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REVIEW ARTICLE

# The paradoxical functions of long noncoding RNAs in hepatocellular carcinoma: Implications in therapeutic opportunities and precision medicine

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**Abstract** Hepatocellular carcinoma (HCC) is among the most aggressive and lethal diseases with poor prognosis, worldwide. However, the mechanisms underlying HCC have not been comprehensively elucidated. With the recent application of high-throughput sequencing techniques, a diverse catalogue of differentially expressed long non-coding RNAs (lncRNA) in cancer have been shown to participate in HCC. Rather than being "transcriptional noise," they are emerging as important regulators of many biological processes, including chromatin remodelling, transcription, alternative splicing, translational and post-translational modification. Moreover, lncRNAs have dual effects in the development and progression of HCC, including oncogenic and tumour-suppressive roles. Collectively, recently data point to lncRNAs as novel diagnostic and prognostic biomarkers with satisfactory sensitivity and specificity, as well as being therapeutic targets for HCC patients. In this review, we highlight recent progress of the molecular patterns of lncRNAs and discuss their potential clinical application in human HCC.

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## Introduction

Hepatocellular carcinoma (HCC) accounts for approximately 90% of all cases of primary liver cancer, and is a common and aggressive human malignancy worldwide.[1] The tumour may be curable by resection or liver transplantation when patients are diagnosed at early stages of the disease; however, most individuals with advanced HCC are not suitable candidates and their treatment options are limited.[2,3] Hepatocarcinogenesis is a multi-stage process that involves changes in numerous gene networks and pathways, many of which have not yet been clarified.[4] Therefore, further insights into the molecular pathogenesis of liver cancer are of great importance for the development of future effective therapeutic approaches.

Intensive investigations over the last few decades have widely explored the roles of protein-coding genes in HCC. Due to the general implementation of deep-sequencing technologies, it is now evident that less than 2% of the human genome can encode proteins, and at least 90% of the genome is actively transcribed into non-coding RNA (ncRNA) that has no protein encoding potential.[5] Previously, ncRNAs were assumed to be exceptional curiosities or 'splicing noise', but accumulating evidence demonstrate their physiological function, as well as their crucial role in the disease context.[6]

The widespread use of microarrays and high-throughput sequencing techniques have facilitated the detection of large quantities of dysregulated long non-coding RNAs (lncRNAs), in human HCC. Using next-generation sequencing technology, Yao and colleagues reported 214 differentially expressed lncRNAs (DELs) detected from 12 HCC tissues and paired adjacent normal tissues. A proportion of lncRNAs were significantly associated with tumour cell differentiation, portal vein tumour thrombosis, and serum or tissue alpha-fetoprotein levels.[7] When categorizing tissues by viral status, 719 lncRNAs were significantly dysregulated in hepatitis B virus (HBV)-HCC tissues compared to paired non-tumour tissues using microarray analysis.[8] Similar to reports on HBV-HCC, the lncRNA transcriptome was also altered in hepatitis C virus (HCV)-infected liver cancer.[9] Recently, the relationship between lncRNAs specificity and metastasis of HCC has been extensively analysed. Comparative expressions of lncRNAs between HCC and portal vein tumour thrombi (PVTT) have identified 107 deregulated lncRNAs associated with metastasis.[10] Additionally, expressions of oxaliplatin-resistant lncRNAs in HCC have been established, with the identification of 120 differentially expressed lncRNAs.[11] Nonetheless, the depository of general transcription is far greater than the molecular properties of the transcripts in liver cancer. This review aims to classify HCC-associated lncRNAs that are closely linked to different biological processes according to their distinct molecular mechanisms. Finally, we discuss and summarize the potential value of lncRNAs as a diagnostic tool and therapeutic goal.

## Classification and structural features of lncRNAs

In general, ncRNAs can be divided into short ncRNAs (sncRNAs) and lncRNAs, defined as ncRNA shorter or longer than approximately 200 nucleotides in length, respectively.[12] MicroRNAs (MiRNAs), piwi-interacting RNAs (piRNAs) and small nucleolar RNA (snoRNA) are the most studied sncRNAs transcripts.[13] Mature miRNAs are approximately 22-nucleotide-long single stranded RNA molecules that target a wide range of mRNA and block translation by binding their seed sequence to the 3′untranslated region (3′UTR) of mRNA.[14] SnoRNAs are small RNAs of 60—300 nucleotides in length that function as guide RNAs for the post-transcriptional modification of ribosomal RNAs and some spliceosomal RNAs.[15] In addition, piRNAs are unique small ncRNAs that form into the piRNA-induced silencing complex (piRISC) to silence transposable elements in germline cells.[16] In comparison to sncRNAs, lncRNAs can be classified according to the location and origin of the genome. Specific lncRNAs exist in the intergenic regions, while other lncRNAs are arranged in the form of antisense, bidirectional or are overlapping with protein-encoding genes. Intriguingly, a subset of lncRNAs originate from the enhancer regions of the genome and have enhancer-like functions (enhancer lncRNA).[17] Two ncRNAs considered as lncRNAs include, circular RNAs (circRNAs) which are back-spliced from a 5′ splice site to an upstream 3' splice site of protein-coding mRNAs or linear ncRNAs[18]; and pseudogenes, which have lost their protein-coding capacity owing to imperfect copies of functional protein-coding genes and various mutations.[19]

LncRNAs analysis shows specific consistency characteristics including paucity of introns, low GC level, poor start codon and open reading frame contexts.[20] In addition to the main features of the sequence, recent research has developed genome-scale approaches and uncovered a variety of secondary lncRNAs structure elements, which can interact with many molecules, such DNA, RNA and/or protein.[21] Emerging studies describe the central role lncRNAs play in many physiological and pathological processes, including differentiation, development and disease.[22,23] Furthermore, lncRNAs, act as a driving factor in tumour inhibition and carcinogenicity in different types of cancer.[24] Over the last ten years, discovery of hundreds of dysexpressed lncRNAs has demonstrated their function as oncogene transcripts or tumour suppressor genes in liver cancer.

