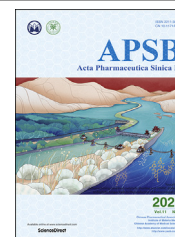




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REVIEW

Long non-coding RNAs: From disease code to drug role



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KEY WORDS

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Abstract Enormous studies have corroborated that long non-coding RNAs (lncRNAs) extensively participate in crucial physiological processes such as metabolism and immunity, and are closely related to the occurrence and development of tumors, cardiovascular diseases, nervous system disorders, nephropathy, and other diseases. The application of lncRNAs as biomarkers or intervention targets can provide new insights into the diagnosis and treatment of diseases. This paper has focused on the emerging research into lncRNAs as pharmacological targets and has reviewed the transition of lncRNAs from the role of disease coding to acting as drug candidates, including the current status and progress in preclinical

Abbreviations: AD, Alzheimer's disease; *ANRIL*, antisense noncoding RNA gene at the INK4 locus; ASncmtRNA, antisense noncoding mitochondrial RNA; ASO, antisense oligonucleotide; *BCAR4*, breast cancer anti-estrogen resistance 4; *BDNF-AS*, brain-derived neurotrophic factor antisense; *CASC9*, cancer susceptibility candidate 9; CDK, cyclin dependent kinase 1; *CHRF*, cardiac hypertrophy related factor; CRISPR, clustered regularly interspaced short palindromic repeats; *DACH1*, dachshund homolog 1; *DANCR*, differentiation antagonizing non-protein coding RNA; DKD, diabetic kidney disease; DPF, diphenyl furan; *EBF3-AS*, early B cell factor 3-antisense; ENE, element for nuclear expression; *ErbB4-IR*, Erb-B2 receptor tyrosine kinase 4-immunoreactivity; FDA, U.S. Food and Drug Administration; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *GAS5*, growth arrest specific 5; *HISLA*, HIF-1 α -stabilizing long noncoding RNA; *HOTAIR*, HOX transcript antisense intergenic RNA; *HULC*, highly upregulated in liver cancer; *lincRNA-p21*, long intergenic noncoding RNA p21; *LIPCAR*, long intergenic noncoding RNA predicting cardiac remodeling; LNAs, locked nucleic acids; lncRNAs, long non-coding RNAs; *MALAT1*, metastasis associated lung adenocarcinoma transcript 1; *MEG3*, maternally expressed gene 3; *MHRT*, myosin heavy chain associated RNA transcripts; MM, multiple myeloma; mtlncRNA, mitochondrial long noncoding RNA; *NEAT1*, nuclear enriched abundant transcript 1; *NKILA*, NF-kappaB interacting lncRNA; *Norad*, non-coding RNA activated by DNA damage; NPs, nanoparticles; *OIP5-AS1*, opa-interacting protein 5 antisense transcript 1; PD, Parkinson's disease; PEG, polyethylene glycol; PNAs, peptide nucleic acids; pHLP, pH-low insertion peptide; PTO, phosphorothioate; *PVT1*, plasmacytoma variant translocation 1; RGD, arginine-glycine-aspartic acid peptide; RISC, RNA-induced silencing complex; *SAL-RNA1*, senescence associated long non-coding RNA 1; sgRNA, single guide RNA; siRNAs, small interfering RNAs; SncmtRNA, sense noncoding mitochondrial RNA; *SNHG1*, small nucleolar RNA host gene 1; *THRIL*, TNF and HNRNPL related immunoregulatory; *TncRNA*, trophoblast-derived noncoding RNA; *TTY15*, testis-specific transcript, Y-linked 15; *TUG1*, taurine-upregulated gene 1; *TWIST1*, twist family BHLH transcription factor 1; *UCA1*, urothelial carcinoma-associated 1; UTF1, undifferentiated transcription factor 1; *XIST*, X-inactive specific transcript.

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Translational medicine;
Clinical trials

research. Cutting-edge strategies for lncRNA modulation have been summarized, including the sources of lncRNA-related drugs, such as genetic technology and small-molecule compounds, and related delivery methods. The current progress of clinical trials of lncRNA-targeting drugs is also discussed. This information will form a latest updated reference for research and development of lncRNA-based drugs.

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1. Introduction

Long non-coding RNAs (lncRNAs) are defined as RNAs that have a transcript length exceeding 200 nucleotides and will not be translated into proteins¹. Although lncRNAs were once thought to be by-products of RNA polymerase II transcription without biological functions, a large repertoire of lncRNAs have been certified to regulate cellular processes, such as chromosome and genome modification, transcription activation and interference, and nuclear transport, thus driving more researchers to explore how lncRNAs influence human biology. LncRNAs can be categorized in terms of length, function, location, and targeting mechanism and currently there is no unified standard for their classification. According to their position in the genome relative to protein-coding genes, they can be classified as sense, antisense, bidirectional, intronic, intergenic, and enhancer lncRNAs, with their functions dependent on their position². Simultaneously, lncRNAs are commonly sorted into bait, scaffold, signal, and guide lncRNAs based on their function mechanisms³. In recent years, several studies have reported that lncRNAs can encode small peptides to fine-tune general biological processes in a tissue-specific manner, further unveiling the value of lncRNAs as well as their complexity^{4–7}.

The mechanisms by which lncRNAs regulate gene expression are rather complicated and have not yet been fully elucidated. They can function through heterogeneous modes of operation, usually being sorted into the following categories: (1) binding to DNA directly or transcription factors so as to achieve gene expression regulation at the transcriptional level; (2) targeting mRNAs, miRNAs, or proteins and modulating their activities and stability to act post-transcriptionally; and (3) interfering with chromatin complexes to repress or activate gene expression in an epigenetic fashion^{8–10}.

The mechanisms of lncRNAs allow their involvement in almost all physiological activities in living cells, and they are associated with a broad range of diseases. LncRNAs have emerged as potential novel molecules shaping disease diagnosis, treatment, and prognosis. Owing to their key roles in disease, scientists are currently developing technologies and tools to target lncRNAs and create lncRNA-based drugs. This represents a major opportunity and new frontier for drug development; however, there are still notable challenges, including the absence of successful reference cases and the lack of clinical data on the safety and efficiency of lncRNA-targeted drugs. In the current review, we have summarized the rapid advances that have occurred in this field in recent years, and examined the remaining challenges.

2. Overview of lncRNAs in diseases

The relationship between lncRNAs and diseases, especially chronic diseases, is explicit. However, cancer is the most intensively studied disease related to lncRNAs.

H19 is one of the first lncRNAs to be associated with various types of cancer, including bladder cancer, colorectal cancer, and hepatocellular carcinoma. The mutually inhibiting interplay between *H19* and *p53*, a master tumor suppressor gene, endows in part the role of *H19* in tumorigenesis¹¹. Mainly *via* the modulation of miRNAs, *H19* can promote angiogenesis, the major element in tumorigenesis¹². In metastasis, ectopic *H19* expression may directly involve in the epithelial–mesenchymal transition through the downregulation of cell-to-cell adhesion molecules, such as E-cadherin¹³. *PVT1*, another highly expressed lncRNA in cancer tissues, appears to be an effective diagnostic and prognostic biomarker^{14,15}. The positive feedback between *PVT1* and c-Myc is well-known; these two molecules act synergistically to promote proliferation, metastasis, and tumor escape¹⁶. Moreover, emerging studies have revealed that *PVT1* is tightly correlated with chemotherapy resistance¹⁷. *NEAT1*, a well-known lncRNA, is also closely related to chemotherapy resistance in various types of cancers, such as triple-negative breast cancer¹⁸. These discoveries support the idea that tumor resistance can be overcome by utilizing lncRNAs. Tumor immunology and tumor metabolism are hotspots in basic medical research and the clinical treatment of cancer, and there is a crosstalk between these two fields and lncRNAs. For example, *NKILA* mediates the apoptotic sensitivity of different subsets of T cells to orchestrate the balance of immune activation and immunosuppressive T cell subsets in the tumor microenvironment, which results in tumor immune escape¹⁹. *HISLA* maintains the continuous activation of HIF-1 α signaling in tumor cells under aerobic conditions to promote lactic acid secretion, which in turn upregulates macrophage *HISLA* expression and forms a positive feedback loop between lncRNA and tumor metabolism²⁰. At present, there exists a lncRNA cascade, including but not limited to *MALAT1*, *HOTAIR*, *UCA1*, *XIST*, *HULC*, and *BCAR4*, which gives rise to comprehensive mechanism networks for carcinogenesis. More details of this topic are discussed elsewhere^{21,22}. Therefore, lncRNAs represent a treasure to excavate carcinogenesis and corresponding drug development.

