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*Review Article*

# Emerging roles of lncRNA in cancer and therapeutic opportunities

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**Abstract:** Cancer is difficult to cure due to frequent metastasis, and developing effective therapeutic approaches to treat cancer is urgently important. Long non-coding RNAs (lncRNAs) have diverse roles in regulating gene expression at both the transcriptional and translational levels and have been reported to be involved in tumorigenesis and tumor metastasis. In this article, we review the emerging roles of lncRNAs in cancer, especially in cancer immunity, cancer metabolism and cancer metastasis. We also discuss the use of novel technologies, such as antisense oligonucleotides, CRISPR-Cas9 and nanomedicines, to target lncRNAs and thus control cancers.

**Keywords:** LncRNA, tumorigenesis, tumor metastasis, ASO, CRISPR-Cas9, virus, nanomedicine

## Introduction

Cancer is among the most life-threatening diseases, and its morbidity and mortality rank first or second among noncommunicable diseases [1]. According to global cancer data from 2018, cancer morbidity and mortality are increasing annually with the rapid growth of the population and the problem of the aging population. The number of cancer deaths worldwide reached 9.55 million, with an incidence rate of 18.08 million [2]. During the past several decades, great success in treating cancer has been achieved. However, the survival time of most cancer patients is still poor, especially that of advanced cancer patients with metastasis. There is an urgent need to understand more about the molecular mechanisms governing tumor progression and to develop more effective clinical strategies for cancer treatment.

RNA-based therapeutics against cancer has gradually changed from concept to reality [3, 4]. Among these therapeutics, non-coding RNA (ncRNA), which refers to a class of RNA that does not encode protein, exerts clinical therapeutic effects against tumors by inhibiting the transcription of mRNA and binding to protein to block its function [5]. According to the molecular size of ncRNA, it can be classified as either small non-coding RNA (sncRNA), measuring under 200 nucleotides in length, or long non-coding RNA (lncRNA), measuring over 200 nucleotides in length [6].

An increasing number of studies have documented that lncRNAs play diverse roles in regulating gene transcription, post-transcription, translation, and epigenetic modification. Aberrant expression or dysfunction of lncRNA is closely associated with various diseases [7-10]. LncRNAs may regulate cell proliferation, apoptosis, migration, invasion and maintenance of stemness during cancer development [11, 12]. Recent studies have shown that lncRNAs may also engage in remodeling the tumor microenvironment and tumor metastasis. In this review, we will discuss the emerging roles of lncRNAs in tumorigenesis, tumor immunity, tumor metabolism and tumor metastasis. Considering the pivotal roles of lncRNAs in cancer, lncRNA-based therapeutics may represent promising approaches in treating cancer.