## Overview of properties of lncRNAs in HCC

Various studies have shown that HCC-related lncRNAs regulate gene expression through different patterns, and categories of lncRNA functions are not single. In liver cancer, the principle routes of lncRNAs activity are divided into four categories.[25] First, lncRNAs are considered as molecular signal markers for important functional biological events through modulating transcription activities or pathways. Decoy lncRNAs can act as molecular decoys to bind and titrate away targeted proteins or RNAs. LncRNAs can also act on near or distant genes as a guide for binding specific proteins, and then locate the molecular complex to specific targets. Finally, lncRNAs serve as central platforms on which different effector molecules can be assembled.

Moreover, lncRNAs referring to Wnt and STAT3 signal transduction in HCC have been separated comprehensively.[26] The Wnt and STAT3 pathways are critical regulators of hepatoma stem cells and are involved in the induction of epithelial-mesenchymal transition (EMT) and metastasis.[27,28] Similarly, Lv and colleagues have highlighted the role of lncRNAs in multiple liver cancer stem cells (LCSCs) regulatory signalling pathways, including the Wnt/β-catenin, STAT3, TGF-β, YAP and cell cycle-related signaling.[29] LncRNAs are further distinguished by its effects on divergent phenotypes in HCC.[30,31] A recent study summarises the basic characteristics and functions of lncRNAs, in relation to the liver cancer microenvironment.[32] However, due to the variety of properties, the functions of lncRNA in HCC remains unclear. Notably, subcellular localization of lncRNA is another means to provide valuable information about its role and mode of action.[33] Thus, most HCC-associated lncRNAs modulate the expression and stability of their downstream targets in relation to the specific cellular compartments through diverse biological processes, including epigenetic, transcriptional, and post-transcriptional levels.

## Functional mechanisms of tumor-promoting lncRNAs in HCC

### Chromatin remodelling regulation

A mechanistic action of lncRNAs is their capacity to induce epigenetic alterations of target genes as a scaffold or guide of chromatin modified complex.[34,35] Polycomb repressive complex 2 (PRC2) is a well-known epigenetic repressor which can inhibit the transcription of various genes through histone H3 lysine 27 tri-methylation (H3K27me3) during the progression of HCC.[36] In recent years, lncRNAs have been shown to control gene expression by binding to PRC2.[37] Fu and colleagues described the function of pro-oncogene lncRNA HOX transcript antisense RNA (HOTAIR) as a bridge to interact with PRC2. This initiates chromatin remodelling and H3K27 trimethylation of miR-218, thus activating oncogene Bmi-1 in HCC. As expected, Bmi-1 targets, tumour suppressors $P16^{Ink4a}$ and $P14^{ARF}$ are downregulated, whereas MDM2 is activated.[38] Additionally, another up-regulated lncRNA CDKN2B antisense RNA 1 (CDKN2B-AS1) has been demonstrated to epigenetically repress tumour suppressor kruppel-like factor 2 (KLF2) transcription in HCC cells by binding with PRC2 and recruiting it to the KLF2 promoter region.[39] Through comparison of HBV-related HCC with paired peritumoral tissues, the level of specific differentially expressed lncRNA High Expression in HCC (HEIH) was analysed, which is significantly linked to recurrence and is an independent prognostic factor for survival. LncRNA-HEIH is also associated with enhancer of zeste homolog 2 (EZH2), a key component of PRC2, and is required for the repression of EZH2 target gene p16, which plays a key role in G (0)/G (1) arrest (Fig. 1A).[40] While lncRNAs have been found to widely interact with PRC2, other chromatin remodellers have been also implicated. Elevated lncRNA translation regulatory long non-coding RNA 1 (TRERNA1) levels can suppress cadherin 1 (CDH1) expression via the recruitment of euchromatic histone lysine methyltransferase 2 (EHMT2) to dimethylate H3K9 in the CDH1 promoter region, thus promoting cell metastasis and invasion of HCC.[41]

Recent evidence showed upregulated LINC0441 being inversely correlated to tumour-suppressor gene retinoblastoma gene 1 (RB1) expression in human HCC samples. Furthermore, RNA pull-down assays demonstrated the decreased RB1 level induced by LINC0441 made in association with the incidental methylation by DNA methyltransferase 3 (DNMT3) recruited by LINC0441, thereby resulting in apoptosis suppression and cell-cycle rearrangement.[42] Highly up-regulated in liver cancer (HULC) is a long non-coding RNA overexpressed in HCC which may elicit methylation of CpG islands in the miR-9 promoter through upregulation of DNA methyltransferase 1 (DNMT1), thus relieving the inhibitory effect on its target peroxisome proliferator activated receptor alpha (PPARA). Therefore, activation of transcriptional factor PPARA activates the acyl-CoA synthetase long chain family member 1 (ACSL1) promoter, inducing the deregulation of lipid metabolism in HCC, and stimulating the accumulation of intracellular triglycerides and cholesterol.[43] Uncovering the role of oncogene lncRNA distal-less homeobox 6 antisense 1 (DLX6-AS1) showed its contribution to induce cell adhesion molecule 1 (CADM1) promoter methylation via increasing the enrichment of methyltransferase (DNMT1, DNMT3a, and DNMT3b) in LCSCs. This ultimately regulates downstream pluripotent genes-related to cancer stem cells including OCT4, SOX2, and Nanog through activating STAT3 signalling pathway (Fig. 1B).[44]

