −22.5 ± 0.44 mV	MDR1 siRNA (0.5 mg/kg/day)	Paclitaxel-resistant ovarian cancer	Inhibited tumor growth in an ovarian cancer animal model.	336
pH-responsive polymer micelle nanoparticles	170 nm/3.2 mV	P-gp siRNA (0.2 mg/kg) and doxorubicin (0.5 mg/kg)	Adriamycin-resistant hepatocellular carcinoma	Reduced liver tumor growth in an orthotopic mouse model.	337
BRBP1-decorated liposomes	110−140 nm/ −2.39 ± 0.28 mV	TWF1 siRNA (0.67 mg/kg) and paclitaxel (1 mg/kg)	Paclitaxel-resistant breast cancer metastasis	Delivery to the brain and reduced brain metastases in a xenograft tumor model.	338
Folate-decorated MSNPs	75 nm ±7 nm/ 18 mV	EGFR1 siRNA (up to 55 μg/mL)	Chemoresistant breast cancer	Decreased the IC50 of carboplatin in MDA-MB-231 cells.	339
Folate-decorated FeONPs	122.4 nm/ 21.5 ± 3.04 mV	GPX siRNA (663 ng/mL) and cisplatin (4 μg/mL)	Chemoresistant GBM	Synergistic therapeutic effect via eliciting apoptosis and ferroptosis in an intracranial xenograft tumor model.	340
pH/redox dual-responsive polyplex	120 nm/≈ 23 mv	MDR1 siRNA and doxorubicin	Adriamycin-resistant breast cancer	Suppressed tumor growth in MCF-7/ADR tumor xenograft mice.	341
Magnetic LNPs	96.37 ± 2.38 nm/ −9.90 ± 0.80 mV	DECR1 siRNA (0.2 mg/kg) and DGLA (5 mg/kg)	Chemoresistant prostate cancer	Under magnetic direction triggered ferroptosis in tumor cells and reduced tumor growth in mice.	342
pH-responsive carboxymethyl chitosan nanoparticles	90.26 nm/−8.6 mv	MVP and Bcl-2 siRNAs (100 nM) and Adriamycin (0.5 μg/mL)	Chemoresistant esophageal cancer	Suppressed tumor growth in the culture of esophageal squamous cell carcinoma KYSE510 cells.	343
Lipid-coated albumin nanoparticles	120 nm/≈7 mv	Paclitaxel (12.5 mg/kg) and sorcin siRNA (312.5 nM/kg)	Paclitaxel-resistant lung cancer	Inhibited tumor growth in A549/PTX tumor xenograft mice.	344
EGFR-targeted chitosan nanoparticles	202.7 ± 2.5 nm/ +21.2 ± 2.4 mv	Mad2 siRNA (3 mg/kg)	Cisplatin-resistant NSCLC	Improved cisplatin-mediated tumor suppression and decreased its effective dosage.	345
RGD-targeted and pH-sensitive ECO nanoparticles	≈ 12 mv	eIF4E siRNA (1 mg/kg)	Paclitaxel-resistant breast cancer	Improved the susceptibility of resistant MDA-MB-231 tumors to paclitaxel *in vivo*, leading to significant tumor regression with minor side effects.	346
PEGylated MSNPs	259.2 nm/ 22.87 ± 1.29 mV	T-type Ca2+ channel siRNA (0.25 mg/kg) and doxorubicin (5 mg/kg)	Adriamycin-resistant breast cancer	Overcome resistance to doxorubicin in MCF-7/ADR tumor xenograft mice.	347
2C5 antibody -decorated mixed dendrimer micelle nanoparticles	155.85 ± 1.34 nm/ −1.48 ± 0.09 mV	MDR-1 siRNA (1.2 mg/kg) and doxorubicin (0.9 mg/kg)	Adriamycin-resistant ovarian cancer	Suppressed tumor growth in A2780 ADR tumor-bearing mice.	348
Aptamer-targeted dendrimer peptide-derived nanoparticles	121.2 ± 7.3 nm/ −19.5 ± 0.7 mV	TRF2 and hTERT siRNAs (200 μg/kg)	Paclitaxel-resistant NSCLC	Increased the sensitivity of cancer cells to taxol in tumor-bearing mice.	349

[a]Abbreviations: AR, androgen receptor; CXCR4, C-X-C chemokine receptor type 4; MDR1, multidrug resistance 1; P-gp, p-glycoprotein; TWF1, twinfilin actin-binding protein 1; MSNPs, mesoporous silica nanoparticles; EGFR, epidermal growth factor receptor; FeONPs, iron oxide nanoparticles; GPX, glutathione peroxidase; GBM, glioblastoma multiforme; LNPs, lipid nanoparticles; DECR1, 2,4 dienoyl-CoA reductase-1; DGLA, dihomo-γ-linolenic acid; MVP, major vault protein; Mad2, mitotic arrest deficient 2; NSCLC, nonsmall-cell lung cancer; eIF4E, eukaryotic translation initiation factor 4E; TRF2, telomeric repeat-binding factor 2; hTERT, human telomerase reverse transcriptase.

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Table 8. siRNA-Based Nanoparticles in the Clinic[a]

Drug moniker	Cancer	siRNA	Carrier	Clinical trial phase/number	Status	Outcomes
NBF-006	Nonsmall cell lung, pancreatic, or colorectal cancer	GSTP	LNPs	Phase 1/NCT03819387	Active, not recruiting	-
EphA2 siRNA	Advanced or recurrent solid tumors	EphA2	Neutral liposome (DOPC)	Phase 1/NCT01591356	Recruiting	-
iExosomes	Metastatic pancreatic cancer with KrasG12D mutation	KrasG12D	Exosomes	Phase 1/NCT03608631	Active, not recruiting	-
Atu027	Advanced solid tumors	PKN3	Liposomes	Phase 1/NCT00938574	Completed	Well tolerated and showed antitumor activity in 41% of patients.[351,356]
DCR-MYC	Solid tumors, multiple myeloma, or lymphoma	MYC	LNPs	Phase 1/NCT02110563	Terminated	Well tolerated and showed promising initial responses across various dose levels.[353]
TKM-080301	Advanced hepatocellular carcinoma	Plk1	LNPs	Phase 1 and II/NCT02191878	Completed	Well tolerated but showed limited antitumor effects.[354]
CALAA-01	Solid tumors	RRM2	PEG-coated and transferrin-decorated cyclodextrin-based polymeric nanoparticles	Phase 1/NCT00689065	Terminated	Safe and induced specific, siRNA-mediated gene silencing.[355]
NU-0129	Recurrent GBM	Bcl-2L12	AuNPs	Phase 0/NCT03020017	Completed	Safe, delivery to tumors, and reduced Bcl-2L12 expression.[352]

[a]Abbreviations: GSTP, glutathione S-transferase P; siRNA, small interfering RNA; LNPs, lipid nanoparticles; EphA2, ephrin type-A receptor 2; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; PKN3, protein kinase N3; Plk1, polo-like kinase 1; RRM2, ribonucleotide reductase M2; Bcl-2L12, GBM, glioblastoma; AuNPs, gold nanoparticles; Bcl2-Like12.

endocytosis, respectively. While CP dissolved in endosomes after endocytosis, siRNA binding to PEI remained unaltered, therefore protecting it from degradation resulting in effective downregulation of MGMT. The results indicated that systemic coadministration of FeONPs with Temozolomide was safe, enhanced apoptosis of GBM stem-like cells, reduced tumor growth, and prolonged survival of mice in a patient-derived xenograft model. These FeONPs were also able to act as an MRI contrast for noninvasive imaging of tumors and evaluation of treatment responses.[244]

