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REVIEW

Stimulus-Responsive Nanodelivery and Release Systems for Cancer Gene Therapy: Efficacy Improvement Strategies

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Abstract: Introduction of exogenous genes into target cells to overcome various tumor diseases caused by genetic defects or abnormalities and gene therapy, a new treatment method, provides a promising strategy for tumor treatment. Over the past decade, gene therapy has made exciting progress; however, it still faces the challenge of low nucleic acid delivery and release efficiencies. The emergence of nonviral vectors, primarily nanodelivery and release systems (NDRS), has resulted in a historic breakthrough in the application of gene therapy. NDRS, especially stimulus-responsive NDRS that can respond in a timely manner to changes in the internal and external microenvironment (eg, low pH, high concentration of glutathione/reactive oxygen species, overexpressed enzymes, temperature, light, ultrasound, and magnetic field), has shown excellent loading and release advantages in the precision and efficiency of tumor gene therapy and has been widely applied. The only disadvantage is that poor transfection efficiency limits the in-depth application of gene therapy in clinical practice, owing to the presence of biological barriers in the body. Therefore, this review first introduces the development history of gene therapy, the current obstacles faced by gene delivery, strategies to overcome these obstacles, and conventional vectors, and then focuses on the latest research progress in various stimulus-responsive NDRS for improving gene delivery efficiency. Finally, the future challenges and prospects that stimulus-responsive NDRS may face in clinical application and transformation are discussed to provide references for enhancing in-depth research on tumor gene therapy.

Keywords: stimulus-responsive NDRS, gene delivery, tumor treatment, delivery and release efficiency, tumor microenvironment, physiological barrier, efficacy improvement strategies

Introduction

Despite improvements in cancer treatment over the past few decades, malignant tumors remain the leading cause of patient deaths. Breakthroughs in diagnostic and therapeutic technologies are crucial for steering humanity away from cancer and achieving good health. Currently, the main treatment strategy for tumors still revolves around chemotherapy, which yields noticeable treatment effects but also brings about significant toxic side effects, poor selectivity, high tumor recurrence rates, and drug resistance, causing unbearable suffering for patients. Therefore, an effective and low-toxicity strategy is of immense significance for cancer treatment.

Tumors typically originate from abnormal gene expressions or mutations. Gene therapy aims to introduce exogenous genes into target cells, altering or modifying defective and/or missing gene sequences to cure acquired or genetic diseases.[1] Therefore, by treating at the genetic level, it is theoretically possible to correct abnormal genes in tumor cells, achieving the goal of treating the source of the disease. Nucleic acid molecules used for cancer gene therapy mainly include plasmid DNA

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(pDNA), short hairpin RNA (shRNA), microRNA (miRNA), messenger RNA (mRNA), small interfering RNA (siRNA), immunostimulatory CpG oligodeoxynucleotides, enzymes, and gene editing systems (such as the CRISPR/Cas9 system).[2,3] In 2018, the US Food and Drug Administration (FDA) approved the first siRNA drug, Patisiran (ONPATTRO), for the treatment of hereditary transthyretin-mediated amyloidosis with multiple peripheral neuropathy.[4] This milestone event has propelled gene therapy from basic research to clinical application. Gene therapy is hailed as the next generation of hope for the treatment of intractable diseases. However, naked nucleic acid molecules are characterized by rapid degradation by nucleases, high renal clearance, low uptake efficiency by intravenously injected cells, and are prone to "off-target" effects, leading to serious side effects.[5,6] Therefore, nucleic acid agents used in clinical settings must be loaded into safe and stable gene vectors and then delivered to tumor cells. It is worth noting that a safe and stable nanodelivery and release systems (NDRS) must meet the following conditions: (1) It must protect nucleic acid molecules from degradation and premature release. (2) To enhance transfection efficiency, it must have the ability to deeply penetrate tumors and reach tumor cells far from the blood vessels for intracellular uptake. (3) They must not interact with biological molecules during the blood circulation and must not cause immune reactions.[7–9]

NDRS can generally be divided into viral and nonviral vectors (for details, see the Nanodelivery and Release Systems). Owing to serious safety issues, such as immunogenicity, inflammatory reactions, and toxicity associated with viral vectors, they have been gradually phased out. Nonviral NDRS have attracted widespread attention from researchers due to their low immunogenicity, simple synthesis, and flexible design, although their transfection efficiency is greatly affected by physiological barriers inside and outside the cells.[10,11] In light of this, researchers have turned their attention to "smart" NDRS to overcome physiological barriers during delivery/release and ultimately achieve efficient gene transfection.[12] The "smart" NDRS can respond to specific tumor microenvironment, such as low pH, high concentrations of glutathione (GSH)/reactive oxygen species (ROS), overexpressed enzyme, as well as physical/chemical reactions that occur under external stimuli, such as light, heat, magnetic fields, and ultrasound, hence they are also known as stimulus-responsive NDRS.[13–15] Furthermore, researchers have developed dual- or multiple-responsive NDRS based on different stimuli to adapt to the complex body environment.[16] Noting, the emergence of Patisiran represents a breakthrough in the field of nucleic acid therapy combining RNA interference (RNAi) and nanotechnology, ushering in a new era of rapid development in gene therapy based on nanomedicine. Gene therapy may be one of the most promising treatment modalities in antitumor research. Considering the unique advantages of stimulus-responsive NDRS, this review aims to summarize the research progress of various stimulus-responsive NDRS for cancer gene therapy and to make reasonable predictions and analyses on the future challenges and prospects in clinical application and translation to provide new strategies to enhance the gene delivery and release efficiency of tumor treatment (Figure 1).

History of Gene Therapy

Gene therapy is the strategy of introducing exogenous nucleic acids (DNA and RNA) into target cells to correct or compensate for diseases caused by genetic defects, mutations, transcription barriers, or translation barriers, thereby achieving therapeutic purposes. For example, overexpression of a certain gene or protein by introducing pDNA/mRNA into target cells or inhibiting the expression of a specific gene using siRNA or RNAi technology.[17]

Since Watson and Crick proposed the double helix model of DNA in 1953, gene therapy has gradually developed.[18] In 1972, Theodore Friedmann and Richard Roblin first proposed a method for replacing defective DNA in patients with genetic diseases, named gene therapy.[19] In 1990, gene therapy was used in the clinical treatment of adenosine deaminase severe combined immunodeficiency (ADA-SCID).[20] In the mid-1990s, Blaese's team successfully treated a severely immunodeficient patient with gene therapy.[21] During the same period, the first gene therapy drug, vitravene, was approved by the US FDA in 1998 for the local treatment of retinitis in patients with immune dysfunction.[22] However, this was accompanied by a historic crisis in gene therapy. In 1999, the first death from gene therapy occurred in the US States because a high-dose adenovirus gene drug was injected for ornithine transcarbamylase (OTC) deficiency.[23] Similarly, in 2002, in a clinical trial for treating immunodeficiency diseases (X-linked severe combined immunodeficiency [X-linked SCID]), two young boys developed T-cell acute lymphoblastic leukemia after a retroviral vector was inserted near the LMO2 gene promoter.[24,25] As a result, the use of retroviral gene therapy was urgently halted, severely impeding the development of gene therapy (Figure 2). Until 2006, when Andrew Fire and Craig Mello discovered that double-stranded RNA could selectively silence genes, gene therapy

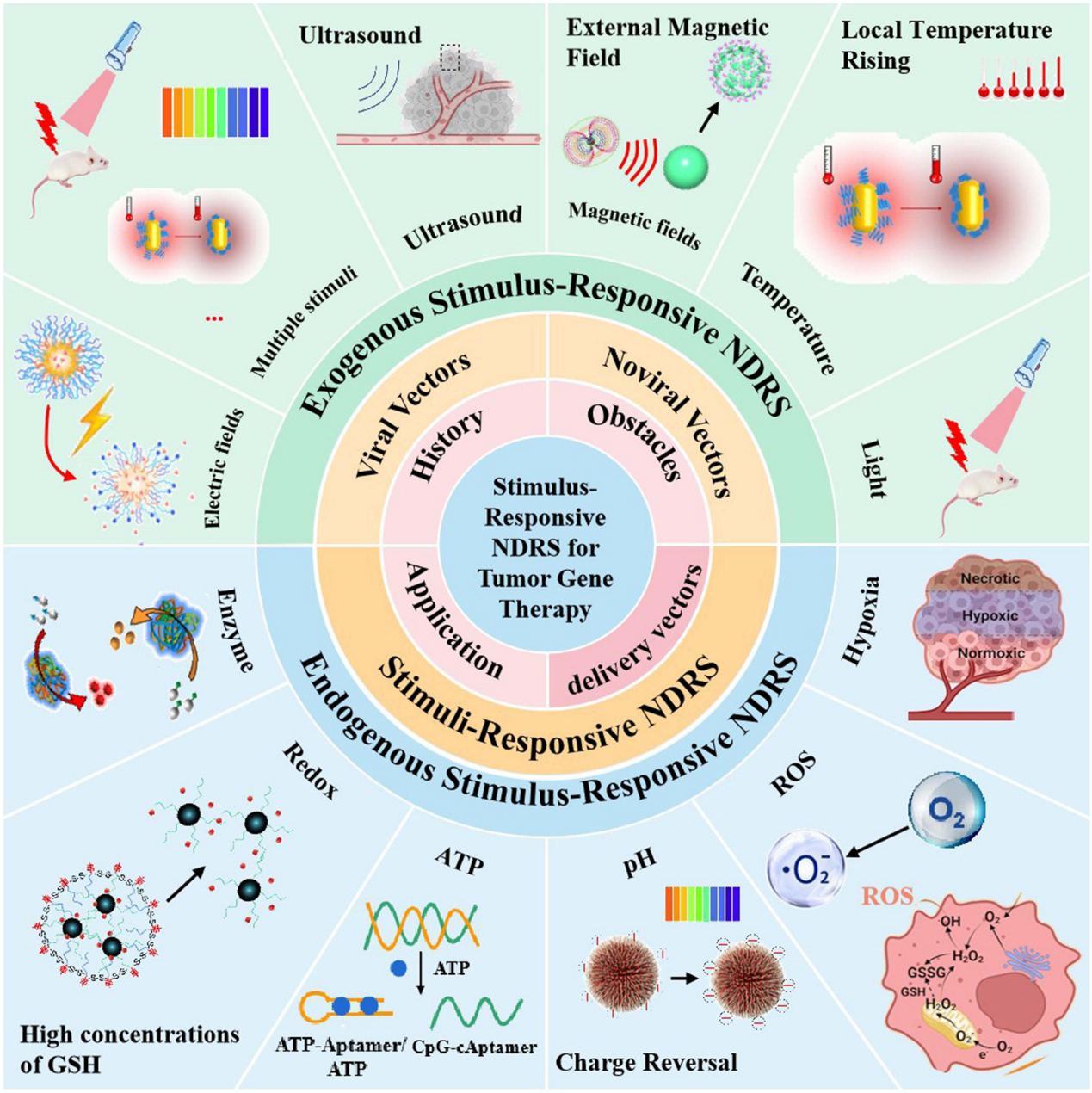

Figure 1 Schematic diagram of this review.

returned to the public eye.[26] In addition, in 2008, three children with Leber's congenital amaurosis successfully improved their vision as a result of gene therapy.[27] In 2012, Glybera, a gene therapy product for lipoprotein lipase deficiency, was approved by the European Medicines Agency, but it eventually withdrawn from the market due to its high price and rare indications in 2017.[28,29] Moreover, the gene agents, Patisiran[30] and Zolgensma,[31] were approved in 2018 and 2019, respectively. In 2020, Emmanuelle Charpentier and Jennifer A. Doudna pushed gene therapy to a new climax with CRISPR-CAS9 gene editing technology.[32] As of 2020, more than 4000 clinical trials for gene therapy were collected in ClinicalTrials.gov (https://classic.clinicaltrials.gov/), indicating that gene therapy technology is gradually transformed into clinical applications. Most gene therapy clinical trials have focused on malignant tumors, genetic diseases, and cardiovascular diseases.