More recently, amounting evidence has begun to emphasize lncRNAs modulation in other diverse physiological and pathological processes. Among these, neurological and cardiovascular diseases are two areas of particular interest. LncRNAs are engaged in the pathogenesis of various neurological diseases, such as Parkinson's (PD), Alzheimer's (AD), Huntington's diseases, and lateral spinal cord sclerosis, and the associated mechanisms have gradually been elucidated²³. Among these, multiple lncRNAs (*e.g.*, *H19*, *MALAT1*, *TncRNA*, *lincRNA-p21*, and *SNHG1*) related to synapse formation and neural cell survival are differentially expressed in PD, which is indicative of their potential as drug targets and biomarkers²³. In addition, in AD, there is a cluster of lncRNAs that regulate neuronal apoptosis, such as *MALAT1*²⁴, *H19*²⁵, *BDNF-AS*²⁶ and *EBF3-AS*²⁷, suggesting that the regulation

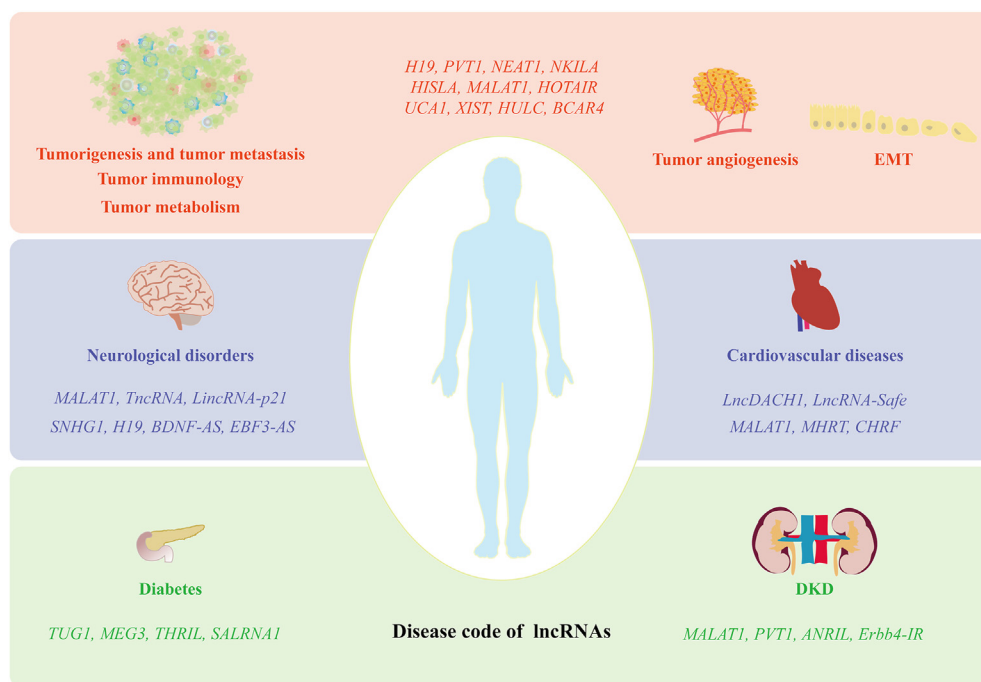


Figure 1 Representative well-studied lncRNAs involved in the different diseases mentioned in this review. lncRNAs participate in multifaceted process in tumorigenesis and tumor metastasis such as tumor angiogenesis and EMT, involving tumor immunology and metabolism. In addition, lncRNAs have been reported to be associated with many other diseases, especially chronic diseases, including but not limited to neurological disorders, cardiovascular diseases, diabetes, and DKD, which provides a basis for drug research and development targeting lncRNAs. lncRNAs, long non-coding RNAs; EMT, epithelial mesenchymal transition; DKD, diabetic kidney disease; *H19*, imprinted maternally expressed transcript; *PVT1*, plasmacytoma variant translocation 1; *NEAT1*, nuclear enriched abundant transcript 1; *NKILA*, NF-kappaB interacting lncRNA; *HISLA*, HIF-1 α -stabilizing long noncoding RNA; *MALATI*, metastasis-associated lung adenocarcinoma transcript 1; *HOTAIR*, HOX antisense intergenic RNA; *UCA1*, urothelial carcinoma-associated 1; *XIST*, X-inactive specific transcript; *HULC*, human universal load carrier; *BCAR4*, breast cancer anti-estrogen resistance 4; *TncRNA*, trophoblast-derived noncoding RNA; *LincRNA-p21*, long intergenic noncoding RNA p21; *SNHG1*, small nucleolar RNA host gene 1; *BDNF-AS*, brain-derived neurotrophic factor antisense RNA; *EBF3-AS*, early B cell factor 3 antisense RNA; *LncDACHI*, dachshund homolog 1; *MHRT*, myosin heavy chain associated RNA transcripts; *CHRF*, cardiac hypertrophy related factor; *TUG1*, taurine upregulated gene 1; *MEG3*, maternally expressed 3; *THRIL*, TNF and HNRNPL related immunoregulatory; *SALRNAI*, senescence associated long non-coding RNA 1; *ANRIL*, antisense non-coding RNA in the INK4 locus; *Erbb4-IR*, Erb-B2 receptor tyrosine kinase 4-immunoreactivity.

of lncRNA networks exerts unneglectable influence on the pathology of AD and that lncRNAs may shed new light on the unclear etiology of AD and the current unsatisfactory drug therapy.

lncRNAs are reported to participate in cardiovascular system development and biology, as well as in cardiovascular diseases²⁸, so the functional characterization of lncRNAs may aid in the prevention, monitoring, and treatment of diseases, including hypertension, heart failure (HF), cardiac hypertrophy, coronary artery disease, and myocardial infarction. For example, *DACHI*, a newly discovered lncRNA that regulates cardiac function, was upregulated in patients with HF, whereas *lncDACHI* knockout/knockdown in HF mice impeded the development of HF²⁹. *LncRNA-Safe* (AK137033) promoted myocardial fibrosis during myocardial infarction, and the inhibition of *Safe* in fibroblasts improved cardiac function; thus, *Safe* may be a new target for antifibrotic therapy³⁰. To date, *MALTAI*, *MHRT*, *CHRF*, and other lncRNAs have been studied in cardiovascular fields². However, whether the detection or modulation of these lncRNAs is helpful for the diagnosis, prevention, treatment, and prognosis calls for more data. Undoubtedly, the functions of lncRNAs in cardiac

biology and disease are largely unclear and should be explored to identify novel cardiovascular biomarkers and therapeutic targets.