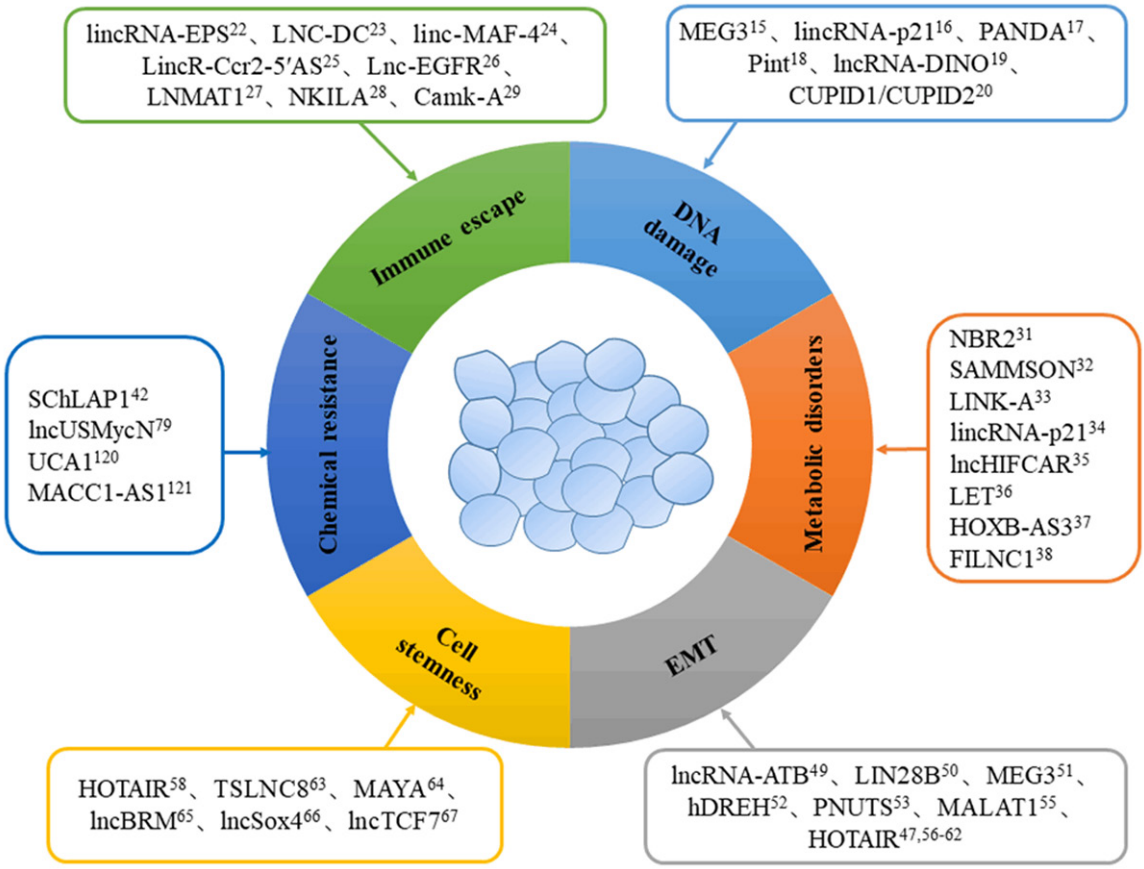


**Figure 1.** LncRNA in tumorigenesis and tumor metastasis. Divided into six subtypes: Immune escape, DNA damage, Metabolic disorders, EMT, Cell stemness, Chemical resistance.

## LncRNAs in tumorigenesis and tumor progression

Tumor formation is not only marked by the unrestricted expansion of tumor cells but also includes many biological processes, such as genomic mutation, DNA damage, immune escape, and metabolic disorder. LncRNAs are distributed in both the nucleus and cytoplasm and serve as important post-transcriptional translational regulators in these processes (**Figure 1**).

*DNA damage*

DNA damage caused by exogenous factors and chemicals or its abnormal cellular localization could transmit dangerous signals in cells [13] (**Figure 1**). The accumulation of DNA damage is one of the symbols of tumor progression. Many molecules participate in facilitating or inhibiting DNA damage.

P53, which has been extensively studied as a tumor suppressor, plays important roles in DNA damage. The lncRNA *MEG3* activates p53 to exert its anti-cancer effect [14]. Many other lncRNAs are associated with the downstream activities of p53 [15-17]. When DNA is damaged, the transcription of the lncRNA damage induced noncoding (*DINO*) is activated *via* p53, thereby controlling the stress response after DNA damage [18]. Additionally, specific expression of lncRNA-*DINO* activates the impaired signaling pathway and cell cycle arrest in the absence of DNA damage [18] (**Figure 1**). In addition, lncRNA *CCND1*-upstream intergenic DNA repair 1 and 2 (*CUPID1* and *CUPID2*) involves in the progression of breast cancer by modulating the stress response to DNA damage [19]. These studies indicate that lncRNAs respond to DNA damage and may be involved in DNA repair, which is a fundamental issue for carcinogenesis.