By reading the developmental history of gene therapy, three crucial aspects in the process of gene therapy can be identified: (1) selection of target genes, (2) construction of safe and efficient gene vectors, and (3) expression of target

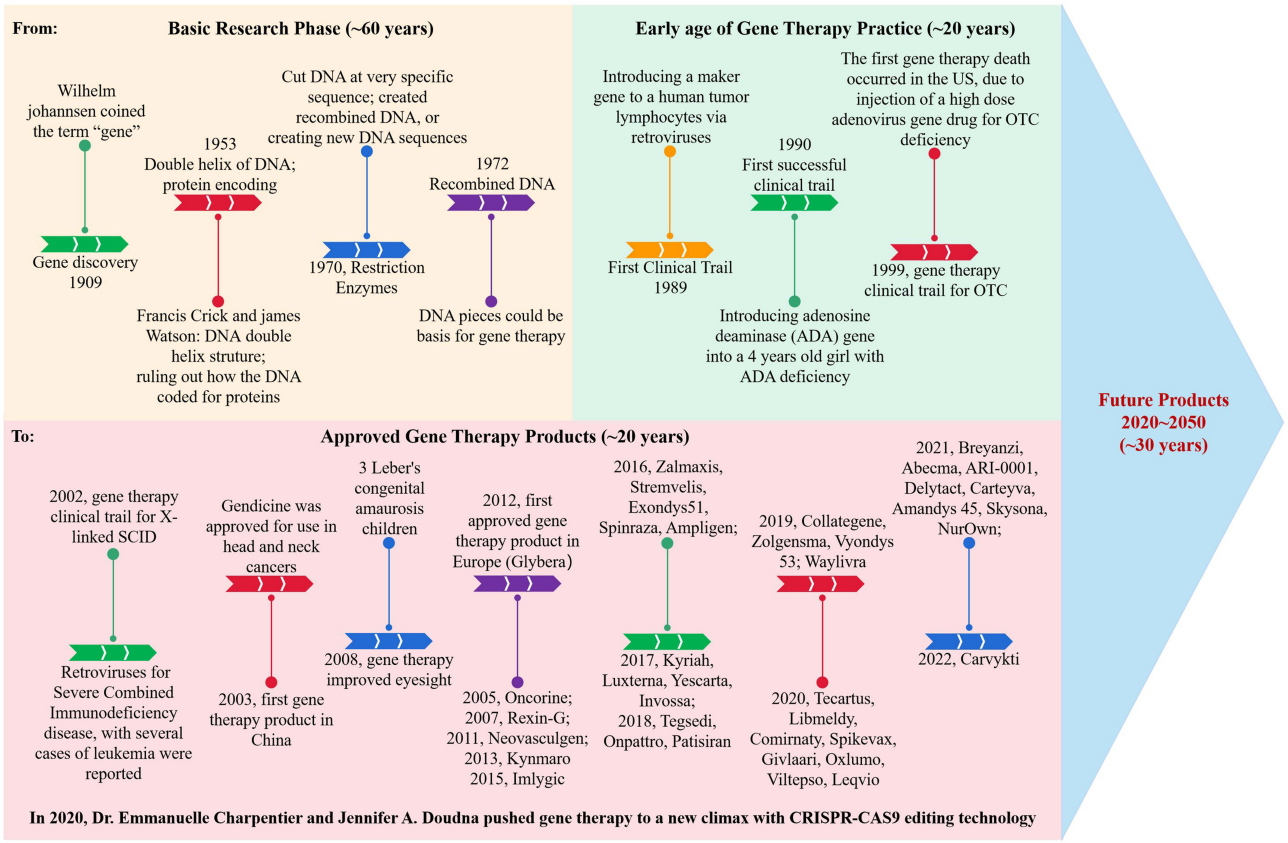

Figure 2 Development schedule of gene therapy.

genes in cells. These three components constitute a complete gene therapy system and are indispensable. The lack of a safe and effective vector remains a bottleneck restricting the development of gene therapy. Although viral vectors are still praised for their efficient transfection efficiency, nonviral vectors are gradually occupying an advantageous position owing to their excellent characteristics.

Obstacles and Solving Strategies of Gene Delivery

Various physiological barriers are the most important factors that affect the delivery/release efficiency in the process of gene therapy, which may lead therapeutic nucleic acids to be prematurely captured or destroyed before reaching the target, such as blood circulation, clearance of reticuloendothelial system, enhanced permeability and retention (EPR) effect, deep tissue penetration, difficulty in target cell uptake and intracellular transport, endosome escape, and early release of nucleic acid cargoes.[33] The above elements have a vital impact on the gene delivery/release efficiency. Therefore, the construction of a safe and effective NDRS is a promising and important strategy to overcome the bottleneck that makes gene therapy difficult to use clinically.

After years of development, researchers have proposed strategies for solving these problems. For example, (1) improving the NDRS space stability by shielding the surface charge shielding effect of polyethylene glycol (PEG), thus greatly prolonging blood circulation time of nucleic acid shipments.[34] However, repeated injections of PEGylated NDRS will lead to accelerated blood clearance.[35] (2) Tumors gene therapy usually relies on the EPR effect, which is considered to be the main reason for NDRS accumulating in the tumor site.[36] However, the EPR effect has been doubted in recent years, because the total coverage between endothelial cells is only 0.048 of the vascular surface area and the actual value is 60 times less than the theory.[37] The latest evidence suggests that trans endocytosis of endothelial cells may be the key mechanism of NDRS aggregation in tumor sites.[38] The new NDRS for gene delivery/release should take full advantage of this new mechanism. (3) Adding cationic polymers such as polyethyleneimine (PEI) and polyamidoamine (PAMAM) to NDRS to prevent nucleic

acid cargo from being degraded by acid hydrolase promotes endosome/lysosome escape and release into the cytoplasm by the proton sponge effect, leading to improved transfection efficiency.[39] However, the cytotoxicity and non-biodegradability of PEI limit its application in vivo. In addition, lysosome escape can also be achieved by adding reagents that can promote lysosome rupture, such as chloroquine, sucrose, photosensitizer and polyvinyl pyrrolidone.[40,41] (4) Promoting cellular uptake of NDRS carrying nucleic acid cargoes by means of endocytosis mediated by endosome-Golgi apparatus-endoplasmic reticulum pathway.[42] This pathway can effectively avoid the degradation of nucleic acid caused by "endosome-lysosome", and significantly increase the release of nucleic acid in the cytoplasm, enhance the efficiency of gene expression/silencing and antitumor effect. (5) To prevent the early release of nucleic acids, researchers have proposed two alternative strategies: adding nuclear localization signals or constructing nucleic acid complexes to promote the entry of genes into the nucleus or to maintain continuous DNA transcription and translation.[43–45] Based on the above strategies, the ideal gene vector should closely protect nucleic acid molecules from degradation before reaching the target cells and release nucleic acids in time for continuous transcription and translation.

Nanodelivery and Release Systems

Although some achievements have been made in the development of gene therapy, most clinical projects have been terminated because of negative immunization-related events. Whether teenagers in the US died of adenovirus gene therapy in 1999, two boys suffered from T-cell acute lymphoblastic leukemia due to retroviral vectors in 2002, or severe allergic reactions triggered by Pfizer's mRNA COVID-19 vaccine in 2020, all highlight the important role of a qualified vector in gene therapy.[46,47] A qualified gene vector must possesses the following characteristics: low cytotoxicity, good biocompatibility, no immune rejection, and high transfection efficiency (Figure 3). Gene vectors can be divided into viral and nonviral vectors. The development of gene vectors (Table 1) with low cytotoxicity, high transfection efficiency, and multiple functions according to requirements has become a focus of research in gene therapy.

