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Advanced gene therapy system for the treatment of solid tumour: A review

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ABSTRACT

In contrast to conventional therapies that require repeated dosing, gene therapy can treat diseases by correcting defective genes after a single transfection and achieving cascade amplification, and has been widely studied in clinical settings. However, nucleic acid drugs are prone to catabolism and inactivation. A variety of nucleic acid drug vectors have been developed to protect the target gene against nuclease degradation and increase the transformation efficiency and safety of gene therapy. In addition, gene therapy is often combined with chemotherapy, phototherapy, magnetic therapy, ultrasound, and other therapeutic modalities to improve the therapeutic effect. This review systematically introduces ribonucleic acid (RNA) interference technology, antisense oligonucleotides, and clustered regularly interspaced short palindromic repeat/CRISPR-associated nuclease 9 (CRISPR/Cas9) genome editing. It also introduces the commonly used nucleic acid drug vectors, including viral vectors (adenovirus, retrovirus, etc.), organic vectors (lipids, polymers, etc.), and inorganic vectors (MOFs, carbon nanotubes, mesoporous silica, etc.). Then, we describe the combined gene therapy modalities and the pathways of action and report the recent applications in solid tumors of the combined gene therapy. Finally, the challenges of gene therapy in solid tumor treatment are introduced, and the prospect of application in this field is presented.

1. Introduction

Small molecule drugs have traditionally been the focus of attention in the pharmaceutical industry due to good spatial dispersion, drug-forming properties, and pharmacokinetic properties, with the advantages of widespread use and well-established theory. The advances in biology have broadened our understanding of the human genome and accelerated the identification of therapeutic targets. Gene therapy is a biological technique that aims at replacing and/or repairing defective genes in target cells or introducing normal or therapeutic foreign genes to treat diseases [1]. Broadly speaking, gene therapy is a method of treating a specific disease by transferring specific genetic material into the body of patients so that it can be expressed in the body of patients [2]. These include mRNA therapeutics [3], RNA interference (RNAi) [4–6], antisense oligonucleotides (ASOs) [7,8], and CRISPR/Cas9 [9,10].

As gene modification technology advances, researchers can modify genes precisely at the molecular level. Different therapeutic modalities differ in targeting molecular disease mechanisms and/or the ability to effectively reach specific locations. ASO [11], RNAi, and CRISPR/Cas9 genome editing [12] expand the universe of druggable targets, including those that are challenging to target with small molecules and proteins, such as transcription factor targets and compensation of dysfunctional proteins within cells [13]. These therapeutic modalities cover a wide range of targets and mechanisms, and binding modalities (e.g., small molecule conjugation to antibodies) can expand this range.

However, nucleic acid drugs are prevented from entering cells by cell membranes and are highly susceptible to breakdown by nucleases [14]. Therefore, carriers that encapsulate, shield, and deliver nucleic acid molecules are essential in the practical application of gene therapy [15]. Nanoparticles and viruses have been developed as carriers for nucleic acid molecules to protect them from degradation and to improve stability as well as transfection efficiency and safety. The most commonly used vectors include viral vectors (e.g., adenovirus, retrovirus), organic

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vectors (e.g., lipids, polymers), and inorganic vectors (e.g., MOF, carbon nanotubes, mesoporous silicon). These vectors should be safe, highly efficient, specific, biocompatible, and easy to produce.

In clinic, traditional therapies include phototherapy [16] (photodynamic and photothermal), chemotherapy [17], ultrasound [18], and magnetic hyperthermia therapy [19]. On top of these, efficient and cascade-amplifying gene therapy has been developed: to further aid more specific and targeted treatment. To obtain better treatment results, researchers have tried to combine gene therapy with traditional treatment modalities. And apply it to diseases that are currently more difficult to overcome and are expected to be cured.

This article reviews and compares different nucleic acid drugs, discusses their properties and mechanisms of action, and describes their delivery systems. The mechanisms of combination therapy with nucleic acid drugs and their application in solid tumors are further discussed.

2. Classification of gene therapy

A wide range of nucleic acid drugs, including mRNA therapeutics, small interference RNAs (siRNAs), microRNAs (miRNAs), and antisense oligonucleotides (ASOs), inhibit target genes by pairing with target RNA. On the other hand, the clustered, regularly spaced short palindromic repeats (CRISPR)/Cas system adds more multiplexing, cost-effectiveness, and high-accuracy to gene editing. This section looks at the therapeutic approaches and designs of many nucleic acid drugs, including mRNA, siRNA, miRNA, ASO, and CRISPR/Cas systems. Key recent advances in gene therapy are shown in Table 1.

Table 1
Characteristics of nucleic acid drugs.

Therapeutic	Characteristic	Ref.
mRNA	Easily synthesized by an *in vitro* transcription (IVT) process, transiently expresses the encoded protein in the cytoplasm, but is structurally unstable and susceptible to enzymatic degradation.	[143–145]
siRNA	A double-stranded RNA molecule synthesized *in vitro*, about 21–23 nt in length, dependent on the processing of the Dicer enzyme, can be applied to any part of mRNA, and silencing of target genes through the RNA interference pathway.	[79,146]
miRNA	Endogenous, widely found in eukaryotes, a kind of RNA molecule with a length of about 22 nt (21–24 nt), depending on Dicer enzyme processing, mature miRNA is single-stranded RNA, mainly acts in the 3′-UTR region of target genes and regulates the expression of target genes by degrading the target mRNA or inhibiting the translation of proteins.	[147,148]
shRNA	A short double-stranded RNA structure (19–25 nt) produced by a stem-loop-dependent sequence, which is enzymatically cleaved in cells to form siRNAs that regulate target genes via the RNA interference pathway.	[5,149]
ASO	A single-stranded oligonucleotide, usually containing 15–25 nucleotides, binds to a target RNA by the principle of base-pairing and inhibits or blocks the expression of the target gene at the level of replication, transcription, splicing, mRNA transport, and translation through a site-blocking effect or by inducing degradation of RNase activity	[150,151]
CRISPR/Cas9	Needs to be introduced into the cell together with sgRNA, is an RNA-DNA complex, needs to be introduced into the cell together with sgRNA, needs to be co-mediated by crRNA and tracrRNA	[37,151, 152]
CRISPR/ Cas12a	Nucleic acid endonuclease mediated by a single crRNA that specifically recognizes and shears double-stranded DNA with a PAM	[153,154]
CRISPR/ Cas12b	A class of nucleic acid endonucleases co-mediated by crRNA and tracrRNA, Cas12b specifically shears ssDNA targets independently of PAM sequences	[47,155]

2.1. mRNA therapeutics

Messenger RNA (mRNA) is an essential single-stranded ribonucleic acid responsible for transporting genetic information from DNA to ribosomes in the cytoplasm for protein synthesis. Over the years, significant improvements in mRNA stability, translatability, and immunogenicity have been achieved. Various types of mRNAs, including conventional mRNAs, self-amplifying mRNAs (saRNAs), and circular mRNAs (circRNAs), have been studied for the construction of mRNA nanodrugs. The mRNAs can be designed to encode different immune-stimulating/enhancing proteins to trigger or enhance immune responses for disease immunotherapy, including encoding antigens to construct mRNA vaccines and stimulate active immune responses, encoding immunomodulatory proteins to modulate immune responses, encoding therapeutic monoclonal antibodies to enhance immunity, and encoding antigen receptors to reprogram immune cells and treat disease [20].

Non-amplified, linear, conventional mRNAs have a linear structure similar to that of natural eukaryotic mRNAs and consist of five basic structural elements, namely, a 5′ cap, a 5′ untranslated region (UTR), an open reading frame (ORF), a 3′ UTR, and a poly(A) tail. By optimizing mRNA structure, synthesis, and purification strategies, the stability and translatability of traditional mRNAs can be improved [21]. However, the rapid degradation and transient protein expression of conventional mRNAs is detrimental in some applications that require persistent protein expression, such as protein replacement therapy, and the need for repeated administration may lead to nanocarrier accumulation and potential toxicity.

Self-amplifying mRNAs (saRNAs) can self-replicate, which can address the transient and limited protein expression drawbacks of non-amplified conventional mRNAs (Fig. 1A) [22]. In addition to the five basic structural elements that can be found in conventional mRNAs, saRNAs have replica coding sequences in the ORF region and subgenomic promoters [23]. The replica coding sequence allows for the production of RNA-dependent RNA polymerase (RDRP) and self-amplification of the mRNA, thus enhancing the efficacy of mRNA therapy, as only low or single doses of saRNA lead to prolonged protein production and avoiding the need for high doses or repeated administration [24].

Single-stranded circRNAs are another attractive alternative to conventional mRNAs with a unique continuous closed loop and no exposed 5′ or 3′ ends, thus avoiding recognition by nucleic acid exonucleases and exhibiting greater enzyme resistance, higher stability, and longer protein expression durations [25]. cicrRNAs can be prepared from linear mRNA precursors by intramolecular loop-forming reactions, and protein expression can be achieved directly in host cells by utilizing internal ribosome entry site (IRES) sequences or N6-methyladenosine without conversion to a linear structure. In addition to prolonging protein expression due to increased circRNA stability, it is believed that the circular structure of RNAs allows for continuous loop translation and can improve overall translation efficiency [26]. In addition, cyclization can greatly reduce the recognition of TLRs and RIG-1 and decrease immunogenicity in the absence of chemical substitution. The fragility and negative charge of mRNA molecules make them susceptible to enzymatic degradation and unable to cross cell membranes. The use of appropriate delivery systems to translocate mRNA into target cells is critical. While the stability and translatability of the therapeutic mRNA itself is a prerequisite, the protection and assistance of the nanocarrier are also crucial as it determines the actual efficacy of the mRNA therapy in practice [3].

2.2. RNA interference (RNAi)

RNA interference (RNAi) designs a sequence of nucleic acid that is complementary to the mRNA of the gene of interest to promote degradation and/or inhibit the downstream process. Small interfering RNA

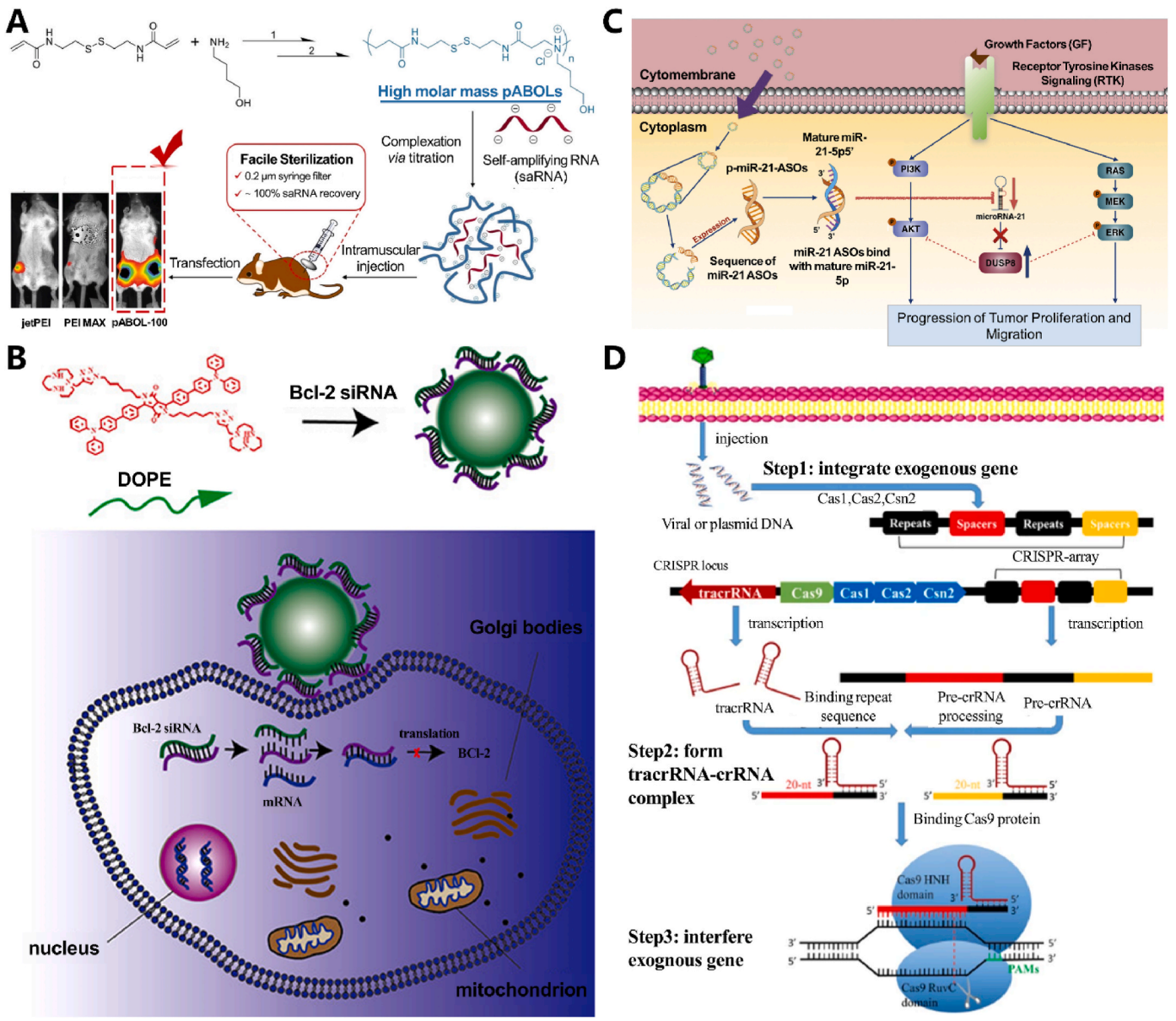

Fig. 1. A) The saRNA delivery system enhances protein expression *in vitro* and *in vivo* [22]. Copyright 2020, American Chemical Society. B) Targeted delivery of Bcl-2 siRNA down-regulates the expression of Bcl-2 protein in cancer cells [31]. Copyright 2020, American Chemical Society. C) Proposed model for the regulation of antitumor by ASOs against miR-21 [36]. Copyright 2018, Elsevier. D) Mechanism of CRISPR/Cas9 gene editing tool [41]. Copyright 2020, BioMed Central.