Apart from gene transcription modulation through histone methylation and DNA methylation, histone acetylation is another important way of modifying chromatin. P300/CBP-associated factor (PCAF) is a well-known histone acetyltransferase, playing a major role in transcription elongation.[45] Acting as an antisense RNA of Glypican 3 (GPC3), GPC3-AS1 overexpression and co-expression with GPC3 in HCC tissues has been shown through microarray analysis.[46] RNA pull-down, RIP, and ChIP assays suggested GPC3-AS1 could directly bind with PCAF and recruit PCAF to the GPC3 gene body region, subsequently inducing an increase in euchromatic histone marks and activating GPC3 transcription (Fig. 1C).[47]

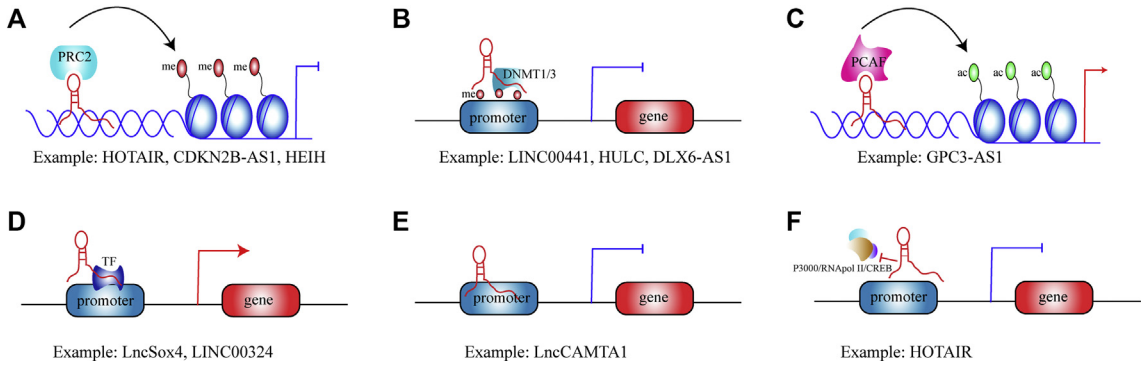


**Figure 1** Chromatin remodeling and transcriptional regulation of lncRNAs in HCC. **(A)** LncRNAs can inhibit target gene expression via recruiting chromatin modifying factors like PRC2 and regulating H3K27me3. **(B)** LncRNAs could epigenetically suppress gene expression by recruiting methyltransferases, including DNMT1/3. **(C)** LncRNAs associate with PCAF complex and direct its localization to specific sites to acetylate histone of promoter. **(D)** LncRNAs could promote transcription by recruiting TF to its target gene promoter. **(E)** LncRNAs can inhibit target gene transcription through binding to their target promoters directly. **(F)** LncRNAs could reduce P300/RNA pol II/CREB to the promoter thereby suppressing gene expression. LncRNA, long non-coding RNA; PRC2, polycomb repressive complex 2; H3K27me3, histone H3 lysine 27 tri-methylation; DNMT1/3, DNA methyltransferase 1/3; PCAF, P300/CBP-associated factor; TF, transcriptional factor; CREB, cAMP responsive element binding protein 1.

## Transcriptional regulation

Generally, lncRNAs regulate transcription through direct chromatin looping, recruitment or prevention of multiple transcription factors or disrupting the Pol II transcription machinery at target genes.[48–51] Chen and colleagues discovered a role of lncSox4 in the self-renewal of liver tumour-initiating cells (TICs), tumour initiation, and propagation. Moreover, the specific binding of lncSox4 and STAT3 remarkably increases STAT3 enrichment at Sox4 promoter and induces Sox4 activation, leading the acquisition of LCSCs.[52] Similarly, LINC00324, another lncRNA overexpressed in HCC tissue, contributes to the maintenance of LCSCs biological properties by upregulating the expression of fas ligand (FasL) through recruitment of transcription factor PU.1 to the FasL promoter region (Fig. 1D).[53]

Additionally, lncRNAs regulate target gene transcription through binding to their loci promoters directly. LncRNA calmodulin binding transcription activator 1(CAMTA1) is vital to obtain CSC characteristics of HCC cells by regulating the expression CAMTA1. Chromatin isolation by RNA purification assay confirmed that the direct combination of lncCAMTA1 and CAMTA1 promoter alters chromatin structure and prevents the transcription of CAMTA1. Therefore, cancer stem cell-like properties could be maintained owing to the repression of CAMTA1 (Fig. 1E).[54]

Recent data suggested lncRNA HOTAIR can stimulate malignant proliferation of human LCSCs. SET Domain-Containing Protein 2 (SETD2) is a vital target of HOTAIR, and SETD2 expression at the transcriptional level is reduced through blocking RNA polymerase II (RNA pol II) catalytic function and by dissociating the cAMP responsive element binding protein 1 (CREB)-P300-RNA pol II complex from the promoter of SETD2. Decreased SETD2 level interferes with mismatch recognition and DNA damage repair, potentially causing tumourigenesis in LCSCs (Fig. 1F).[55]