In other chronic diseases, such as diabetes, lncRNAs are involved in pancreatic β -cell disorder (e.g., *TUG1*³¹), insulin resistance (e.g., *MEG3*³², *THRIL*, and *SALRNAI*³²), and its complications. One of the major diabetic complications, diabetic kidney disease (DKD) has become the leading cause of chronic kidney disease and end-stage renal disease³³, thus innovative biomarkers and therapies are urgently needed. Inflammation and fibrosis are fundamental steps in the onset and progression of DKD. In recent years, lncRNAs such as *MALATI*³⁴, *PVT1*³⁵, *ANRIL*³⁶ and *Erbb4-IR*³⁷ have been found to regulate ECM proteins and the expression of pro-fibrosis factors in DKD. *MALATI* is also able to govern inflammation in DKD through the regulation of inflammatory genes and cytokines. More studies are being conducted to clarify the role of lncRNAs in DKD.

The well-studied lncRNAs involved in different diseases mentioned in this review are summarized in Fig. 1. Information on lncRNAs and their functions in diseases is continuously being decoded, which should provide a theoretical basis to utilize lncRNAs to treat diseases, provide abundant potential drug target

candidates for subsequent drug design, and finally pave the way for a new drug arena of lncRNAs.

3. Current prevalent approaches to lncRNA targeting

3.1. Small interfering RNAs

Small interfering RNAs (siRNAs) complementary to the target lncRNAs can be applied to target lncRNAs. siRNAs recruit the RNA-induced silencing complex (RISC) containing arginine to induce lncRNA degradation. In lncRNA research, siRNAs have been used successfully in several preclinical models to study the therapeutic significance of targeting lncRNAs in various diseases³⁸. For example, in the *in vivo* and *in vitro* functional analysis of lncRNA *CASC9*, siRNA was used to target different sites within *CASC9*, and two sites, *CASC9-2* and *CASC9-3*, were determined to have the highest knockout efficiency. In subsequent experiments, *CASC9-2* siRNA cloned into a lentiviral vector significantly reduced the invasion and migration of esophageal squamous cell carcinoma³⁹. Moreover, tumor progression was conspicuously impeded when 1 mg/kg *DANCR* siRNA nanoparticles (NPs) was systematically administered in a triple-negative breast cancer mouse model⁴⁰. siRNA (1 mg/kg) targeting *Linc00311* and lncRNA AK141205 could effectively relieve neuropathic pain in rats by inhibiting the activation of the STAT3 signaling pathway⁴¹. siRNA-mediated LINC01296 knockdown in a non-small cell lung cancer model reduced the number of cancer cells *in vitro* and shrank the tumor mass *in vivo*⁴², demonstrating the ability of siRNA to systematically target specific lncRNAs.

Definitely, there is still one major challenge for siRNA now: off-target. Although this will be mitigated by lower doses and modifications of siRNA or delivery systems (overviewed later), the off-target effects have not been completely addressed^{43–45}. With the world's first siRNA drug Onpattro (patisiran) being used to treat patients with polyneuropathy caused by hereditary parathyroid amyloidosis and another siRNA drug, Givlaari (givosiran) being approved for the market last year for acute hepatic porphyria in adults, the investigation of lncRNA-based siRNA drugs may enter a new era.

3.2. Antisense oligonucleotides

Antisense oligonucleotides (ASOs) are a type of chemically synthesized short single-stranded oligonucleotides that have a 15- to 25-nucleotide DNA sequence. They can bind to complementary RNA and recruit RNase H, provoking RNA degradation and altering the expression of downstream proteins. Moreover, they can also inhibit transcription without the assistance of RNase H cleavage, such as by the spatial blocking of ribosomes⁴⁶. The chemical modification of ASOs has allowed the disadvantages of low cell permeability and poor stability to be overcome, and remarkable progress has been made in the improvement of their pharmacological properties. ASOs can be divided into three generations according to the chemical modification. The first generation is represented by phosphorothioate (PTO) oligonucleotides. PTO is nuclease-resistant and highly soluble; however, its specificity and affinity are insufficient and sometimes incur off-target effects, such as non-specific protein downregulation and cell apoptosis^{47,48}. The second-generation 2'-O-substituted ASOs have stronger binding ability⁴⁹, greater stability⁵⁰, lower toxicity, and lower immunostimulatory effects⁵¹ but a lower ability to

induce cleavage by RNase H⁵². The third generation ASOs, peptide nucleic acids (PNAs), phosphorodiamidate morpholino oligomers, and locked nucleic acids (LNAs), were designed for decent affinity and stability, but still have disadvantages. For example, RNase H1 recognition and cleavage specificity are not qualified enough, thus inducing hepatotoxicity along with the off-target effects. In addition, there are still difficulties with the delivery of third-generation ASOs, as they cannot be orally administered, mostly by injection^{53–55}. Thus, each generation has its own problems to some degree.

In the past few years, ASO-based therapies have led to clinical breakthroughs. The U.S. Food and Drug Administration (FDA) has approved three ASOs drugs for the treatment of neurodegenerative or muscular dystrophy. Nusinersen was approved for treating multiple forms of spinal muscular atrophy, eteplirsen for treating Duchenne muscular dystrophy, and inotersen for treating familial amyloid polyneuropathy, suggesting that ASOs may form the next generation of cutting-edge drugs for the treatment of neurological diseases⁵⁴. The application of ASOs is mainly focused on the treatment of PD, AD, amyotrophic lateral sclerosis, Huntington's disease, and cancer. Furthermore, a large number of ASO-based drugs are undergoing clinical trials.

With the use of ASO technology, research into lncRNAs targeted by ASOs has burgeoned, indicating the clinical prospects of lncRNA-targeted therapy. The subcutaneous delivery of *MALAT1* phosphorothioate-modified ASO successfully inhibited primary tumor differentiation and reduced lung metastasis in a mouse model of breast cancer, which offers preliminary evidence that *MALAT1* ASO is a therapeutic drug able to inhibit breast cancer progression⁵⁶.

In addition, the most recent third generation ASOs are often used to target lncRNAs. PNAs with modified nucleotide furanose rings are artificially synthesized DNA mimics resistant to nuclease degradation that can be further modified for systematic applications⁵⁷, and researchers have tried to use them for targeting various non-coding RNAs, including lncRNAs⁵⁸. For example, cross-linking pH-low insertion peptide (pHLIP) with PNAs can resist the acidic tumor microenvironment, effectively inhibiting *HOTAIR* activity both *in vitro* and *in vivo*, hence the proposal of pHLIP-PNA targeting lncRNAs for solid tumors treatment systematically⁵⁹. The combination of PNAs with cholesterol groups can increase hydrophobicity, thus promoting cell absorption and enhancing tissue targeting. The attachment of lysine groups can reinforce the release of endoplasmic bodies after the target cells taking up PNA⁵⁸.