*Immune escape*

Immune escape has long been considered as one of the hallmarks of cancer. Tumor cells may train macrophages and regulatory T cells (Treg),

forming an inseparable "fortress", to escape from threats of killer T cells. LncRNAs have been documented to involve innate and adaptive immune responses by modulating the functional status of immune cells [20-24]. For instance, lnc-*EGFR* promoted immune escape of hepatocellular carcinoma cells by stimulating the differentiation of Treg cells [25]. In the tumor microenvironment, tumor-associated macrophages display limited phagocytosis function and promote the progression of cancer. LncRNA lymph node metastasis associated transcript 1 (*LNMAT1*) was involved in the regulation of CCL2 recruiting macrophages into the tumor [26] (**Figure 1**).

In tumor-specific cytotoxic T lymphocytes (CTLs) and type 1 helper T (TH1) cells, an NF-κB-interacting long noncoding RNA (*NKILA*) may enhance T cell sensitivity to activation-induced cell death by mechanically inhibiting the NF-κB signaling pathway [27]. In addition, lncRNA *CamK-A* participates in the remodeling of the tumor microenvironment *via* activation of $Ca^{2+}$-triggered signaling [28] (**Figure 1**).

Taken together, these evidences suggest that lncRNAs may be pivotal regulators in remodeling the tumor immune microenvironment.

*Metabolic disorders*

Cellular metabolic disorder is one of the most prominent characteristics of cancer. Abnormal cellular metabolic processes not only provide energy for the proliferation of cancer cells, but also maintain cellular redox homeostasis by inhibiting reactive oxygen species production. Notably, the proportion of cellular metabolites ATP/AMP is altered by various stimulations. Energy stress may increase the ratio of AMP/ATP which activates AMP-activated protein kinase (AMPK) [29] (**Figure 1**).

Under energy stress, the lncRNA neighbor of BRCA1 gene 2 (*NBR2*) activated AMPK via direct binding. Knockdown of lncRNA-*NBR2* lead to cell metabolism disorders and subsequently promoted cell proliferation [30]. Mitochondria are the center of energy metabolism, and their homeostasis is also affected by lncRNA. The lncRNA-*SAMMSON* bound to the major mitochondrial regulator p32 protein in melanoma cells and enhanced its cancer-promoting function [31] (**Figure 1**).

In addition, glycolysis replacing oxidative phosphorylation is the principal mode of energy metabolism in cancer cells. Hypoxia-inducible factor 1-alpha (HIF-1α) plays an important role in this process. Recent studies have reported an interaction between HIF-1α and lncRNAs. Long intergenic non-coding RNA for kinase activation (*LINK-A*) regulated the phosphorylation of HIF-1α, maintained its stability, and activated the transcriptional program of HIF-1α to promote tumorigenesis of triple-negative breast cancer (TNBC) [32]. HIF-1α up-regulated the expression of lincRNA-*p21* and participated in tumor formation by regulating the Warburg effect [33]. Long noncoding HIF-1α co-activating RNA (*LncHIFCAR*) is a co-activator of HIF-1α that drives the progression of oral cancer [34] (**Figure 1**).

The redox reaction is inhibited during the proliferation of tumors, and ATP is mainly formed by the decomposition of pyruvate by lactate dehydrogenase, creating a hypoxia microenvironment inside of the tumor. In a hypoxic environment, histone deacetylase 3 inhibits the expression of lncRNA low expression in tumor (*LET*) by reducing histone acetylation of the lncRNA-*LET* promoter region, and the low expression of lncRNA-*LET* is a key step in stabilizing the nuclear factor 90 protein, thereby promoting cancer cell invasion [35] (**Figure 1**).

In addition, lncRNA *HOXB-AS3* participated in the metabolism of cancer by affecting the expression of a conserved 53-amino acid peptide [36], while lncRNA FoxO-induced long noncoding RNA 1 (*FILNC1*) functioned as a tumor suppressor. The down-regulation of *FILNC1* enhanced glucose metabolism and lactic acid production by increasing the expression of c-Myc [37] (**Figure 1**).