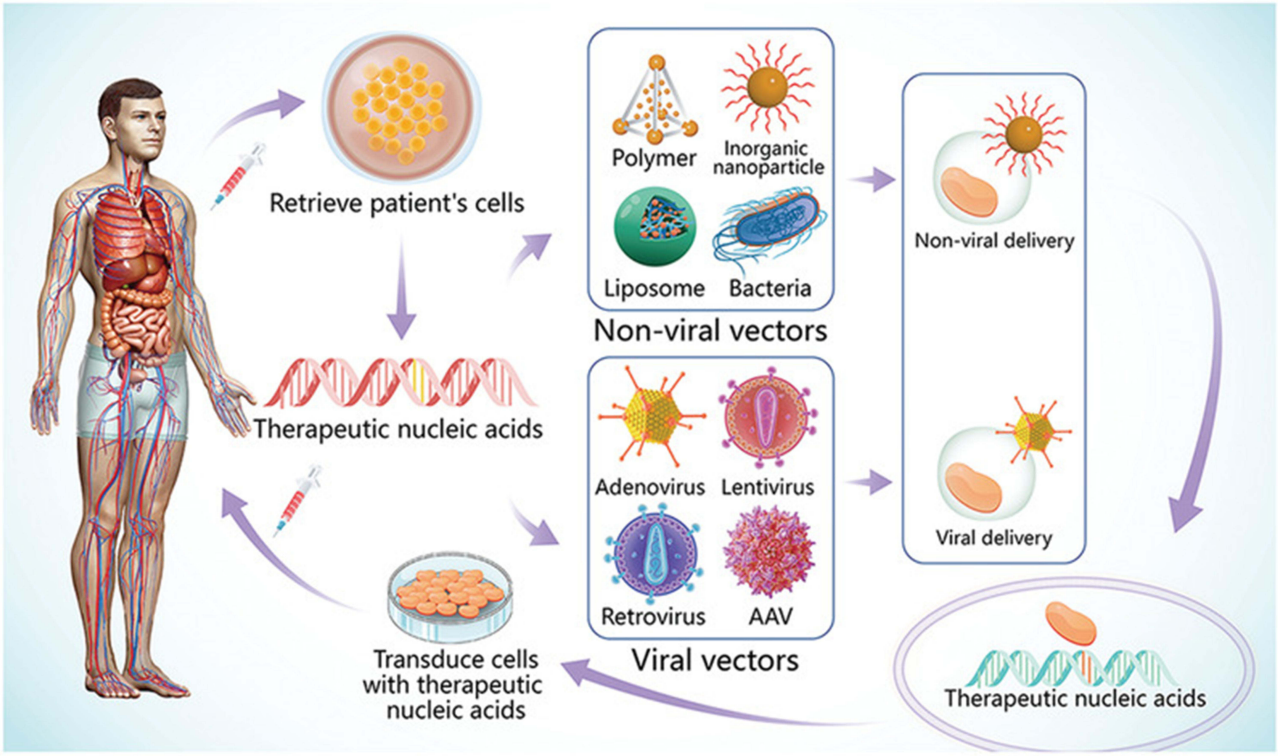

Figure 3 Overview of gene therapy strategies. Cells are retrieved form the patient. Therapeutic nucleic acids are introduced into the retrieved cells via viral or nonviral vectors. The modified cells are then readministered genetically and amplified, and subsequently reinfused into the patient. Finally, therapeutic nucleic acids are delivered directly to the patient's target cells by viral or nonviral vectors. Reprinted with permission from C Yu, L Li, P Hu, et al. Recent Advances in Stimulus-Responsive Nanocarriers for Gene Therapy. Adv Sci. 2021; 8(14): 2100540. Copyright (2022) Wiley-VCH GmbH.[14]

Table 1 Comparison Between Kinds of NDRS for Tumor Gene Therapy

Types		Advantages	Disadvantages
Viral Vectors	Adenovirus	The immunogenicity has been reduced significantly in second- and third generation adenovirus vectors; provide persistent extrachromosomal transgene expression	With strong immunogenicity for the first-generation adenovirus vectors
	Adeno-Associated Virus (AAV)	Do not cause toxic or pathogenic responses	Repeated administration has generated strong immune responses, reducing the efficacy of delivery and transgene expression
	Retrovirus	Reverse transcriptase activity allows the production of dsDNA copies of the RNA genome for integration into the host genome	Random integration has been of concern, even resulting in leukemia development in treated SCID-X1 patients
	Lentivirus	Show low cell cytotoxicity and due to their "semi-random" chromosomal integration provide improved biosafety for clinical applications	The low titers obtained, and residual toxicity have compromised their utilization
Nonviral Vectors	Cationic Liposomes	Are convenient for large-scale production with stable in performance	The primary obstacles are short half-life and obvious acute toxicity/immune reactions
	Cationic Polymers	More closely and more stable than liposome complexes; simple/controllable synthesis process, modifiable structure, and low immunogenicity	Nondegradability, cytotoxicity, and poor transfection efficiency
	Inorganic Nanoparticles	Easily controlled size, good biodegradability, and excellent biocompatibility	Fast elimination by the immune system, low accumulation in tumor sites, and severe toxicity to the organism
	Stimulus-Responsive NDRS	Structure or properties can change in response to specific signals, thus achieving more precise release of nucleic acid cargoes	A single stimulus response error could lead to the failure of the entire process; the design and preparation of multiple components is challenging

Viral Vectors

Currently, there are three types of viral vectors for gene therapy, adenoviruses, AAV, and retroviruses.[1] They are widely used because of their high transfection efficiency. Adenoviruses and AAV are well known for their wide host range, low pathogenicity to humans, lack of insertion mutagenicity, and simultaneous expression of multiple genes.[7,48] As a result, adenovirus-based gene therapy clinical trials account for 50% of the global trials and are mainly applied in cancer treatment and novel vaccines.[49,50] Notably, the strategy of adenovirus-based gene therapy for cancer therapy is to induce the expression of tumor-associated antigen and/or promote antitumor immune responses through adenovirus-mediated gene delivery. Additionally, retroviruses such as RNA viruses can cause lifelong infections in host cells and can be transmitted to daughter cells during cell division. Among them, third-generation self-inactivating lentivirus vectors are the most widely used, playing a crucial role in correcting primary immunodeficiencies and modifying chimeric antigen receptors.[51] In the development of gene therapy over the past 40 years, significant progress has been made in the research of delivery/release vectors. However, viral vectors still have drawbacks such as high immunogenicity, low encapsulation efficiency, and difficulties in large-scale production (especially for genes with numerous base pairs). Therefore, nonviral vectors have emerged as a research hotspot.

Nonviral Vectors

Compared with viral vectors, nonviral vectors have attracted widespread attention from researchers owing to their good safety, controllability, low cost, and ease of large-scale production. Nonviral vectors can be divided into organic nanoparticles (eg, cationic polymers, liposomes, or stimuli-responsive NDRS) and inorganic nanoparticles (eg, gold, Fe_3O_4, graphene, and SiO_2 nanoparticles).[10,11,52] They mainly rely on physical adsorption or positive-negative charge binding to encapsulate nucleic acids and then introduce cargoes into target cells. An excellent nonviral vector should meet the following criteria: (1) nucleic acids

can be carried across the cell membrane, (2) can protect nucleic acids from enzymatic degradation, (3) can degrade rapidly inside the cell to release nucleic acids, (4) can be cleared from the body, and (5) is nontoxic to cells. Although nonviral vectors have made satisfactory progress, their transfection efficiency remains much lower than that of the viral vectors. The design of safe and efficient nonviral gene delivery and release vectors remains a significant challenge.

Cationic Liposomes

Since their appearance in 1980, liposomes have been among the most common nonviral gene vectors. They can be subdivided into cationic, neutral, anionic, and liposomal complexes. Among them, cationic liposomes, which can provide positive charges to attract, encapsulate, and compress nucleic acids, are the most commonly used gene delivery/release vectors.[53] Cationic liposomes are generally positively charged amphiphilic lipid bilayer self-assembled vesicles composed of various cationic lipid molecules alone or together with neutral auxiliary lipids (eg, DOPE or cholesterol) to stabilize the bilayer, reduce reagent toxicity, and enhance endosomal escape capability).[54] They bind to negatively charged nucleic acids through electrostatic adsorption, and then enter cells for nucleic acid release through either membrane fusion or endocytosis. Cationic liposomes are convenient for large-scale production with stable performance, and currently, there are many available commercial products (eg, DOSPA, DOGS, DOTAP, and DOTMA). Cationic liposomes have been successfully used for nucleic acid delivery and release in tumors and in the brain, lungs, and muscles. However, the primary obstacles to cationic liposome-mediated transfection are their short half-lives and acute toxicity/immune reactions, which greatly affect their therapeutic effects.

Cationic Polymers

In addition to being more stable than liposome complexes, cationic polymers can bind nucleic acids more closely. Similar to the mechanism of cationic liposomes, cationic polymers form nanosized complexes by binding to negatively charged nucleic acids through electrostatic adsorption, and timely escape from endosomes via the "proton sponge" effect, avoiding rapid degradation of nucleic acids.[55,56] As gene carriers, the advantages of cationic polymers include simple/controllable synthesis process, modifiable structure, flexible/diverse functions, low immunogenicity, and high transfection efficiency. Among them, polyethylenimine (PEI), poly (L-lysine) (PLL), and chitosan are the most representative cationic polymer carriers for gene therapy.[57–59]

Owing to its excellent "proton sponge" effect, the transfection efficiency of 25k Da branched PEI is much higher than that of ordinary materials, and is known as the gold standard for gene transfection. However, the nondegradability and cytotoxicity of PEI are fatal flaws.[60] It usually needs to be combined with polylactic acid to endow PEI biodegradability, or with PEG to enhance the stability and biocompatibility of the complex formed by PEI and DNA, thus achieving biodegradability and improving haemolysis to accomplish safe and effective gene delivery/release. Similarly, chitosan has good biocompatibility, biodegradability, and adhesion properties that can prevent nucleic acid degradation.[57] In addition, the amino groups of chitosan can induce a "proton sponge" effect similar to that of PEI, which helps the endosomal escape of the complex. In contrast, with poor transfection efficiency, PLL requires the help of lysosomotropic agents, such as chloroquine, for endosomal escape. Unmodified PLL has significant cytotoxicity and must be modified with PEG to minimize nonspecific interactions with some serum components, thereby increasing blood circulation time.[61]

Inorganic Nanoparticles

One of the main factors affecting gene delivery/release efficiency is the insufficient number of target genes that reach lesions. As a gene carrier, inorganic nanoparticles possess unique advantages.[62] The controllable size allows target genes to remain in the tumor tissue through the EPR effect or to target various lesion tissues with blood circulation. Therefore, inorganic nanoparticles are promising candidates for gene therapy. AuNPs are the most widely used inorganic nanocarriers.[63] They can precisely control their size and shape, and can also be functionalized and modified by different biologically active targeting molecules through thiol-gold bond connections on the surface, thereby guiding their enrichment in specific tissues/cells to improve the uptake rate and gene expression efficiency. In addition, gold nanoparticles have photothermal capabilities, enabling synergistic effects between gene therapy and photothermal therapy.[64] Similarly, magnetic nanoparticles can achieve the same gene delivery/release effect using an external magnetic field.[65]

However, with a deeper understanding of complex diseases and nucleic acid delivery/release, single-function gene carriers can no longer satisfy this demand. It is imperative to develop new types of delivery/release carriers, and stimulus-responsive NDRS with multiple functions have emerged for gene delivery and release.