(siRNA), microRNA (miRNA), small hairpin RNA (shRNA), and dicer siRNA (dsRNA) dependent on dicer enzymes are available for RNA interference techniques [27]. miRNA is a single -chain RNA, while siRNA and shRNA are dual -chain RNA. Among them, the action path of shRNA is at the top, and its effectiveness requires nuclear processing; followed by dsRNA, which requires an internal enzyme treatment. Amongst these techniques, miRNA and siRNA are the most effective and widely used ones. A key distinction between miRNA and siRNA is that the former is an endogenous molecule, a purposefully expressed product of the organism's origin from virus and transposons; whereas siRNA is believed to have an exogenous origin from virus and transposons. The way of its role is to directly transport them to the RNA-Induced SilenCing Complex (RISC). Sequence pairing, this difference causes miRNA and siRNA to play different gene silencing effects; at the same time, miRNA suppresses translation, while siRNA induces Argonaute-2 protein(AGO2) degradation [28]. While siRNA may affect any section of mRNA, miRNA mostly affects the 3′-non-translation region of the target gene. While siRNA is produced symmetrically from the two sides of the dual-chain RNA front body, miRNA is formed asymmetrically. Due to the effectiveness and selectivity of RNA interference, it has become the preferred method to silence the expression of a gene of interest in mammalian cells. Various human diseases caused by abnormal expression of one or several genes are suitable for using RNA-based treatment strategies, including explicit genetic diseases, virus infections, cancer, and autoimmune diseases. The fragility and negative charge of mRNA molecules make them susceptible to enzymatic degradation and unable to cross cell membranes. The use of appropriate delivery systems to translocate mRNA into target cells is critical. While the stability and translatability of the therapeutic mRNA itself is a prerequisite, the protection and assistance of the nanocarrier are also crucial as it determines the actual efficacy of the mRNA therapy in practice [29].

2.2.1. *siRNA*

The siRNA is a chemical synthesis dual-chain RNA (dsRNA), which contains 19–23 base pairs, of which 2-nucleotide is not paired at the end of 5′-phosphorylation and non-phosphorylation 3′ [4]. The siRNA is

mixed with a silent complex (RISC) induced by RNA, which separates the dsRNA from the passenger (sense) chain which will be discarded. Then, guide the (antisense) chain to guide RISC annealing and cutting target mRNA or blocking its translation. Suzuki et al. [30]. Reported a protocol for TNF-α siRNA in intracellular photochemical internalization (PCI), endosomal vesicle rupture, followed by the release of siRNA in the cytoplasm. Sun et al. [31] reported a delivery system for Bcl-2 siRNA and achieved excellent anti-tumor efficacy of Bcl-2 siRNA. However, mRNA can potentially use both the sense strand and the antisense strand as guide strands. As a result, the target mRNA won't be damaged by siRNA since the antisense strand will be destroyed (Fig. 1B). However, if the loading direction is off, the prosthetic strand will function as a guide strand to identify the target mRNA, which will have off-target consequences. It is, therefore, necessary for the guide strand of the siRNA to bind correctly to the RISC. By combining an unstable antisense chain with a more stable sense chain at the 5′ end, the unstable antisense chain may be combined with RISC. A significant limitation of siRNA in clinical applications is the nature of RNA being unstable and easily degraded. The modification of siRNA contributes to its resistance to nuclease degradation. The location of the siRNA modification is one of the most important factors to consider. For RNA-RISC interactions, the 5′ phosphate, 5′ proximal region, and central placement of the guide strand are essential. Therefore, chemical changes should be avoided in these areas. The 3′ proximal region and the 3′ protrusion of the guide strand, however, had no impact on the activity of siRNA. Common chemical modifications of siRNA are the modification of the ribose 2′-OH group, the locking and unlocking of nucleic acids, and the modification of the phosphorothioate acid (PS).

2.2.2. miRNA

miRNA is an endogenous non-coding RNA that regulates the transcription of gene expression. The mechanism of miRNA silencing a target gene is similar but not identical to that of siRNA. In the three non-translational regions of the target mRNA, miRISC is often hybridized with certain complementary binding sites, or it combines the target mRNA and encourages its cutting. The miRNA gene is transformed into miRNA (18–25 nt double-stranded RNA, 2 nucleotides 3′ protrusion) by many biological activities. miRNAs join together to create miRISC complexes after engaging with RISCs. In contrast to siRNA, when the miRNA double strand is broken, AGO2 does not destroy the sense strand but releases it. The miRNA antisense strand directs the remaining miRISCs to bind to mRNA. In contrast to siRNA, which demands that the antisense strand be complementary to the target RNA, miRNA simply needs partial complementarity with the mRNA. Consequently, a single miRNA sequence can silence several genes by binding to different mRNA sequences. Additionally, after recognizing the target mRNA, miRISC generally silences the gene by methods including translation inhibition and degradation [32]. Rarely, mRNA is highly complementary to miRISC and works similarly to siRNA to mediate the cleavage of mRNA by AGO2.

2.2.3. shRNA

In transfected/transduced cells, shRNA with a short hair rack is synthesized in the nucleus, forming paired antisense and sense strands of stem regions that are linked by unpaired cyclic nucleotides. shRNA is converted into siRNA using the same RNAi mechanism as miRNA processing. By using bacterial or viral vectors, shRNA is delivered to the target cell's nucleus, and in certain instances, the vector can be permanently incorporated into the genome. RNA polymerase II or III may be used to catalyze the transcription of shRNA, depending on the promoter driving the expression. Before being carried into the cytoplasm by Exportin-5, the early precursor structures must be processed by Drosha and its double-stranded RNA-binding partner DGCR8. The pre-shRNA is then broken down by Dicer and TRBP/PACT to release the hairpin structure and create a 20–25 nt double-stranded siRNA with two free bases on either end. The silencing complex is subsequently completed by the active siRNA. Once shRNA and siRNA are incorporated into RISCs, the mechanism of target mRNA identification and breakdown is essentially the same. The transformation of shRNA can cause long-term gene silencing of mammals. Ohno and others have developed a single-chain RNA with a 30 nucleotide, called the guidance hairpin RNA (ghRNA). Its physiological function is similar to miRNA and siRNA, which do not cause congenital cytokine reactions *in vitro* or body [33]. Alsing et al. [5] prepared agshRNA targeting VEGFA and microRNA-embedded agshRNA (mir-agshRNA) to silence retinal genes.

2.3. Antisense oligonucleotides(ASO)

Antisense oligonucleotide is based on the principle of base complementarity. The expression of the target gene is inhibited or blocked at the levels of replication, transcription, splicing, mRNA transport, and translation by combining artificially or biosynthetically complementary DNA or RNA fragments (or their chemical modification products) with the nucleic acid of interest sequence by steric hindrance effect or induced degradation of RNase activity [34]. ASOs are oligonucleotide drugs with a length range of 8∼50 bp, similar to single-stranded DNA.

According to different modes of action, antisense oligonucleotide technology can be divided into three categories: (1) antisense DNA technology: the synthesized antisense oligodeoxynucleotide is ingested into the target cell and binds to the target mRNA, interfering with the transcription of mRNA or blocking protein translation; (2) antisense RNA technology: the antisense oligodeoxynucleotide sequence is attached to the vector (virus, plasmid) and introduced into the target cell, and the loaded oligonucleotide transcribes the antisense RNA, crosslinks with the target mRNA, blocking the mRNA protein translation process; (3) ribozyme technology: ribozymes are a class of RNA molecules with enzymatic properties that specifically cleavage substrate RNA molecules by catalyzing the cleavage of phosphodiester bonds in the RNA strands at the target site inhibiting the expression of genes as a result [35].

Although ASOs belong to the category of short nucleic acid sequences, they have many differences from other RNA therapies (such as small interfering RNA) in terms of structure, molecular weight, mechanism of action, and other characteristics. ASO is a single-stranded structure with hydrophobic bases and hydrophilic nucleotide links. The rigid double-stranded structure of siRNA, in contrast, has a molecular weight that is roughly double that of ASO. The siRNA's prosthetic strands act as a guide by preventing the rapid degradation of its antisense strands. ASOs have the added advantage of being able to target immature pre-mRNA in the nucleus, where RNase H1 is called upon to cleave RNA-DNA-like duplexes after ASOs create RNA-DNA-like duplexes with target RNA through complementary base pairing. siRNA, on the other hand, relies on AGO2 on RISC to continuously cleave the sensory strand and target mRNA of siRNA, rather than directly targeting immature pre-mRNA. Consequently, gene silencing induced by siRNA is more complicated than ASO. ASO-associated RNase H1 may also quickly cleave target RNA. In contrast, siRNA-associated AGO2 only partially degrades the target mRNA. To prepare additional target mRNAs for destruction, AGO2 can temporarily attach to the antisense strands of the siRNA. ASO is intended to mediate the degradation of additional non-coding RNAs involved in the therapy of diseases, such as miRNAs, in addition to facilitating the cleavage of mRNA. In contrast, siRNA is still predominantly employed in the treatment of disorders through the mediation of mRNA degradation. Ding et al. [36] reported a eukaryotic vector encoding ASOs against miR-21, inhibiting the growth of human CRC cells *in vivo*. miR-21 can regulate the growth of CRC cells through various signaling pathways, and the ASO plasmid targeting miR-21 enters the cytoplasm of tumor cells through the cell membrane and expresses the miR-21 ASOs sequence bound to mature miR-21-5p, thereby inhibiting its expression. Due to the downregulation of miR-21 expression, the upregulation of DUSP8 expression is activated, which in turn weakens the transduction of AKT and ERK pathways, thereby inhibiting

the proliferation and migration of CRC cells *in vivo* (Fig. 1C).

2.4. CRISPR/Cas9 genome editing

CRISPR is a repetitive sequence in the prokaryotic genome that was initially discovered in *E. coli* in 1987. As one of the most popular gene editing methods, CRISPR can perform multiple gene editing as well as gene knock-out and in Refs. [9,10]. In this system, single-guide RNA (sgRNA) precisely directs Cas9 endonuclease to a target site and then causes a DNA double-strand break (DSB), leading to site-specific genomic modification [37]. Compared to conventional gene editing techniques like ZFN (zinc-finger nuclease) and TALEN (transcriptional activator-like effect nuclease), CRISPR/Cas9-mediated gene editing is more adaptable, effective, and accurate [38]. There are two primary classifications for CRISPR/Cas (I and II). In class I CRISPR/Cas systems, several Cas proteins are needed to enable their applications. But for a class II system, it requires only one protein. Therefore, Class II systems are more popular in gene editing. CRISPR/Cas9 is the most popular Class II system among the several varieties because of its simplicity [39,40].

The Cas9 protein, CRISPR RNA (crRNA), and *trans*-activated crRNA (tracrRNA) combine to generate CRISPR type II. Six key domains, including recognition (REC)1, recognition (REC)2, bridge helix (BH), PAM interaction (PI), HNH, and RuvC, are present in the Cas9 endonuclease (Fig. 1D) [41]. A 20-nt initial spacer sequence and adding fractions for tracrRNA complementary pairing are often present in crRNAs. TracrRNA contains two functional portions in its structure, one for binding to crRNA and the other for Cas9 protein binding. Additionally, it is simple to create crRNA-tracrRNA complexes as a single guide RNA (sgRNA) (Fig. 1D). Briefly, the REC1 domain can connect to sgRNA and activate Cas9 endonuclease. The HNH and RuvC domains of the Cas9 protease are activated by the sgRNA's attachment to the target DNA near the protospacer adjacent motif (PAM), which causes the Cas9 protease to shear the double-stranded DNA. There are three ways that sgRNA and Cas9 proteases can work: co-encoded into the same pDNA, Cas9 mRNA and sgRNA, and Ribonucleic complexes (RNPs) containing Cas9-sgRNA [42]. The Cas9 protein's PI domain is essential for starting the endonuclease activity that allows it to bind with the target DNA early in the process. Overall, CRISPR/Cas9 has strong gene-editing capability thanks to the synchronization of numerous functional areas [43,44]. As research develops, Cas12, Cas13, and Cas14 nucleases have been found as additional gene editing components of the CRISPR family. Given their smaller size, these nucleases are easier to administer than Cas9 nucleases, which considerably extends the range of applications for this technique.

Another DNA-targeting CRISPR system for genome editing is Cas12a (also known as Cpf1). Unlike Cas9, Cas12a can generate staggered ends with its cleavage pattern and PAM sequences, thus facilitating precise integration of DNA. More importantly, the Cas12a protein is a single crRNA-guiding enzyme without tracrRNA assistance and is itself responsible for pre-crRNA processing. In addition, Cas12a can cleave crRNA arrays to generate its crRNAs. This crRNA processing capability facilitates the use of a single customized crRNA array, simplifying multiplexed genome editing using multiple crRNAs. The first Cas12a homologs to demonstrate activity in mammalian cells recognized the PAM sequence 5′-TTTV upstream of the target sequence [45]. To increase the targeting range of Cas12a, AsCas12a variants have recently been designed to recognize PAMs 5′-TYCV and 5′-TAT or PAMs 5′-VTTV, 5′-TTTT, 5′-TTCN, and 5′-TATV [46]. The unique functionality and cleavage mechanism of Cas12a provide an expanded genome editing tool for the CRISPR toolbox. Similarly, Cas12b (also known as C2C1), Cas12b recognizes distal 5′-T-rich PAM sequences [47]. However, the cleavage activity of Cas12b requires both crRNA and tracrRNA, a feature that contrasts with Cas12a, which requires only crRNA; most significantly, Cas12b has minimal off-target effects, providing a safer alternative for genome editing and diagnostic applications [48].