Another crucial component of DNA damage responses is breast cancer type 1 susceptibility protein (BRCA1), which preserves genomic integrity and impedes tumorigenesis by repairing DNA double-strand breaks (DSB) through the homologous recombination (HR) pathway.[245] However, in malignant cells, BRCA1 plays a role in reducing chemosensitivity via repairing chemotherapy-mediated DNA lesions. To address this, pH-sensitive shell−core nanoparticles were designed to transport BRCA1 siRNA and the cisplatin prodrug to triple-negative breast cancer (TNBC) cells. The biomineralization method yielded nanoparticles with cisplatin prodrug-loaded hydrophobic DSPE as the dense inner core and the hydrophilic plasminogen activator analog (uPA)-PEG and calcium phosphate absorbing BRCA1 siRNA generating the porous external shell. The high surface area (215.49 m^2 g^{-1}), as well as the suitable total pore volume (0.65 cm^3 g^{-1}) of nanoparticles, conferred a strong physical adsorption force on siRNAs. Further, the produced nanoparticles were positively charged in the aqueous solution, which improves electrostatic interactions with the siRNAs and promotes cell internalization through negatively charged plasma membranes. These attributes make these nanoparticles an ideal system for co-loading chemotherapy drugs and siRNAs with high encapsulation efficacy. PEG chains were used as a shield protecting the nanoparticles against serum protein precipitation and nuclease inactivation. Further, tuning the particle size and enlisting the uPA, which recognizes its receptors on target cells, provided suitable targeting capabilities for the nanoparticles. Following targeted delivery to malignant cells, the calcium phosphate outer shell disintegrated due to the acidic environment of endosomes, permitting the sequential release of the siRNA and cisplatin prodrug. Therefore, BRCA1 was downregulated, and cisplatin was activated to induce permanent DNA damage and kill TNBC cells.[246]

In contrast, defects in DNA damage pathways may diminish cancer cell sensitivity to chemotherapy. These defects force cancer cells to seek a different repair process, which may be addressed using synthetic lethal strategies.[238] Supporting that notion, the poly(ADP-ribose) polymerase 1 (PARP1) function has been shown to repair DNA damage in breast cancer cells with a BRCA1 or BRCA2 mutation that have a faulty HR DNA repair mechanism.[247] To address this, Sharp et al. synthesized lipidoid nanoparticles using NC100 lipidoid, ceramide-PEG, and cholesterol for PARP1 siRNA delivery to BRCA1-deficient ovarian cancer cells. In acidic conditions, e.g., during the nanoparticle preparation process or inside endosomes, ionizable cationic lipidoids acquire a positive charge that allows for higher encapsulation efficiency and enhanced intracellular siRNA release via endosomal escape, respectively. Meanwhile, ionizable cationic lipidoids obtain a neutral charge at physiological pH, eliminating safety concerns about cationic lipids. Accordingly, safe and efficient delivery of PARP1 siRNA to BRCA1-deficient ovarian cancer cells

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through lipidoid nanoparticles suppressed tumor growth *in vitro* and *in vivo*.[248,249] BRCA2 in-frame deletions have been found to enhance the DNA repair capacity and protect BRCA2 mutant tumors from PARP1 inactivation.[250] Therefore, integrating multiple siRNAs targeting various DNA repair components into a single nanoparticle-based delivery platform may induce a chemosensitizing effect.

While targeting components engaged in the DNA damage response has shown promise, it is also important to manipulate elements indirectly involved in DNA damage repair pathways. Overexpression of Rac1 small GTP binding protein, for instance, upregulates glycolysis and, in particular, the nonoxidative pentose phosphate pathway, both of which are implicated in MDR to neoadjuvant chemotherapy in breast cancer. In this respect, Li et al. designed endosomal pH-responsive mPEG-*b*-poly(2-(diisopropylamino) ethyl methacrylate) (mPEG-*b*-PDPA) polymeric nanoparticles to efficiently transport Rac1 siRNA and cisplatin prodrug to chemoresistant breast cancer xenografts. The siRNA encapsulation efficiency was about 80%. Since hydrophobic cisplatin was inefficiently encapsulated into nanoparticles, a cisplatin prodrug was created by adding two hydrophobic tails to the drug structure. After being administered, the nanoparticles extravasated into tumor tissue and entered cells via endocytosis. The pH-sensitive polymer incorporated in the fabrication of nanoparticles had a pK_a (~6.24) close to the endosomal pH (6.0−6.5), thereby responding to the acidic endosome environment and enabling endosomal escape as well as the cytosolic release of Rac1 siRNA and cisplatin prodrug. In the cytoplasm, reductive agents like GSH cleave the two hydrophobic tails of the cisplatin prodrug, resulting in the production of intact cisplatin. Accordingly, the nanosystem attenuated Rac1 expression and synergized with cisplatin to generate DNA damage and eradicate malignant cells.[251] To sum up, substantial evidence points to the role of siRNAs in improving chemosensitivity by suppressing tumor cell repair capacities.

5.1.3. Promoting Apoptosis. Integrating cellular damage caused by chemotherapeutics with underlying cell death mechanisms, especially apoptosis, is paramount for killing cancer cells. However, pro-survival mechanisms and impediment of apoptosis pathways are well documented concerning the evolution of cancer chemoresistance.[252] Emerging data highlight the engagement of diverse anti-apoptotic proteins, particularly pro-survival Bcl-2 family members (e.g., Bcl-2, Bcl-xL, and MCL1), in malignant cell longevity and poor response to the death-promoting mechanisms of chemotherapy drugs.[253,254] Anti-apoptotic protein levels are primarily associated with mutation, amplification, or overexpression of encoding genes; oncogenic induction of kinases may also contribute to this process by encouraging the function of pro-surviving transcription factors, such as nuclear factor-κB (NF-κB) and STAT3.[255−257] Moreover, mutations in tumor suppressor gene p53 are implicated in the uncontrolled cell cycling and apoptosis evasion of malignant cells.[258]

Targeting of factors implicated in the inhibition of chemotherapy-induced apoptosis has shown considerable potential for eliminating chemoresistance. Several studies have proposed nanoparticle-based delivery methods for co-delivery of chemotherapeutics and siRNAs addressing anti-apoptotic genes to malignant cells. Reddy et al. examined administration of paclitaxel and Bcl-2 siRNA using kojic acid−based cationic liposomes to treat malignant melanoma. Kojic acid is a known melanin inhibitor with whitening properties for melanocytes functioning through inhibiting tyrosinase without being toxic to normal melanocytes. Accordingly, the formulation was shown to be safe and did not produce any toxicity. Further, kojic acid was functionalized with imidazolium to provide nanocarriers with pH-sensitivity. The electrostatic interaction between the cationic lipid and siRNA made the liposomes stable. Notably, the liposome formulation enabled 90% entrapment efficiency with paclitaxel and siRNA. Following endocytosis, the imidazole head groups of cationic lipids acquired a proton in the acidic environment of endosomes, resulting in cytosolic release. The nanoparticles enabled efficient and targeted distribution of payloads, leading to induction of apoptosis by Bcl-2 siRNA, which increased the activity of paclitaxel *in vitro* and *in vivo*.[259] These advances made the cancer cells more sensitive to chemotherapeutic drugs, lowering the necessary dose and avoiding side effects. Another study by Kumar et al. employed MSNPs for codelivering doxorubicin and Bcl-2 or Bcl-xL siRNA to chemoresistant TNBC cells. The surface of MSNPs was modified with poly-L-arginine and PEG, conferring considerable siRNA binding ability and improving their stability and biocompatibility, respectively. Further, functionalizing nanoparticles with the antinucleolin aptamer provided the nanosystem with active targeting functionality, allowing uptake of payloads by tumor cells. Notably, up to a 40-fold drop in doxorubicin IC50 was observed, highlighting the important of the nanoparticles in overcoming chemoresistance.[260] A further investigation by Zhang et al. developed self-assembled polymeric nanocarriers composed of N-(2-hydroxypropyl) methacrylamide (HPMA) and a positively charged trimethyl-2-methacroyloxy ethylammonium chloride (TMAEMC) monomer in their hydrophilic end and the methyl methacrylate (MMA) in their hydrophobic end named PPEMMRA. The nanocarriers showed excellent safety and stability and allowed for efficient co-delivery of paclitaxel and siRNAs targeting Bcl-xL and MCL1 anti-apoptotic mRNAs to chemoresistant breast cancer cells. The hydrophobic drug paclitaxel was loaded inside the carrier, while siRNAs were attached through electrostatic interactions. The nanocarrier protected payloads, and after entering the tumor cells, enabled the rapid release of siRNAs followed by the slow release of paclitaxel within 72h. Results showed the increased sensitivity of cancer cells to paclitaxel-induced apoptosis and a 2−5-fold reduction in the IC50 value. *In vivo* experiments also confirmed the synergistic antitumor effect.[261]