These evidences suggest that lncRNA is involved in many aspects of cell metabolism, such as ATP production, the hypoxic environment, and Warburg effect regulation. Therefore, these lncRNAs may serve as potential therapeutic targets through inhibiting tumor energy production and reprogramming its growth microenvironment.

## LncRNA in tumor metastasis

*EMT*

Epithelial-mesenchymal transition (EMT) is a complex multi-step biological process that is

orchestrated by a variety of EMT-inducing transcription factors. Briefly, epithelial-like cells transdifferentiate into mesenchymal-like cells, facilitating their invasion and migration into blood vessels and lymphatic vessels, thereby participating in the metastasis of a variety of cancers [38-41]. Previous studies have also found that lncRNAs are involved in the regulation of EMT in tumors [42] (**Figure 1**).

Transforming growth factor β (TGF-β) acts as an initial agonist in EMT. It promoted cell migration and invasion by inducing the occurrence of EMT [43]. LncRNA activated by TGF-β (lncRNA-*ATB*) induced distant metastasis of liver cancer by up-regulating the levels of zinc finger E-box-binding homeobox (ZEB1 and ZEB2) to stimulate the EMT cascade [12]. In addition, TGF-β induced the production of *LIN28B*, which participated in the development of pancreatic ductal adenocarcinoma (PDAC) [44]. LncRNA *MEG3* participated in the TGF-β signaling pathway *via* RNA-DNA triplex structures [45] (**Figure 1**).

In addition, lncRNA human ortholog RNA of Dreh (hDREH) was down-regulated by hepatitis B virus X protein (*HBx*), which is an inhibitor of EMT in hepatocellular carcinoma (HCC) [46]. Further studies have revealed that the transcription factor PNUTS has its corresponding lncRNA-*PNUTS*, which is involved in the metastasis of breast cancer by affecting the EMT process [47] (**Figure 1**).

HOTAIR is also known to promote the metastasis of various cancers, such as breast cancer, liver cancer, and pancreatic cancer [42, 48-50]. TGF-β secreted by carcinoma-associated fibroblasts stimulated the expression of *HOTAIR* in cancer cells to activate the SMAD cascade signaling pathway and subsequently induced the EMT process and promoted cancer metastasis [51] (**Figure 1**).

Collectively, many lncRNAs have been documented to regulate the EMT process during tumor metastasis. However, EMT is an intricate multi-cascade process. The roles of lncRNAs in the trans-vascular migration process and vascular circulation require more in-depth research. On the other hand, it is known that spread tumor cells may undergo mesenchymal-epithelial transition (MET) when they arrive at the distant organ, which facilitates the growth of overt metastasis lesions. Whether lncRNAs also participate in the MET process remains an intriguing issue.

*Cancer cell stemness*

Stemness is an important property of tumor metastasis-initiating cells. After colonization of spread tumor cells into distant tissues, tumor metastasis-initiating cells with high stemness can survive and form micrometastases and subsequent overt macrometastases. This step is critical for tumor cell colonization and metastasis and is coordinated by various signaling pathways.

Various studies have shown that lncRNAs are involved in stemness-related signaling pathways. For example, lncRNA tumor suppressor long noncoding RNA on chromosome 8p12 (*TSLNC8*) exerted a tumor suppressor function by inhibiting the STAT3 signaling pathway [52], and lncRNA MST1/2-antagonizing for YAP activation (MAYA) participated in the Hippo-YAP signaling pathway [53]. These lncRNAs might be directly regulating cell stemness [52, 53] (**Figure 1**). *LncBRM* stimulated YAP1 to regulate the self-renewal of liver cancer stem cells [54]. *LncSox4* participated in the self-renewal of liver cancer-initiating cells through the STAT3 pathway [55]. The expression of *lncTCF7* recruited the SWI/SNF complex and further activated the Wnt signaling pathway based on the activation of the TCF7 transcriptional promoter, thereby promoting the stemness of cancer cells [56] (**Figure 1**).