Overall, nucleic acid medications are superior to conventional small molecule and protein therapies because of their wider therapeutic target range, improved target specificity, and stronger, longer-lasting therapeutic effects. Drugs based on nucleic acids, therefore, offer a lot of potential for treating illness. Unmodified nucleic acid drugs are susceptible to degradation by extracellular nuclease enzymes, and their negatively charged nature hinders their ability to cross hydrophobic cytoplasmic membranes, negatively affecting their stability and pharmacokinetics. The most important challenges extracellular barriers for nucleic acid drugs include rapid systemic clearance, making them susceptible to nuclease-mediated degradation. In addition, the stability of nucleic acid drugs is an important factor affecting their degradation. To address this issue, a large number of experiments have been used to investigate chemical modifications and encapsulation measures for gene drugs. Also, nucleic acid drugs are usually highly anionically charged, difficult to cross cell membranes, and not targeted. Therefore, it is essential to create a high-quality carrier that effectively distributes nucleic acid medications in the body. Different kinds of nucleic acids require various loading methods due to variations in molecular weight and structure. For instance, oligonucleotide medications can be chemically altered to become more stable and to bind to ligand molecules to encourage the accumulation of these molecules in lesions. Contrarily, nucleic acid medications with relatively large molecular weights are difficult to chemically modify and are suited for loading and administration *in vivo* using vehicles having substantial cavity structures, such as liposomes.

3. Vectors for nucleic acid drugs

Given the nature of instability and disintegration of nucleic acid, its delivery to the target cell has been one of the hurdles impeding its wider application. Loading nucleic acid drugs into a vector is an instrumental assembly to the successful delivery of the effective drug to the target cell. In this section, the vectors of gene therapy will be introduced from three aspects: viral vectors, organic nanocarriers, and inorganic nanocarriers. Vectors' characteristics are summarized in Table 2.

3.1. Viral gene therapy vector

Current delivery of therapeutic genes via viral vectors includes: retrovirus(RV) [49], adenovirus (Ad) [50], adeno-associated virus (AAV) [51], recombinant adeno-associated virus (rAAV) [52] based vectors. Both lentiviruses and retroviruses belong to the retrovirus family, they are single-stranded RNA viruses with an envelope and a spherical shape of around 100 nm. Its typical feature is that the RNA genome can be reverse-transcribed into a cDNA copy, and the cDNA copy can be stably integrated into the host cell genome. Lentiviruses are a genus in the family Retroviridae. Retroviruses can be classified as either simple(e.g., γ retrovirus) or complex(e.g., lentivirus HIV-1) viruses based on their genomic organization. Effective treatments for single-gene diseases of the blood system, such as primary immunodeficiency and β-thalassaemia, based on corrected hematopoietic stem and progenitor cell (HSPC) gene therapy using lentiviral and gamma retroviral vectors, have been reported [53]. Alternatively, therapeutic transgenes can be delivered *in vitro* to extract the patient's cells and culture them *in vitro*. Lentiviral vectors with high packaging capacity and high levels of gene expression are being integrated to allow long-term transgene expression, making them suitable for transgene delivery for therapeutic purposes. Delviks-Frankenberry et al. [54] report an effective delivery of the HIV-1 Vif resistant mutant A3G-D128K to a target cell lentiviral vector (Fig. 2A). Erendor et al. [55] investigated a novel lentiviral vector (LentiINS) that carries an insulin booster that targets glandular β cells to maintain pancreatic β cell-specific insulin gene expression. The cells are genetically modified by introducing therapeutic transgenes and reintroduced into the patient [54,55].

Unlike retrovirus, adenovirus is a non-enveloped virus, it, cannot be

Table 2
Characteristics of nucleic acid drug vectors.

Vectors	Characteristic	Ref.
Retrovirus	Stable integration into the host cell genome, usually transcribed at the 5′ end	[49]
Lentivirus	Stable integration into the host cell genome, multiple integrations into sites far from the transcription start site	[156, 157]
Adenovirus	Wide range of infection, high infection efficiency, no genomic integration, high titers	[50]
Adeno-associated virus	Classified in the genus Microviridae, requiring other viruses to complete the life cycle, relatively low protein expression, late onset of expression, very low level of immune response	[51, 65]
Recombinant adeno-associated virus	Complete removal of a viral coding sequence, non-pathogenic, great packaging capacity, low cytotoxicity, and immunogenicity	[158]
Lipid nanoparticle	Good biocompatibility, and drug and packaging rates, as well as the advantages of high stability, low toxicity, and enhanced efficacy	[159, 160]
Solid-lipid nanoparticle	Good stability, control the release of drugs and effectively avoid the degradation and leakage of drugs	[77]
Polymer nanoparticle	Utilizing the proton sponge effect, nucleic acid drugs can be efficiently loaded and endosomes/lysosomes can escape, but toxicity is high	[79]
Chitosan	Easily modified, low toxicity, transfection efficiency may be affected due to tight entanglement when delivering large molecular weight nucleic acid drugs	[67]
Magnetic nanoparticle	Easily targeted and capable of integrated diagnosis and treatment	[119]
Gold nanoparticle	Delivery is efficient and may cause a non-specific inflammatory response	[161, 162]
Metal-organic framework	Excellent drug-carrying capacity and responsive release, and capable of efficient drug encapsulation	[88, 91]
Covalent organic framework	Good drug adsorption capacity and environmental stability	[94, 95]
Inorganic nonmetallic nanoparticle	simple functionalization, biocompatibility, highly specific surface area, superior thermal and optical characteristics	[96]
Carbon nanotube	Excellent optical properties, thermal and electronic conductivity, easy functionalization, and high drug-loading capacity	[70, 163]
Mesoporous silica	Higher loading capacity and chemical and thermal stability of guest molecules	[164]
Clay mineral	Good environmental stability, longer blood circulation life, and accumulation in bladder cancer tumors	[100, 101]

integrated into the chromosomes and is only expressed transiently. Thanks to high transduction efficiency and the broadness of different tissue targets as a carrier for gene therapy [56], it is widely used in genetic modification. First-generation adenovirus vectors fail to replicate in host cells, and transducer cells are cleared due to *de novo* expression of adenoviral proteins, activating the host immune response. As a result, the researchers developed a second-generation vector by deleting regions of earlier genes, which also provided more space for their transgenic expression to be greatly extended. However, the deletion of some of these genes negatively affects adenovirus vector amplification, resulting in reduced titers.

In third-generation adenoviral vectors, most of the viral sequence is deleted and is called high-capacity adenovirus vectors (HCAds), but adenovirus helper viruses are required. HCAds have reduced immunogenicity, prolonged transduction time in host cells, and significantly larger carrying capacity [52]. However, the main issue facing HCAd production is to ensure the elimination of accessory adenovirus from the vector formulation to ensure the efficacy and safety of the vector *in vivo*. Lee et al. [57] reported the production of enterovirus-free adenovirus (GLAd) in the absence of helper adenovirus, which can efficiently deliver target genes and show therapeutic potential in various genetic diseases. Stephens et al. [43] reported an adenovirus-based platform for CRISPR/Cas9 gene insertion, which can treat diseases such as hemophilia. In response to numerous promoter and microRNA inputs, Huang et al. [58] reported programmable and modular synthetic gene circuits that govern adenovirus replication and immune effector release in hepatocellular carcinoma cells might improve the specificity and efficacy of immune responses against cancer. Han et al. [59] replacing RNA-specific targeted suicide gene activity with hepatocyte-specific telomerase reverse transcriptase (TERT) achieves effective anti-hepatocellular carcinoma and minimizes hepatotoxicity by adenovirus-mediated Tetrahymena group I *trans*-splicing nucleases (Fig. 2B). These sum up the advantages of using adenoviral vectors: (1) effective proliferation and high titers; (2) a wide range of hosts and low pathogenicity to humans; (3) infection and expression of genes in proliferating and non-proliferating cells; (4) it is not integrated into chromosomes hence does not cause germline mutations in the genome.

Adeno-associated virus vectors can be stably integrated into the host genome and have the potential for long-lasting gene expression. The main disadvantages of AAVs are their small packaging capacity for the gene of interest, relatively low protein expression, and late onset of expression (2–7 days *in vitro*, 3–21 days *in vivo*), however, the level of immune response generated by this delivery system is very low. AAVs lack the essential genes needed to replicate and express their genomes, instead, they rely on Ad, or any virus that can provide auxiliary functions to complete their life cycle, and are therefore classified in the genus Microviridae. AAV has unique advantages for clinical applications including broad vectoriality, low immunogenicity, non-pathogenic, rarely integrates into the chromosome, and leads to long-term expression of transgenes [60]. AAV has been successfully applied to treat diseases such as hemophilia and eye diseases. However, because of the limited packaging capacity of ments used(including the promoter) must be optimized [61,62]. According to Yoo et al. [63], a multipurpose AAV cross-linking platform could allow AAV vector systems to alter their cellular tropisms and go on to form self-assembling AAV-DTSSP deposits that could imitate polymeric gene delivery systems while also inducing solid-phase delivery, which could enable sustained gene delivery (Fig. 2C). Takatsuka et al. [51] reports an on-demand AAV release system that can be triggered by the near-infrared (NIR) using alginate hydrogel beads encapsulated with alginate hydrogel beads that can release viruses with a diameter of tens of nanometers. The genetic transfection of AAV to cells is verified without losing viral activity by exposing Fe_3O_4 beads encapsulating Fe with AAV to NIR radiation. The NIR-triggered AAV release device expands the range of gene therapy medication delivery options.

The recombinant adeno-associated viruses (rAAVs) consist of the same capsid sequence and structure found in wild-type adeno-associated viruses (wtAAVs). However, the rAAVs-packed genome deletes the entire AAVs protein-coding sequence and adds a therapeutic gene expression cassette. Only one viral source sequence (ITR) that is known during vector production is necessary for genome replication and packaging. Complete removal of the virus-coded sequence maximizes the packaging capacity of rAAVs while favoring reduced cytotoxicity and immunogenicity when delivered *in vivo*. The rAAVs gene therapy platform utilizes powerful and ubiquitous promoters to enable efficient transgene expression [64]. Being one of the most often utilized viral vectors for therapeutic gene delivery *in vivo*, rAAV is not pathogenic, cannot multiply on its own, and can infect both dividing and non-dividing cells [65]. rAAV is often produced by transfecting cells with a “helper-free” (HF) plasmid or employing a “helper” adenovirus. Su et al. [52] reported an improved transfection-free helper adenovirus system. The transport of the rAAV genome, AAV representation and cap genes, and helper function of the adenovirus were all proven. These techniques produced up to 30 times more rAAV vectors than helper plasmid-free techniques did, and considerably increased particle infectivity across a variety of serotypes.

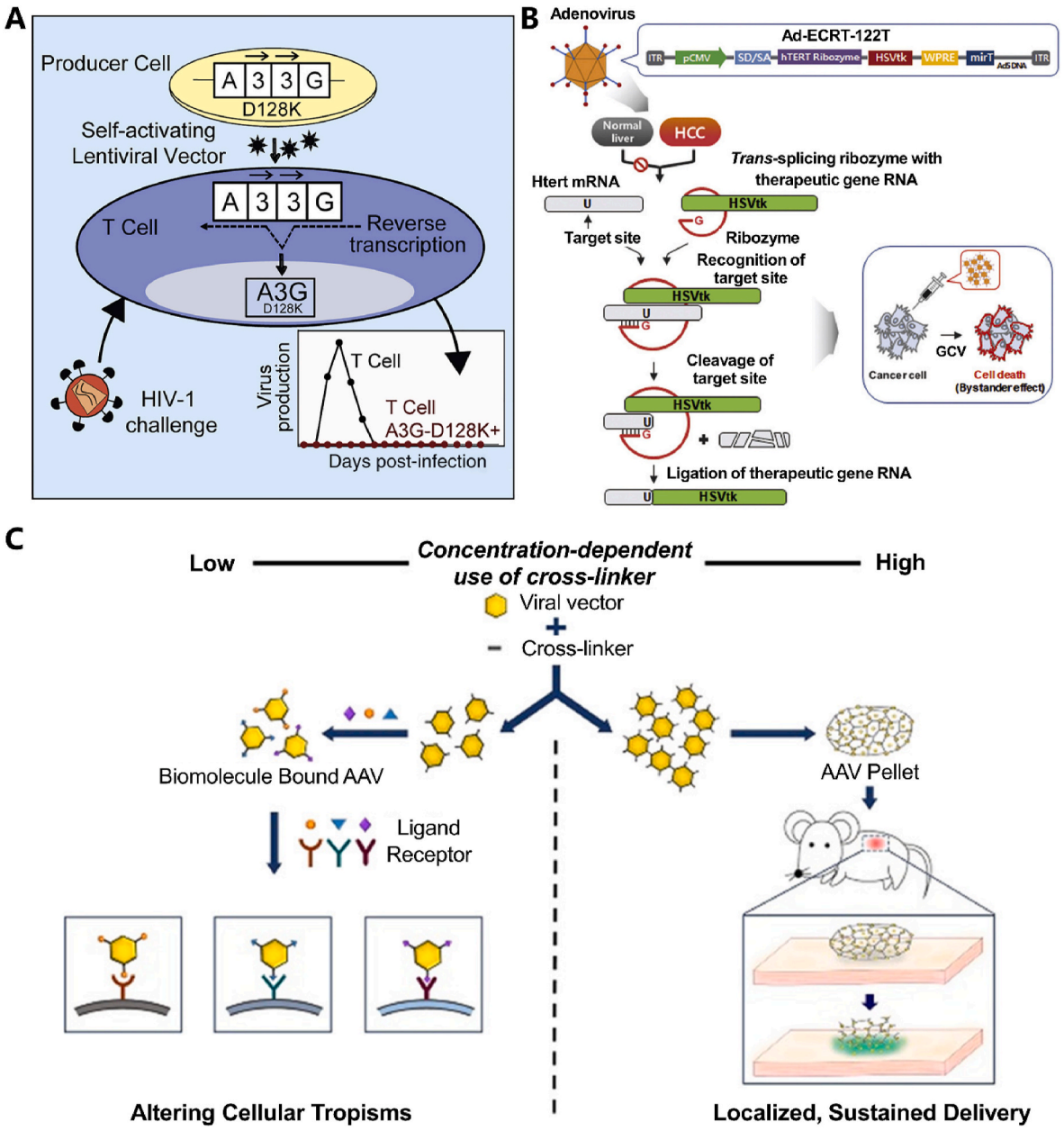

Fig. 2. A) Autoactivating lentiviral efficient transduction to deliver anti-HIV genes [54]. Copyright 2019, Elsevier. (B) Adenovirus-mediated nucleases with high cancer targeting, potent anti-cancer effects, and minimal hepatotoxicity [59]. Copyright 2020, Elsevier. C) Modification and application of adeno-associated virus-based gene therapy vectors [63]. Copyright 2020, American Chemical Society.