Addressing multiple resistance components with targeted RNAi strategies can improve elimination of chemoresistance. With this in mind, administration of siRNAs for specific silencing of MDR1 and Bcl-2 genes using PLGA nanoparticles has been explored for eradicating paclitaxel and cisplatin resistance in ovarian cancer. The siRNAs were first complexed with cationic PLL to produce positively charged complexes and improve their encapsulation efficacy inside the negatively charged PLGA nanoparticles. Silencing the MDR1-mediated drug efflux along with the EPR effect gave PLGA nanoparticles an improved passive targeting ability that is appropriate for their intended drug delivery. Furthermore, after uptake by tumor cells, these nanoparticles can escape from endosomes and slowly release their siRNA cargo into the cytoplasm. Assessments showed an initial burst release followed by a sustained siRNA release for up to 10 days. Given the interdependence of drug efflux and apoptosis

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evasion in the evolution of chemoresistance, the dual RNAi delivery system facilitated chemosensitization compared to each intervention alone. Further, the nanoparticles encapsulated with siRNAs were given 1 day before the paclitaxel and cisplatin to eliminate chemoresistance since their concurrent administration showed minimal, if any, suppression of chemoresistance.[262] The mentioned research stands out since it employed PLL as the complexing reagent rather than the more common PEI to improve encapsulation efficiency. As previously noted, the high positive charge density of PEI raised concerns about possible cellular toxicity.[263]

Cancer cell apoptosis may be induced not only directly by targeting anti-apoptotic proteins but also by addressing factors implicated in cell cycle progression. For instance, overexpression of Plk1, a key mediator of mitotic growth, is associated with malignant transformation and drug resistance.[264,265] Silencing Plk1 expression using siRNAs was shown to impede cell cycle advancement and trigger apoptosis in malignant cells, making it a potential target for overcoming chemoresistance.[266] To this end, nanocarriers composed of cationic micelles and pH-responsive endosomolytic copolymers were fabricated for targeted delivery of Plk1 siRNA to doxorubicin-resistant ovarian cancer cells. Cationic micelles were generated from diblock copolymers of dimethylaminoethyl methacrylate (pDMAEMA) and butyl methacrylate (pDbB). Doxorubicin was loaded into the hydrophobic butyl methacrylate core of pDbB. The pDMAEMA corona of micelles condensed siRNAs, and the pH-responsive copolymer of poly(styrene-*alt*-maleic anhydride) (pSMA) that was electrostatically complexed to the siRNA/micelle complex enabled endosomal escape. Indeed, endosomal acidic pH protonates the carboxylate ions of pSMA, thereby changing the hydrophilic and inert state of the polymer to a hydrophobic and membrane-destabilizing state. Such polymers are potentially suitable and safe alternatives for cationic lipids. This system facilitated a reduction in Plk1 expression and doxorubicin sensitization by promoting apoptosis as indicated by p53-dependent upregulation of caspases.[265]

Xue et al. designed AuNP-oligonucleotides core/shell nanovehicles for Plk1 siRNA transport and controlled release in cancer cells.[267] As previously stated, AuNPs have been incorporated for siRNA delivery and gene silencing in different cancer cells, tissues, and organs without producing apparent toxicity.[211] Of note, the immunogenicity of AuNPs can be lower than that of their lipid-based counterparts, making them promising nanocarriers.[268] To improve the siRNA loading efficiency and stability and increase the targeting capacity of nanoparticles, the AuNP core comprises siRNA-encapsulated DNA self-assembled coating, and the outer layer consists of aptamer-incorporated Y-shaped backbone-rigidified triangular DNA bricks. The siRNA avoided enzymatic degradation in the bloodstream and delivered to target cells through aptamer-mediated endocytosis, where the nanosystem escaped the endosomes and released Plk1 siRNA in response to intracellular miRNAs-mediated hybridization of anchored DNA strands of the interior shell. Accordingly, the nanoparticles exhibited proficient serum stability, tumor targeting capability, extended circulation, and regulated siRNA release. *In vitro* and *in vivo* evaluation on MCF-7 breast cancer cells and nonsmall cell lung cancer (NSCLC) xenografts in nude mice showed robust apoptosis induction and complete (100%) tumor growth inhibition, respectively, complementing the precise delivery of Plk1 siRNA.[267]

Fei et al. encapsulated temozolomide into channels of benzaldehyde-functionalized MSNPs and capped Gint4.T aptamer-hepatoma-derived growth factor (HDGF) siRNA chimera on the surface using a pH-sensitive benzoic-imine bond. The nanosystem exhibited a spherical nucleic acid-like structure consisting of a nanoparticle core decorated with densely packed nucleic acids, enhancing the stability of HDGF siRNA and boosting blood-brain barrier permeability through scavenger receptors expressed on vascular endothelium. Downregulating HDGF expression is recognized to inhibit the aggressive behavior of GBM cells and improve temozolomide-induced apoptosis. Following systemic administration, the nanoparticles demonstrated delivery to the brain, where they specifically bound to and entered glioblastoma cells due to the Gint4.T aptamer. In the acidic lysosome environment, the chimera released quickly once the benzoic-imine link on the surface of MSNPs cleaved to repress HDGF expression, increasing the cytotoxicity of temozolomide. Experimental results showed that these nanoparticles effectively reduced tumor growth and extended the survival of GBM model mice.[269]

As discussed, many different approaches may be implemented to improve the effectiveness and reduce the adverse effects of siRNA-based therapeutics for promoting cancer cell apoptosis. These include the incorporation of biocompatible nanocarriers with suitable compositions supported by surface engineering. Future cancer therapies could utilize these promising strategies to overcome drug resistance.