LNA gapmeR, an ASO designed specifically for lncRNA and mRNA^{60,61}, has become one of the most widely used ASO-targeting lncRNAs in preclinical research. In the case of *NEAT1*, this technology was used to develop a new lncRNA function suppressor, g#N1_E LNA gapmeR, to trigger the RNase H-dependent degradation of lncRNAs. The main mechanism was that the LNA-gapmeRs sequence was complementary to the 5' region of *NEAT1*, which led to the *NEAT1* degradation. After *in vitro* delivery was optimized, g#N1_E LNA gapmeR resulted in 80%–90% of *NEAT1* downregulation. CD138+ cells from patients with multiple myeloma (MM) showed downregulation of *NEAT1* after 6 days of exposure to g#N1_E, and finally, a large number of cells died. Later, *in vivo* experiments in mice revealed that #N1#E LNA gapmeR could induce striking antitumor activity, simultaneously showing the best tumor absorption without systemic toxicity, consistent with previously documented data⁶¹. It was found that *MALAT1* has a tumor-promoting effect in MM

through the inhibition of proteasome activity. LNA gapmeR ASO, which was used to degrade *MALAT1*, displayed superior antitumor activity in a humanized mouse MM model and could induce cytotoxic effects through the destruction of the *MALAT1*/RF1 interrelation cycle and in combination with bortezomib, which provided preclinical evidence for the use of this new effective ASO-targeting lncRNA for the treatment of MM⁶². In addition to cancer, ASO-targeting lncRNAs have been applied in other diseases such as bone disease. It was discovered that *Lnc-ob1* (its human homolog is *LNC-OBI*) was rich in osteoblasts and significantly reduced during the loss of mouse and human bones. In lnc-ob1 knocked-in mice, bone density and bone formation rate were upregulated. An osteoblast-targeted drug delivery system⁶³ was used to package and deliver lnc-ob1 ASO intravenously in lnc-ob1 knockdown mice, which drastically inhibited the osteogenic process⁶⁴. Osteoblast-targeted *Lnc-ob1* can ameliorate ovariectomy-induced bone loss in mice, demonstrating the potential of targeted delivery of specific lncRNAs to treat osteoporosis in humans.

Existing randomized clinical trial results disclose the stable pharmacokinetics and safety of apolipoprotein-targeting ASOs⁶⁵. However, ASO, as a special nucleic acid drug, may also have potent off-target effects and difficulties associated with highly specific delivery as previously mentioned; thus, despite the numerous preclinical applications for targeting lncRNAs, further technological progress is required.

3.3. Clustered regularly interspaced short palindromic repeats

Clustered regularly interspaced short palindromic repeats (CRISPR) are found in many bacteria, and its related proteins (CRISPR-associated proteins, the Cas family) are involved in protection against mobile genetic elements. Essentially, the CRISPR/Cas system is an immune defense system for prokaryotes. One type of CRISPR system, the type II system, relies on a Cas protein that anchors to a defined DNA sequence, making it the basis for a genome-editing tool. The type II Cas protein in *Streptococcus pyogenes* has been shown to conduct RNA-guided DNA cutting in mammalian cells, opening a new stage in which CRISPR/Cas9 has become a widely used gene-editing tool.

The CRISPR/Cas9 system includes a single guide RNA (sgRNA) and a Cas9 enzyme; sgRNA guides the Cas9 nuclease to specific sites in the genome *via* complementary base pairing, and Cas9 cleaves the DNA sequence followed by the protospacer-adjacent motif. Owing to its outstanding accuracy, efficiency, permanence, and ease of programming^{66,67}, CRISPR/Cas9 has been successfully used to target lncRNAs and has stimulated research into lncRNAs. *Norad* is a non-coding RNA activated by DNA damage. To study the function and mechanism of lncRNA *Norad* at the whole animal level, a mouse model of lncRNA *Norad* deletion was created using the CRISPR/Cas9 system. *Norad*-deficient mice showed clear similarities in the multisystem degenerative phenotype of premature aging, causing severe genomic instability and mitochondrial dysfunction⁶⁸. Hence, CRISPR/Cas9 has been employed to explore lncRNA function. In addition, CRISPR/Cas9 is often used for the screening and annotation of lncRNAs⁶⁹.

Furthermore, the therapeutic effect of CRISPR/Cas9-targeting lncRNAs is still limited to basic research owing to the unpredictable clinical risk. However, according to *in vitro* and *in vivo* preclinical data, it is still a potential therapeutic tool. *TTY15* is a lncRNA highly expressed on the Y chromosome. In a knockdown

experiment aimed to confirm that *TTY15* promoted the proliferation and migration of prostate cancer cells, a double-sgRNA guided CRISPR/Cas9 system was used to achieve complete *TTY15* knockout and simultaneous CRISPR/Cas9-mediated transcription terminator knock-in to stop *TTY15* transcription. These different CRISPR/Cas9 strategies successfully knocked out *TTY15 in vitro*, resulting in the substantially reduced growth of *TTY15* knockout clones and weakened tumor phenotype in a xenograft mouse model⁷⁰. Another example is the use of a dual sgRNA CRISPR/Cas9 system to knockout *NEAT1*, which led to glioblastoma cells apoptosis and metastatic phenotype suppression⁷¹. These results indicate that CRISPR/Cas9 represents a possible brand-new technology to translate lncRNAs into clinical therapy.

In a genome-wide analysis, however, CRISPR/Cas9 was found to safely manipulate only 38% of 15,929 lncRNA *loci* without deregulating the expression of their divergent neighboring genes, which occurred mainly due to their intragenic or bidirectional promoters⁷². While this issue didn't happen when using siRNA or ASOs, hence adding the deficiency and limitation of CRISPR/Cas9. Globally, off-target effects, caused by inaccurate recognition and cleavage of similar sequences, are the first major hurdles for CRISPR/Cas9 bench-to bedside transition, as off-target effects will induce undesired genetic modifications. The current antidotes are off-target prediction, detection, and prevention, and there is no complete solution so far. Different algorithms and continuously improving technologies have been developed to predict and detect off-target respectively, such as the kinetic biophysical model of sequential target recognition established by Klein et al.⁷³ for off-target determinants prediction as well as off-target rate calculation, and the recently proposed prominent genome-wide off-target analysis to detect off-target mutations *via* whole genome sequencing without individual single-nucleotide variant interference⁷⁴. Modifications of Cas9 or sgRNA⁷⁵, as well as sgRNA truncation, are viable to reduce off-target effects^{76,77}, and the design of sgRNA with high-specificity for lncRNAs is not always easy but is a key component. Considerable software and online tools have been developed to assist in the design of sgRNAs⁷⁸.

Similar to siRNA and ASO, *in vivo* delivery for CRISPR/Cas9 is different from traditional medicine. Compared with *in vitro* or *ex vivo* systems, the *in vivo* delivery of CRISPR/Cas9 is immature. Thus, the clinical applications, especially to treat human disease, will be unachievable, unless we prevent off-target effects and solve systematic delivery and other potential thorny technical problems.

3.4. Selected applications of siRNA, ASOs, and CRISPR/Cas9

The cellular location of lncRNAs should be considered when choosing siRNA, ASOs or CRISPR/Cas9 for lncRNA targeting in preclinical models. ASOs can more effectively inhibit nuclear lncRNAs such as *MALAT1* and *NEAT1*, and siRNA can more effectively inhibit cytoplasmic lncRNAs such as *DANCR* and *OIP5-AS1* (for which RISC is present mainly in the cytoplasm). CRISPR/Cas9 can be applied for lncRNA knockout regardless of cellular location, but this technology has a relatively narrow lncRNA spectrum, as mentioned above. Therefore, for lncRNAs with unclear or dual cellular localization, using these methods alone makes it difficult to achieve effective interference. At this time, using both ASOs and siRNA concurrently can inhibit dual-localized lncRNAs. The recently improved Smart Silencer, a hybrid approach combining ASOs and siRNA reagents, is a mixture containing three siRNAs and

three ASOs, and has been successfully applied in countless experiments involving lncRNA knockdown *in vivo* and *in vitro*^{79–82}. For example, given the uncertain localization of lncRNA *RP11-544D21.2* and *XLOC_014288*, some researchers believed the expected knockout effect was difficult to achieve with traditional siRNAs, so they used Smart Silencer at a concentration of 50 nmol/L, which resulted in massive downregulation of the target lncRNAs⁷⁹. The optimal application of siRNA, ASOs, and CRISPR/Cas9, based on lncRNA intracellular localization, is shown in Fig. 2.