3.2. Non-viral nano vectors

In comparison to viral vectors, non-viral vectors have the following advantages: affordable, ease to manufacture in batches, safe, and can be administered repeatedly. They are also easier to store and have a longer shelf life due to their stability. Materials with nanoparticles range in size from 10 to 1000 nm. They are particularly sought after in studies on non-viral vector delivery because of their improved permeability and retention. Additionally, nanoparticles have longer blood circulation durations, a greater surface area-to-volume ratio, improved biocompatibility, generally acceptable stability, and adjustable surface charges, all these features making non-viral vectors a promising candidate as a carrier for gene delivery. According to their composition, non-viral vectors can be further categorized into organic and inorganic materials. Liposomes [66], polyethyleneimine (PEI) [67] and its derivatives, and chitosan [68] and its derivatives are a few examples of organic materials. Gold nanoparticles [69], carbon nanotubes [70], up-conversion nanoparticles, and silica nanoparticles [71] are the most widely used and representative examples of inorganic materials for the vector.

3.2.1. Organic nano vectors

Lipid nanoparticles (LNPs) are a new type of lipid-loading system. It mainly uses natural or artificial synthetic lipids as gene vectors [72], including liposomes, micelles, and solid lipids. By packaging the gene in a lipid core, the lipid vector will fuse with the cell membrane and be released into the cytoplasm, bypassing lysosomal capture for superior transfection efficiency [73]. Gene expression therapeutic protein or correct genetic defects to provide opportunities for the treatment of diseases (Fig. 3A) [74]. LNPs have good biocompatibility, and drug and packaging rates, as well as the advantages of high stability, low toxicity, and enhanced efficacy. Singh et al. [66] reported that hyaluronic

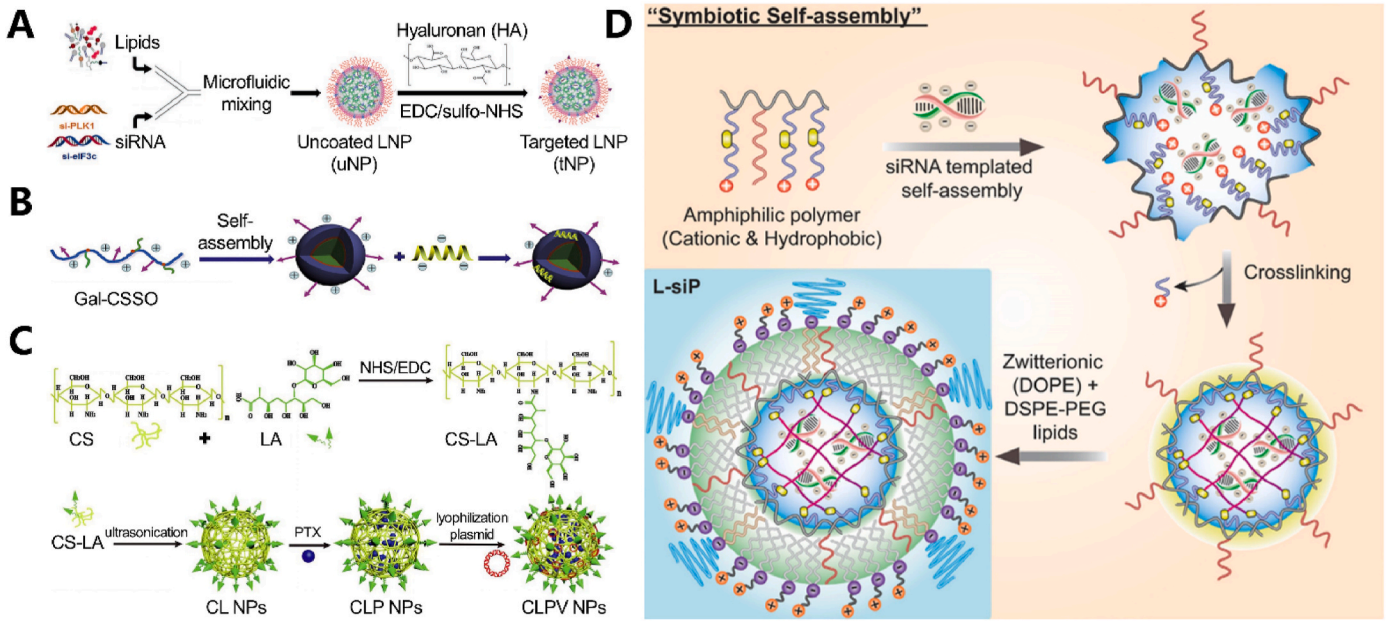

Fig. 3. A) Schematic diagram illustrating the construction of CLZU nanoparticles and their use in anti-tumor gene therapy [74]. Copyright 2021, John Wiley and Sons. B) Preparation of responsive glycolipid-like self-polymerizing micelles [76]. Copyright 2021, Elsevier. C) Preparation of chitosan (CS) composite nanoparticles loaded with PTX and sg-VEGFR2/Cas9 (VC) therapeutic systems [137]. Copyright 2020, Elsevier. D) Symbiotic self-assembly strategy for constructing L-siP nano assemblies and their constituent molecules [80]. Copyright 2019, American Chemical Society.

acid-modified lipid nanoparticles targeting CD44-expressing cell clusters, specific tumor targeting, and robust gene silencing in tissues involved in ovarian cancer pathophysiology led to apoptosis in 85 % of breast cancer cells and a 60 % increase in overall survival in treated mice. Mo et al. [75] reported porphyrin-LNPs, which can generate reactive oxygen species (ROS) upon near-infrared (NIR) light irradiation and achieve endosomal release via photoactivated siRNA with a 2-fold increase in escape efficiency, significantly improving knock-down efficacy.

When the concentration of the surfactant reaches a certain level, molecules in an aqueous solution will self-assemble to create an organized arrangement of thermodynamically stable colloidal aggregates, known as micelles. Because of the presence of hydrophobic groups, the attraction between water molecules and surfactants is stronger than repulsion, which causes the excess surfactant to be dispersed in the aqueous solution. The hydrophobic groups then join together under the influence of van der Waals forces to form the micelle kernel, and the hydrophilic groups face outward to form the octamer. Micelles are associated with low molecular weight surfactants, forming a structurally stable aggregate, which is key to the successful delivery of micelles in the body, and the morphology of micelles can be designed to achieve slow and controlled release of drugs. Given the small size of micelles, ranging from 10 to 100 nm, it will be hard for the endothelial reticular system (RES) in the circulation system to identify and capture them, which made it possible for the carrier to keep circulating in the body for longer if it hadn't been taken up by the cell. They may develop a spherical vesicular shape because they are amphiphilic, which is ideal for drug administration (Fig. 3B). Miao et al. [76] designed a glycolipid-like polymer micelle that targets hepatocytes and microenvironment in response to rapid drug release.

Solid-lipid nanoparticles (SLNs), also known as solid liposomes, are natural or synthetic liposomes or lipid-like nanoparticles that are solid at room temperature [77]. The research of SLNs began in the late 20th century, characterized as a solid lipid particle delivery system with a diameter of 50~1000 nm using stearic acid, lecithin, triacylglycerol, and other lipid raw materials as the matrix, and wrapping the drug in lipid nuclei. SLNs are solid at room temperature and have the following four characteristics: (1) good biocompatibility; (2) They can effectively control the release of drugs and effectively avoid the degradation and leakage of drugs; (3) It is suitable for multiple routes of administration; (4) Good stability, can stabilize of unstable drugs. In addition to their physicochemical advantages and manufacturing advantages, SLNs help with targeted intracellular transport to subcellular compartments [78].

In general, polymer nanoparticle (PNP) is a kind of nanoparticle produced by interactions between polymers and nucleic acid medicines, including electrostatic, hydrophobic, and/or hydrogen bonding. Easy synthesis, great chemical structural tunability, and accessibility of multifunctional capabilities are three of polymers' remarkable benefits. PNPs show great potential in therapeutic diagnostics and nanomedicine applications, where they play an important role in the targeted delivery of large volumes of therapeutic and imaging contrast agents [37,79,80]. Combining the benefits of biocompatible lipids with structurally robust polymers to efficiently encapsulate and deliver siRNAs [80], the lipid-siRNA-polymer was evaluated for delivering four different siRNA molecules across six different cell lines, demonstrating effective gene knockdown (Fig. 3D). Zhang et al. [79] reported photoactivatable Pt(IV) pre-drug backbone polymer nanoparticle systems to achieve controlled drug/gene release as a cancer treatment strategy. By electrostatically adsorbing medicines that are anionic to nucleic acids, cationic polymers, such as PEI, create PNP. By using the proton-sponge effect, nucleic acid medications may be loaded effectively and endosome/lysosomal escape can be accomplished [81]. Park et al. [67] developed PEI-grafted copolymers and investigated their efficiency as gene carriers.

Chitosan (CS), a chitin-derived linear basic polysaccharide with good qualities including biodegradability, biocompatibility, stability, and solubility, serves an essential function [82]. Through chemical alterations, CS nanoparticles can be used as a platform for the simultaneous administration of anticancer drugs and siRNA (Fig. 3C). In the context of treating bladder cancer, Ye et al. [68] developed a low molecular weight chitosan-based combination with gene medication to create nanoparticles and a CD44-targeted siRNA delivery system.

3.2.2. *Inorganic nano vectors*

The structure of metal nanoparticles as the carriers in gene therapy is usually based on the structure of the metal being the core and functional materials being the shell. Such structure is proven to have, good

biocompatibility [69], storage stability [83], easy preparation, versatility [84], and few side effects [85]. By coupling with other materials, such as gold nanoparticles, it can achieve tissue-specific targeting, improved controllability, and medical imaging. Combining gene therapy with photothermal therapy (PTT) strategies, guided by clinical imaging diagnosis, a more comprehensive overview of diseases has been made possible during diagnosis and treatment [83,84,86]. In recent years, magnetic nanomaterials have received a lot of attention because their magnetic property makes them easy to enrich and separate, or directional mobile positioning. Biomedical applications of magnetic nanoparticles *in vivo* are broadly classified into two categories: therapeutic and diagnostic; the former includes, magnetic resonance imaging (MRI), and the latter includes mechanical chemotherapy, magnetofluid hyperthermia, and antibacterial. Previous studies have shown that converting metal dopants such as manganese into magnetic nanoparticles (MNPs) [19] can significantly improve MRI sensitivity and are more suitable as an ideal MRI contrast agent. Functional self-assembly based on MNP can be easily combined with small molecule drugs such as indocyanine green (ICG). Multifunctional iron oxide magnetic nanoparticles (IONPs) [87] are a rising potential candidate in several biomedical applications due to their small particle size, high biosafety, large surface curvature, high relaxation performance, superparamagnetic, and easy surface modification and functionalization (Fig. 4A).

Due to its adaptability, gold nanoparticles (GNPs) can be useful in PTT and gene delivery multifunctional nanotherapeutic systems. One exceptional feature is that Au nanoparticles can cross the blood-brain barrier, which can then be used to deliver particular siRNA delivery systems into the central nervous systems to inhibit gene expression, such as DARPP-32 [69,85]. It has been demonstrated that several synthetic multifunctional gold nanorods (GNRs)-siRNA complexes may be employed to treat various cancer types, including breast cancer, pancreatic adenocarcinoma, and head and neck cancer [85]. GNPs-siRNA complexes are used in this research as photothermal or imaging agents for therapeutic diagnostic reasons as well as nanocarriers for siRNA and chemotherapeutic medication delivery (Fig. 4B). The synthesized GNRs-siRNA nano complexes demonstrated excellent ability in delivering siRNA to cancer cells, with high silencing efficiency, and made cancer cells sensitive to PTT under moderate laser irradiation, improving PTT efficiency.

Metal-organic frameworks (MOFs) are hybrid substances made of organic bridging ligands and metal cations [88]. MOFs can be used as medication delivery systems or diagnostic-free systems because of their porous structure, tunable composition, and pore size [89,90]. Additionally, MOF-based structures have been created as Sound sensitive agents to induce reactive oxygen species (ROS) or oxygen in the tumor environment and nanocatalysts to accelerate the creation of oxidative •OH radicals within cancer cells. MOF nanosheets have been created in the context pf facilitating the photothermal and chemodynamics therapy to treat cancer, as it has been suggested that MOFs can serve as siRNA nanocarriers because of the strong affinity of the metal composition for nucleic acid (Fig. 4D) [91–93].