5.1.4. Attenuating Adaptive Responses. Adaptive mechanisms are key drivers of chemoresistance. Common adaptive resistance responses are triggered by the disintegrin and metalloproteinase enzymes known as ADAMs, which cleave and activate the ligands for different growth factor receptor tyrosine kinases (RTKs), hence triggering pro-survival signaling such as phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPKs).[270] For instance, in colorectal cancer, chemotherapeutic-mediated upregulation of ADAM17 leads to the activation of EGFR signaling and chemoresistance.[271] In this respect, cancer cells may be more sensitive to chemotherapeutics by inhibiting either ADAMs or RTKs.[272,273] To address the EGFR-associated chemoresistance in NSCLC cells, LHRH-functionalized PEGylated NLCs coloaded with EGFR siRNA and paclitaxel were developed. Notably, α-tocopherol was used as a liquid lipid phase of the NLCs to improve their stability in aqueous medium. The hydrophobic drug paclitaxel was loaded into the core structure of NLCs with an entrapment efficacy of greater than 90%. The siRNA attached noncovalently to the cationic lipid DOTAP incorporated in the NLC membrane with a conjugation efficiency of more than 85%. The NLCs remained stable during short-term and long-term storage in an aqueous solution with various ranges of pH, temperature, and freezing−thawing circumstances. After 60 days of storage at physiologic pH and 4 °C, the entrapment efficacy of paclitaxel was maintained. Only 10−15% of siRNAs leaked out of the nanoparticles, and siRNAs remained fully functional, supporting the great capacity of NLCs in preserving the stability of siRNAs. The LHRH ligand that recognizes associated receptors on the surface of cancer cells enhanced the targeting capabilities of the nanocarrier to deliver to target cells, where they enabled endosomal escape of siRNAs to downregulate

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EGFR transcript and increase the sensitivity of malignant cells to paclitaxel.[274]

However, EGFR blockage may be rendered ineffective due to a number of downstream adaptive reactions. In many patients with EGFR-driven lung cancer, mesenchymal-epithelial transition factor (MET) proto-oncogene amplification facilitates RTK erbB-3 (also known as HER3)-dependent activation of PI3K and decreases susceptibility to EGFR inhibition by gefitinib.[275] Hence, one strategy to alleviate chemoresistance is the specific targeting of EGFR and MET using nanoparticle-based delivery platforms.[276] Recently, it has been established that suppressing bromodomain-containing protein 4 (BRD4), a transcriptional and epigenetic regulator, lowers MET expression in tumor cells.[277] In this regard, GALA and CREKA peptide-modified redox-sensitive PEG−PEI polymeric nanoparticles were constructed to deliver siRNAs targeting EGFR and BRD4 to tumor cells in a xenograft mice model of breast cancer. These nanoparticles accumulated in the tumor area due to CREKA targeting fibrin in the tumor vessels. After cellular uptake, the GALA peptides facilitated endosomal release of the siRNA. GALA is a synthetic pH-responsive amphipathic peptide that is water-soluble at neutral pH while binding to bilayer membranes at acidic pH, promoting membrane permeabilization. The expression of EGFR and BRD4 was substantially reduced, accompanied by a marked decrease in tumor growth.[278−280] The use of such interventions is highly regarded for fighting drug resistance and increasing the sensitivity of cancer cells to chemotherapy.

The oncogenic bypass of EGFR inhibition is also explained by the acquisition of the epithelial-mesenchymal transition (EMT) phenotype. According to experimental evidence, EMT may provide a subset of cancer cells with stem-cell-like traits, resulting in the development of resistance.[281,282] NSCLC cells unresponsive to EGFR tyrosine kinase inhibitors (TKIs) frequently exhibit EMT commonly associated with the overexpression of AXL RTK.[283] Such changes are related to the suppression of apoptosis and enhancement of survival; thus, targeting AXL may be a promising approach.[284] To that end, Suresh et al. designed EGFR antibody (cetuximab)-conjugated enzymatically cleavable porous gelatin nanoparticles loaded with AXL siRNA to sensitize NSCLC cells to EGFR TKIs. AXL siRNAs were covalently conjugated to gelatin nanoparticles with a conjugation yield of 90% using maleimide chemistry, which may provide greater stability for siRNAs compared to electrostatic attraction. The steric bulk of EGFR antibody further masked siRNAs and improved their stability. The unique affinity of cetuximab to EGFR, overexpressed on malignant cells, increased the targeted delivery of siRNAs. Following endocytosis by target cells, gelatin nanoparticles disintegrated within endosomes enabling the cytosolic siRNA release. As a result, silencing AXL diminished EMT and apoptosis resistance by downregulating mTOR signaling and elevating expression of the tumor suppressor p53, rendering cancer cells more vulnerable to EGFR TKIs.[285] Despite AXL, the cytoskeleton-related protein PDZ and LIM domain 5 (PDLIM5) are also essential for the induction of EMT through promoting transforming growth factor-beta (TGF-β) signaling, which confers resistance to EGFR TKIs in NSCLC cells. Cancer cells were made more susceptible to the chemotherapy drug gefitinib after being treated with PDLIM5 siRNA-adsorbed PVA-stabilized MSNPs, which were shown to effectively downregulate PDLIM5 transcripts and reduce TGF-β expression.[286] PVA served as a surfactant, reducing the surface charge of the nanoparticles and making them more stable in water.

Another example of malignant adaptive responses is the ocurrance of secondary resistance to BRAF inhibitors in patients with BRAF-driven cancers via induction of growth factors, activation of other RAF isoforms, and additional mutations in the KRAS and MEK1 oncogenes.[287,288] Targeting these elements can help eradicate chemoresistance. For example, resistance to RAF inhibitor sorafenib in hepatocellular carcinoma is correlated with the overexpression of midkine growth factor, triggering anti-apoptotic, angiogenic, and metastatic responses.[289] With this in mind, Younis et al. designed ultrasmall LNPs for targeted codelivery of sorafenib and midkine siRNA to hepatocellular carcinoma cells in a xenograft mice model. Optimizing the size and lipid composition of the nanoparticles improved their pharmacokinetic properties and biological stability following intravenous administration. The nanoparticles were functionalized with hepatocellular carcinoma cell-selective SP94 peptide for active targeting. The formulation also employed a pH-sensitive fusogenic lipid, YSK05, which possesses a neutral charge at physiological pH and a cationic charge at the lower pH values of endosomes. The LNPs efficiently targeted tumor cells and delivered payloads to the intracellular space to silence midkine and eradicate resistance to sorafenib *in vitro* and *in vivo*.[290]

Cao et al. also synthesized poly(N, *N*′-dimethylamino-2-ethyl methacrylate (PDMAEMA)-*co*-poly(*N*-isopropylacrylamide) (PNIPAM)-*co*-poly(2-methacryloyloxyethyl) (PMPC) (PDNM) nanoparticles through photoinitiated free radical polymerization for KRAS siRNA delivery. PDMAEMA with high positive charge condensed the siRNA through electrostatic adsorption and exhibited low immunogenicity and satisfactory loading capacity. PNIPAM modification enhanced the biocompatibility and reduced the cytotoxicity of PDMAEMA while also providing thermal responsiveness and pH sensitivity to the carrier. This helps in achieving targeted and controlled drug release in tumor cells. PMPC has a phospholipid polar group that resembles phosphatidylcholine lipids found in cell membranes, allowing nanoparticles to pass through the cell membrane and reach the cytoplasm. As a result, the PDNM/KRAS siRNA nanoparticles exhibited excellent cellular uptake and biocompatibility and effectively inhibited cell cycle progression and increased apoptosis in pancreatic cancer cells, leading to an antitumor effect both *in vitro* and *in vivo*. This demonstrates an effective approach to enhancing siRNA loading capacity and creating biocompatible nanocarriers with targeting abilities and controlled release features.[291] Another study conducted by Qian et al. showed that bifunctional nanoparticles made by combining radical cations (P6●+) with KRAS siRNA could inhibit oncogenic KRAS and mutant p53, leading to a substantial reduction in tumor growth, invasion, and drug resistance in pancreatic ductal adenocarcinoma with p53 and KRAS double mutations *in vivo*. Furthermore, by repressing mutant p53 and KRAS, the TME was remodelled, which resulted in an increased antitumor immune response by recruitment and infiltration of antitumor immune cells.[292]