4. Small-molecule compounds

Similar to proteins, lncRNAs are equipped with multidimensional architecture; these secondary and tertiary structures are complicated but attractive targets awaiting understanding and exploitation. It is envisioned that specific lncRNA structures can be bound or blocked by small molecules. Therefore, in addition to nucleic acid technology, small-molecule compounds identified by library screening have been proven to be effective entities. They disrupt lncRNA spatial structure or lncRNA–protein interaction and will overcome the extra obstacles to the delivery of ASO/siRNA. These are based on the decipherment of lncRNAs functions in a definite disease, the appropriate structure within the exact lncRNA, and favorable “pockets” that can be stably bound by ligand molecules. Earlier, Pedram Fatemi et al.⁸³ developed ALPHA screening technology to quantify lncRNA–protein interactions and identify a small molecule, ellipticine, that could inhibit the interaction of *BDNF-AS*–*EZH2* and *HOTAIR*–*EZH2*, which demonstrated the use of high-throughput screening to identify lncRNA–protein interactions and target lncRNAs with small molecules⁸³. Here, we first used *MALAT1*, a lncRNA with notable drug potential^{84,85}, as an example of the roadmap to the discovery of small-molecule modulators based on their chemical structure, to illustrate the tremendous potential of small molecules as lncRNA-targeted drugs.

There is a highly conserved element for nuclear expression (ENE) at the 3' end of *MALAT1*. The triple helix structure of ENE, which ensures that *MALAT1* is not degraded, is necessary for the

physiological function of *MALAT1*^{86–88}. Recently, Donlic et al.⁸⁹ confirmed that the triple helix structure at the 3' end of *MALAT1* could be selectively targeted by small molecules. In their study, a series of diphenyl furan (DPF)-derivates based on RNA-binding scaffolds were synthesized, and molecules with different structural shapes, which are essential for lncRNA recognition, were screened to assess the binding strength with the *MALAT1* triple helix and stem loop *via* fluorescence-based screening. Rod-like DPFs were found to be the most promising ligands from principal moments of inertia calculations. This work encouraged the exploration of other small molecules for lncRNAs⁸⁹. Abulwerdi et al.⁹⁰ used small-molecule microarray and molecular docking technology to select two specific compounds that could specifically bind the *MALAT1* 3' triple helix structure with different mechanisms and binding modes, as measured with docking poses analysis using AutoDock 4, and compound 5 presented better regulation of *MALAT1* downstream gene *Csn2* than ASO⁹⁰. This further displayed the development prospects of small lncRNA-targeting molecules.

The second example is lncRNA *GAS5*, whose decrease is closely related to insulin resistance and diabetes^{91–93}, and it has been tested as a drug target through compound library screening. Briefly, Shi et al.⁹⁴ synthesized a γ -AApeptide combinatorial library and employed a one-bead-two-compound screening strategy with the help of fluorescein-tagged oligonucleotide, which identified hit small molecules that bound and stabilized lncRNA *GAS5* through disruption of the interaction between *GAS5* and *UTF1*, a transcriptional factor downregulating *GAS5*. Finally, they tested the ability of NP-C86 to competitively bind to *GAS5* transcript with high affinity and specificity, thus restoring the *GAS5* level to that of normal physiological levels without causing *GAS5* overexpression, which was confirmed at the tissue level⁹⁴. This is an elegant example of translating the idea of using small-molecular compounds targeting lncRNAs into practice. Among the existing small-molecular drugs, there are also some molecules acting prominently on lncRNAs to exert pharmacological effects. For example, in Ewing sarcoma cells, *HULC* acted as a sponge for miR-186 to promote the expression of the

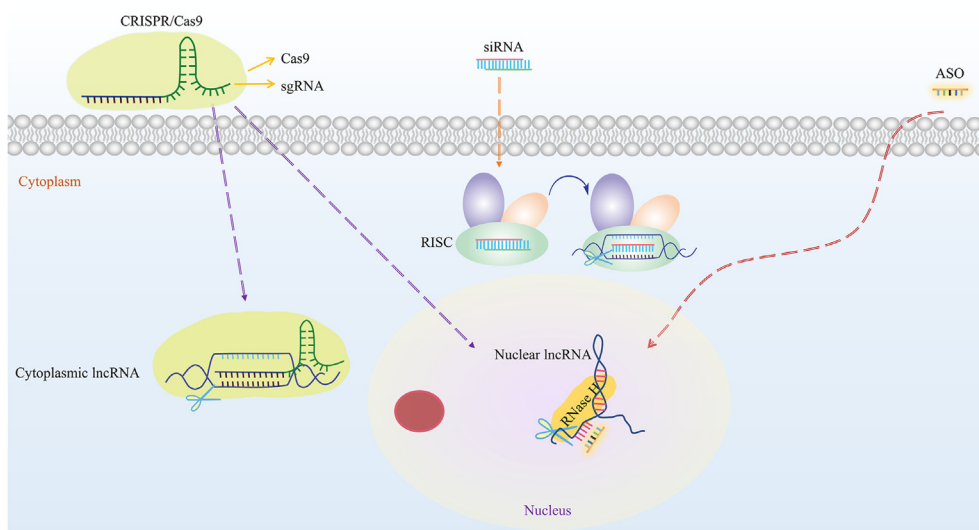


Figure 2 Respective optimal applications of siRNA, CRISPR/Cas9, and ASOs based on lncRNA intracellular localization. siRNAs are appropriate for cytoplasmic lncRNAs, ASOs for nuclear lncRNAs, and CRISPR/Cas9 for dual-localized lncRNAs or those with unknown cellular localization. CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR associated protein 9; sgRNA, single guide RNA; ASO, antisense oligonucleotide; RISC, RNA-induced silencing complex.

Table 1 Examples of applications of targeting strategies in lncRNAs-based intervention as mentioned in this account.

Approach	Mechanism/origin	Targeted lncRNA	Condition	Ref.
siRNA	Being complementary to the target lncRNAs and recruiting RISC to induce lncRNA degradation	<i>CASC9</i>	Esophageal squamous cell carcinoma	39
		<i>DANCR</i>	Triple-negative breast cancer	40
		<i>LncRNAAK141205</i>	Neuropathic pain	41
		<i>LINC01296</i>	Non-small cell lung cancer	42
ASO	Binding to complementary RNA and recruiting RNase H to degrade lncRNAs	<i>MALAT1</i>	Breast cancer	56
		<i>MALAT1</i>	Multiple myeloma	62
		<i>HOTAIR</i>	Breast cancer	59
		<i>NEAT1</i>	Multiple myeloma	61
		<i>Lnc-ob1</i>	Osteoporosis	64
CRISPR/Ca9	sgRNA recognizing targeted lncRNA sequence Cas9 nuclease conducting lncRNA degradation	<i>Norad</i>	Normal physiology and aging	68
		<i>TTY15</i>	Prostate cancer	70
		<i>NEAT1</i>	Glioblastoma	71
Small molecule	ALPHA screening quantifying lncRNA–protein interactions and identifying small molecules ellipticine Synthesizing a small molecule library based on an RNA-binding scaffold and then screening Small molecule microarray and molecular docking technology selecting two specific compounds One-bead-two-compound screening strategy identifying NP-C86 Existing small molecule YK-4-279 inducing lncRNA downregulation	<i>BDNF-AS&HOTAIR</i>	–	83
		<i>MALAT1</i>	–	89
		<i>MALAT1</i>	–	90
		<i>GAS5</i>	Diabetes	94
		<i>HULC</i>	Ewing sarcoma cells	95
Natural molecule	Resveratrol Curcumin Berberine	<i>MALAT1</i>	Parkinson's disease	99
		<i>NEAT1</i>	Multiple myeloma	102
		<i>H19</i>	Gastric cancer cells	103
		<i>MALAT1</i>	Colon cancer cells	104
		538 lncRNAs	Non-alcoholic fatty liver disease	105

CASC9, cancer susceptibility candidate 9; *DANCR*, differentiation antagonizing non-protein coding RNA; *Norad*, non-coding RNA activated by DNA damage; *TTY15*, testis-specific transcript, Y-linked 15; *GAS5*, growth arrest specific 5.