Stimulus-Responsive NDRS

Nonviral vectors do not exhibit high transfection efficiency of viral carriers because various physiological barriers need to be overcome during the delivery/release process before reaching the nucleus or cytoplasm of the target cells. One of the most effective ways to improve the carrier delivery/release efficiency is to adequately utilize cellular and extracellular environmental signals. Based on this, researchers have focused on "smart" gene carriers, also known as stimulus-responsive NDRS, to overcome physiological barriers during delivery/release and ultimately achieve efficient gene transfection.[66] Compared to conventional nanocarriers, stimulus-responsive NDRS exhibit stronger dynamic activity. Their structure or properties can change in response to specific environments, physicochemical factors in the body, or external signals, thus achieving a more precise release of nucleic acid cargo. Stimulus-responsive NDRS can respond to specific environments within the cells, such as pH, GSH, ROS, enzymes, adenosine triphosphoric acid, as well as physical/chemical reactions that occur under external stimuli, such as light, heat, magnetic fields, and ultrasound (Figure 4).[67–70] Furthermore, researchers have also developed multiple "smart" carriers that can respond to different stimuli based on the specific physiological environment of the lesion (For details, see the section Multiple Stimuli-Responsive NDRS and Table 2). Since nucleic acids are easily degraded, the premature release of nucleic acids may lead to loss of therapeutic function. Therefore, the design of a stimulus-responsive NDRS for gene delivery/release is not the same as that for drug delivery/release.

Application of Stimulus-Responsive NDRS

Endogenous Stimulus-Responsive NDRS

Tumors are typically accompanied by an abnormal TME, such as low pH, high concentrations of GSH/ROS, overexpressed enzyme.[100] For tumors gene therapy, the ideal carrier should have excellent stability and unrecognizable characteristics by the RES in the body. Nucleic acid agents should be promptly and efficiently released when a gene carrier reaches a tumor site. Therefore, stimulus-responsive NDRS based on endogenous TME has emerged as the time required. In recent years, TME endogenous stimulus-responsive NDRS have achieved significant progress in gene therapy.

Enzyme-Responsive NDRS

Unlike normal tissues, excessive enzyme are usually secreted in TME, such as matrix metalloproteinases (MMPs), hyaluronidase (HAase), intracellular tissue proteases, β-glucuronidase, and esterases.[101–103] These enzymes are highly selective and specific, acting only on specific substrates. Enzyme-responsive NDRS relies mainly on the cleavage of esters or short peptide sequences by enzymes, achieving specific rupture of carriers in tumor tissues or cells, thus accomplishing highly efficient nucleic acid delivery/release at target sites.

Components that play important roles in tumorigenesis, development, metastasis, and invasion are usually selected for the construction of MMPs-responsive NDRS, such as MMP2 and MMP9. Upregulated MMP2 is considered a diagnostic and prognostic biomarker for many cancers.[104] MMP2-responsive NDRS provides a new strategy for targeted delivery in tumor gene therapy. Lu et al developed a MMP2-sensitive NDRS camouflaged with red blood cell membrane for miR-126-3p delivery (REMAIN).[71] Compared to the control group, REMAIN not only significantly increased transfection efficiency (26-fold) in vivo but also demonstrated longer circulation lifespan, lower toxicity, better biocompatibility, and immune evasion (Figure 5). In addition, the targeting effect of REMAIN promoted the accumulation and retention of miR-126-3p in tumors, showing effective inhibition and anti-angiogenesis in cancer cells. Simultaneously, REMAIN induced significant down-regulation of target genes in tumor tissues.

In general, the most common hyaluronic acid (HA) receptor, CD44, is distributed on the tumor cell membrane.[105] Therefore, gene carriers modified by HA, HAase-responsive NDRS, can specifically target tumor cells overexpressing CD44. Then, under the action of excessive HAase, HA on the outer layer of the nanocarrier is enzymatically degraded and taken up by cells, ultimately leading to the release of gene cargo. Chen et al constructed an HAase-responsive NDRS

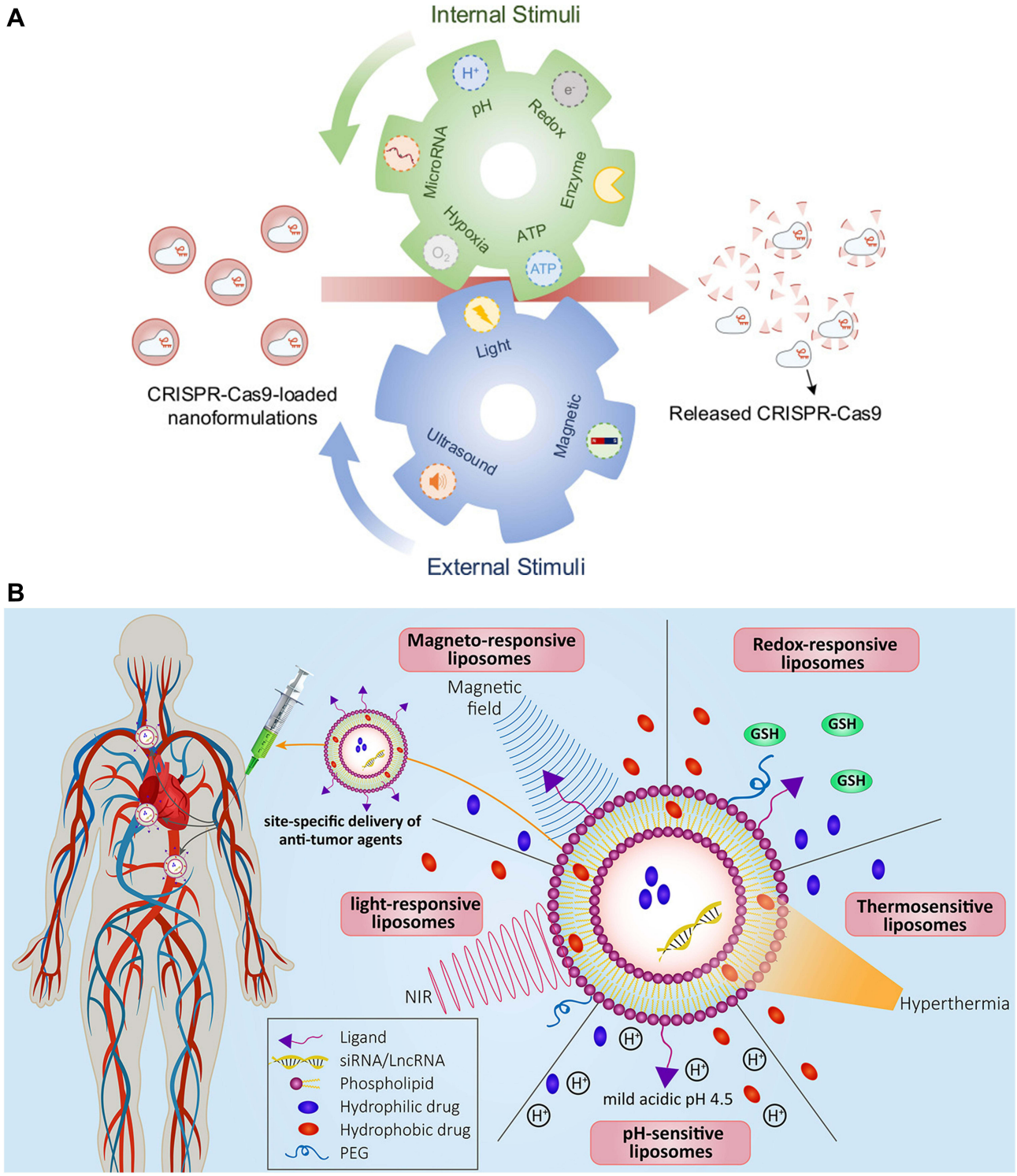

Figure 4 Scheme of endogenous and exogenous stimuli-responsive NDRS. Endogenous stimuli-responsive NDRS are based on changes in the tumor microenvironment (TME), such as pH, ROS, and redox status. For the precise control of cargo release by NDRS, exogenous stimuli, such as light-responsive NDRS, have been developed to appropriately release genes at tumor lesions to suppress cancer progression. (**A**) Type of the stimulus signal. Reprinted with permission from Fang T, Cao X, Ibnat M, et al. Stimuli-responsive nanoformulations for CRISPR-Cas9 genome editing. J Nanobiotechnology. 2022;20(1):354. Copyright (2022) BioMed Central.[69] (**B**) An case of a stimulus-responsive NDRS. Reprinted with permission from X. Zhang, B Qin, M Wang, et al. Stimuli-responsive liposomal nanoformulations in preclinical and clinical approaches for cancer therapy. J Control Release. 2022; 35150–80. Copyright (2022) Elsevier.[70]

Table 2 Strategies for Enhancing the Gene Delivery/Release in Tumor Therapy of Stimulus-Responsive NDRS

Types	Strategies		Cancer	Cell line	Mechanisms	Ref.
	Before	Behind				
Endogenous Stimulus-Responsive NDRS						
Enzyme	miR-126-3p	MMP2-sensitive NDRS (RMmiR)	Lung adenocarcinoma	H460, NCI-H1299, A549 cells	Significantly increased transfection efficiency and target gene downregulation with lower toxicity, better biocompatibility, and immune evasion	[71]
	shR-survivin	HAase-responsive NDRS	Breast cancer	MDA-MB -231 cells	Exhibited efficient HAase-based response release; significantly inhibited tumor growth by silencing survivin while maintaining low toxicity	[72]
	siRNA	MMP7-sensitive NDRS (pVLN)	Cervical cancer	Hela cells	Helps the siRNA escape from the lysosomes, resulting in a final silencing efficiency of 92%	[73]
Redox	pDNA-EGFP	PEG-SS-PEI-loaded microbubbles	Ovarian cancer	A2780 cells	Promote the uptake of plasmids by tumor cells, achieve enhanced release of nucleic acid cargoes	[74]
	siR-Bcl2	Disulfide-bond-inserted DNA nanodevice (DRD)	Breast cancer	MCF-7 cells	Completed structure cleavage and siRNA release, effectively knocking out key genes in cancer progression	[75]
	miR-30a-5p	Redox-responsive nanospheres (rMMNs)	Melanoma	MUM2B, CRMM2, CM 2005.1 cells	Enhance the miRNA payload and enable miRNA release under GSH-dominant TME	[76]
	siRNA	RGD-PEG-PLys colloid	Glioma	U87 cells	Exhibited potent in vivo RNAi to the targeted glioma cells and antitumor efficacy via systemic administration	[77]
	siR-PLK1	cRGD-PEG-PAsp (MEA) -PAsp (C=N-DETA)	Prostatic cancer	PC-3 cells	Achieved siRNA-mediated silencing of PLK1 gene without cationic-related toxic side effects	[78]
pH	pDNA	pH-responsive multi-chain micelle	Murine neuroblastoma	Neuro-2A cells	Can undergo charge conversion and decomposition, enhance cellular uptake and promoting endosomal escape, thereby achieving efficient gene transfection	[79]
	siR-VEGA	CHCE/siRNA NDRS	Melanoma	SK-MEL-28 cells	Improve siRNA delivery/release efficiency, effectively silenced VEGA	[80]
	siRNA	pH-responsive NDRS (CPNPs)	Breast cancer	MCF-7 cells	Facilitate the siRNA endo/lysosome escape and cytoplasm delivery	[81]
	siR-FGL1	Hybrid biomimetic membrane-poly (lactic-co-glycolic acid) nanoparticles	Breast cancer	4T1 cells	Can effectively silence the FGL1 gene, promoting T-cell-mediated immune responses and enhancing antitumor immunity	[82]

(*Continued*)

Table 2 (Continued).