In summary, lncRNA plays a pivotal role in cancer metastasis. Emerging findings in this field have revealed more previously unknown functions of lncRNA in tumor progression and metastasis. Thus, targeting lncRNAs as a promising approach to treat cancer has attracted interest from researchers in recent years.

## Therapeutic opportunities of lncRNA in controlling cancer

Non-coding RNAs play a significant role in tumorigenesis and tumor progression [57]. LncRNAs may be promising targets for controlling cancer. Some efforts have been paid to the lncRNA therapy in animal models via various methods. In this section, we list four types of approaches targeting lncRNAs in cancer treatment (**Figure 2**).

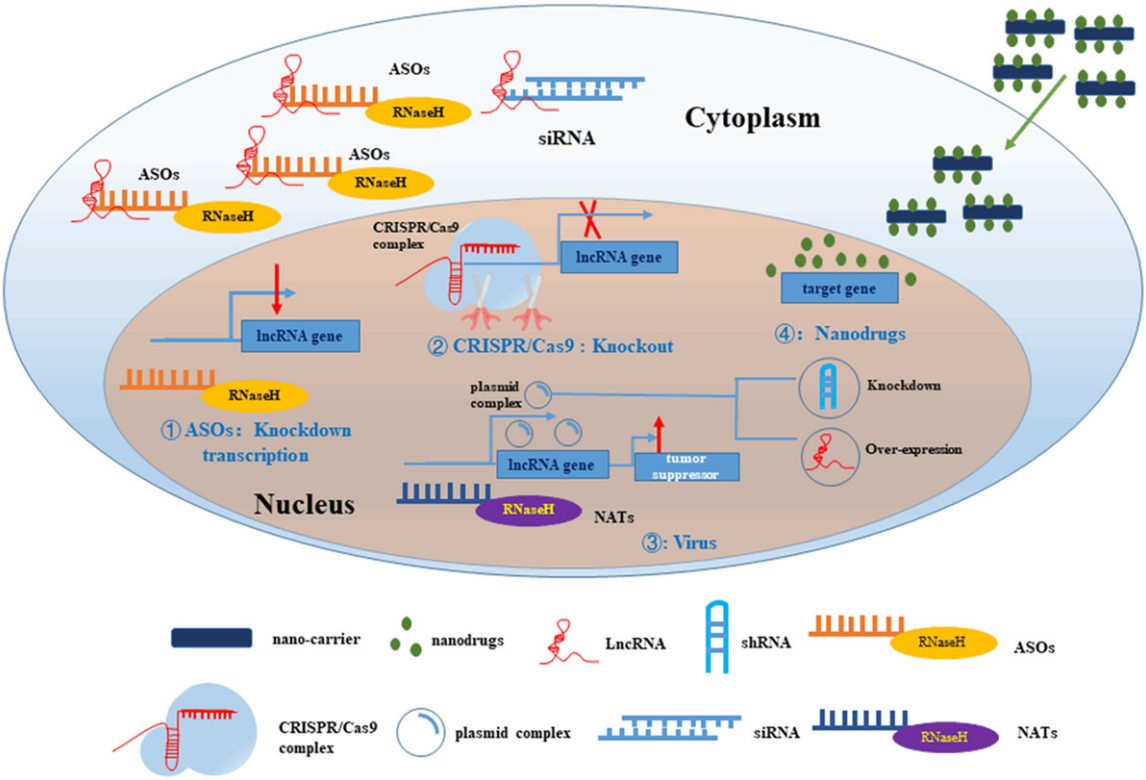


**Figure 2.** Targeting lncRNA to treat cancer. ① ASOs-mediated knock down of cytoplasmic and nuclear lncRNA transcription levels through RNaseH-dependent degradation. ② CRISPR-cas9 based knock-out strategy of lncRNA via specific gDNA. ③ Two methods of virus therapy, encapsulated shRNA or lncRNA-mediated knock down or up-regulation of targeted lncRNA, or NATs-mediated down-regulation of lncRNA elevating the expression of neighboring tumor suppressor genes. ④ Nano-carrier-absorbed on nanodrugs detached within cytoplasm after specific stimulation.