–Not applicable.

oncogene *TWIST1*, whereas the small-molecule YK-4-279 can inhibit this axis by releasing miR-186 from *HULC*; however, the exact mechanism remains to be determined⁹⁵. These achievements, as stated above, will support the use of lncRNA-targeting small molecular compounds.

In this respect, it is necessary to acquire information on three principal stages: elucidating the action mechanisms of lncRNAs, analyzing the functional structure pocket of lncRNAs, and finding molecules that can form specific binding sites with the pocket. Among them, the development of techniques for lncRNAs structural analysis and the recognition of interactions between lncRNAs and other molecules is essential⁹⁶. Then, establishing screening methods and libraries is helpful for the rapid identification of drug-like molecules and highly complex lncRNA motifs to improve binding specificity and affinity^{88,97}. On the other hand, the compounds obtained by this approach require further *in vitro* and *in vivo* model tests to confirm their structure–function relationships and actual pharmacological effects.

Based on currently published literature, research into small-molecule inhibitors of lncRNAs is still relatively limited, so it is uncertain whether small molecules will outperform nucleic acid technology for the development of lncRNA-based drugs. However, compared with the nucleic acid drugs described above, small-molecule inhibitors have the advantages of lower cost and more convenient administration modes, so it is reasonable to develop them as lncRNA-targeted drugs, either alone or in combination with other techniques. More critically, small molecules target lncRNAs in a structure-specific manner rather than in a

sequence-complementary manner and may orchestrate lncRNA functions without changing their expression, which cannot be achieved *via* siRNAs, ASOs, and CRISPR, because small molecules can only disrupt the binding/interaction between lncRNAs and other biomolecules. Naturally, to determine the qualified fine-tuning of small molecules, extensive research into lncRNAs is required.

5. lncRNA regulators derived from natural plants

A large number of studies have shown that in models of diseases such as cancer, non-alcoholic fatty liver disease, osteoporosis, and PD^{98–101}, plant-derived natural compounds have a credible regulatory effect on lncRNAs, including *MALAT1*, *PVT1*, *HOTAIR*, *H19*, and *NEAT1*. For example, resveratrol ameliorates PD through the regulation of *MALAT1* and its downstream signaling pathways⁹⁹. Besides, resveratrol inhibits the proliferation and migration of MM cells *via* *NEAT1*/Wnt/ β -catenin, showing the lncRNA-related mechanism of resveratrol in cancer treatment¹⁰². However, this should be confirmed *in vivo*. Curcumin, another natural molecule with anticancer effects, can downregulate *H19* and promote the expression of *p53* in a time- and concentration-dependent manner¹⁰³. When combining curcumin with si-*MALAT1*, downregulation of *MALAT1* was more effective *in vitro*¹⁰⁴, suggesting the potential of phytochemicals to regulate lncRNA. *In vivo*, berberine altered the expression of 538 lncRNAs in a rat model of non-alcoholic fatty liver disease¹⁰⁵, indicating a new possible mechanism for the effect of berberine⁹⁸.

By searching website “ClinicalTrial.gov”, several natural compounds mentioned above have been tested in clinical trials, such as curcumin against colorectal cancer, and resveratrol and berberine have moved into phase II or phase III clinical trials for some metabolic related diseases (e.g., NCT02439385, NCT03597568, NCT02114892, and NCT03251716). However, the lncRNAs are not claimed as major targets of these natural compounds at beginning, but are novel possible intracellular targets that can be referred to explain their pharmacological activities to some extent.

Optimistically, phytochemicals come from a wide range of herbal sources and are relatively safe with a long history in traditional Chinese medicine. The lack of accurate targets and mechanisms is a shortcoming of phytochemicals. Traditionally, proteins in the human body are the most possible targets for phytochemicals. However, targeting lncRNAs currently provides an innovative perspective to explain their bioactivities and to possibly extend their usage in clinical trials. Approaches targeting lncRNAs and their examples are summarized in Table 1.

6. Delivery systems

The delivery system is the key to the envisioned drug role for lncRNAs. It must have a satisfactory specificity, stability, cell permeability, and low immunogenicity. Classical carriers are mainly divided into viral vectors and non-viral vectors.

6.1. Exogenous vectors

6.1.1. Viral vectors

Lentiviral vectors are the most widely used lncRNAs-carrying systems. They have a high transfection efficiency and long expression time. As a typical tool, lentiviral vectors are used in most studies to interfere with lncRNAs, such as by loading specific lncRNAs or siRNAs, to achieve targeted lncRNA cellular overexpression and knockdown^{106,107}.

Adenoviral vectors are also commonly used for lncRNA overexpression or interference^{29,108}. An adenovirus is an unencapsulated linear double-stranded DNA virus, with an approximate diameter of 80–120 nm, which can rapidly infect dividing and non-dividing cells. The knockdown models of the *LncLGR* gene can be effectively constructed by the injection of an adenovirus carrying *LncLGR* siRNA into mice¹⁰⁸. By modifying the wild-type adenovirus, preserving the packaging signal sequence, and removing the viral protein-coding sequence, the loading capacity for foreign genes increases while cytotoxicity and immune response simultaneously reduce in the body; overall, this results in a safer system for the introduction of foreign nucleic acids.

In light of the controversy over the safety of viral vectors, they have been gradually replaced by other vectors in systemic drug administration, which we discuss as below.

6.1.2. Non-viral vectors

Liposomes are ideal materials for the targeted delivery of lncRNAs, especially given the development of nanomaterials. Nanoliposomes have become increasingly popular in preclinical trials in which the function and therapeutic potential of lncRNAs are studied. Liposomes have many desirable qualities¹⁰⁹. In addition to reduced toxicity and immunogenicity, liposome encapsulation has also improved drug stability and targeting to liver tissues, which can ensure that the encapsulated material, such

as siRNA, is not filtered and removed by the kidney, and is gradually absorbed by target cells in the liver during blood circulation. Several liposome-encapsulated DNA (NCT01502358) or mRNA (NCT03382405) *in vivo* delivery systems have entered into clinical trials, and they are also frequently used to deliver lncRNAs in preclinical experiments *in vitro* and *in vivo*^{110–112}. Liposomes can be cationic or cationic-anionic exchangeable up to certain pH values, allowing flexibility in the increase of drug-carrying efficiency, as well as the control of drug delivery and release^{113–116}.

Lipid NPs are similar to liposomes; however, they have greater diversity and are more suitable for encapsulating a variety of nucleic acid drugs, and have become the most popular non-viral delivery system used in gene therapy^{117,118}. In lncRNA delivery, RGD-PEG-ECO/siRNA *DNCR*-targeted NPs have been proven to effectively inhibit tumor growth in preclinical animal trials⁴⁰, with no obvious side effects, showing the high efficiency and safety of lncRNA nanoparticle administration. Intranuclear lncRNAs are more difficult to target because of the nuclear envelope protection. Nevertheless, the recent application of ASO-gold-TAT NPs targeting the nuclear lncRNA *MALAT1* in a mouse model of xenotransplanted lung cancer noticeably suppressed tumor metastasis, and the survival time of mice was prolonged by 80%. This was a result of the highly specific nuclear localization ability of ASOs conducted by NPs¹¹⁹. Therefore, gold NPs bounded with transmembrane peptide (or TAT), which have good biocompatibility, chemical inertia, multivalent effect, and easy functionalization, can promote nuclear-lncRNA-targeted ASO applications. Moreover, rod-like supramolecular nanoassemblies, combining the unique characteristics of CNC, PGEA, and PAsp, when cotransfected with *MEG3* and miR101, can be condensed into nanocomposites with a particle size less than 200 nm to achieve cooperative delivery of non-coding RNAs with different sizes and enhance drug targeting and therapeutic efficacy¹²⁰.