## Alternative splicing regulation

At the post-transcriptional level, lncRNAs may play a key role in alternative splicing processes, which can actively participate in tumourigenicity.[56] Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a nucleus-enriched lncRNA and its close proximity to nuclear SC35 speckle actively controls pre-mRNA processing through modulating oncogenic alternative splicing factors.[57] MALAT1 induces serine and arginine rich splicing factor 1 (SRSF1) to mediate its target cleavage, increasing the occurrence of anti-apoptotic splicing isoforms and activating the mTOR pathway by regulating the selective splicing of S6 kinase 1 (S6K1).[58] Expression of lncRNA nuclear enriched abundant transcript 1 (NEAT1) is reportedly higher in HCC than normal tissues, and acted as a cancer driver promoting proliferation, invasion and migration.[59] NEAT1 can form a protein complex with U2 small nuclear RNA auxiliary factor-2 (U2AF2), an essential splicing factor of polypyrimidine tract pre-mRNA, thus modulating heterogeneous nuclear ribonucleoprotein (HNRNP) A2 expression.[59,60] HNRNPA2 is associated with the poor prognosis of hepatoma patients, and is an essential cleavage factor in facilitating the proliferation and invasion of liver cells (Fig. 2A).[61]

## mRNA stability regulation

LncRNAs are also implicated in the control of mRNA stability through diverse post-transcriptional regulatory pathways.[62] The antisense lncRNAs, including those located antisense to tumour related genes, can affect the stability of mRNAs in complicated and accurate gene-net of malignant diseases.[63,64] The up-regulated antisense lncRNA, PCNA antisense RNA 1 (PCNA-AS1), and proliferating cell nuclear antigen (PCNA) form a double-stranded RNA to increase

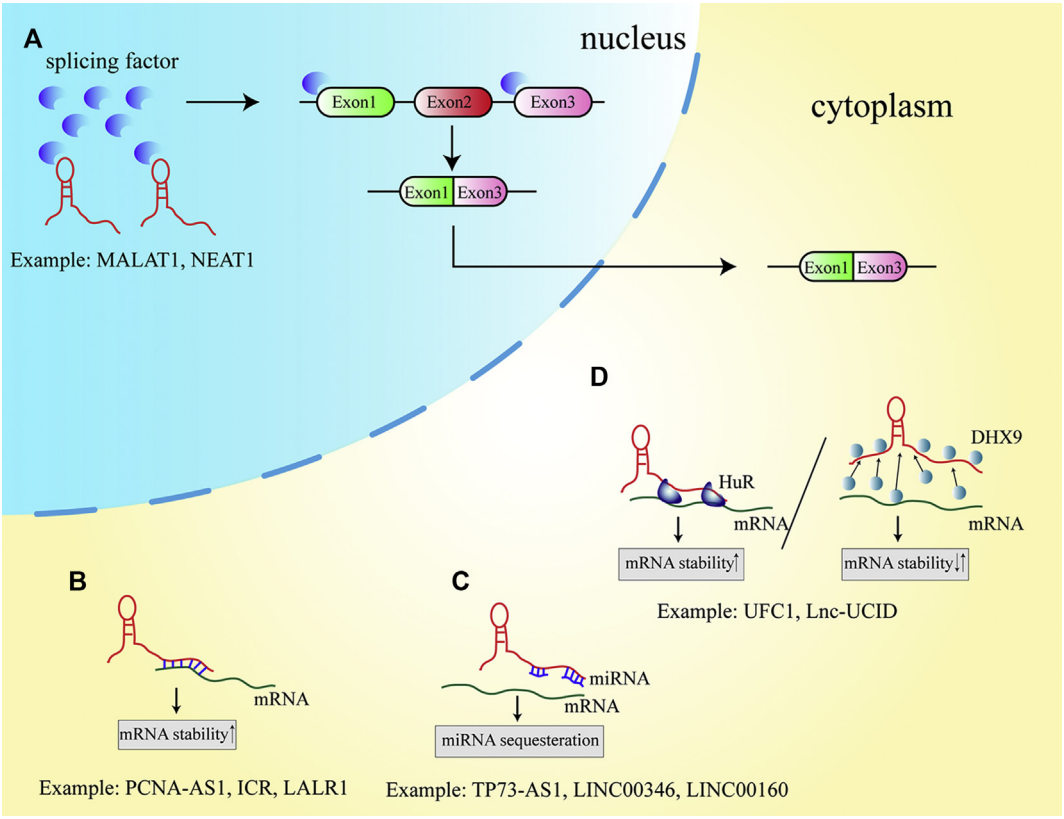


**Figure 2** Post-transcriptional regulation of lncRNAs in HCC. **(A)** LncRNAs could modulate alternative splicing of various pre-mRNAs through influencing the splicing factors. **(B)** LncRNAs could form a RNA duplex with mRNA to increase its stability. **(C)** LncRNAs can function as a competing endogenous RNA to release the miRNA-mediated suppression of target genes. **(D)** LncRNAs interact with mRNA binding protein to change the levels of mRNA. HuR, Hu antigen R; DHX9, DEAH (Asp-Glu-Ala-His) box helicase 9.