Types	Strategies		Cancer	Cell line	Mechanisms	Ref.
	Before	Behind				
Other Endogenous Stimulus	siR-Bcl2	ATP-responsive NDRS	Melanoma	B16F10 cells	siR-Bcl-2 can be effectively released from the NDRS in response to intracellular ATP and interfere with Bcl-2 expression	[83]
	siR-CDC20	Hypoxia-responsive NDRS	Breast cancer	MCF-7 cells	Specifically reduced the expression of breast cancer cell-related genes, thereby enhancing the antitumor effect	[84]
	mR-p53	ROS-responsive polymer	Lung cancer	H1299 cells	Allowing rapid release and translation of mRNA, thereby inducing p53 expression to promote apoptosis of lung tumor cells	[85]
Exogenous Stimulus-Responsive NDRS						
Light	siRNA	Light-responsive NDRS (LPGN)	Pancreatic cancer	Panc-1 cells	Responds to NIR light, achieving selective siRNA delivery and controlled release	[86]
	siRNA/pASO	Photosensitive spherical nucleic acid	Cervical cancer	Hela cells	Can simultaneously facilitate the release of siRNA and pASO to achieve cytoplasmic targeting; inhibit the genes expression of HIF-1α and Bcl-2	[87]
	Mitochondrial RNA (mtRNA)	NIR fluorescent probe f-CRI	Breast cancer	4T1 cells	Dominantly accumulate in cellular mitochondria and could be covalently conjugated onto mtRNA upon 808 nm irradiation	[88]
Temperature	Single-stranded oligonucleotides (ssDNA)	Thermoresponsive pNIPAAm-co-pAAm polymer	/	/	Regulate the accessibility of sequence-specific hybridisation between complementary DNA, thereby improving the cytotoxicity of NDRS	[89]
	CRISPR-Cas9 Ribonucleoprotein (RNP)	NIR light-triggered thermo-responsive copper sulfide	Melanoma	A375 cells	Achieve controlled release of RNP and doxorubicin for tumor synergistic combination	[90]
	Cas9-sgPlk-1	Lipid-encapsulated AuNPs	Melanoma	A375 cells	Can enter tumor cells and release plasmids into the cytosol, enabling effective knock-outs of Plk-1 of melanoma and inhibition of the tumor both	[91]
Other Exogenous Stimulus	siR-VEGF	Magnetic-responsive NDRS	Breast cancer	MDA-MB 231 cells	Facilitated the accumulation of siRNA and enhanced the silencing of VEGF in cancer cells at the gene and protein levels (60% and 40%)	[92]
	pDNA	Mesoporous silica nanoparticle loading microbubbles	Ovarian cancer	SKOV3 cells	Exhibited stable pDNA release, effectively protected pDNA from enzymatic degradation, ultimately enhancing the efficiency of pDNA delivery/release.	[93]
	mRNA	PLGA-based NDRS	Breast cancer	4T1 cells	Promote mRNA escape from endosome, and augments antigen presentation	[94]

(*Continued*)

Table 2 (Continued).

Types	Strategies		Cancer	Cell line	Mechanisms	Ref.
	Before	Behind				
Multiple Stimuli-Responsive NDRS						
pH/GSH	CRISPR-Cas9/sg-PD-L1plasmids	HMnMPH nanoplatform	Breast cancer	4T1 cells	The released CRISPR-Cas9 plasmid could knockdown the PD-L1 immune checkpoint and restart immunosuppressive T cells	[95]
	SiR-IRAK4	Biomimetic nanodrug	Pancreatic cancer	PANC-1, SW1990 cells	Exhibit sensitive GSH and pH-dependent drug release profiles and enhance the inhibitory effects on the proliferation and migration of tumor cells	[96]
GSH/enzyme	siR-Plk1	HPAA-peptide-HPG	Breast cancer	MDA-MB -231 cells	Could form the compact nanocomplex with siR-Plk1, thus confirming the stable load of genes and subsequent targeted gene delivery	[97]
pH/ROS	miR155	MiR@PCPmP	Triple-negative breast cancer	4T1 cells	Exhibited effective endosome escape and efficient cytoplasmic miR155 release, with no apparent systemic toxicity	[98]
pH/GSH/TAP	pDNA	Cationic cross-linked polymer	/	/	Showed more effective DNA condensation and selectively released complex DNA in TME	[99]

for the delivery/release of survivin-shRNA.[72] Surprisingly, the experimental results confirmed that the HAase-responsive NDRS exhibited strong stability in blood circulation, efficient release rate, improved penetration in lesions, and enhanced accumulation in tumors through targeted recognition. Additionally, HAase-responsive NDRS significantly inhibited tumor growth by silencing survivin while maintaining low toxicity.

Redox-Responsive NDRS

Redox-responsive NDRS are designed based on different reducing substances between intracellular, extracellular, or normal environments/TME, such as nicotinamide adenine dinucleotide phosphate, GSH, and oxygen/superoxide.[106,107] The rapid proliferation of tumor cells consumes a large amount of oxygen, while insufficient oxygen delivery due to abnormal blood vessel perfusion directly leads to decreased oxygen pressure, causing a significant accumulation of reducing substances inside the cells (with GSH being the most representative). The concentration of GSH in tumor cells is 7–10 times higher than that in normal cells, whereas GSH in normal cells is 100–1000 times higher than that in the extracellular environment.[108] Therefore, GSH is often used as a stimulating factor to trigger rapid gene release within tumor cells in gene therapy. Researchers have designed a series of NDRS capable of undergoing redox reactions to deliver or release anticancer nucleic acids or drugs. Typically, the gene release from redox-responsive NDRS is relatively low because of the low GSH concentration in the blood, which significantly reduces its toxicity to other normal tissues and cells. The disulfide bond is the most important and widely used linker for constructing redox-responsive NDRS. Under the action of a high concentration of GSH in tumor cells, GSH, as a reducing agent, breaks the disulfide bond into sulfhydryl groups and breaks the structure of the carrier, thus accelerating the release of nucleic acid cargo and improving transfection efficiency, whereas GSH itself is oxidized into oxidized glutathione. However, the disulfide bond is quite stable in blood circulation and the extracellular environment, which means that redox-responsive NDRS exhibit excellent stability.[109]

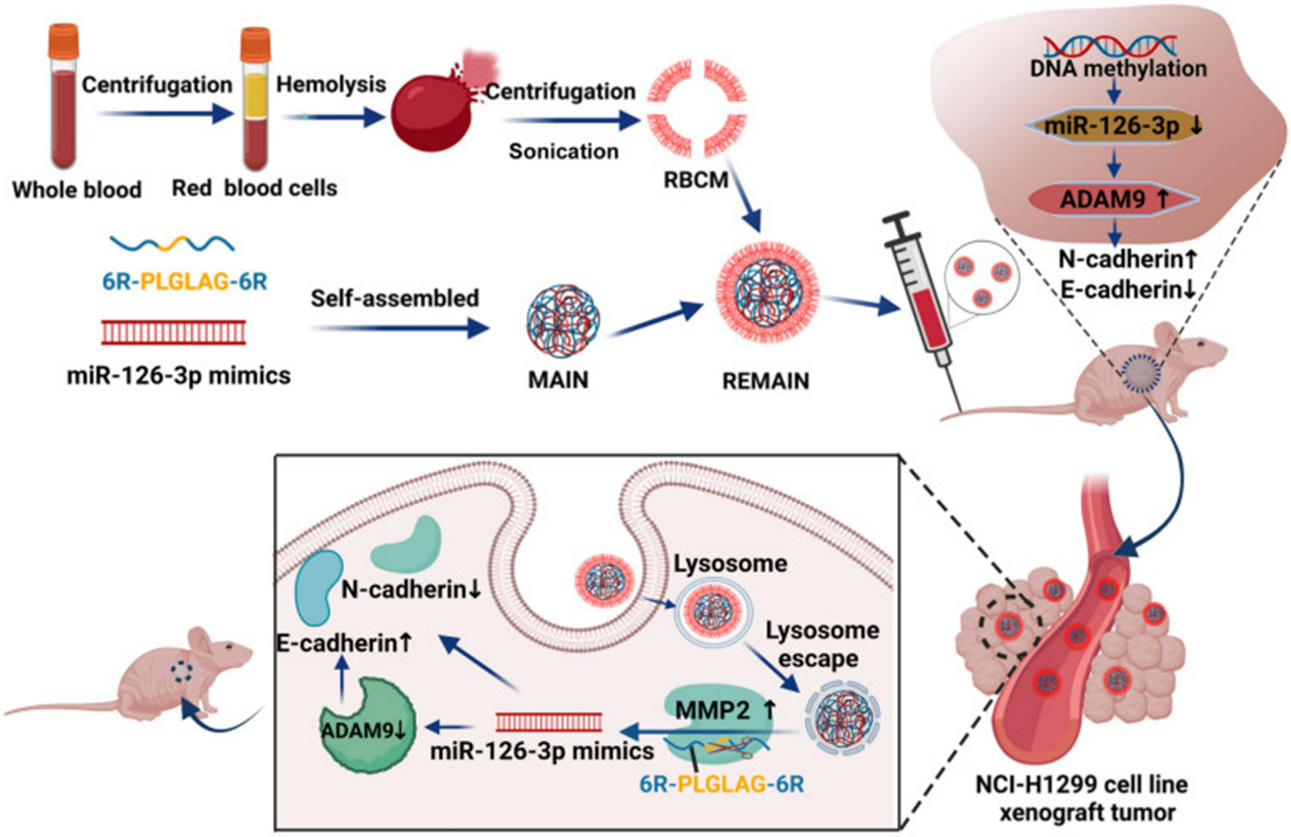

Figure 5 Schematic diagram of preparation and miR-126-3p "smart" delivery/release process of REMAIN for lung adenocarcinoma gene therapy. Reprinted with permission from L Liang, H Cen, J Huang, et al. The reversion of DNA methylation-induced miRNA silence via biomimetic nanoparticles-mediated gene delivery for efficient lung adenocarcinoma therapy. Mol Cancer. 2022; 21(1): 186. Copyright (2022) BioMed Central.[71]

Research has shown that cationic polymers containing disulfide bonds are more suitable for nucleic acid delivery and release. Researchers have introduced disulfide bonds into traditional materials, such as PAMAM, PEI, and PLL, and have developed numerous reduction-responsive cationic polymers for gene delivery. Chun et al established a gene delivery/release system called PEG-SS-PEI-loaded microbubbles (PSP@MB).[74] PSP@MB can promote the uptake of plasmids by tumor cells, achieve enhanced release of nucleic acid cargoes, and only exhibit minimal toxicity to normal tissues. In addition, Wang et al used DNA origami technology to construct a disulfide-bond-inserted DNA nanodevice (DRD) for siRNA and doxorubicin delivery/release.[75] Triggered by disulfide bonds and GSH, DRD completed structure cleavage and siRNA release, effectively knocking out key genes in cancer progression. Furthermore, by combining RNAi and chemotherapy, DRD induces potent cytotoxicity and tumor growth inhibition without systemic toxicity.