Since its creation in 2005, the covalent organic framework (COF), a novel kind of organic porous material, has been thoroughly investigated. COF has been applied in numerous disciplines, such as catalysis, energy, and water treatment, and has obtained outstanding research results thanks to its distinctive porosity structure and strong stability in severe settings. The intrinsic porosity nature of COF materials provides them with natural benefits in drug loading in the field of biomaterials (Fig. 4C) [94]. Hao et al. [94] grafted polyethylene glycol (PEG) onto the surface of COF nanoparticles and designed a small lipoic acid-based molecule to facilitate cellular endocytosis of COF nanoparticles. The prepared cationic COF nanoparticles (CLZU NPs) had excellent gene transfection ability and good biocompatibility. Using polyacrylic acid (PAA) as a defect amplifier, Gao et al. [95] attached dense amino-terminated hairpin DNA to porphyrin COF nanoparticles (NPs) to create the first COF-based spherical nucleic acid probe (SNAP). The resultant SNAP is successfully utilized in cancer cell imaging and can successfully light particular targets *in vitro*.

Inorganic particles and biodegradable polycations are combined to create inorganic non-metallic nanoparticles (INPs). Metal oxides, carbon-based substances, and silicon-based substances are examples of typical inorganic non-metallic nanoparticles. Amongst all, given their homogeneous porosity, simple functionalization, biocompatibility,

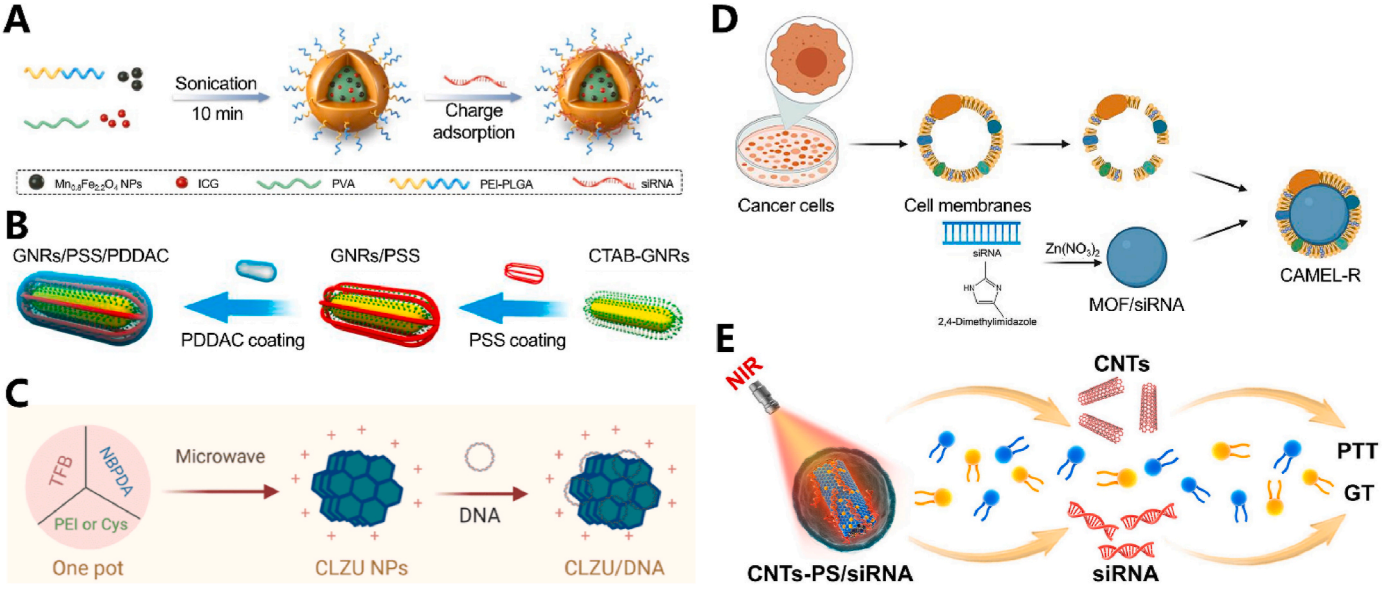

Fig. 4. A) MRI/NIR-guided mild PTT and YAP1 regulation using manganese-doped magnetic nanocarriers combined with YAP1 siRNA for hepatocellular carcinoma [83]. Copyright 2021, Elsevier. B) A cancer cell membrane camouflaged zeolitic imidazolate framework 8 (ZIF-8)-based metal-organic frameworks (CAMEL) nanoparticles for targeted delivery of siRNA to knockdown Plk1 gene in tumors has been fabricated [138]. Copyright 2016, Elsevier. C) Schematic diagram illustrating the construction of CLZU nanoparticles and their use in anti-tumor gene therapy [91]. Copyright 2021, Elsevier. D) An excellent gene transfection capability and good biocompatibility have been demonstrated with cationic COF nanoparticles (CLZU NPs) [94]. Copyright 2020, Springer. E) The delivery of siRNA is performed by carbon nanotubes with excellent temperature sensitivity and photothermal performance [70]. Copyright 2021, American Chemical Society.

highly specific surface area, large pore volume, and biodegradability, mesoporous silica nanoparticles (MSN) have been one of the most popular choices [96]. Because of their superior thermal and optical characteristics, carbon nanotubes (CNTs) are often utilized in biological disciplines. For instance, carbon nanotubes are now utilized as bio-imaging probes and medication transporters [70,97]. Carbon nanotubes are good light absorbers for photothermal ablation of cancer cells due to their great near-infrared (NIR) absorption. Sentinel lymph nodes can be significantly cleared of tumors and cancer cells utilizing imaging-guided PTT with CNT. CNTs can also be partially bound to carriers like magnetic nanoparticles and anticancer medications by being covalently or non-covalently modified with various functional groups. Thus, it is possible to enhance these medications' photothermal capability and create complementary treatments (Fig. 4E) [70].

Mesoporous silica NPs (MSNs) are one of the most widely used NPs, generally considered non-cytotoxic and highly biocompatible *in vitro* and *in vivo* (Fig. 5A). MSNs are solid inorganic nanoparticles having advantageous structural characteristics, such as variable pore size and shape, a large surface area, and a chemically active surface. Functionalization of amines on the MSN, generating a positive charge between 30 and 40 mV, resulted in efficient loading of Cas9/gRNA RNP as well as Cas9 plasmid and gRNA, with an MSN to cargo ratio of 20:1 (w/w) [98]. Their inorganic features provide significant advantages over organic nanoparticles, including higher loading capacity and chemical and thermal stability of guest molecules (Fig. 5B).

Clay minerals are inorganic products obtained from the chemical weathering of sedimentary rocks [99]. Clay minerals have nanoscale stratification and are often named nano clay only. Natural nano clays can be divided into two subgroups: crystalline and amorphous. The most popular medicinal clay minerals include kaolinite (Kaol), halloysite nanotubes (HNTs) [100], montmorillonite (MMT), laponite (Lap), etc. Compared to plate-like nano clays such as MMT, Kaol, and Lap, HNTs

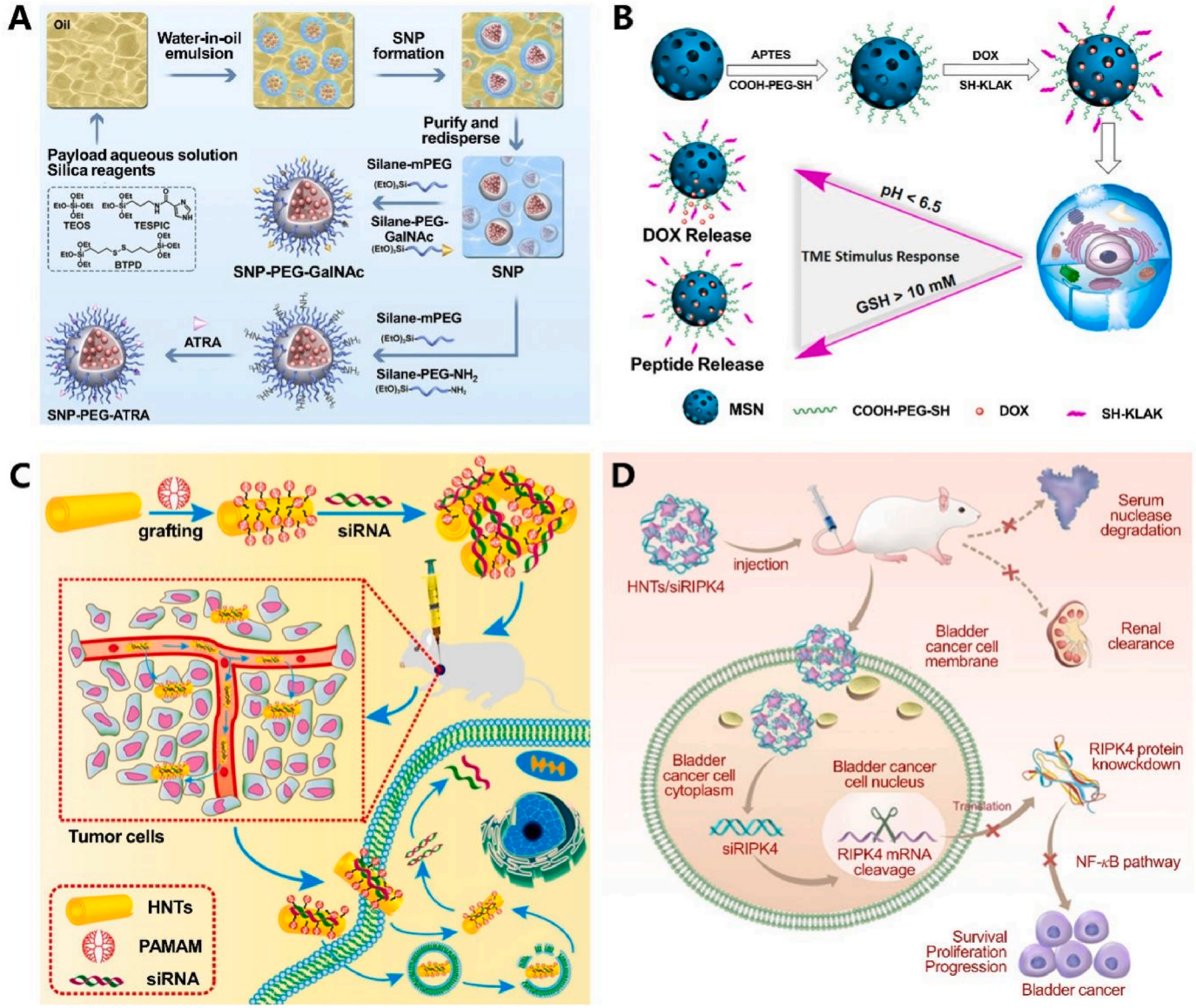

Fig. 5. A) Schematic representation of stimulus-responsive silica nanoparticle delivery of nucleic acids and CRISPR genome editor [139]. Copyright 2021, Elsevier. B) Diagram illustrates the preparation of DMK nanoparticles and pH/Glutathione (GSH)-sensitive drug release for breast cancer chemotherapy [96]. Copyright 2019, American Chemical Society. C) Schematic Illustration for the PAMAM-g-HNTs/siRNA Complex Preparation in Tumor Cells and Intracellular Process [102]. Copyright 2018, American Chemical Society. D) The HNTs/siRIPK4 complex protects RIPK4 siRNA from serum degradation by nuclease and clearance by the kidney, and promotes accumulation of RIPK4 siRNA in tumor cells, thereby silencing RIPK4 and down-regulating RIPK4 expression inhibits the proliferation and spread of bladder cancer [101]. Copyright 2019, American Association for the Advancement of Science.

are more widely employed in biomedical applications than plate-like nano clays like MMT, Kaol, and Lap because of their distinctive tubular structure, nanoscale size dispersion, selective drug loading, high mechanical strength, and superior biocompatibility. Liu et al. [101] reported a halloysite nanocluster that effectively delivers siRNA, which can target the expression of RIPK4 in silencing bladder cancer and inhibit bladder cancer tumor growth. The outcomes demonstrated that bladder cancer cells more readily absorbed HNT-encapsulated siRNA, which is more stable in serum has a longer blood circulation life, and accumulates in bladder cancer tumors. HNTs/siRNA complexes significantly knocked down the expression of RIPK4 in bladder cancer cells and effectively inhibited the tumorigenesis and progression of bladder cancer (Fig. 5C). Long et al. [102] reported grafting polyamide amines on HNTs, loading siRNA, silencing VEGF genes, and realizing the treatment of breast cancer (Fig. 5D).

4. Gene therapy combined with other therapy

There are still many shortcomings with traditional treatment methods. Chemotherapeutic drugs can produce drug resistance, phototherapy is not completely effective, and the targeting effect of traditional treatment is low, which can be lethal for normal cells [17]. Traditional chemotherapy shows limited anti-tumor effects after repeated treatment, which may aid the development of drug resistance [103]. To improve the efficiency of treatment, gene therapy is used in combination with other treatment modalities. A combination of chemotherapy drugs and gene therapy is used to improve treatment outcomes by silencing resistant genes during cancer treatment. Phototherapy combined with gene therapy can work on deep-seated cancer cells and improve the treatment effect. The application of ultrasound technology combined with gene therapy can significantly improve the efficiency of gene/drug delivery on tumor cells. The uptake of genetic material itself can be enhanced by ultrasound, and the addition of ultrasound-focused techniques activates these ultrasonic particles and enables targeted gene delivery to exposed sites [104].

4.1. Phototherapy

Specific therapeutic methods such as photothermal therapy (PTT) and photodynamic therapy (PDT) were developed based on specific factors such as acidic pH, endogenous H_2O_2, and overexpressed enzymes at high levels in the tumor microenvironment (TME). Phototherapy, including photodynamic therapy (PDT) and photothermal therapy (PTT) [17], has been developed as a new anti-tumor technology due to its non-invasiveness, high selectivity, and low toxicity. However, limits such as single function, poor water solubility, easy aggregation, weak targeting, and poor ability to cure diseases are still limiting wider applications. Therefore, the combination of phototherapy and gene therapy is expected to become an effective means to cure diseases. For instance, with N-CDs as the photothermal agent, heme as the photodynamic agent, and histidine as the targeting agent, this multifunctional nanocomposite material of one component synthesized by amidation design has FRET-enhanced photothermal and photodynamic characteristics and realizes dual phototherapy of cancer cells. The obtained CC NCs can serve as effective gene vectors to facilitate gene transfection in cancer cells (Fig. 6C).