PCNA stability, consequently prompting HCC growth.[65] Moreover, lncRNA ICAM-1-related (ICR) that is specifically highly expressed in liver PVTT forms an RNA duplex with ICAM-1 to maintain its expression through approximately 800 bp complementary to the ICAM-1 mRNA sequence, thereby promoting the CSC characteristics of HCC cells.[66] In comparison to tumour adjacent tissues, lncRNA associated with liver regeneration (LALR1) dramatically increases in HCC tissues. RNA pulldown analyses revealed lncRNA-LALR1 could upregulate oncogene ID2 expression through interaction with ID2 mRNA in HCC cells, highlighting an ID2-dependent effect of LALR1 on tumourigenicity (Fig. 2B).[67]

Contrarily, lncRNA can act as legitimate bona fide microRNA competitor thereby actively competing with their parent protein-coding genes for the same pool of microRNAs through sets of conserved microRNA response element, thus enhancing the translation of target RNAs.[68] TP73 antisense RNA 1 (TP73-AS1) is a type of lncRNA highly expressed in liver cancer, which shares a similar binding site of miR-200a with high mobility group box 1 (HMGB1). TP73-AS1 competes with HMGB1 for miR-200a binding, thus attenuating the inhibitory effect of miR-200a on HMGB1 expression.[69] By sponging and competitively binding to miR-199a-3p, LINC00346 releases the miR-199a-3p-mediated suppression of CDK1/cyclin B1, thereby affecting p53 signalling pathways and regulating apoptosis, invasion and cell cycle of HCC cells.[70] The newly discovered role of lncRNA LINC00160 in HCC showed LINC00160 silencing suppresses autophagy and drug resistance in HCC by decreasing the expression of phosphoinositide-3-kinase regulatory subunit 3(PIK3R3) via miR-132 promotion (Fig. 2C).[71]

Aside from RNA-duplex formation and miRNA sponges, mRNA stabilizing protein Hu antigen R (HuR) can be guided by lncRNA-UFC1 to interact with β-catenin mRNA, ultimately enhancing β-catenin levels to promote HCC proliferation and inhibit apoptosis.[72] In contrast, Lnc-UCID (lncRNA up-regulating CDK6 by interacting with DHX9) increases CDK6 expression in HCC cells by competitively binding to DEAH (Asp-Glu-Ala-His) box helicase 9 (DHX9) and sequestering DHX9 from CDK6-3′UTR. DHX9 is an RNA helicase, which could post-transcriptionally suppress CDK6 expression by binding to the 3′UTR of CDK6 mRNA (Fig. 2D).[73]

## Post-translational regulation

Regarding post-translational modification, lncRNAs modulate protein ubiquitination positively or negatively and influence the degradation of proteins mediated by ubiquitin proteasome. It is widely recognized that degradation of intracellular proteins by the ubiquitin proteasome system regulates a broad array of cellular processes.[74] Higher lncRNA-PVT1 expression indicates a poor HCC clinical

prognosis. Fang and colleagues confirmed that lncRNA-PVT1 up-regulates nucleolar protein 2 (NOP2) by preventing the degradation of ubiquitin proteasome system. Moreover, the role of lncRNA-PVT1 depends on the presence of NOP2.[75] In addition, the frequent DNA-gain regions of HCC produces a tumour-promoting lncRNA, LINC01138, which exerts its oncogenic activity through physical interaction with protein arginine methyltransferase 5 (PRMT5) and stabilizes PRMT5 through blocking ubiquitin/proteasome-dependent degradation in HCC (Fig. 3A).[76] In HBV replicating cells, HOTAIR may serve as a ubiquitination scaffold through interaction with PRC2 and E3 ligases Mex-3 RNA-binding family member B (Mex3b) simultaneously. This facilitates the degradation of PRC2 and results in de-repression of PRC2 targets, including epithelial cell adhesion molecule (EPCAM) and pluripotency genes (Fig. 3B).[77]

In addition to the above, lncRNAs can also modify protein localization or activity, as well as the composition of protein complexes to exert various biological effects.[78] Recent evidence demonstrated HCC cells grown together with HCC Mesenchymal stem cells (MSC) switch to a more aggressive phenotype. Subsequently, lncRNA MSC-upregulated factor (MUF) is activated by HCC-MSCs, and accountable for the formation of tumour sphere and EMT processes. Mechanistic investigations showed higher interaction between ANXA2 and glycogen synthase kinase 3β (GSK-3β) due to the combination of lncRNA-MUF and ANXA2, reducing GSK-3β-mediated phosphorylation of β-catenin and preventing its degradation of ubiquitin-proteasome system. Eventually, β-catenin translocates to the nucleus activating the Wnt cascade (Fig. 3C).[79] A new lncRNA, gastric cancer metastasis associated long non-coding RNA (GMAN), is evidently responsible for anti-apoptosis and increasing the invasion and migration potential in HCC. Mechanistic analysis implied that GMAN and eukaryotic translation initiation factor 4B (eIF4B) directly combine to stabilize phosphorylation of eIF4B at serine-422 by preventing the binding and dephosphorization of eIF4B from the protein phosphatase 2 regulatory subunit B-alpha (PPP2R2A), thus increasing translation and expression of anti-apoptotic mRNA (Fig. 3D).[80] Moreover, Ni and colleagues identified that oncogenic lncRNA uc.134 combines with cullin 4A (CUL4A) to prevent its nuclear export without changing its protein level in HCC cells, thus impeding the CUL4A-mediated ubiquitination of large tumour suppressor kinase 1 (LATS1), and increasing yes associated protein 1 (YAP) S127 phosphorylation to silence the YAP target genes (Fig. 3E).[81]