Moreover, research by Chen et al. reported the development of ultrasonic-propelled folic acid-functionalized cationic liposomes loaded with BRAF siRNA to reverse the BRAF V600E mutation-induced resistance to trametinib, a MEK1/MEK2 inhibitor, in NSCLC. Notably, ultrasonic waves can

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penetrate deep into the body, thereby expanding the permeability of cells and tissues, which in turn increases the delivery of liposomes. The folic acid decoration of nanoparticles as an active targeting strategy in combination with ultrasonic-improved permeability facilitated intratumoral accumulation as well as targeted delivery of trametinib and BRAF siRNA to tumor cells. *In vitro* and *in vivo* experiments showed that the nanoparticles considerably reduced chemoresistance and enhanced the sensitivity of tumor cells to trametinib.[293] These findings highlight the incorporation of ultrasound as a safe and cost-effective approach to improve the effectiveness of liposomal drug delivery.[294]

Overall, the formation of adaptive responses, in particular the acquisition of the mesenchymal phenotype, involves a wide variety of signals and molecules. Besides applying siRNAs as powerful tools in targeting adaptive response elements, cancer chemoresistance may be overcome with the help of new molecular insights uncovered by siRNA-based RNAi screening.[295,296] This requires nanocarriers for efficient and tailored delivery of siRNAs to target cells. As discussed, different techniques and formulations have been developed to achieve this goal. Future research using lessons learned from these breakthroughs may help develop promising drug candidates for overcoming cancer chemoresistance.

5.1.5. Suppressing Altered Drug Targets. Alteration (i.e., mutation or overexpression) in drug targets plays a key role in chemoresistance. Mutations in the target molecule may sometimes lessen its susceptibility to chemotherapy drugs. For instance, mutations in the echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion gene have been associated with resistance to targeted therapies such as EGFR inhibitors in a considerable number of NSCLC patients, making it an important therapeutic target.[297] To reduce resistance in such a situation, Li et al. established a gold nanoshell-based controlled release delivery technology that targets the EML4-ALK mutation. ALK siRNAs were conjugated to the surface of hollow gold nanoshells. After, doxorubicin was attached to the surface of siRNA-loaded nanoparticles by electrostatic adsorption. Nanoshells densely coated with siRNA were shown to absorb more doxorubicin. Thiol-PEG-COOH was also incorporated as a surface stabilizer and a linker to attach the targeting peptide RGD. The nanoparticles accumulated in the tumor region during laser irradiation due to the photothermal conversion action of gold nanoshells, which locally improved the vascular permeability of tumors. Uptake of nanoparticles was observed in target cells given their dense spheroidal structure and RGD-based targeting, where the photothermal impact of gold nanoshells permeabilized endosomes and enabled the release of siRNAs and doxorubicin. Improved doxorubicin therapeutic efficacy with fewer off-target effects was shown *in vitro* and *in vivo*.[298] Another example is the mutation in the gatekeeper residue of the oncogenic breakpoint cluster region-Abelson (BCR-ABL) kinase in chronic myeloid leukemia (CML), which limits its interaction with imatinib while sustaining oncogene activity.[299] BCR-ABL is involved in the development and spread of CML, resulting in the excessive expansion of immature myeloid cells in the bone marrow and the blood. Various nanoparticle-based strategies have been established to downregulate BCR-ABL in CML cells and thereby increase their susceptibility to chemotherapy.[300−302] A recent investigation demonstrated the application of lipopolymers derived from low molecular PEI substituted with thioester-containing lipid, synthesized through combining linoleic acid and mercaptopropionic acid, in the targeted delivery of BCR-ABL1 siRNA. Such modification of PEI improves interaction with cellular membranes. The study showed that a high degree of propionic acid substitution on PEI had a negative impact on the surface hydrophobicity and cationic charge of the complexes, hindering siRNA uptake and its silencing impact. Conversely, moderate substitutions improved the mentioned parameters, resulting in improved cellular siRNA uptake and silencing effect. The optimized cationic lipopolymers reduced BCR-ABL transcripts and improved the therapeutic response to imatinib in patient-derived CML CD34+ cells.[301]

In addition, overexpression of certain targets may diminish the efficacy of chemotherapeutics that inhibit these targets since a significant fraction of target molecules will continue to evade inhibition. For instance, bicalutamide and other antiandrogen receptor medications have been shown to upregulate androgen receptors in prostate cancer cells.[303] Therefore, instead of inhibiting the androgen system, docetaxel-based chemotherapy is the most efficient and first-line treatment for castration-resistant prostate cancer (CRPC). Even so, therapy resistance occurs due to amplified androgen receptor signaling, which must be sufficiently suppressed to maximize the therapeutic efficacy of docetaxel.[304] Accordingly, PEI−PLGA polymeric micelles with docetaxel encapsulated in the hydrophobic interior layer of PLGA and androgen receptor siRNA condensed by PEI in the hydrophilic exterior layer have been constructed. These micelles were coated with PSMA-targeted anionic PEG-polyaspartic acid to prolong their circulation and improve their ability to deliver payloads to target cells. Linking PEI to PLGA reduced its toxicity but did not affect its ability to transport siRNAs. These nanoparticles selectively silenced androgen receptor signaling and improved the anticancer effect of docetaxel in a xenograft mouse model of prostate cancer.[305]

5.1.6. Targeting Tumor Microenvironment (TME) Elements. Different TME components are essential for the development of cancer drug resistance. In several types of cancer, including breast and hematological malignancies, chemoresistance is linked to the overexpression of integrins, i.e., surface adhesion receptors attaching cells to ECM, such as β1- and β3-integrins. Integrin-mediated adherence to ECM alters cell response to chemotherapies by suppressing apoptosis and activating pro-survival signals such as the PI3K/Akt and NF-κB pathways.[306,307] Consistently, increased expression of β3-integrin is a critical determinant of breast cancer spread and resistance to chemotherapy. Parvani et al. incorporated β3-integrin siRNA into (1-aminoethyl)iminobis-[N-oleicylsteinyl-1-aminoethyl)propionamide] (ECO) cationic lipid-based nanoparticles for silencing β3-integrin and attenuating tumor growth in an orthotopic mouse model of malignant breast cancer. Self-assembly of ECO with therapeutic siRNAs generated stable nanoparticles that can be easily modified with targeting moieties, and PEGylation with RGD-PEG_{3400}-maleimide improved their biocompatibility and enabled active targeting. These nanoparticles were delivered to tumor cells, where the pH-sensitivity of the ECO nanoparticle increased endosomal escape. As expected, the nanosystem facilitated a selective and efficient down-regulation of β3-integrin and reduced tumor metastasis and relapse.[308] Overexpression of β1-integrin has also been attributed to regorafenib resistance in advanced colorectal

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cancer. In light of this, β1-integrin siRNA and regorafenib-loaded biodegradable dimethyldidodecylammonium bromide (DDAB)-mPEG−PCL hybrid nanoparticles have been designed. The presence of DDAB cationic lipid on the nanoparticle shell improved their biocompatibility and enabled them to condensate β1-integrin siRNA and deliver to cells, while the hydrophobic PCL core allowed the encapsulation and controlled release of regorafenib. Further, the mPEG layer improved the stability and circulation lifetime of nanoparticles. The nanoparticles preferentially delivered payloads to tumor cells, reducing β1-integrin gene expression in tumor cells and leaving them sensitive to regorafenib.[309]