In many cases, liposomes and lipid NPs are coupled with additional molecules, including nucleic acid aptamers, antibodies, peptides, protein ligands, polymers, or small molecules, to form an active or passive targeted drug delivery system (Fig. 3). Similarly, sometimes non-coding RNA drugs themselves are modified; for example, in the paper of Das et al.¹²¹, the S1 aptamer is fused with lncRNAs to increase the amount of lncRNA LOC284454 in target cells. Moreover, through the use of diverse organic polymers, especially plant-derived polymers, polyplex/polymer micelles^{122–124} and polymer NPs^{125,126}, can also contribute to better drug delivery. For example, it has been proposed that polyurethane nanocapsules act differently from traditional lipid NPs as they encapsulate small nucleic acids *via* electrostatic interactions. Preclinical results have validated their good biocompatibility, low toxicity, and good mechanical elasticity⁶³, some of which are also suitable for lncRNAs¹²⁷.

6.2. Endogenous vector: Exosomes

Exosomes are 30–200 nm membrane-bound vesicles naturally secreted by cells and contain lipids, proteins, DNA, mRNAs, miRNAs, and lncRNAs¹²⁸. Evidence is accumulating to confirm that exosomes have instrumental value in disease diagnosis and targeted therapy. As endogenous nano-vesicles, exosomes are of paramount priority for the delivery of protein and nucleic acid drugs, and even exceed liposomes in loading efficiency¹²⁹.

Going back to two decades ago, Alvarez-Erviti et al.¹³⁰ injected self-exosomes secreted from dendritic cells and

engineeringly loaded with *GAPDH* siRNA into mice, demonstrating that siRNA encapsulated in targeting ligand-expressing exosomes can pass through the blood–brain barrier to specifically target brain tissue, and knockdown specific genes. This was the first proposed concept of exosomes as nucleic acid drug delivery vehicles. Recently, Haney et al.¹³¹ delivered exosomes *via* nasal injection into a PD mouse model and identified specific neuroprotective effects. Kamerkar et al.¹²⁹ designed exosomes from normal fibroblast-like mesenchymal cells as a vector carrying specific siRNAs or shRNAs, and observed a drastic reduction in tumor volume and improved survival rate in mice with pancreatic cancer.

Therefore, extracellular vesicles, represented by exosomes, are very promising delivery vectors and can be engineered to restore or knockdown pathogenic lncRNAs expression in lncRNA-dominating diseases. Compared with viral and non-viral nanocarriers, exosomes have lower immunogenicity and higher stability *in vivo*; specially, patient-derived tissues are suitable as a source of individualized and biocompatible drug-packaged exosomes. Due to the mediocre efficiency of exosomes in packaging large nucleic acids, exosomes can now be integrated with liposomes (Fig. 3), organic NPs, or polymer NPs to improve the specificity and controllability of drug delivery systems. For example, exosome–liposome hybrids have been used successfully for *in vivo* and *in vitro* delivery in CRISPR–Cas9 systems, symbolizing a new direction for precision medicine^{132,133}. Parallel to exogenous NPs, surface modification is also a practical tactic for exosome delivery optimization; these can be cataloged as genetic engineering or chemical modifications¹³⁴. Most essentially, the

exosome membrane is always incorporated specific peptides which bind their receptors within the target tissue^{130,135,136}, as shown in Fig. 4.

The described delivery systems have their own fine features and defects, which suggests that it is a preferable choice to allow their comprehensive and conjunctive use^{134,136}. In contrast, the purification and stability of exosomes are paradoxical. To this end, exosome drug delivery, especially for RNAs with special structure, is still in its infancy and requires further improvement.

7. Clinical trials: Mitochondrial lncRNAs

Although most of the studies on lncRNA targeting are still in the preclinical stage, research into mitochondrial long non-coding RNA (mtlncRNA) has progressed rapidly.

When the two strands of mitochondrial DNA are fully transcribed, the light chain carrying only seven tRNA genes and the gene encoding ND6 protein generates and releases large non-coding sequences, including lncRNA, during transcription processing¹³⁷. Mitochondrial energy metabolism and functions related to the induction of apoptosis, inflammation, and metastasis are mediated, to a certain extent, by mtlncRNA. The lncRNAs produced by mtlncRNA mainly include sense mtlncRNA (sense non-coding mitochondrial RNAs, SncmtRNA) and antisense non-coding mitochondrial RNA (ASncmtRNA)¹³⁸. Undoubtedly, the regulatory property of mtlncRNA in diseases and its clinical application are of great value. For example, mtlncRNA *LIPCAR* is a new biomarker for cardiac remodeling

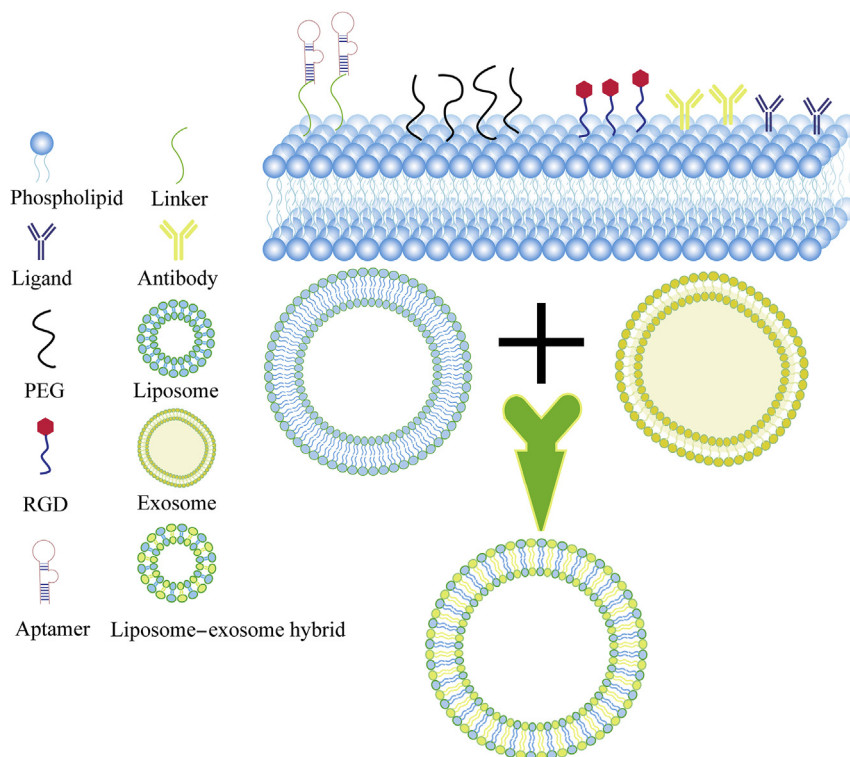


Figure 3 Mainstream surface modifications for liposomes/lipid nanoparticles, as well as liposome–exosome hybrids in lncRNA drug delivery. Liposomes/lipid nanoparticles can be moderated by ligands, PEG, RGD, aptamers, linkers, or antibodies to improve delivery performance. In addition, liposome–exosome hybrids are new drug delivery agents that can be successfully applied in preclinical experiments. PEG, polyethylene glycol; RGD, arginine-glycine-aspartic acid peptide.