## Tumor-suppressive effect of lncRNAs in HCC

Increasing reports focus on the characterization of oncogenic lncRNAs in HCC; however, there are lncRNAs, which

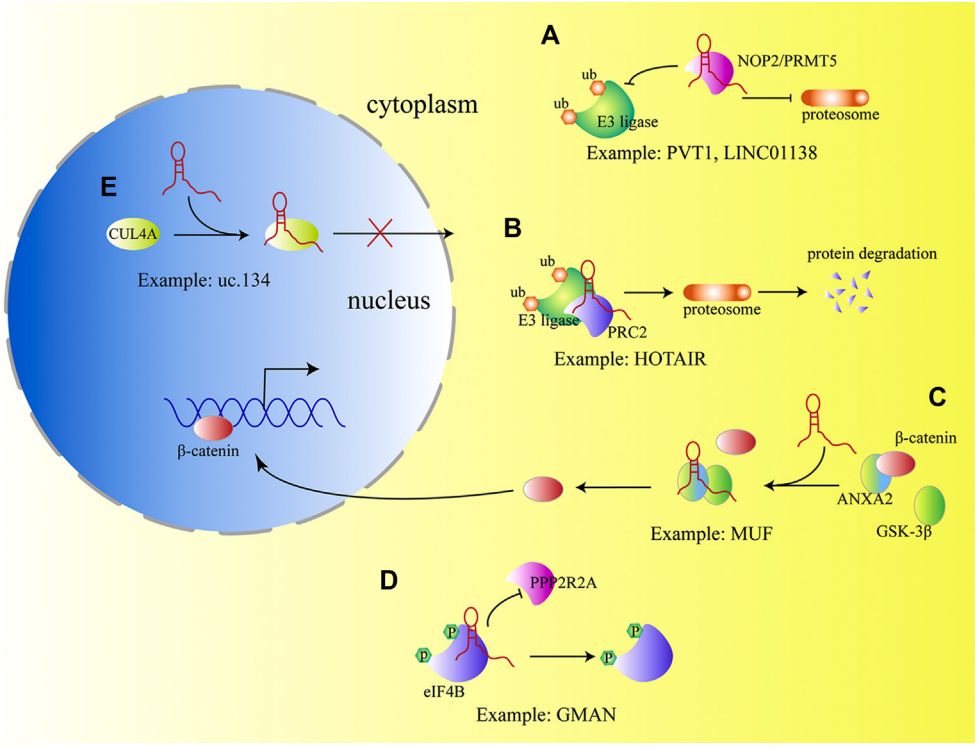


**Figure 3** Post-translational regulation of lncRNAs in HCC. **(A)** LncRNAs interact with proteins and enhance their stability by preventing the degradation of ubiquitin proteasome system. **(B)** LncRNA could induce proteins degradation via the ubiquitin-proteasome pathway. **(C)** LncRNA could modify protein complex assembly to exert various biological function. **(D)** LncRNA promotes phosphorization of target proteins by preventing dephosphorization of phosphatase. **(E)** LncRNA binds to target protein and inhibits its nuclear export. NOP2, nucleolar protein 2; PRMT5, protein arginine methyltransferase 5; PRC2, polycomb repressive complex 2; ANXA2, Annexin A2; GSK-3β, glycogen synthase kinase 3β; PPP2R2A, protein phosphatase 2 regulatory subunit Balpha; eIF4B, eukaryotic translation initiation factor 4B; CUL4A, cullin 4A.

play a suppressive role in liver cancer. These may influence distinct biological processes, such as pro-oncogene lncRNAs in HCC, being dependant on their cellular distribution. Regarding their modes of action, lncRNAs may be an important regulator of nuclear mechanisms once localized into the nucleus. Subsequent exportation of lncRNAs into the cytoplasm can affect mRNA turnover and controls protein stability (Table 1).

## Roles of lncRNAs in the nucleus

The lncRNA MAGI2 antisense RNA 3 (MAGI2-AS3) is very prominent in the nucleus of HCC cells and markedly promotes H3K4me2 demethylation at the Rac GTPase activating protein 1 (RACGAP1) promoter through recruitment of lysine demethylase 1A (KDM1A), ultimately suppressing the expression of RACGAP1 and restraining tumourigenesis.[82]

Early studies supported the notion of a stimulatory role of lncRNA H19 in HCC. Zhang and colleagues have provided an explanation for the hitherto puzzling literature on the relationship between H19 and HCC. H19 associates with the protein complex hnRNPU/PCAF/RNAPolII and initiates the transcription of the miR-200 family by enhancing the histone H3 acetylation, thus impairing the aggressive and metastatic properties of HCC.[83]

Maternally expressed gene 3 (MEG3) encodes lncRNA whose expression is lost or downregulated in major human cancers. MEG3 compound is formed by association with p53 DNA binding domain, and is then recruited to its specific site where it influences the expression of partial p53 target genes and implements a suppressive role in hepatoma cells.[84]

LncRNA wilms tumour-associated antisense RNA (WT1-AS) may possess a tumour-inhibitory role by reversing WT1-mediated resistance to doxorubicin-based chemotherapy in HCC cells. Bioinformatics analysis revealed binding of WT1-AS to the TATA region of the WT1 promoter could inhibit WT1 transcription, which can negatively regulate HCC