*Antisense oligonucleotides*

Antisense oligonucleotides (ASOs), which may form a DNA-RNA structure with target RNA through base pairing rules, can trigger RNase-H-mediated RNA degradation. ASOs have been clinically tested for targeting mRNA in cancer [58]. As we have reviewed above, the aberrant expression of lncRNAs regulates tumorigenesis and tumor progression [59-63]. Targeting lncRNA by ASO may be a promising method for treating cancer.

Knocking down metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) by ASOs significantly inhibited tumor growth and metastasis, including breast cancer [64] and lung cancer [65]. Prostate cancer is mild in most patients, while only a small percentage of patients have a definite deterioration in symptoms [66, 67]. One study has found that a small percentage of patients with high expression of the lncRNA second chromosome locus associated with prostate-1 (*SChLAP1*) showed significantly limited tumor formation and metastasis after ASO-mediated down-regulation of lncRNA-*SChLAP1* [57], providing idea for the clinical treatment of malignant prostate cancer. In addition, targeting lnc-*USMycN* by LNA-ASO markedly inhibited tumor formation in mice with neuroblastoma [68].

Due to the poor membrane permeability of ASOs, ASOs are mainly constricted within the cytoplasm and it is difficult for ASOs to manipulate sub-nucleus lncRNAs [69]. Although recent studies have claimed the abundance of RNase-H inside nucleus, it is still difficult to obtain accurate therapeutic effects. Linking ASOs with nanotechnology may be a latent method and we will discuss them in detail in the following sections (**Figure 2**).

*CRISPR/Cas9 genome editing technique*

As a technology for specific DNA modification of targeted genes, CRISPR/Cas9 has also received extensive attention in the treatment of

cancer. Recent studies have found that CRISPR/Cas9 can successfully silence the transcription of the lncRNA-expressing loci [70, 71]. CRISPR/Cas9 targeted the transcriptional site of a gene promoter to silence transcription [72]. Studies have found that more than 16,000 lncRNA promoters in the human genome could be targeted by guide RNAs [73].

The CRISPR/Cas9 system has been used to target genomic DNA in cancer cells and animal cancer models. For example, knockout of lncRNA-*NEAT1* and lncRNA-*MALAT1* dramatically inhibited the metastasis of cancer cells [74, 75]. LncRNA-*NEAT1* is involved in the regulation of the replication stress response and chemosensitivity of cancer cells. Knockout of LncRNA-*NEAT1* induced the sensitivity of precancerous cells towards DNA damage-induced cell death and promoted the lethality of chemotherapy drugs on cancer cells [74]. Previously, we reported that lncRNA-*GMAN* is a gastric cancer metastasis-associated long non-coding RNA. *GMAN* is highly expressed in gastric cancer cells and is associated with poor prognosis [76]. A well-designed proof-of-concept animal experiment found that the delivery of a CRISPR/Cas9 system targeting *GMAN* significantly suppressed the metastasis of gastric cancer cells and improved overall survival in mice [76].

Many lncRNAs are expressed specifically in different tissues and even different people. Therefore, it is clinically possible to make personalized treatment depending on the situation of patients. Although CRISPR/Cas9 has broad adaptability and target specificity as a genome editor theoretically, off-target cleavage events can still occur in practical applications [77, 78]. Therefore, oncologists should be more cautious in designing gene-editing therapy. Now, the clinical application of the CRISPR/Cas9 system targeting lncRNA to treat cancer can be vague. Moreover, developing more specific gene-editing tools is important [79] (**Figure 2**).