Meanwhile, researchers have begun to focus on developing noncationic NDRS owing to the cytotoxicity limitations of cationic materials. Huang et al prepared noncationic polymer-siRNA nanocapsules with disulfide bond (cRGD-PEG-PAsp[MEA]-PAsp[C=N-DETA]).[78] cRGD-PEG-PAsp[MEA]-PAsp[C=N-DETA] not only exhibited satisfying performance (eg, efficient siRNA encapsulation, outstanding stability in serum, excellent targeted delivery effect, and high-performance GSH-triggered siRNA release), but also significantly inhibited tumor growth in vivo. Most importantly, cRGD-PEG-PAsp[MEA]-PAsp[C=N-DETA] achieved siRNA-mediated silencing of polo-like kinase 1 (PLK1) without toxic side effects.

pH-Responsive NDRS

In general, normal cells rely on oxidative phosphorylation for energy production. However, rapidly proliferating tumor cells lead to insufficient oxygen supply, causing a shift in the energy supply mechanism to a high rate of glycolysis, resulting in lactate accumulation and pH decrease.[110,111] It is well known that the purpose of pH-responsive targeting can be achieved through ionizable pH-sensitive functional groups or acid-labile chemical bonds. Therefore, researchers have

designed pH-responsive NDRS based on the pH differences between healthy and tumor tissues and organelles (eg, normal tissues: pH 7.2–7.4, tumor tissues: pH 6.5–6.9, cytoplasm: pH 7.4; lysosomes: pH 4.5–5.0, and endosomes: pH 5.5–6.0). As one of the most commonly used gene delivery/release vectors, pH-responsive NDRS exhibit advantages in tumor gene therapy, such as good biocompatibility, high tumor cell uptake rate, long blood circulation time, and low cytotoxicity.

The Introduction of acid-labile chemical bonds into materials is a common method for constructing pH-responsive NDRS, such as imine bonds, acetal bonds, and orthoester bonds. These chemical bonds are stable at physiological pH but undergo cleavage at acidic pH, leading to the disintegration of pH-responsive NDRS and the specific release of nucleic acid agents. Shen et al developed a stepwise pH-responsive multi-chain micelle using ethylenediamine polycarboxybetaine for pDNA delivery/release.[79] This pH-responsive multi-chain micelle can undergo charge conversion and decomposition when transitioning from circulating blood to tumor and endo/lysosomal, thereby enhancing cellular uptake and promoting endosomal escape, achieving efficient gene transfection. Zhang et al prepared a tumor-targeting and pH-responsive dual-functional siRNA delivery/release NDRS (CHCE/siRNA NDRS) to overcome various biological barriers, improve siRNA delivery/release efficiency.[80] CHCE/siRNA NDRS effectively silenced vascular endothelial growth factor A (VEGA), induce tumor cell apoptosis, and inhibit cell proliferation, leading to improved antitumor effects (Figure 6).

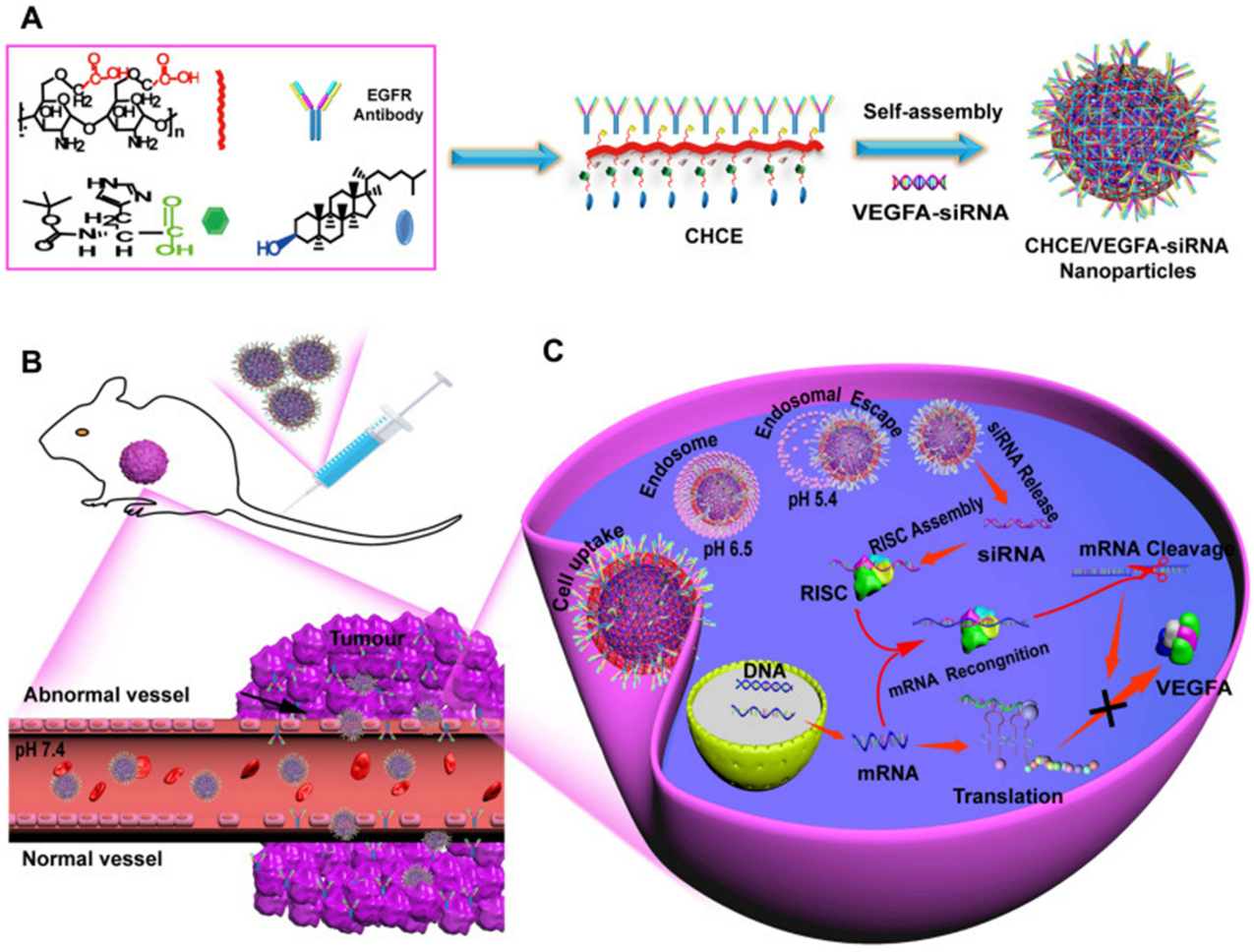

Figure 6 The preparation and siRNA delivery of CHCE/siRNA NDRS. (**A**) Synthesis of CHCE/siRNA NDRS. NDRS backbone carboxymethyl chitosan (red), cholesterol (blue), and helper histidine (Green). The CHCE forms stable NDRS through self-assembly. (**B**) The CHCE/siRNA NDRS target delivery to the tumor. (**C**) The endosomal escape and RNAi-induced gene silencing. Reprinted with permission from X Zhang, B Qin, M Wang, et al. Dual pH-Responsive and Tumor-Targeted Nanoparticle-Mediated Anti-Angiogenesis siRNA Delivery for Tumor Treatment. Int J Nanomedicine. 2022; 17: 953–967. Copyright (2022) Dove Medical Press.[80]

Other Endogenous Stimulus-Responsive NDRS

In addition, endogenous factors that can be used for stimulus-responsive NDRS construction include the following. (1) Adenosine triphosphate (ATP) is one of the most important sources of energy for cellular metabolism and has a much higher intracellular concentration than the extracellular.[112] (2) Hypoxia: the rapid proliferation of tumor cells leads to increased oxygen consumption and insufficient oxygen supply caused by vascular abnormalities.[113,114] Hypoxia is inevitably present within tumors. Common hypoxia-sensitive compounds include 2-nitroimidazole and azobenzene. (3) ROS: highly active molecules or free radicals present in cells are important molecules in signal transduction and metabolism, including hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide (O^{2-}), and hydroxyl radicals (•OH).[115] Common ROS-responsive chemical bonds include sulfides, disulfides, and phenylboronic acid/esters. Therefore, researchers have focused on these factors in an effort to develop new gene delivery and release strategies. Qiao et al constructed an ATP-responsive NDRS loaded with copper complexes and siRNA targeting B-cell lymphoma-2 (Bcl-2).[83] According to the experimental results, siR-Bcl-2 can be effectively released from the NDRS in response to intracellular ATP and interferes with Bcl-2 expression, thereby overcoming the drug resistance of B16F10 melanoma cells and significantly enhancing the therapeutic effect. Li et al developed a hypoxia-responsive NDRS for siRNA delivery/release (cell division cycle 20, CDC20) by combining 2-nitroimidazole-modified peptides and cationic lipids.[84] Hypoxia-responsive NDRS specifically reduced the expression of breast cancer cell-related genes, thereby enhancing the antitumor effect. Zhou et al reported an ROS-responsive polymer NDRS platform for the co-delivery of mRNA and photosensitizers onsite for the first time.[85] After ROS triggering, the NDRS decomposes, allowing rapid release and translation of mRNA, thereby inducing p53 expression to promote apoptosis of lung tumor cells, demonstrating an effective and safe antitumor effect, and significantly improving the efficiency of lung cancer treatment.