Other key TME components which play a role in chemoresistance include cytokines such as interleukins (ILs) and tumor necrosis factor-alpha (TNF-α) and growth factors such as EGF, fibroblast growth factor (FGF), and hepatocyte growth factor (HGF). These factors dampen apoptosis while activating pro-survival signaling pathways, including STAT3, PI3K/Akt, and MAPKs, to confer resistance to chemotherapeutics.[310,311] By inducing the NF-kB signaling, the IL17 receptor B (IL17RB) plays a pivotal role in tumor growth and spread. In this regard, carboxymethyl dextran-containing cationic chitosan nanoparticles were synthesized to encapsulate doxorubicin and adsorb IL17RB siRNA electrostatically. The negatively charged carboxymethyl dextran forms electrostatic interaction with positively charged chitosan nanoparticles to improve their physicochemical and biological properties as nanocarriers. The nanosystem was shown to increase doxorubicin sensitivity in MDA-MB361 TNBC cells by selectively silencing IL17RB, as evidenced by increased apoptosis and repression of invasion in comparison to doxorubicin-loaded nanoparticles.[312] Another example is targeting human EGFR type 2 (also called HER2) using siRNA to chemosensitize HER2 monoclonal antibody (trastuzumab)-resistant HCC1954 ovarian cancer cells. To that end, PEI−PEG copolymer-coated MSNPs were loaded with HER2 siRNA and functionalized with trastuzumab. PEI coating of MSNPs improved their capacity to deliver siRNAs and reduced toxicity compared to PEI-siRNA complexes. The PEG layer provided further protection for siRNAs against blood enzymes and prevented cationic nanoparticle aggregation and immunogenicity. Given the optimized physicochemical properties of polymeric nanoparticles as well as the specific interaction of trastuzumab with HER2-positive cells, HER2 siRNA remained intact in the circulation and delivered to cancer cells. The nanosystem decreased HER2 expression, triggered apoptosis, and repressed tumor development with fewer adverse effects and fewer immune responses than trastuzumab.[313] With these advancements, siRNA combined with delivery platforms might address critical TME constituents to reduce cancer chemoresistance. Although discovering therapeutic targets and tackling known undruggable targets has been enabled through the use of siRNA, developing safer and more targeted nanocarriers to reach the full potential of siRNAs in cancer treatment *in vivo* remains a challenge.

5.1.7. Immunomodulation. Programmed death-1 (PD-1), an immune checkpoint, is critical in repressing the activated T cell immune response. Tumor immune escape is facilitated by the binding of PD-1 on the surface of T cells to PD-L1 in cancer cells. Accordingly, PD-L1 expression in tumor tissues has been identified as a mechanism of chemoresistance following therapy with standard medications (e.g., doxorubicin and sorafenib) and even other therapeutic candidates (e.g., volasertib).[314−316] Thus, improving chemotherapies could be achieved by reviving antitumor immunity by addressing the PD-1/PD-L1 relationship.[317]

One example of this approach was the design of polydopamine nanoparticles wrapped in stem cell membranes to codeliver doxorubicin with PD-L1 siRNA to treat prostate cancer bone metastasis. PDA nanoparticles were generated from the spontaneous polymerization of dopamine with high hydrophilicity, biocompatibility, stability, and biodegradability. With numerous catechol and amino functional groups, these nanoparticles can efficiently bind various functional molecules to their surface, making them an excellent material for drug delivery. In this study, doxorubicin and PD-L1 siRNA were efficiently loaded into PDA nanoparticles via $\pi-\pi$ stacking interactions. Interestingly, coating nanoparticles with mesenchymal stem cell membrane provided tropism toward the TME mainly through chemokine-receptor interaction. Indeed, tumor cells and tumor-associated stromal cells produced chemokines, attracting mesenchymal stem cells. Further, the immune masking capabilities of the stem cell membrane coating improved the safety and passive targeting capacity of nanoparticles. The coating also enhanced the cellular uptake efficiency. The study demonstrated that lower pH levels accelerated drug release, likely due to the protonation of amino groups on the PDA nanoparticles or in doxorubicin molecules, which weakened the $\pi-\pi$ stacking interactions. As a result of targeted and efficient drug delivery as well as the pH-dependent release behavior, bone metastases were reduced, highlighting the usefulness of the nanosystem in combating cancer chemoresistance.[316,318] Importantly, upregulation of TGF-β and cyclooxygenase-2 (Cox-2) in the TME inhibits T cell infiltration, leading to a lack of exposure to tumor antigens and rendering PD-L1 inhibitors ineffective for stimulating the T cell response. Regarding this matter, nanoparticles composed of histidine and lysine-containing branched polypeptides were synthesized for transporting and delivering TGF-β and Cox-2 siRNAs to hepatocellular cancer cells. Following cell internalization, the acidic pH of endosomes protonated the histidine moieties of nanoparticles, enabling siRNA release.[319] Of note, nanoparticles with additional histidine have shown several benefits, including rapid siRNA release in acidic conditions and low cytokine stimulation.[320−322] PD-L1 suppression was enhanced in syngeneic orthotopic hepatocellular carcinoma model mice by silencing TGF-β and Cox-2, which enabled CD4+ and CD8+ T cells to penetrate deeper into tumors. Such approaches enhance the effectiveness of PD-L1 inhibition by transforming an immune-excluded tumor into a T cell-inflamed tumor.[319]

Incorporating targeting ligands may result in increased targeting capabilities of the nanoparticles as well as the efficacy of PD-L1-targeting strategies. As smart nanocarriers for codelivering PD-L1 siRNA and doxorubicin to 4T1 breast cancer cells, Wan et al. developed T7 peptide-decorated redox-responsive trimethyl chitosan-based nanoparticles. Trimethylation was carried out to enhance the water-solubility of chitosan and boost the positive charge for siRNA electrostatic adsorption. Further, the amino groups of nanoparticles were reacted with 4-nitrophenyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzyl carbonate (NBC), which provided the nanoparticles with a hydrophobic core to load doxorubicin. Since the T7 peptide is specific for

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overexpressed transferrin receptors on tumor cells, the nanoparticles delivered to target cells where ROS-mediated NBC oxidation allowed PD-L1 siRNA and doxorubicin release. A marked reduction in tumor growth was observed in mice bearing breast tumors.[323] In another investigation, Cao et al. synthesized GE11 peptide-functionalized PEGylated calcium phosphate core-lipid shell nanoparticles for efficient encapsulation and transportation of the FOLFOX regimen (miriplatin, 5-Fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP), and calcium folinate) and PD-L1 siRNA to overcome drug resistance in colorectal cancer and esophageal squamous cell carcinoma models. The GE11 peptide efficiently targeted EGFR on tumor cells, enhanced nanoparticle uptake, and facilitated drug accumulation within tumors. Although the GE11 peptide has a lower affinity for EGFR than the EGF, it lacks mitogen activity. Notably, the calcium phosphate inner core was highly responsive to acidic environments, leading to efficient drug release from endosomes, and enhanced drug accumulation in the cytoplasm. In addition, a higher PEGylation ratio was attained in calcium phosphate core−shell lipid nanoparticles compared to normal liposomes (20% vs 5%), which resulted in a prolonged circulation time. Interestingly, the miriplatin, a precursor of oxaliplatin, induced immunogenic cell death (ICD)-mediated release of damage-associated molecular patterns and subsequently promoted dendritic cell maturation and antitumor T cell immune response. FdUMP induced large amounts of ROS to further enhance ICD, while calcium folinate functioned as the FdUMP sensitizer. This nanoformulation resulted in a superior long-term antitumor effect compared to the free drug regimen.[324,325]