4.1.1. Photothermal therapy (PTT)

A new non-invasive tumor therapy procedure called PTT employs a substance called a photothermal agent (PTA) to destroy tumor cells by converting light energy into heat when exposed to external light sources like near-infrared light (NIR) (Fig. 6A). A new method for the therapy of cancer is near-infrared illumination using nanomaterial-mediated PTT. NIR light may penetrate tissue with enough intensity and high spatial precision because of the tissue's low light scattering and absorption of the intrinsic chromophore. Thus, PTT offers an efficient way to transform photon energy into deadly heat to extremely selectively eliminate cancer cells, especially in crucial locations. In comparison to conventional cancer therapies, PTT with a high level of local heat transfer is less invasive, quicker, and may be coupled with chemotherapy and drug/gene delivery [105]. PTT has received a lot of research interest due to instant results, high potency, and little or no side effects [106]. However, given the limitation on the depth in which a tissue light can penetrate, it is still difficult to completely remove tumor cells, leading to cancer recurrence and metastasis [17]. Heat energy does not only kill cancer cells, it also causes considerable damage to neighboring normal tissues [85]. Materials that undergo photothermal conversion can successfully attach to negatively charged genes, enhance the stability of siRNA in serum, target tumor areas, and regulate the release of siRNA using the vector's innate features. Therefore, PTT in combination with GT can be a potentially effective strategy for cancer therapy. Zhao et al. [70] reported that a lipid-coated carbon nanotube delivery system with good temperature-sensitive properties can lead to significant tumor suppression *in vitro* and *in vivo* with precisely temperature-controlled gene release and silencing protein expression in tumor cells. A NIL-triggered heat-responsive nanotherapeutic device was described by Chen et al. [107] accomplished photothermal regulated release of Cas9 RNP and DOX to lower tumor heat tolerance, and boost mild PTT effect.

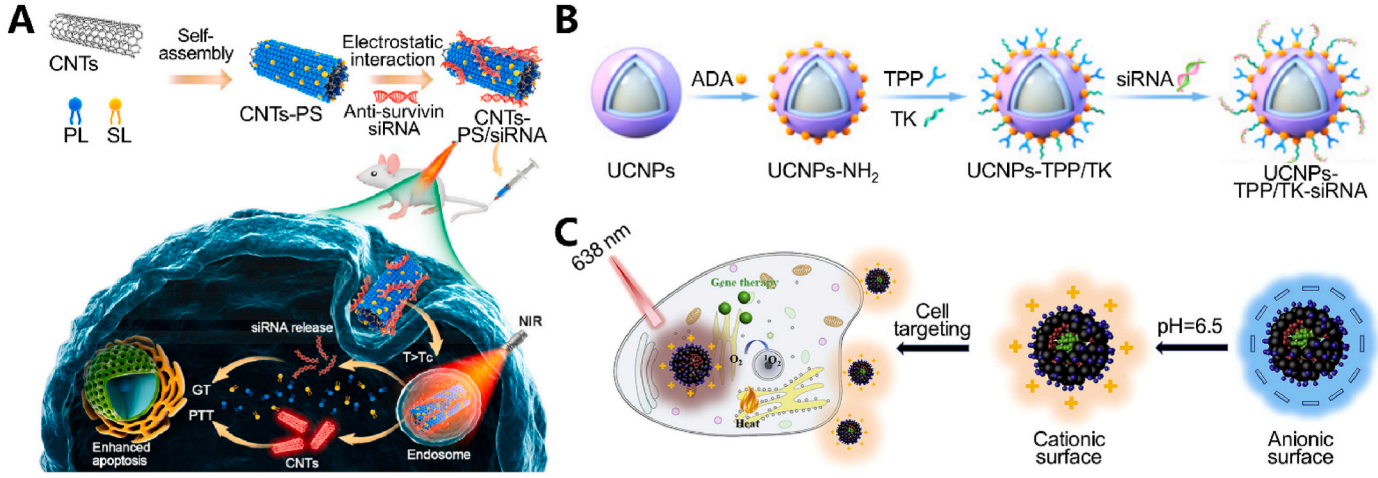

Fig. 6. A) Temperature-sensitive CNT-PS/siRNA nanoparticles for cancer cells in synergistic PTT and GT [70]. Copyright 2021, American Chemical Society. B) Assembling the upconversion nanoprobe for use in photodynamic therapy and cascade gene therapy, along with the associated optogenetic nanosystem [111]. Copyright 2023, Elsevier. C) Photothermal and photodynamic synergistic gene therapy targets for cancer therapy [109]. Copyright 2018, Elsevier.

4.1.2. Photodynamic therapy (PDT)

PDT is a novel non-invasive treatment for tumors, It uses irradiation released from lights at a given range of wavelength to activate the photosensitizer in tumor tissue, this produces biotoxic monomorphic oxygen species (ROS), which then causes oxidative damage on tumors, upregulates immune response and triggers apoptosis [108]. Since the core of PDT is photoexcitation, the application target can be controlled precisely and minimize the damage to the surrounding normal tissues (Fig. 6B). Most clinically used photosensitizers are excited by UV–Vis light, which has very limited tissue penetration, hence impeding its application in eliminating deep tumors [109]. Combining gene therapy with photodynamic therapy allows for precise targeting and inhibition of antioxidant regulatory factors, near-infrared photodynamic therapy, and cascade gene therapy on target cells [110]. Song et al. [111] reported that under near-infrared irradiation, the emission of UCNPs can excite the expressed protein sensitizer to produce ROS, thereby stimulating the release of siRNA in a controlled manner to enable PDT and cascade gene therapy. Bazylińska et al. [112] evaluated the effects of cellular uptake and NIR-induced photodynamic therapy with a photosensitive delivery system on human cutaneous melanoma (MeWo) and ovarian (SKOV-3) cancer cells. Tang et al. [113] report a novel strategy to integrate gene delivery, single-photon/TP luminescence tracking, and PDT into single-molecule designs to simultaneously achieve triple synergistic effects that can be imaged in real-time and efficiently kill cancer cells.

4.2. Chemotherapy

Chemotherapy is a form of systemic therapy, regardless of the route of administration (oral or intravenous), chemotherapy drugs will enter the circulation system in the body and affect most organs. Chemotherapy drugs are cytotoxic drugs, and most of the adverse reactions and toxic side effects of chemotherapy are reversible and may cause damage to liver and kidney function. Chemotherapy combined with gene therapy can accumulate in tumors in a targeted manner to reduce the overall toxic side effects of chemotherapy drugs on the entire body [114]. This circumvents the side effects of traditional chemotherapy and also silences drug-resistant genes to improve therapeutic efficacy [115], with high specificity and therapeutic effect (Fig. 7A). Li et al. [96] reported a pro-apoptotic peptide/DOX double-loaded MSN nanoplatform that efficiently penetrates the nucleus and produces antitumor effects. Wang et al. [90] reported metal MOF nanoparticles targeting DNase, which acts as a tool for gene silencing to inhibit the proliferation and metastasis of cancer cells, effectively blocking tumor metastasis. Yue et al. [115]

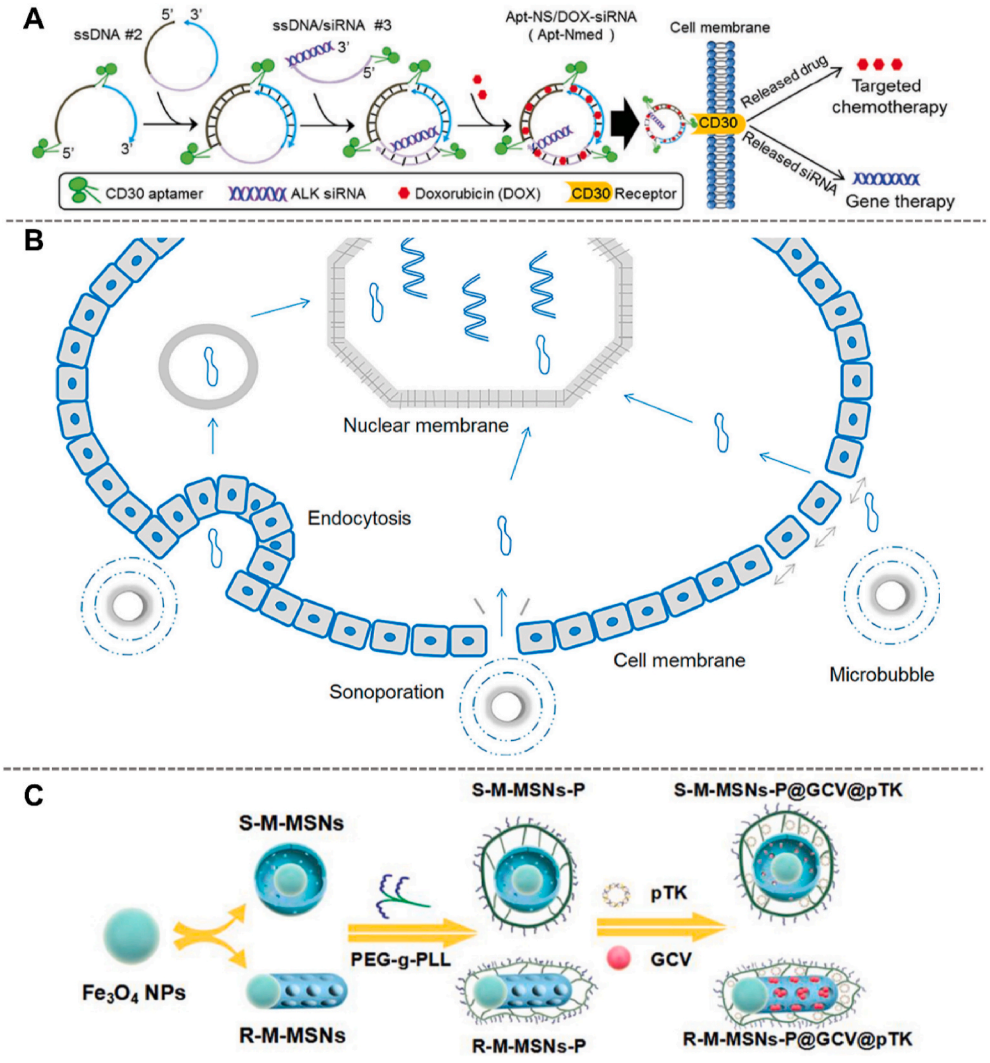

Fig. 7. A) The formation of Apt-NS/DOX-siRNA (Apt-NMed) inducing targeted chemotherapy and gene therapy through self-assembly of aptamer nanostructures carrying gene-specific and cell-specific entities [140]. Copyright 2017, John Wiley and Sons. B) Gene delivery through ultrasound microbubbles modulates the integrity of vascular endothelial cells and stimulates the absorption of internal cells [141]. Copyright 2017, Elsevier. C) Hepatocellular carcinoma suicide gene therapy with sphere-like and rod-like M-MSNs for MRI-guided, magnetically targeted, and hyperthermia-enhanced delivery [118]. Copyright 2018, Elsevier.

used the overexpression of two miRNAs in breast cancer cells to design them as molecular triggers for siBcl-2 generation and Dox release in living cells, a combination of cancer treatment with high specificity and therapeutic effect.

4.3. Ultrasonic

Ultrasound therapy is ultrasound drug penetration therapy that uses ultrasound to push drugs into the body. Technologies based on ultrasound have been created to improve the pharmacokinetics and effectiveness of medications for treating illnesses. Exogenous nucleic acid transfection in particular has the potential to activate immune responses in the tumor microenvironment [18,104]. The area of ultrasound-mediated gene transfection is expanding, and ultrasound has long been a popular medical diagnostic tool. Ultrasound can be combined with other therapies to treat a variety of diseases, including cancer [89]. The combined imaging and local therapeutic actions of ultrasound increase spatial selectivity, which lowers systemic toxicity (Fig. 7B). Gene delivery can be achieved directly through ultrasound-mediated or targeted to achieve the aggregation of nucleic acid drugs at the target site. In addition, ligands on ultrasound particles can selectively bind biomarkers by conjugation targeting, further enhancing cell and disease specificity. Wang et al. [116] reported an ultrasonic microbubble as a carrier, loaded with miRNA, for molecular imaging and targeted therapy.

4.4. Magnetic hyperthermia

Clinical trials have introduced magnetic hyperthermia (MH) as an alternative to the treatment of tumors locally [19]. When subjected to alternating magnetic fields (AMFs), magnetic nanoparticles (MNPs) generate heat [117]. It has become an important topic in the field of nanomedicine due to the many advantages of effective anti-tumor therapy in the field of nanomedicine, such as high biosecurity, deep tissue penetration, and targeted selective tumor killing (Fig. 7C). Wang et al. [118] reported gene delivery vectors MNPs applied to magnetic targeting and hyperthermia for hepatocellular carcinoma (HCC). Wang et al. [119] described the synergistic effect of immunogenic nanoparticles mediated combination of PDT and magnetic hyperthermia to enhance the anti-metastatic efficacy of immunotherapy to combat cancer metastasis.