Exogenous Stimulus-Responsive NDRS

In addition to endogenous biological stimuli, exogenous substances with unique properties, such as light, heat, ultrasound, and magnetic fields, can be used to construct gene delivery/release carriers and exogenous stimulus-responsive NDRS for effective delivery and selective release of nucleic acids. Compared to endogenous stimulus-responsive NDRS, the characteristic of exogenous stimulus-responsive NDRS is the ability to control the timing and site of nucleic acid release, thereby achieving excellent gene delivery/release effects that can be manipulated by humans.[116] Currently, various types of gene delivery/release carriers that can change their physicochemical properties in response to exogenous stimuli have been developed.

Light-Responsive NDRS

Light is widely used to stimulate nucleic acid release from NDRS for noninvasive and precise control over time and space. Among them, with higher energy and efficiency, short-wavelength light (eg, ultraviolet light) is easily absorbed by skin and damaged tissue. Hence, short-wavelength light is not suitable for exciting NDRS.[117] In contrast, long-wavelength light (eg, near-infrared [NIR] light) is characterized by lower absorption and scattering, better penetration in human tissue (approximately 10 cm), and less cell damage owing to its lower energy.[118] Therefore, NIR light has become a research hotspot in the field of light-responsive NDRS. Polymers, cationic liposomes, and gold nanoparticles are the most commonly used nanomaterials for constructing light-responsive NDRS. Generally, light-cleavable molecules (eg, nitrobenzyl and azobenzene) must be combined with nanomaterials to confer light-responsive properties, thereby directly or indirectly changing the NDRS structure to release nucleic acid cargo. Jia et al addressed the shortcomings of low gene delivery/release efficiency and the inability to trigger release on demand by creating an intelligent light-responsive NDRS called liposome-coated Prussian blue@gold nanoflower (LPGN).[86] LPGN not only responds to NIR light, achieving selective siRNA delivery and controlled release but also efficiently converts absorbed NIR light into heat, enabling gene-photothermal synergistic therapy in vitro and in vivo. Similarly, Chen et al developed a photosensitive spherical nucleic acid NDRS (PSNA) for siRNA and antisense oligonucleotide (ASO) delivery/release.[87] PSNA can simultaneously facilitate the release of siRNA and pASO to achieve cytoplasmic targeting via lysosomal escape. PSNA also inhibited the expression of hypoxia-inducible factor-1α (HIF-1α) and Bcl-2, thereby inhibiting tumor cell growth (Figure 7).

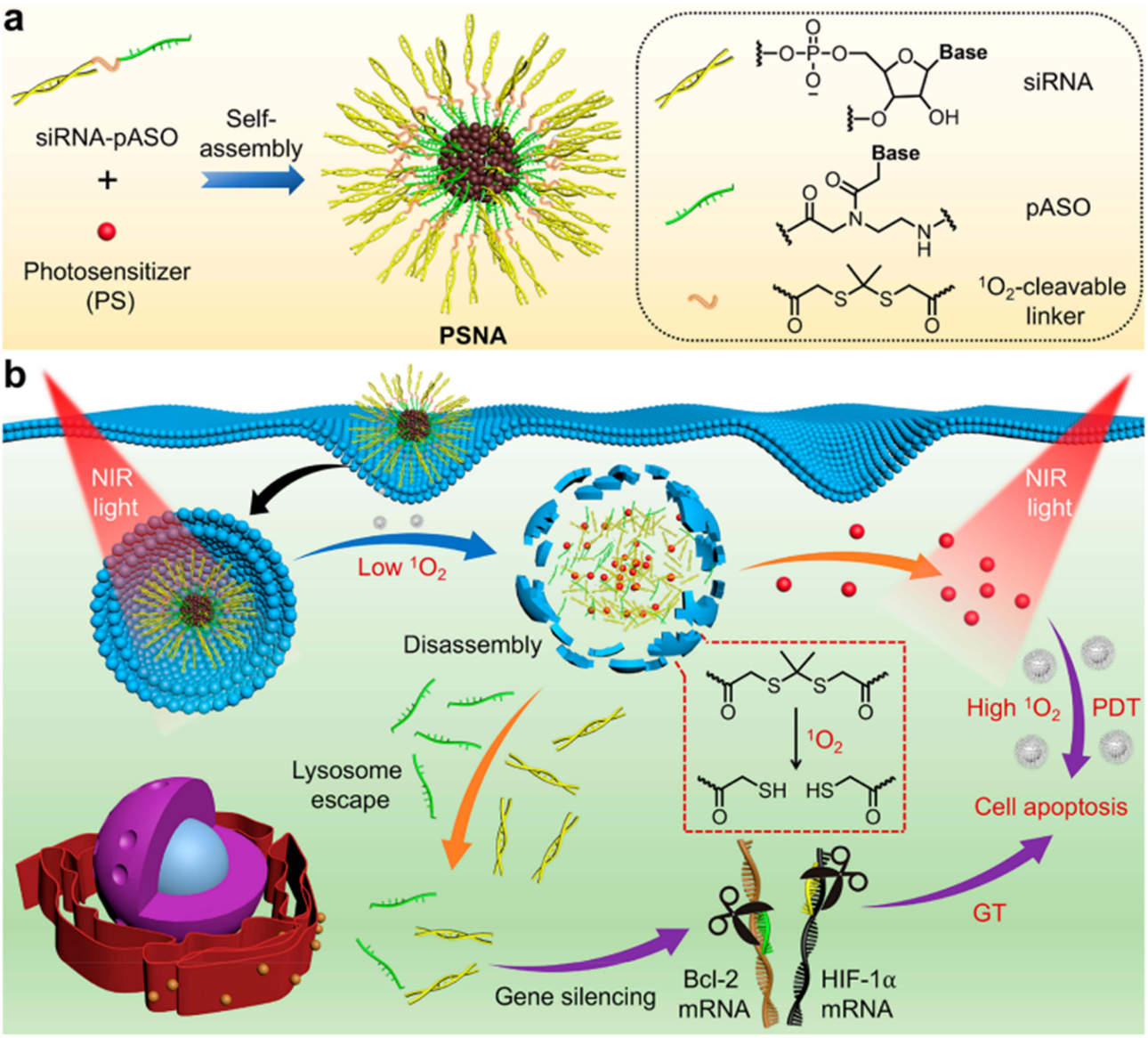

Figure 7 Design of PSNA. (**a**) Illustration of the preparation of PSNA. (**b**) Schematic representation of the use of PSNA to deliver siRNA, pASO, and PS for combination cancer therapy. Reprinted with permission from L Chen, G Li, X Wang, et al. Spherical Nucleic Acids for Near-Infrared Light-Responsive Self-Delivery of Small-Interfering RNA and Antisense Oligonucleotide. ACS Nano. 2021;15(7): 11929–11939. Copyright (2021) American Chemical Society.[87]

Temperature-Responsive NDRS

Temperature can serve as an exogenous stimulus when the external temperature changes, or as an endogenous stimulus when the temperature changes in the diseased area. Typically, the local temperature of tumor tissue increases and exceeds that of normal tissue owing to rapid cell proliferation and abnormal vascular morphology. Hydrophilic and biocompatible polymers have attracted much attention, such as poly(N-isopropylacrylamide), poly(N-vinylcaprolactam), and polyphosphonitrile or polyether with side chains of PEG monomethyl ether, are used.[119,120] These polymers have the characteristic that the hydrophilic segments can form hydrogen bonds with water molecules when the temperature is below the critical solution temperature, while the hydrogen bonds are disrupted or even disappear when the temperature is above the critical solution temperature.[121] The hydrophilic–hydrophobic balance in the polymer is broken, leading to hydrophobic collapse and disintegration. Therefore, researchers have designed temperature-responsive NDRS (polymer micelles, vesicles, and thermosensitive liposomes) by utilizing this controllable condition. Hamner et al prepared a temperature-responsive polymer for the delivery/release of DNA-encoded drugs.[89] The results indicated that the

thermal response behavior of the polymer regulated the accessibility of sequence-specific hybridization between complementary DNA, thereby improving the cytotoxicity of NDRS. However, thermosensitive materials have limitations (eg, as low thermal triggering efficiency and poor biodegradability). Therefore, temperature-responsive NDRS is often used in combination with other stimuli (for details, see the section Multiple Stimuli-Responsive NDRS).

Other Exogenous Stimulus-Responsive NDRS

Other exogenous stimuli used for gene therapy include magnetic fields, ultrasound, or electric fields. Among these, magnetic fields are considered one of the best choices for exogenous stimulus-responsive NDRS because of their minimal physical interaction with the body. The use of magnetic fields to transfer genes loaded in a magnetic-responsive NDRS to target sites is called magnetofection. Research has shown that magnetic-responsive NDRS in the presence of an external magnetic field can target specific organs, enhance gene transfection efficiency, and reduce toxicity.[122] Dalmina et al designed a novel magnetic-responsive siRNA NDRS (SPION) using superparamagnetic iron oxide nanoparticles coated with calcium phosphate and biocompatible PEG-polyanion block copolymers.[92] Nucleic acid quantification results demonstrated that SPION-carrying siRNA agents moved towards an external magnetic field, indicating that SPION facilitated the accumulation of siRNA in the target tissue through its magnetic capability. Furthermore, in vitro studies showed that SPION enhanced the silencing of vascular endothelial growth factor (VEGF) in breast cancer cells at the gene and protein levels (by approximately 60% and 40% respectively) without associated toxicity.

Ultrasound-responsive NDRS is also a highly anticipated strategy for cancer gene therapy, with ultrasound microbubbles being the most commonly used carriers. Under certain ultrasound intensities, ultrasound microbubbles undergo transient bursting due to acoustic response, creating a "cavitation effect" that temporarily enhances cell membrane permeability, thereby assisting in gene delivery/release.[123] By creating an ultrasound environment at the tumor site, ultrasound-responsive NDRS can effectively deliver/release the target gene to the target site, and overcome "off-target" effects. Du et al designed mesoporous silica nanobubbles (M-MSN@MBs) for ultrasound-mediated gene delivery/release.[93] M-MSN@MBs exhibited excellent biocompatibility, ultrasound responsiveness, and stable DNA release. PEI-modified M-MSNs effectively protected the pDNA from enzymatic degradation and significantly reduced cytotoxicity. Following ultrasound stimulation of tumor lesions, the microbubble structure of M-MSN@MBs was disrupted, promoting the opening of the blood-tumor barrier and increasing cell membrane permeability, ultimately enhancing the efficiency of pDNA delivery/release.