Rather than directly targeting PD-L1, several studies have considered targeting the molecular factors involved in PD-L1 upregulation. For instance, oncogenic induction of STAT3 was demonstrated to coordinate the immunosuppressive activity of tumor-associated myeloid cells by triggering PD-L1 expression.[326] With this in mind, Yan et al. fabricated lipid-coated calcium phosphate nanoparticles and liposome-protamine-hyaluronic acid (LPH) nanoparticles to enhance the *in vivo* delivery of gemcitabine and STAT3 siRNA, respectively.[327] Co-administration of the nanoparticles effectively reduced tumor growth in a syngeneic mouse melanoma model compared to monotherapy with gemcitabine-loaded nanoparticles via endogenous antitumor immunity exhibited by downregulating immunosuppressive mediators, eliminating myeloid-derived suppressor cells, and improving T cell effector functions in tumors and lymphoid tissues.[327,328] Furthermore, the emergence of secondary resistance mechanisms may render PD-L1-based interventions ineffective.[317] For example, upregulation of the constitutive WNT signaling mediated by β-catenin stabilization has been shown to exclude T cells from tumors, thus disabling PD-L1 blocking effects.[329] Accordingly, several investigations have addressed the Wnt/β-catenin pathway to reinstate the therapeutic function of PD-L1 blockade in cancer therapy. For this purpose, Ganesh et al. recruited DCR-BCAT, an optimized Dicer substrate siRNA (DsiRNA) formulated in tumor-selective LNPs, silencing the CTNNB1 gene that encodes β-catenin. Intravenous administration of DCR-BCAT synergized with anti-PD-L1 and anti-CTLA4 antibodies in terms of T cell infiltration and suppressed tumor growth in immunotherapy-refractory malignancies *in vitro* and *in vivo*.[330]

In addition to PD-L1 function, several other mechanisms may be involved in immune dysfunction-mediated chemoresistance. For example, treatment with doxorubicin has been shown to increase CD73 in the TME, resulting in chemoresistance due to an increase in adenosine level, which promotes cancer progression and inhibits the activity of infiltrating immune cells upon binding to its receptor.[331] In a recent study using 4T1 breast tumor-bearing mice, the therapeutic efficacy of liposomal doxorubicin was enhanced by CD73 siRNA-loaded smart cationic liposomes. GE11 peptide with a high affinity for EGFR on the surface of tumor cells was used to functionalize liposomes through conjugation with maleimide-PEG_{2000}-DSPE. Compared to nontargeted liposomes, GE11-functionalized liposomes increased tumor cell sensitivity to liposomal doxorubicin as well as CD73 silencing.[332] Moreover, the interaction of fibrinogen-like protein 1 (FGL1) with lymphocyte-activation gene 3 (LAG-3) forms an essential signaling pathway that adversely regulates immunological responses in the TME. Wan et al. used polymeric nanoparticles to codeliver siRNAs targeting FGL1 and PD-L1 to lung carcinoma cells. The nanoparticles were generated through the self-assembly of *cis*-aconitate (CA), PLL, and the ROS-responsive group thioketal (TK) and siRNAs via electrostatic interaction. The siRNA-loaded nanoparticles were coadministered with iRGD peptide intravenously to tumor-bearing mice. Targeting the $\alpha v\beta 3$ integrin receptor, which is overexpressed in tumor vasculature, by iRGD peptide triggers a sequence of processes that allows nanoparticles to leak out of tumor blood vessels and enter tumor tissue. After entering target cells, CA was protonated at the acidic pH of endosomes, facilitating the escape of nanoparticles. High intracellular ROS then degraded the structure of nanoparticles and released siRNAs to silence FGL1 and PD-L1. This strategy ameliorated the immunosuppressive TME, as demonstrated by increasing the infiltration of effector CD4+ and CD8+ T cells, and is potentially promising for combating resistance to various chemotherapy drugs.[333]

Another gene target, iRhom1, exhibited a negative correlation with prognosis in TNBC and colorectal cancer patient cohorts treated with chemotherapy, indicating that iRhom1 potentially plays a role in chemosensitivity. By attenuating endoplasmic reticulum aminopeptidase 1 (ERAP1)-mediated antigen processing and presentation, iRhom1 suppresses CD8+ T cell responses. In light of this, Luo et al. created chondroitin sulfate-coated PEG-chitosan−lipid polymeric nanocarriers, which were then loaded with iRhom pre-siRNA and doxorubicin through electrostatic and hydrophobic interactions, respectively. Chondroitin sulfate is a natural ligand of CD44 that is upregulated in tumor cells. Furthermore, by reducing CD44 cleavage on the cell membrane, iRhom1 silencing enhanced CD44-mediated tumor targeting. The efficient delivery of iRhom pre-siRNA and doxorubicin improved antitumor efficacy compared to doxorubicin-loaded nanocarriers by activating the tumor immune microenvironment in TNBC and colorectal cancer models in female mice.[334] According to the presented data, immunomodulation of the TME via siRNA therapeutics and nanoparticle delivery platforms is a promising approach to address the immunosuppressive mechanisms of chemoresistance.

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6. NANOPARTICLE-BASED SIRNA DELIVERY FOR CANCER THERAPY IN CLINICAL TRIALS

According to data gathered from ClinicalTrials.gov, there are multiple completed and ongoing clinical trials investigating siRNA therapy for patients with advanced malignancies. Different carriers have been investigated for siRNA delivery, including peripheral blood mononuclear cells (NCT03087591 and NCT02166255), dendritic cells (NCT00672542), and viral vectors (NCT00257647). There are also a number of clinical trials investigating the impact of siRNA-based nanotherapeutics in chemoresistant and malignant neoplasms (Table 8).

Among various siRNA nanocarriers, lipid-based nanoparticles have attracted significant attention for clinical cancer investigations. The liposomal siRNA silencing protein kinase N3 (PKN3) in the vascular endothelium, known as Atu027, inhibiting tumor invasion and metastasis, has shown favorable safety, tolerability, and efficacy in patients diagnosed with metastatic pancreatic adenocarcinoma in Phase I and II clinical trials.[350,351] Another phase 0 clinical trial used spherical nucleic acids, AuNP cores chemically conjugated with radially oriented and densely packed siRNAs, to target the GBM oncogene Bcl2-Like12 (Bcl-2L12). Spherical nucleic acids were shown to be a safe and potentially brain-penetrant precision medicine method for the systemic therapy of GBM.[352] Further, anti-MYC siRNA-loaded LNPs were shown to be well tolerated and exhibited promising initial clinical and metabolic responses in patients with advanced solid tumors.[353] A phase I dose escalation clinical study with a phase II expansion cohort evaluated the safety, pharmacokinetic, and antitumor impact of TKM-080301, Plk1 siRNA-loaded LNPs, in patients with advanced hepatocellular carcinoma. TKM-080301 was well tolerated; however, early phase studies demonstrated limited antitumor effects.[354]

Another phase I clinical investigation utilized polymeric nanoparticles, comprised of cyclodextrin-based polymer, coated with PEG, and actively functionalized with transferrin targeting ligand, for systemic delivery of RRM2, an endogenous ferroptosis suppressor, siRNA in patients with solid tumors. Outcomes showed that the nanoparticle system, CALAA-01, was safe and induced specific siRNA-mediated gene silencing.[355] There are also a number of ongoing clinical trials. A Phase I/Ib clinical trial is evaluating the safety, pharmacokinetics, and preliminary efficacy of glutathione S-transferase P (GSTP) siRNA-encapsulated LNPs in patients with pancreatic, colorectal, and nonsmall cell lung cancers (NCT03819387). In addition, two clinical studies are underway to assess the impact of iExosomes (mesenchymal stromal cells-derived exosomes with KrasG12D siRNA) and ephrin type-A receptor 2 (EphA2)- siRNA-encapsulated 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) in treating metastatic pancreatic cancer patients with KrasG12D mutation and advanced or recurrent solid tumors, respectively (NCT03608631, NCT01591356).