**Table 1** Tumor-suppressive role of lncRNAs in HCC.

| LncRNA | Classification | Expression in HCC | Effect on HCC | Molecular mechanism | Reference |
|---|---|---|---|---|---|
| **Functions of lncRNAs in the nucleus** | | | | | |
| MAGI2-AS3 | antisense | down | inhibits cell growth, migration, invasiveness, and promote cell apoptosis | promotes H3K4me2 demethylation at RACGAP1 promoter through recruitment of KDM1A | [82] |
| H19 | intergenic | down | inhibits EMT and tumor metastasis | recruits HnRNP U/PCAF/ RNAPolII complex to activate miR-200 family through histone acetylation | [83] |
| MEG3 | intergenic | down | inhibits proliferation and induces apoptosis | interacts with p53 DNA binding domain to activates p53-mediated transcriptional activity | [84] |
| WT1-AS | antisense | down | negatively regulates HCC chemotherapy resistance | binds to the WT1 promoter to inhibit transcription | [85] |
| **Functions of lncRNAs in the cytoplasm** | | | | | |
| FTX | overlapping | down | inhibits EMT, cell metastasis and invasion | competitively sponges miR-374a and upregulates WIF1, PTEN and WNT5A | [86] |
| LINC01093 | intergenic | down | suppresses cell proliferation and metastasis | disrupts the association between IGF2BP1 and GLI1 mRNA, resulting in the degradation of GLI1 mRNA | [87] |
| LINC01554 | intergenic | down | abolishes aerobic glycolysis, inhibits cell growth, colony formation, foci formation | promotes the ubiquitin-mediated degradation of PKM2 and inhibits Akt/mTOR signaling pathway | [88] |
| MIR503HG | intergenic | down | inhibit HCC invasion and metastasis | induce HNRNPA2B1 degradation via the ubiquitin-proteasome pathway | [89] |

LncRNA, long non-coding RNA; HCC, hepatocellular carcinoma; EMT, epithelial—mesenchymal transition.

chemotherapy resistance through JAK2/STAT3 and MAPK signalling.[85]

## Roles of lncRNAs in the cytoplasm

In additional to their functional role in the nucleus, lncRNAs also govern gene expression post-transcriptionally following exportation into the cytoplasm. One study demonstrated the reduction of epithelial marker E-cadherin upon lncRNA-FTX interference, accompanied with increased mesenchymal markers N-cadherin, Snail, Vimentin, ZEB1, and Twist in SMMC 7721 cells. FTX acts as a repressive factor of HCC metastasis and invasion by competitively sponging miR-374a and upregulating multiple negative regulators of the Wnt/β-catenin signalling cascade, including WIF1, PTEN, and WNT5A.[86]

A novel liver-enriched lncRNA LINC01093 competitively pairs with oncofoetal protein insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1), preventing glioma-associated oncogene homolog 1 (GLI1) mRNA binding to IGF2BP1 and promoting GLI1 degradation. Decreased level of GLI1 is crucial to repress its downstream molecules, thus avoiding HCC cell proliferation.[87]

*In vitro* assay and nude mice xenograft models have confirmed the role of LINC01554 as a tumour suppressor in HCC. Subcellular fractionation assay showed the cytoplasmic accumulation of LINC01554 in hepatoma cells. Mechanistic analyses suggested that LINC01554 downregulation impedes the ubiquitin-mediated degradation of pyruvate kinase M2 (PKM2) and facilitates Akt/mTOR signalling pathway, thereby empowering cancer cells to acquire high aerobic glycolysis to sustain cell growth.[88] The host gene of miR503, lncRNA MIR503HG, interacts with HNRNPA2B1 to expose concealed ubiquitination sites and mediates protein degradation via the ubiquitin—proteasome pathways. Simultaneous formation of miR503HG-HNRNPA2B1 complex anchors to p52 and p65, making the mRNAs fragile and ultimately inactivating the NF-κB signalling pathway in HCC cells.[89]

## Putative diagnostic and prognostic lncRNAs in HCC

Accumulating evidence supported the notion that the wide range of lncRNA expression patterns in various cancers holds significant promise for the discovery of novel cancer biomarkers.[90] Differential expressions and mutations of lncRNAs in HCC are turning into reliable tools for predicting cancer prognosis and patients outcome.[91] The majority of lncRNAs display strict tissue-specific and neoplasm-specific expression traits, opening up a new range of possibilities for the precise classification of distinct subcategories of tumours, and to predict therapeutic action.[92,93]

A retrospective study showed HCC-expressed lncRNA, called lncRNA regulator of Akt signalling associated with HCC and RCC (LNCARSR), is up-regulated in HCC patients and cell lines. High-incidence LNCARSR in patients correlates with large tumour volume, advanced clinical stage, and poor prognosis.[94] Another study identified the strong correlation of lncRNA small nucleolar RNA host gene 20 (SNHG20) expression with multiple clinicopathological characteristics in HCC patients.[95] Moreover, screening deregulated genes in 46 HCCs, 4 focal nodular hyperplasia, and 7 cirrhosis, using cDNA arrays, characterized HULC with highly specific up-regulation in HCC tissues.[96] HULC is detected at higher frequency in the plasma of early stage HCC compared to healthy controls.[97] LncRNA ZEB1 antisense RNA 1 (ZEB1-AS1) is abnormally overexpressed in HCC samples, especially in metastatic tumour tissues. Patients with high ZEB1-AS1 expressions have shorter survival rates and higher recurrence ratio.[98] In addition to those mentioned above, dysexpressed LINC00974, LINC01225 and lncRNA downregulated in liver cancer stem cells (DILC) have been variably shown to correlate with clinicopathologic parameters, and/or poor outcome in HCC patients.[99—101] Furthermore, combination of lncRNAs as candidate biomarkers in HCC has been proposed. Increased expression of lncRNA urothelial cancer associated 1 (UCA1) and WD repeat containing antisense to TP53 (WRAP53) is associated with advanced HCC clinical stage. It is noteworthy that two types of lncRNAs in combination with alpha-fetoprotein have surprisingly increase sensitivity to 100%.[102] Additionally, the sensitivity and specificity values of a 2-lncRNA signature (PVT1 and uc002mbe.2) for distinguishing HCC patients from the healthy group reached 60.56% and 90.62%, respectively.[103] A group of three up-regulated lncRNAs in liver cancer, RP11-160H22.5, XLOC_014172 and LOC149086, showed improved diagnosis efficiency and credible clinical potential, with a merged area under the curve training set and validation set of 0.999 and 0.896, respectively.[104]