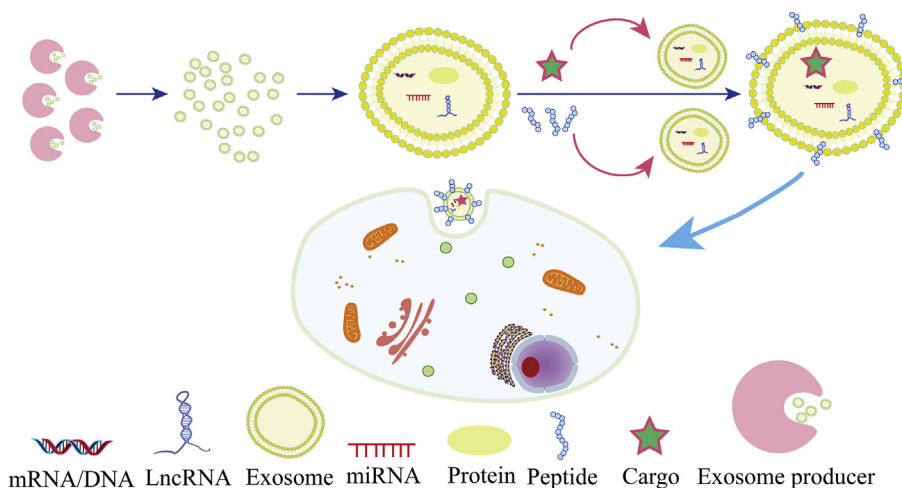


Figure 4 Schematic illustration of peptide-decorated exosomes for targeted lncRNA delivery. Exosome producers can be living cells, in particular, cells from patients themselves. The exosome membrane is always modified with specific peptides binding to their receptors within the target tissue, thus enhancing the specificity of exosome delivery.

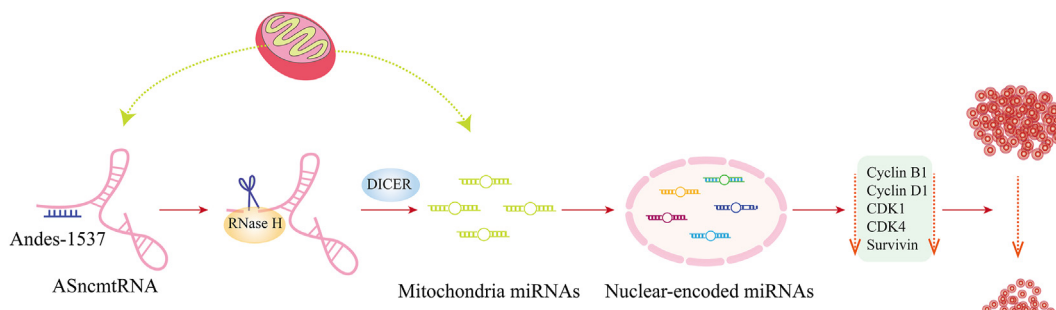


Figure 5 Schematic illustration of the mechanism of Andes-1537. The binding between Andes-1537 and ASncmtRNA activates RNase H degradation in this binding region. DICER then releases mitochondrial miRNAs, mainly hsa-miR-4485, inducing an increase in some specific nuclear-encoded miRNAs. These miRNAs (such as hsa-miR-5096 and hsa-miR-3609) downregulate cell cycle progression factors including cyclin B1, cyclin D1, CDK1, CDK4, and survivin, and consequently reduce cell cycle and tumor cells. Further details can be found in the previous study¹⁴⁵ [Adapted with modification from Ref. 145 © The Author(s) 2019]. ASncmtRNA, antisense non-coding mitochondrial RNA; CDK, cyclin dependent kinase.

that can predict the survival of patients with heart failure¹³⁹. In recent years, two types of ASncmtRNAs have been identified: ASncmtRNA-1 and ASncmtRNA-2 (hereinafter collectively referred to as ASncmtRNAs), which are downregulated in various tumor tissues¹⁴⁰. It was then reported that ASOs targeting ASncmtRNAs could induce death in a large area of cancer cells without affecting normal cells. Subsequent *in vivo* experiments in immunoreactive mice showed that targeted therapy of ASncmtRNAs could prevent lung metastasis after melanoma resection¹⁴¹. The lentiviral construct targeting ASncmtRNAs obviously blocked B16F10 primary tumor proliferation¹⁴². These results supported the clinical application of ASncmtRNAs-targeting ASOs for the treatment of melanoma. Similarly, *in vivo* studies in isogenic mouse renal cancer models manifested that ASncmtRNAs-targeting ASOs could completely reverse tumor growth¹⁴³. Based on these animal experiments, ASOs targeting specific mtlncRNAs may be a safe and effective tumor treatment strategy, providing a reliable basis for subsequent clinical trials.

To this end, FDA has approved a clinical trial of Andes-1537, a short single-stranded phosphorothioate ASO, which binds to ASncmtRNAs through complementary base pairs, for the targeted treatment of solid tumors, including a phase I clinical trial recruiting patients with advanced metastatic cancer (NCT02508441). The phase I clinical results showed that Andes-1537 was well-tolerated¹⁴⁴. Although it has been discontinued (the second part was terminated to redefine a new regimen), the first part of the study was completed and the maximum tolerated dose was determined. Another clinical trial of Andes-1537 for multiple solid tumors (NCT03985072) was started last year. Furthermore, to a deeper degree, the related research group carried out mechanistic experiments to explain how Andes-1537 realize its anti-tumor effects¹⁴⁵; for details see Fig. 5, presented according to the results of Fitzpatrick et al.¹⁴⁵

However, the roles and mechanisms of mtlncRNAs in tumors, especially tumor metabolism and other functions, have not been unraveled completely; as such, further research is indisputably

needed, which will lay the foundation for the reasonable clinical application of mtIncRNA.

8. Summary and outlook

Although the future is bright, some concerns remain about the possible adverse effects of gene therapy targeting lncRNAs as it is still an emerging concept and strategy compared with the traditional drug targets and proteins. The greatest risk is that basic research on the function of druggable lncRNAs and the potential downstream effects is insufficient currently; therefore, unexpected risks and inappropriate pathological effects may occur when lncRNA-targeted drugs are used clinically. Specifically, off-target effects may lead to adverse effects. Highly specific targeting approaches and delivery systems require improvement to guarantee that only the selected lncRNA is affected. Owing to the lack of sufficient data on clinical trials, the efficacy and safety of lncRNA drugs in humans remain inconclusive.

Target selection is a key element of drug development; therefore, identifying the most potential lncRNAs is the first step and the most important process. All the possible interactions between druggable lncRNAs and the intracellular miRNAs or proteins they regulated should be elucidated to clarify their role in the physiological activity *in vitro* and *in vivo*. Further advances in lncRNA-targeted drugs are clearly dependent on the in-depth basic research into the function and mechanisms of lncRNAs. Of course, accurate approaches to the lncRNAs targeting and efficient delivery systems can significantly speed up this progress.

lncRNA-targeted drug design provides a plethora of opportunities and challenges for the drug industry. Current gene therapy aims to treat disease by artificially controlling gene expression and is considered to be the “third generation” of therapeutic drugs, following chemical small-molecule drugs and biological macromolecular drugs. This type of therapy is promising for modulating diseases at the genetic level, and able to overcome the limitations of incompatible proteins. From the perspective of the clinical breakthrough in targeted mRNA drugs and the cumulative recruitment for clinical trials of miRNAs, it is conceivable that targeting lncRNAs will likely play a pivotal role in gene therapy in the near future, enabling new options for precision medicine.

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Author contributions

Yuanyuan Chen collected literature and wrote this review under the guidance of Sen Zhang. Zhaojun Li and Xiaoguang Chen helped to revise the manuscript.

Conflicts of interest

The authors declare no conflicts of competitive interest.

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