5. Application in solid tumour

Due to gene therapy's high efficiency and precision, it is widely used in the treatment of various diseases. In particular, it plays an important role in clinical trials of solid tumors. Gene therapy combined with other treatment methods can greatly improve the treatment effect, and the combination of different treatment methods can promote each other and synergize. Solid tumors transmit information through tight and gap junctions. In contrast to liquid tumors, solid tumors form a mass when cells proliferate, they usually do not contain pockets of fluid, pus, air, or other substances. Solid tumors can be noncancerous (benign), precancerous (cells that have the potential to become malignant), and cancerous (malignant). Solid tumors account for about 90 % of adult cancers. They can develop in many parts of the body, including the breast, lungs, prostate, colon, bladder, and kidneys [120]. Of these, breast cancer alone accounts for 31 % of cancers in women, and lung cancer causes approximately 350 deaths per day. The mortality rates for lung cancer and breast cancer are significantly higher compared to other cancers. Additionally, glioma is difficult to treat with conventional methods because they are primarily intracranial, and the blood-brain barrier significantly hinders substance exchange, affecting drug delivery to the tumor site. Consequently, gene therapy approaches for these three cancers are highlighted in this section.

The term "tumor micro-environment" (TME) refers to the microenvironment around tumor cells, which may include extracellular matrix (ECM), nearby blood arteries, immune cells, fibroblasts, myelogenic inflammatory cells, and numerous signaling chemicals. Due to the fast development of the tumor tissue, the excessive swelling, and the imperfect vascular system inside the tumor tissue, these factors will cause an inadequate supply of oxygen to the tumor tissue, and the tumor microenvironment hypoxic which in turn facilitates angiogenesis and tumor growth.

Tumor cells can only metabolize energy by anaerobic digestion due to insufficient oxygen supply, which will cause the accumulation of lactic acid. The ion exchange protein on the tumor cell membrane is also continuously transporting H^+ inside the cell to the outside of the cell to prevent its acidosis. Additionally, the tumor microenvironment's pH decreases as a result of these cellular reactions, contributing to the tumor's overall acidic environment. In the microenvironment of tumor occurrence and hypoxia and acidity, tumor tissue and peripheral tissue cells will undergo myriad apoptosis, releasing cell debris and chemokines, resulting in inflammatory cell infiltration and secretion of inflammatory factors. Additionally, the formation and occurrence of the tumor itself will set off an immune reaction in this region, leading to an accumulation of inflammatory cells and a virulent inflammatory response. According to the particularity of the tumor microenvironment, gene therapy methods targeting tumor sites can be realized.

5.1. Breast cancer

One of the probable causes of tumor-dependent mortality in females is breast-related carcinoma (BC), which is predicted to account for 31 % of new tumor cases [120]. It is a disease with a wide range of manifestations, and various signaling chemicals and cascades support its initiation and development. Different breast cancer subtypes, including luminal, $HER2^+/ER^+$ [103], and basal-like, have been identified by molecular investigations based on gene expression profiles [121]. About 70 % of basal-like cancers (ER-/PR-/HER2-negative) [122] are triple-negative. A breast tumor subtype known as triple-negative breast cancer (TNBC) has a poor prognosis because it lacks the expression of hormone receptors and HER2 gene amplificatio [123]. Compared to other kinds of BC, it is the most aggressive and has the highest risk of metastasis. Additionally, it has been shown that TNBC is more common when there is a somatic (5–10 %) or germline (20–25 %) deficiency in the BRCA DNA repair associated (BRCA) gene, which protects the TP53 mutation that inactivates the p53 protein [121]. Generating PARP1 defects in TNBC cells using CRISPR/Cas9 technology can increase the sensitivity of TNBC cells to chemotherapeutic agents [124].

It was shown that tumor-associated adipocytes (TAAs) aggravate tumor progression and exacerbate immunosuppressive TME. Liu et al. [125] designed nanoparticles targeting the tumor microenvironment to specifically deliver plasmid DNA and successfully inhibit TAAs. Chlorin e6 (Ce6), a photosensitizer, docetaxel (DTX), a microtubule stabilizer, and an anti-Twist siRNA-containing nanoparticle (CDTN) were reported by Meng et al. [121] to exert combined PDT/CT/GT against metastatic breast cancer and to inhibit the growth of primary tumors by >80 % (Fig. 8A). Li et al. [10] reported a DNA-based nano-delivery system based on plasmonic activation. They generated ultra-long single--stranded DNA by rolling circle amplification (RCA) as a scaffold for the nano-system, programmed repetitive sgRNA recognition sequences, DNAzyme and Hhal cleavage sites in the process, and introduced Mn ions as DNAzyme cofactors to assemble the nano delivery system. Ultimately, the combination of CRISPR/Cas9 and DNAzyme is achieved through lysosome-mediated internalization into cancer cells (Fig. 8B). Lu et al. [126] reported a vortexed iron tetraoxide nanorod with DOX and EZH2 siRNA loaded on the surface by coupling with PEI and PEG and coating the particles with macrophage membranes for targeting. As EZH2 siRNA significantly inhibited the growth, metastasis, and chemoresistance of adriamycin (DOX)-resistant TNBC cells, the combination treatment modality significantly improved antitumor efficacy and

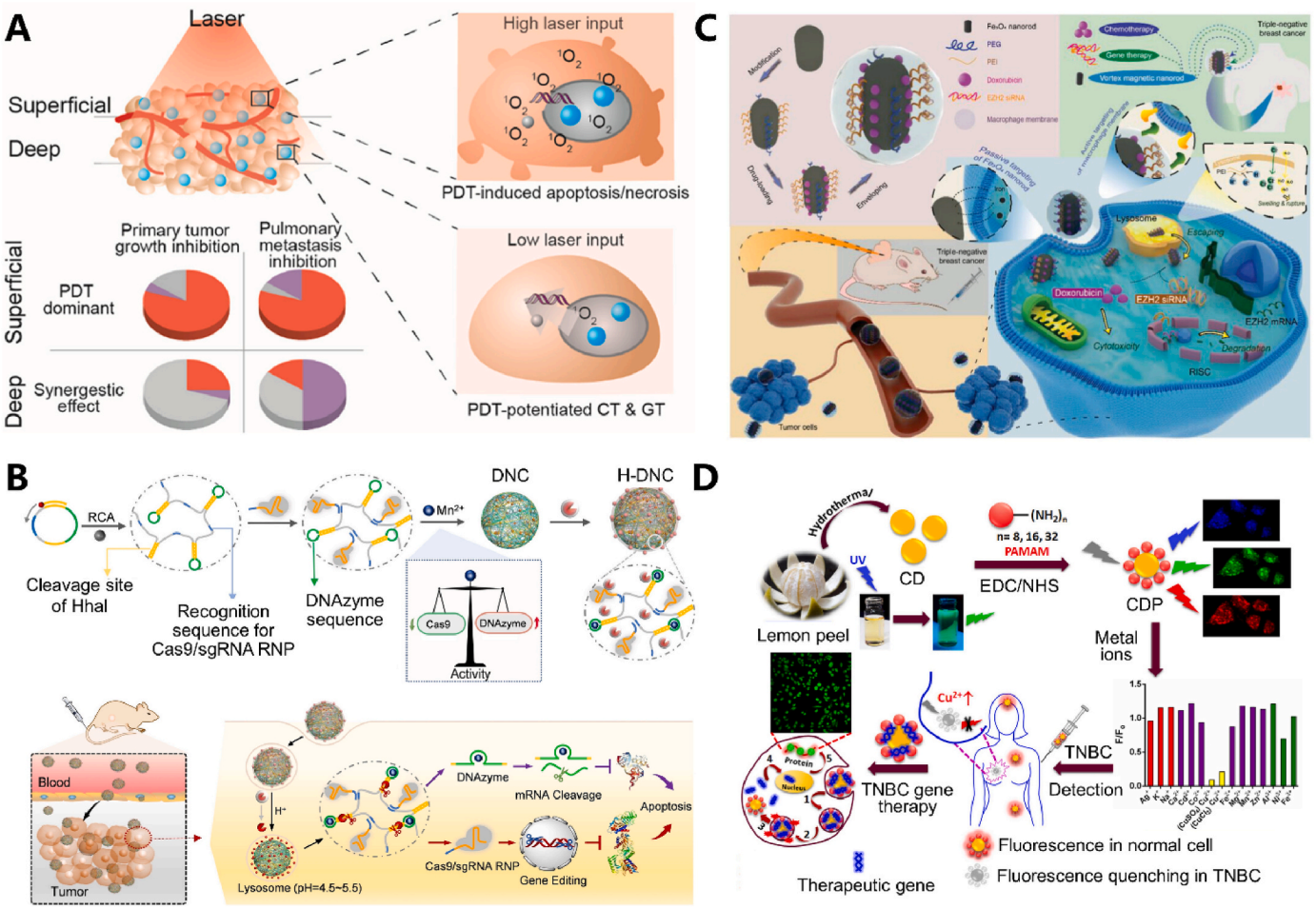

Fig. 8. A) Application of CRISPR/Cas9 combined with phototherapy in lung cancer [123]. Copyright 2018, American Chemical Society. B) The proton-activated DNA nanosystem enables the co-delivery of Cas9/sgRNA RNP and DNAzyme for combined gene therapy [131]. Copyright 2022, John Wiley and Sons. C) Vortex ferroferric oxide nanorods load DOX and EZH2 siRNA to inhibit the growth of drug-resistant TNBC cells, transfer, and chemical resistance [126]. Copyright 2023, John Wiley and Sons. D) CDP synthesis methods and the treatment of TNBC diagnostics [103]. Copyright 2019, Elsevier.

reduced systemic toxicity (Fig. 8C). Ghosh et al. [103] synthesized CD and conjugated it with PAMAM dendrimers. After this, CDP bound with pDNA, among all, CDP3 showed excellent resistance to gene synthesis and enzyme digestion of gene protection ability. In addition, CDP3 showed highly selective detection of Cu(II)ion which could be useful to identify the metastases phase of triple-negative breast cancer which exhibits a higher level of Cu(II)ion (Fig. 8D).

5.2. Malignant glioma

The characteristic feature of glioblastoma is a widespread invasion into healthy brain tissue. Significantly invasive is the cause of recurrence after surgical resection. Invasion and resistance to chemoradiotherapy are fundamentally caused by the existence of glioma stem cells (GSCs), which can initiate tumors and self-renew, in addition to differentiated glioma cells. Ryota Tamura et al. [127] showed that neural stem cells (NSCs) derived from CRISPR/Cas9-edited human induced pluripotent stem cells (hiPSCs) expressing suicide genes have a higher tumor nutrient migration capacity compared to mesenchymal stem cells (MSCs), resulting in significant *in vivo* antitumor effects (Fig. 9A). Wang et al. [128] proposed a lipoprotein-biomimetic nanoparticle ptHDL/siHIF-ICG that mimics the native structure and function of HDL nanoparticles while delivering ICG and siHIF to deep regions of solid tumors for combination therapy of PDT, PTT, and siRNA gene silencing against gliomas. The designed lipoprotein-biomimetic nanoparticles are efficient solutions for precise tumor localization and early detection, targeted, long-acting, and combination therapy of glioma. They may also optimize the synergy of multiple therapies and diagnostics [127]. Li et al. [129] reported magnetosome-like ferrimagnetic iron oxide nano chains (MFIONs) were applied to MSCS to achieve high expression of the HSV-tk suicide gene. An increased expression of Cx43 was detected, promoting the gap junction intercellular communication, and effectively inducing tumor cell apoptosis (Fig. 9B). Zheng et al. [130] successfully synthesized atMO-21-coated NPs (B1L@SpAcDex-ATMO-21 NPs) using the liquid method. As a gene delivery platform, SpAcDex NPs achieved high Atom-21 loading efficiency (>90 %) and high delivery efficiency. The use of the surface of B1L-functionalized SpAcDex NPs enabled efficient and intelligent penetration of the NPs across the BTB for brain tumor targeting, showed satisfactory anti-tumor efficacy in orthotopic human GBM bearing mice, and could significantly inhibit the expression of tumor vascularrelated genes (HIF1α and VEGF) by up-regulating PTEN expression (Fig. 9C).