*Virus*

As a superior RNA interference (RNAi) transfection method, viral vectors mainly include recombinant vectors of adenovirus, lentivirus and retrovirus. RNAi is a biological process of specific gene knockdown *via* neutralizing targeted RNA by exogenous double-stranded RNA, first found in Caenorhabditis elegans [80-82], which includes short interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs). Despite its specificity, siRNA's efficiency is transient due to its instability, while stem-loop shRNA may provide a durable and long-lasting effect *in vivo* [83-86].

In particular, the application of adenovirus vectors is far more extensive, and there have been emerging clinical trials [87-89]. Adeno-associated viruses (AAVs) are structures of uncoated, single-stranded DNA [90]. The AAV-based vector is an efficient gene delivery system, mainly due its non-pathogenicity, free from immune response and stability within live cells [91]. After large-scale screening, more ideal AAVs have been developed for human cancer cells [92]. AAVs have laid a solid foundation for the clinical treatment of tumors by targeting lncRNA.

There have been many reports on the use of shRNAs to target lncRNAs in treating cancer. In a recent study, the lncRNA-*BCAR4* knockdown cell line constructed by lentiviral transfection significantly inhibited the formation of metastases in breast cancer *in vivo* in mice [93]. In addition, transfecting the *HOTAIR* shRNA with a retrovirus in a gastric cancer cell line significantly inhibited cells from spreading in the peritoneal dissemination [94]. It was also found that knockdown of lncRNA-*PNUTS* by an adenovirus system could reduce the formation of primary breast cancer and metastases *via* inhibiting the expression of metastatic Ki-67 [47] (**Figure 2**).

On the other hand, some lncRNAs with tumor suppressor function are expressed at low levels in tumors. It becomes feasible to upregulate the expression of these lncRNAs to achieve cancer treatment. Virus transfection, as the main method for accurately transmitting the shRNA plasmid to the target site, can also be used to transfect exogenously synthesized lncRNA plasmids into cancer cells to upregulate corresponding lncRNAs. However, solid experimental data are required to verify the feasibility and practicability of this method (**Figure 2**).

There is also a small class of special non-coding RNAs in the human genome, natural antisense RNAs (NATs), which belong to non-coding RNAs and are antisense to the overlapped protein-coding gene [95]. Studies have shown that

inhibition of NATs expression can upregulate the expression of neighbor/overlapping coding genes [96, 97]. Therefore, it is possible to apply a method such as ASO to knock down NATs that are adjacent to or overlap with certain tumor suppressor genes, such as *CDKN2B* (*ANRIL*) and *CDKN1A* (*P21-AS*) [98, 99], and then upregulate their expression to treat cancer (**Figure 2**).

Although viral transfection has achieved excellent therapeutic results in basic research, the complexity of clinical trials is considerable. It is noteworthy that the off-target effect has species differences [100, 101]. Therefore, dose control and improved accuracy of viral infections should be seriously considered in future applications.

*Nanomedicine*

After being proposed in the 1990s, nanotechnology gradually gained prominence due to its small size, biodegradability, ability to covalently combine with a large variety of small molecule drugs, and ability to reach sub-nucleus targets. As a consequence, its potential in treating cancer and related diseases has gradually developed.