7. CONCLUSION AND FUTURE PROSPECTS

Cancer researchers are seeking out therapeutic strategies to keep malignant cells sensitive to chemotherapy. Integrating cutting-edge gene therapy methods with recent advances in nanotechnology may attain these objectives. The inherent characteristics of siRNAs necessitate their delivery *in vivo* through vector-dependent mechanisms. By equipping bio-

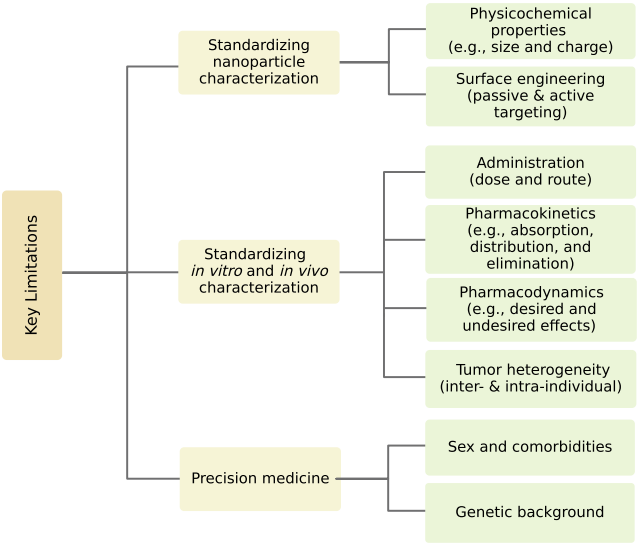

Figure 9. Limitations of small interfering RNA (siRNA) nanoparticles for clinical translation. Standardizing the characterization methods for evaluating nanoparticles is critical for predicting robust and reproducible clinical effects. Moreover, integrating these standard methods with principles of precision medicine is a promising application of siRNA-based cancer therapy. Created with BioRender.com.

compatible nanoparticles with targeting abilities and improved architecture, they are able to protect and deliver siRNAs efficiently to a specific target. Optimizing the features of vectors and implementing modifications to regulate their interactions with siRNAs as well as tissues, cells, and biological molecules requires a deep understanding of the many physiological obstacles facing the vectors. Strategies designed to overcome obstacles should precisely consider the pros and cons of different vectors, their preparation and functionalization methods, and potential chemical interactions (e.g., hydrophobic and electrostatic as well as covalent and hydrogen bonds).

It is important to acknowledge that in chemoresistant malignancies with intricate pathophysiologies, such as glioma, siRNAs may not fully meet therapeutic purposes; hence, codelivering siRNAs with conventional drugs within a delivery platform is of great interest. Nanoparticle-based delivery platforms for combined siRNA and chemotherapy achieved outstanding success during the past decade, specifically in overcoming cancer chemoresistance. In such approaches, achieving accurate drug release in a spatiotemporally regulated way is crucial to maximize multidrug delivery. This necessitates thorough investigation into the microenvironment at various targeted locations, as well as assessing the sensitivity of functional groups that govern drug delivery. Comprehensive validation and screening processes are also key. Furthermore, integrating different carriers (e.g., liposomes and hydrogels) can enhance the coloading efficiency and enable delivery of multiple therapeutic agents.[357] Thus, it is essential to acquire an in-depth knowledge of the physicochemical characteristics and techniques for functionalizing diverse vectors.

While extensive studies are needed to optimize nanostructures as therapeutic candidates, future implementation could rely on significant refinements in personalized nanomedicine according to a patient's genetic background. Given the

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Table 9. Significant Challenges Associated with the Clinical Application of siRNA-Based Cancer Nanotherapeutics

Challenges	Description
Intricate pathophysiology of malignancies	- siRNAs may not fully meet therapeutic purposes, underscoring the need for codelivering siRNAs with chemotherapy drugs within nanotherapeutics.
	- Accomplishing a planned and spatiotemporally regulated release of siRNAs and drugs tailored to the specific characteristics of each type of cancer is a difficult task.
Safety concerns	- Current nanoparticle-based delivery systems lack enough specificity and may potentially transfect other organs, especially the liver, after systemic administration.
	- Charged nanoparticles with a high surface area may interact with various macromolecules and signaling pathways that are not yet fully understood.
	- The possibility of immunogenicity due to foreign siRNAs must be considered.
Extrapolation of preclinical results	- The dose−response relationship from mouse models used in preclinical studies is not directly applicable to humans and does not offer a precise toxicity assessment.
	- Nonprimate models do not have enough similarity in genomic sequences with humans to forecast pharmacodynamic effects accurately.
Personalized nanomedicine	- Given the inherent variations among patients, achieving successful therapy for the same malignancies using a single vector and siRNA against an isolated target is challenging.
Built-in real-time imaging capability	- Creating a platform with excellent delivery capabilities that offers immediate access to precise siRNA delivery and release data continues to be challenging.
Production scale-up	- Complex delivery systems can lead to reproducibility concerns even at the laboratory level, which could pose difficulties during large-scale manufacturing.
	- Numerous components result in higher production costs, potentially making the treatment plan unaffordable for patients.

inherent variations among patients, achieving successful therapy for the same malignancies using a single vector and siRNA against an isolated target would be challenging. Despite the extensive research on multiple vectors, siRNAs, functionalization methods, and combination therapies, reliable and rapid screening of patients in clinical settings remains complex. Nanoparticles that facilitate the codelivery of siRNAs and contrast agents or recruiting siRNA vectors possessing magnetic properties have revolutionized the field by allowing for *in vivo* tracking of vectors and prompt evaluation of therapeutic outcomes. This breakthrough can potentially lead to more precise treatment of malignancies; however, real-time monitoring of siRNA release and pharmacodynamics remains challenging. Research and development in nanotheranostics remain focused on increasing the imaging sensitivity and accuracy of contrast agents. In this regard, a comprehensive grasp of the fundamental concepts of medical imaging is necessary, and innovative techniques employed for introducing contrast agents into vectors are needed. Furthermore, achieving additional diagnostic information confers the potential for more accurate screening of vectors and medications appropriate for patients, hence facilitating the advancement of precision therapy for malignancies.

In short, engineering nanoparticles for siRNA delivery to the site of action holds great promise in treating malignancies and overcoming chemoresistance. Optimizing vector preparation and functionalization methods, as well as innovating real-time monitoring approaches, is paramount to this goal. Importantly, standardizing nanoparticle characterization methods, as well as their *in vitro* and *in vivo* testing, and integrating these standard protocols with principles of precision medicine will accelerate siRNA-based nanotherapeutics toward clinical use (Figure 9). These clinical needs are described in Table 9.

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Notes

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VOCABULARY

Cancer group of diseases characterized by the uncontrolled growth and spread of abnormal cells, which can invade and damage surrounding tissues and organs.

Chemotherapy type of cancer treatment that uses drugs to kill or inhibit the growth of rapidly dividing cancer cells throughout the body.

Chemoresistance ability of cancer cells to resist the effects of chemotherapy, leading to treatment failure and cancer progression.

Small-interfering RNA (siRNA) short, double-stranded RNA molecule that silences specific gene expression by degrading mRNA, preventing the production of target proteins.

Nanotechnology technology that involves designing and using materials and devices at the nanoscale (1−100 nm) for various applications, including medicine, electronics, and energy.

Gene Therapy medical technique that treats or prevents diseases by introducing, altering, or silencing genes within malignant cells.

Nanoparticles tiny particles, typically between 1 and 100 nm in size, that can be engineered for applications such as targeted drug delivery, medical imaging, and diagnostics.

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