Multiple Stimuli-Responsive NDRS

The human body has a complex environment, and slight differences between internal microenvironments often render the single-response NDRS insufficiently sensitive. Therefore, researchers have begun to design dual- or triple-responsive NDRS (eg, pH/ROS,[98] pH/GSH,[95,96] GSH/enzyme,[97] hypoxia/ROS/pH,[113,124] and pH/ROS/enzyme[125]) to further enhance the responsiveness of NDRS, increase their targeting to diseased sites, and reduce toxic side effects during treatment (Figure 8). These multi-responsive NDRS utilize the synergistic effects between different stimuli to achieve highly sensitive nucleic acid delivery/release and can intelligently regulate gene transfer processes, overcoming intractable obstacles, such as low gene loading capacity, weak intracellular/lysosomal escape capability, slow gene release, high toxicity, and difficult nuclear transport. Jing et al developed a pH/ROS-responsive NDRS (MiR@PCPmP) by encapsulating miRNA with PEG-carboxymethyl dextran-PEI-peroxycarbonate-poly(ε-caprolactone) and mannose to silence miR155 for efficient gene therapy of triple-negative breast cancer.[98] In TME, MiR@PCPmP exhibited selective cellular uptake, followed by effective endosome escape and efficient cytoplasmic miR155 release, with no apparent systemic toxicity. The results confirmed the perfect delivery of nucleic acid cargoes by the multi-responsive NDRS. Moreover, Sahoo et al designed and synthesized a cationic cross-linked polymer (CLP) with triple pH, GSH, and ATP responsiveness using disulfide bonds and reversible boronic ester bonds.[99] Compared to traditional cationic polymers, CLP showed more effective DNA condensation and selectively released complex DNA in the TME, demonstrating its enormous potential as an effective nonviral gene delivery and release carrier.

Although multi-responsive NDRS can improve transfection efficiency and enhance therapeutic effects, the design and preparation process is challenging, with complex coordination and unification among multiple components. A single

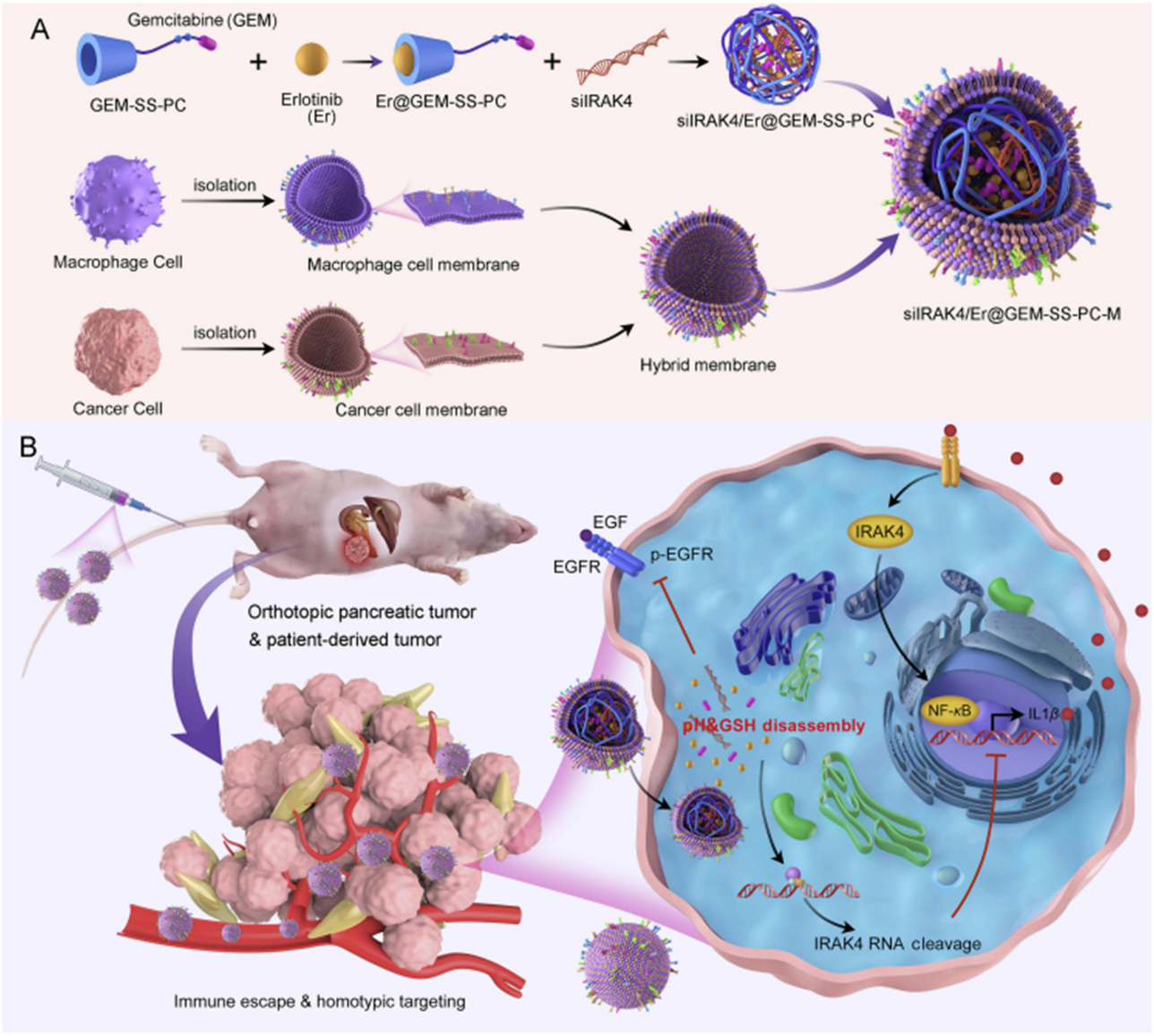

Figure 8 Construction of cancer cell-macrophage hybrid membrane-coated drug-delivery nanosystem for pancreatic cancer treatment. (**A**) Schematic illustration for the preparation of siIRAK4/Er@GEM-SS-PC-M: gemcitabine (GEM) is conjugated with PC by a GSH-responsive linker to form GEM prodrug, followed by encapsulating the Erlotinib (Er) via host–guest molecular interaction and loading with the siRNA to form cell membrane-coated nano-drug (siIRAK4/Er@GEM-SS-PC-M). (**B**) Schematic illustration of targeted nanoparticles to deliver to GEM, Er and siIRAK4 against pancreatic tumors including orthotopic pancreatic tumor and patient-derived tumor (PDX). Reprinted with permission from H Tang, Y Xue, B Li, et al. Membrane-camouflaged supramolecular nanoparticles for co-delivery of chemotherapeutic and molecular-targeted drugs with siRNA against patient-derived pancreatic carcinoma. Acta Pharm Sin B. 2022;12(8): 3410–3426. Copyright (2022) Elsevier.[96]

stimulus response error could lead to failure of the entire gene delivery/release. Therefore, multi-responsive NDRS present higher demands and greater challenges for researchers.

Concluding and Perspectives

Genes guide protein expression, which is a fundamental control of all biochemical activities in living cells. The direct cause of various diseases is disruption of essential protein expression; however, the ultimate reason undoubtedly traces back to genetic defects. Hence, compared to traditional therapies that only alleviate disease symptoms, gene therapy aims to eradicate diseases by repairing or replacing the patient's genetic code, especially in cancer treatment. Gene therapy has attracted widespread attention in the field of cancer treatment, owing to its outstanding selectivity and effectiveness. However, delivering gene agents to target cells and achieving effective release are challenging because naked nucleic acid molecules are prone to

volatility and degradation by nucleases in the body, leading to "off-target" effects. To protect nucleic acid drugs from premature degradation, prolong their blood circulation time, and achieve targeted delivery to tumors, researchers have developed various specialized NDRS to enhance the efficiency of gene therapy. In particular, stimulus-responsive NDRS are increasingly being used for gene delivery/release, owing to their outstanding safety and ease of development. Therefore, based on the diverse internal/external microenvironments within tumor tissues, including low pH, high concentrations of GSH and ROS, and overexpressed enzymes, a corresponding endogenous stimulus-responsive NDRS has been developed for specific gene delivery/release. Additionally, spatiotemporally controlled stimulus-responsive NDRS have also been designed for on-demand and specific gene delivery/release using external triggers, such as light, heat, ultrasound, and magnetic fields. In summary, this review first introduces the development history of gene therapy, the current obstacles faced by gene delivery, strategies to overcome these obstacles, and conventional vectors, and then focuses on the latest research progress in various stimulus-responsive NDRS for improving gene delivery efficiency.

Over the past few decades, with the cross-disciplinary fusion of polymer materials science, oncology, molecular biology, and pharmacy, thousands of stimulus-responsive NDRS have been designed for tumor gene delivery. However, there have been almost no successful cases of advanced clinical application. Therefore, the primary task for researchers at present is not to continue developing new stimulus-responsive NDRS, but to overcome the obstacles related to clinical translation applications. Future breakthroughs may include the following. First, although the existing stimulus-responsive NDRS can address one or two issues in the nucleic acid delivery/release process, adapting to the complex TME is challenging. Simultaneously, owing to the problems of nonspecific retention or low cellular uptake efficiency of NDRS, passive or active targeting strategies alone cannot meet the needs of complex biological systems. To further improve the ability of precise delivery/release and effectiveness of treatment, a multi-responsive NDRS is expected to emerge. However, these designs are classified as "overdesign" from an industrial perspective, facing the dilemma of difficulty in scaling up production and lack of clinical safety. Therefore, it is essential to balance the functional design. Second, the current development and testing of NDRS relies overly on in vitro or animal experiments (eg, rats, mice); nevertheless, fundamental physiological differences between humans and animals easily lead to clinical translation failures. Local biological stimuli are highly heterogeneous and undergo dynamic changes during different stages of disease progression. This heterogeneity provides potential opportunities for individualized treatment but also poses significant challenges for clinical translation. In the future, with a better understanding of the unique biological signals of tumors, more specific endogenous stimuli should be screened to construct a responsive NDRS for the precise delivery or release of therapeutic nucleic acids to target sites. Finally, the obstacles faced by nonviral gene delivery/release vectors in vivo/vitro vitro need to be better understood, and alternative effective delivery pathways beyond the EPR effect should be developed. The low transfection efficiency prompted us to rethink the current strategies for targeted delivery and release using NDRS. Most research is based on the theory of passive accumulation of NDRS at tumor sites through the EPR effect, but this theory has recently been challenged.[37] Therefore, there is an urgent need to explore new strategies or mechanisms in the future to achieve gene targeting in tumor tissues.

In summary, this review aimed to demonstrate the potential of stimulus-responsive NDRS in biomedical applications and their clinical applicability. We believe that gene therapy based on stimulus-responsive NDRS will bring about significant advances in cancer treatment in the near future.

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Disclosure

The authors report no conflicts of interest in this work.

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