The use of non-coding RNA molecules packaged in exosomes as non-invasive biomarkers are a promising field of research as they are free from degradation by RNases present in plasma or other body fluids.[105] LncRNA focally amplified lncRNA on chromosome 1 (FAL1) is reportedly up-regulated in serum exosomes of HCC patients, and transfer of exosomal FAL1 to HCC cells may trigger non-positive effects.[106] Additionally, major alterations of LINC00161 in extracellular vesicles obtained from hepatoma cells can significantly discriminate HCC patients from healthy individuals.[107] Moreover, recent data from 301 participants showed higher levels of serum exosomal lncRNAs ENSG00000258332.1 and LINC00635 in the liver cancer group, which can be utilized as diagnostic biomarkers for detecting HCC.[108]

## Therapeutic potential of lncRNAs in HCC

Advancement in lncRNA-targeting therapeutics provides an outstanding opportunity to impact various aspects of cancer progression. Currently there are two major approaches employing nucleic acid therapeutics; double stranded RNA-mediated interference (RNAi) and single stranded antisense oligonucleotides (ASOs).[109] Although siRNA holds promise in therapeutic gene silencing, several barriers of *in vivo* systemic siRNA therapy still exists including circulating nuclease degradation, renal clearance, off-target effects and immune stimulation.[110] However, a PEGylated PLGA nanoplatform (NP) loaded with LINC00958 siRNA for HCC systemic administration has been developed and characterized. PLGA-based nanosystem is controlled release, tumour targeting, safe, and presents satisfactory antitumor efficacy.[111] Furthermore, *in vivo* interference with lncRNA

differentiation antagonizing non-protein coding RNA (DANCR) action through shRNA leads to decreased tumour cell vitality, tumour shrinkage, and improved mouse survival.[112] ASOs are single stranded oligonucleotides that offer specific complementarity and degrade mRNA or lncRNA via RNase H.[113] This approach for targeting HCC-related lncRNAs has been reported recently. Targeting oncogenic linc00210 by ASOs substantially attenuate tumour initiating ability of HCC *in vivo*.[114] Interestingly, Tang and colleagues generated an artificial lncRNA (AlncRNA), which is expressed by an adenoviral vector (Ad5-AlncRNA) and simultaneously harbours tandem antisense sequences for multiple miRNAs underlying sorafenib resistance in HCC. Ad5-AlncRNA turns out to have synergistic inhibitory effects with sorafenib on HCC animal models.[115] Another adenovirus-expressed lncRNAi, representing an artificial interfering lncRNA demonstrated an optimal anti-tumor effect on HCC patient-derived xenograft tumour models in nude mice, due to its complementary sequence with oncogenic miRNAs.[116]

## Conclusions and future perspectives

In this review, we comprehensively describe how large amounts of oncogenic lncRNAs in HCC could be involved in gene modulation through epigenetic, transcriptional, and post-transcriptional regulation. In addition to promoting carcinogenesis, lncRNA can also act as negative regulators of HCC progression, impacting similar cellular processes. However, not all HCC-associated lncRNAs participate in gene regulation of molecular patterns proposed above. Particularly, lncRNA associated with microvascular invasion of HCC (MVIH) promotes tumour growth and intrahepatic metastasis by activating tumour-inducing angiogenesis through inhibiting the secretion of PGK1.[117] KRASIM, a generative highly conserved 99-amino acid microprotein encoded by lncRNA NCBP2-AS2, is recognized as the first KRAS-binding protein and decreases KRAS protein level, as well as downstream ERK signalling activity in HCC.[118] These findings point towards the complexity of the lncRNA molecular mechanisms in HCC, warranting further investigations.

Mounting evidence demonstrate the suitability of lncRNAs as a molecular tool in the early diagnosis and prognosis prediction for patients with liver cancer. However, most studies have been conducted in a few cases. Collaborative projects with larger patient cohorts are necessary to identify more specific and sensitive markers for future diagnosis and prognosis. Apart from serving as biomarkers, novel strategies based on lncRNA therapy, such as RNAi and ASO, may provide potential opportunities for treating HCC. These methods can be promising alternatives in advanced liver cancer patients with limited therapeutic options. Considering the efficacy of targeting lncRNAs in animal models, careful application of these in human therapy will be critical in order to validate its stability, side effects and safety.

## Authorship statement

Duguang Li: Conceptualization, Writing-Original draft preparation. Jing Yang and Hui Lin: Writing-Reviewing and Editing. Duguang Li, Xiaoxiao Fan and Yirun Li: Making tables and drawings.

## Conflict of interests

The authors declare that there are no conflicts of interest.

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## Appendix A. Supplementary data

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