Nanomedicine is usually composed of four main structures: drug, targeting agent, imaging agent, and linker. In the classification of third-generation nanomedicines, five types of nanomedicines have been proposed based on the specific resistance of cancer, including the following: (I) Lipid-based nanoparticles (liposomes), which are lipid-based vesicles that are capable of carrying payloads in either an aqueous compartment or embedded in the lipid bilayer and have been previously used in conjunction with paclitaxel to target human epidermal growth factor receptor 2 (HER2) for clinical trials [102]. (II) Polymer-based nanoparticles and micelles are composed of biodegradable or natural multimer covalently cross-linked therapeutic particles. Among these nanoparticles and micelles, two multimers, polylactide (PLA) and poly (lactide-co-glycolide) (PLGA), have been used to synthesize FDA-approved nanomedicines [103]. (III) Dendrimers are well-defined globular structures with a central core composed of multiple branched polymers. Clinical trials using dendrimer complex structures to transport paclitaxel for the targeted treatment of breast cancer and non-small-cell lung cancer have been conducted [104]. (IV) Carbon-based nanoparticles mainly release drug particles into the cytoplasm through pinhole-like penetration. Therefore, the constituent materials of carbon-based nanoparticles must be fine and small in size, as well as biodegradable. Previous studies have used this material in the treatment of cancer [105]. (V) Metallic and magnetic nanoparticles, such as gold nanoparticles, can be used to transport small molecules, such as proteins, and can release covalently bound drugs through photophysical properties, such as tumor necrosis factor α (TNFα) bound to colloidal gold for the treatment of solid tumors [106, 107].

Although scientists have developed diverse nanomedicines, multi-drug resistance (MDR) in cancer cells is still challenging. P-glycoprotein, which is highly expressed on the surface of cancer cells, can activate the efflux of anti-cancer drugs within tumor cells, leading to a significant decrease in drug potency. Similarly, lncRNAs can also regulate cancer cell sensitivity towards different types of drugs. LncRNA urothelial cancer-associated 1 (*UCA1*) is involved in the chemical resistance of bladder cancer by regulating Wnt6 [108], and lncRNA *MACC1-AS1* is involved in the chemical resistance of gastric cancer through fatty acid oxidation [109].

Additionally, a system has been established for the stable transportation of nanomedicine into the nucleus. Mechanically, the nanodrug binds to the nanotruck through an aptamer, which facilitates its cell membrane permeability and then detaches the nanodrug from the nanotruck through near-infrared (NIR)-range (700-900 nm) radiation in the cytoplasm [110, 111], eventually entering into the nucleus [111]. Since the majority of lncRNAs are located in the cell nucleus, *via* this system, sub-nucleus lncRNAs can be accurately and effectively targeted to obtain the desired therapeutic effect (**Figure 2**).

## Conclusions

Numerous studies have documented that lncRNA plays a key role in tumorigenesis and tumor progression. In particular, abnormal lncRNA expression may accompany DNA damage, immune escape as well as cellular metabolic disorders in cancer cells. The diversity and heterogeneity of lncRNAs make the compli-

cated tumorigenesis process even more intriguing. In addition, lncRNA is also strongly associated with EMT, as well as the regulation of cell stemness. These findings together make lncRNA a solid component of tumor metastasis. Therefore, targeting lncRNA could be an opportune clinical approach in cancer treatment.

Combination therapy has made great progress in the clinical treatment of tumors. Combining surgery with chemotherapy, or the emergence of specific targeted-drugs, has further improved the survival rate of patients. Recently, with the significant clinical achievements in immunotherapy of PD1/PD-L1, ideas focusing on the re-stimulation of the suppressed cellular immunity of patients through immunotherapy have been under the limelight of cancer research. As a part of the immune escape of cancer cells, lncRNAs may provide new insight for future cancer treatment combined with immunotherapy.

The rapid development of a new generation of gene-editing tools makes it possible to target lncRNA inside tumor cells. ASOs or CRISPR/Cas9-based therapy has already shown the feasibility of gene editing in treating cancer, but their off-target event or unstable efficiency due to the spatiotemporal specificity of lncRNA should also be carefully evaluated before further application. Moreover, the combination of nanotechnology and bioinformatics accelerates new nanoparticle development as well as its optimization under clinical conditions *via* the deep analysis of lncRNA functions and distribution. The emergence of new cancer therapeutic strategies worldwide exhibits promise in the treatment of cancer, one of the most serious human diseases.

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## Disclosure of conflict of interest

None.

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