5.3. Lung cancer

The most prevalent malignancy in the world is lung cancer. Lung cancer has the highest rate of morbidity and death among malignant tumors worldwide. More than 85 % of diagnosed lung cancer patients have NSCLC [120]. Among the different types of NSCLC, NSCLC expressing mutations in the echinoderm microtubule-associated protein-like 4-mesenchymal lymphoma kinase (EML4-ALK) fusion gene is the most malignant type. Suppressing the expression of target proteins

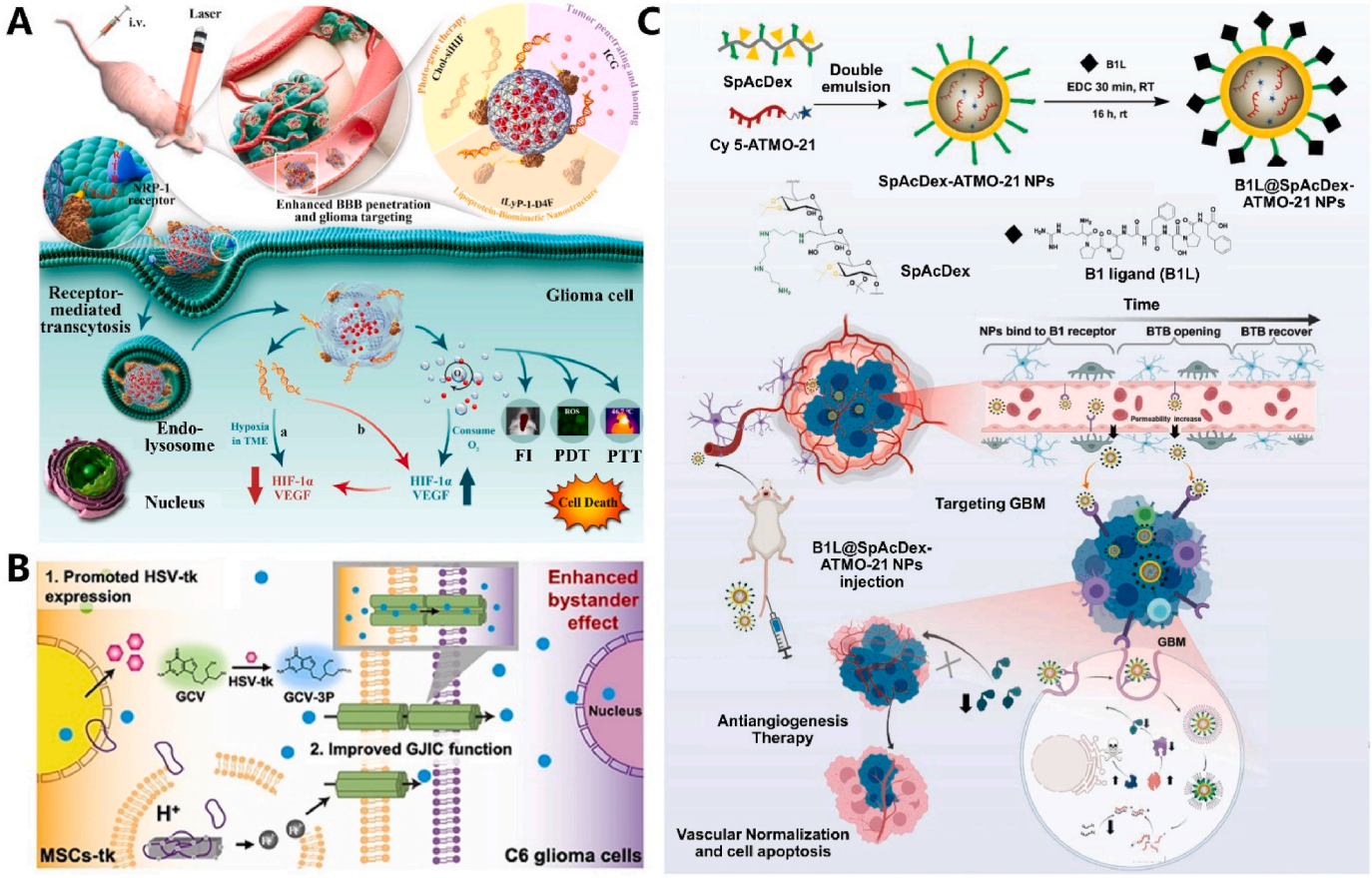

Fig. 9. (A) ptHDL/siHIF-ICG accumulates at tumor sites by targeted peptide guidance and is internalized by receptor-mediated endocytosis. ptHDL/siHIF-ICG is broken down in glioma cells under near-infrared laser irradiation [142]. Copyright 2018, Elsevier. (B) MSCS with HSV-tk suicide gene was genetically engineered using MFION to mediate the treatment of glioma [129]. Copyright 2021, John Wiley and Sons. (C) MicroRNA-21 oligonucleotide inhibition and anti-angiogenesis therapy to treat brain tumors [130]. Copyright 2021, John Wiley and Sons.

by inhibiting their mRNAs is a promising strategy to inhibit targeted ALK mRNAs for cancer therapy. Gold nanoshells are a new type of biocompatible nanocarriers, the unique spatial structure formed by densely packed spherical particles prevents biomolecules from being rapidly degraded by enzymes in the blood circulatio [131]. Combined with the good photothermal conversion efficiency of the gold nanoshells, the elevated temperature will disrupt the thiol-gold bond under the irradiation of near-infrared light, which triggers the subsequent release of siRNAs and chemotherapeutic drugs for combination therapy (Fig. 10A). The epidermal growth factor receptor (EGFR) gene, which is essential for the development of tumors, is linked to a mutation in 15 % of instances of non-small cell lung cancer. Taeyoung Koo et al. [132] demonstrate the use of the CRISPR/CRISPR-associated protein 9 (Cas9) system to discriminate between the oncogenic mutant and wild-type EGFR alleles and eliminate the carcinogenic mutant EGFR allele with high accuracy to treat mutated EGFR-mediated lung cancer [133–135]. FRα and FRβ were highly expressed in tumor cells and stromal TAM, respectively. High expression of interstitial FRβ is associated with poor prognosis of lung adenocarcinoma and lung squamous cell carcinoma. FRβ can be used as an independent prognostic factor for lung squamous cell carcinoma and lung adenocarcinoma [135]. Huang et al. [106] investigated the combination of PIONs-mediated PTT and LNC CRYBG3-mediated gene therapy, so that LNC CRYBG3 could be overexpressed in tumor cells to degrade the actin cytoskeleton and lead to apoptosis. Faced with the challenge of pulmonary delivery, a suitable aerodynamic diameter (1–5 μm) enables successful carrier deposition in the respiratory region. Appropriate particle size (less than 200 nm) reduces or even avoids macrophage phagocytosis and optimally penetrates mucus. The near-neutral surface charge and hydrophilicity allow carriers to gain the ability to penetrate mucus. Due to the specificity of the pulmonary environment, inhalation provides a more invasive, locally targeted route of delivery compared to systemic delivery, bypassing hepatic and splenic clearance and evading the mononuclear phagocyte system, resulting in more efficient uptake by lung cancer cells. A delivery system that combines passive targeting and active targeting via molecular recognition by inhalation not only exhibits high transfection of siRNA stabilization [136], lattice protein-mediated endocytosis, and effective escape into the cytoplasm, providing a superior method of gene delivery (Fig. 10B).

6. Conclusion and future perspectives

Research into the molecular mechanisms of human disease has developed rapidly in recent decades, greatly expanding the global demand for gene therapy. Nucleic acid drug-based therapies have come a long way, with significant improvements in the stability, functionality, and production of nucleic acid drugs. A significant amount of research is currently focused on various applications of nucleic acid drug therapies, with a range of clinical trials underway. How can their inherent instability and degradation be improved by delivery system optimization? How can their activation of the immune system be modulated? Essentially, the clinical translation of nucleic acid drug-based therapies requires delivery technologies that can ensure the stability of nucleic acid drugs under physiological conditions. Optimization techniques to improve the structure of nucleic acid drugs and engineering precision nanoparticles for nucleic acid drug-based therapies are also key points in

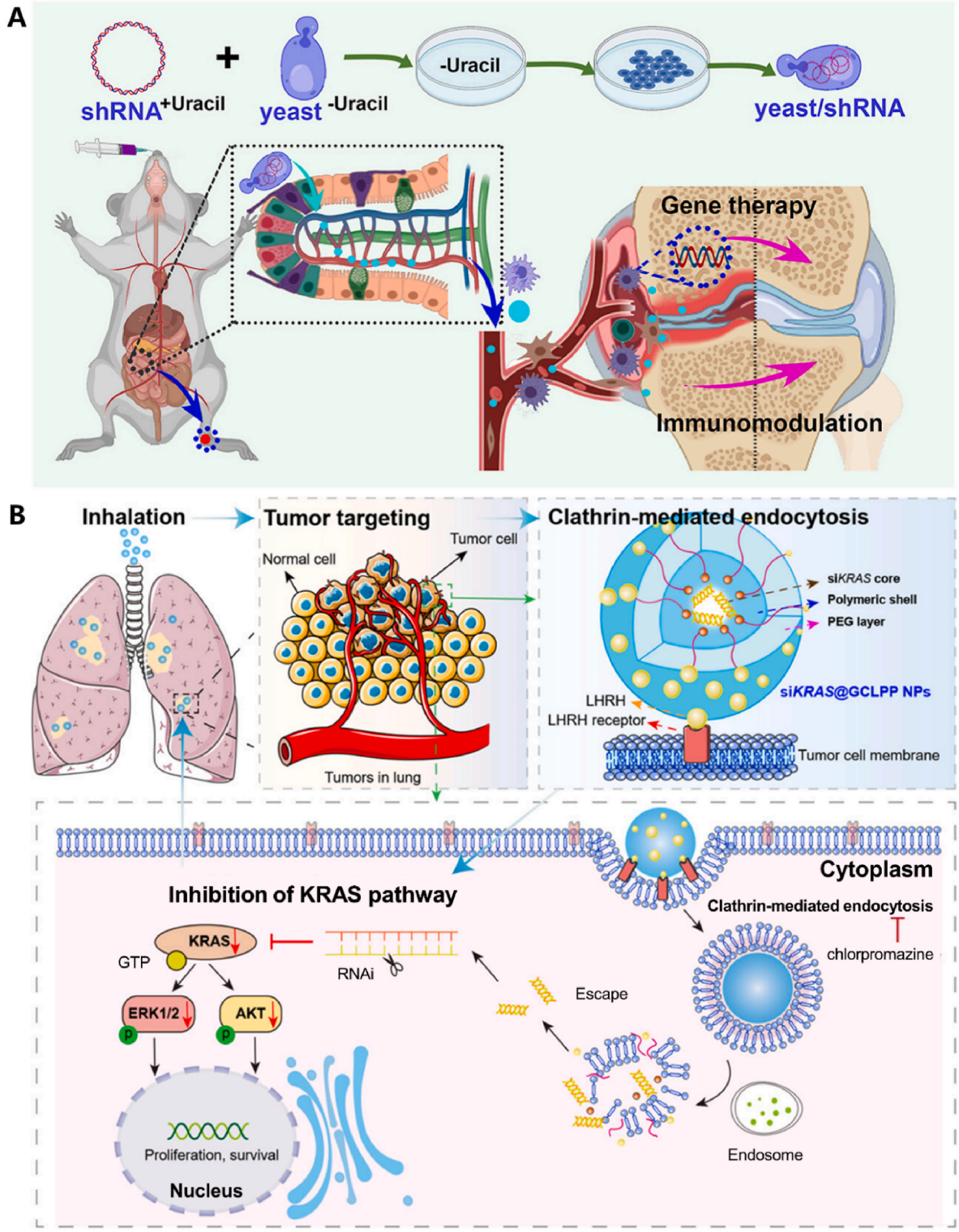

Fig. 10. A) Gold nanoshell-based dual-target gene therapy for non-small cell lung cancer [131]. Copyright 2018, Ivyspring International. B) A noninvasive aerosol inhalation nanoparticle (NP) system, termed "siKRAS@GCLPP NPs," treats KRAS-mutant non-small-cell lung cancer (NSCLC) [136]. Copyright 2023, American Chemical Society. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the development of nucleic acid drug medicines as powerful and versatile disease tools. While gene therapies offer unprecedented opportunities to treat cancers and various other diseases, they have also raised serious concerns about the often unseen risks that come with irreversible one-off treatments. Side effects of nucleic acid-based drugs may be caused by a variety of factors, including off-target effects. They may lead to unexpected gene silencing and deleterious effects, raising many safety concerns. For example, siRNA may accidently target mRNAs with similar sequences, leading to unintended regulation of host gene expression. CRISPR may cleave non-target DNA, etc. More potential problems deserve to be explored, and there are many more challenges for gene therapy.

Gene therapy can treat a wide range of diseases, including but not limited to, cancer, cardiovascular, immune, and genetic diseases as we have discussed in this review. Among these diseases, cancer and single-gene diseases account for the largest share of gene therapy demand. An important part of gene therapy is the vector, but at present, vector technology has been the main obstacle to the clinical application of this treatment model transformation. Although viral vectors have high transfection efficiency, they also have shortcomings that are difficult to solve, such as limited genetic material loading capacity, complex production process, wide tendency, cytotoxicity, immunogenicity, and tumorigenicity. Non-viral vectors are also favored by researchers, but many of these problems still need to be solved. The selection and modification of various carrier systems, the particle size requirements of the load, and the response conditions still need to be explored. The transfection efficiency of non-viral nano vectors remains low. Second, it may be difficult for genes to be fully released in target cells without causing serious off-target effects due to the complex human microenvironment. Finally, the issue of safety and ethics must also be considered. Despite their low immunogenicity, because of the physicochemical properties of nanoparticles, the maximum tolerated dose and long-term toxicity of stimulus-responsive gene delivery systems should be assessed fully.

CRediT authorship contribution statement

Yuhan Ma: Writing – original draft, Methodology, Investigation, Data curation. **Juan Liao:** Methodology, Investigation, Data curation. **Hongxia Cheng:** Methodology, Data curation. **Qian Yang:** Writing – review & editing, Investigation, Conceptualization. **Huaming Yang:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Abbreviation

ASO: antisense oligonucleotide
AGO2: Argonaute-2 protein
DSB: double-strand break
TALEN: transcriptional activator-like effect nuclease
Ad: adenovirus
rAAV: recombinant adeno-associated virus
NIR: near-infrared
COF: covalent organic framework
HSPC: hematopoietic stem/progenitor cell
SERCA2a: sarcoplasmic reticulum calcium ATPase
TNF: tumor necrosis factor
SCD: Sickle cell disease
HbA: hemoglobin A
PEI: polyethyleneimine
PNP: polymer nanoparticle
PTT: photothermal therapy
TME: tumor microenvironment
MRI: magnetic resonance imaging
INP: inorganic non-metallic nanoparticle
Kaol: kaolinite
MMT: montmorillonite
MH: magnetic hyperthermia

ECM: extracellular matrix
NSCLC: Non-small cell lung cancer
RISC: RNA-Induced Silencing Complex
PCI: photochemical internalization
ZFN: zinc-finger nuclease
RV: retrovirus
AAV: adeno-associated virus
LNP: Lipid nanoparticle
ROS: reactive oxygen species
MOF: Metal-organic framework
VEGF: vascular endothelial growth factor
RA: rheumatoid arthritis
LHON: Leber hereditary optic neuropathy
HbS: hemoglobin S
HbF: hemoglobin F
SLN: solid-lipid nanoparticle
CS: chitosan
PDT: photodynamic therapy
ICG: indocyanine green
MNP: magnetic nanoparticles
MSN: mesoporous silica nanoparticle
HNTs: halloysite nanotubes
Lap: laponite
HCC: hepatocellular carcinoma
EGFR: epidermal growth factor receptor