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## REVIEW

# Characteristics of long non-coding RNA and its relation to hepatocellular carcinoma

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**Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide with high prevalence and lethality. However, the underlying mechanism for HCC has not been entirely elucidated. Recent studies have highlighted the roles of long non-coding RNAs (lncRNAs) in carcinogenesis, and it is suggested that they might play critical roles in HCC progression. Here, we will briefly introduce the biology of lncRNAs, emphasizing the mechanisms and emerging roles of HCC-related lncRNAs. To date, HCC-related lncRNAs are demonstrated to influence the life cycle of genes by various means including epigenetic silencing, splicing regulation, lncRNA–miRNA interaction, lncRNA–protein interaction and genetic variation. Moreover, they can participate in diverse biological processes involved in HCC progression through impacts upon cell proliferation, apoptosis, invasion and metastasis and angiogenesis. Since lncRNA can present in body fluid and have good specificity and accessibility, some HCC-related lncRNAs are suggested to be useful as novel potential biomarkers for HCC diagnosis, prognosis and prediction of response to therapy. Those HCC-related lncRNAs may provide potential novel therapeutic targets for HCC and other diseases.**

## Introduction

Hepatocellular carcinoma (HCC) is the most common form of liver cancer, accounting for 90% of primary liver cancers. In the last decade, it has become one of the most frequently occurring tumors worldwide and is also considered to be the most lethal of the cancer systems, accounting for approximately one-third of all malignancies (1,2). The process of HCC involves a series of sequential and complex steps. Intensive investigations over the last few decades have focused on the role of protein-coding genes in the pathogenesis of HCC. Nevertheless, only ~1% of the human genome encodes proteins, leaving another ~4–9% that is transcribed to yield many short or long RNAs with limited protein-coding capacity (3). Among them, those transcribed RNA molecules >200 nucleotides in length are defined as long non-coding RNAs (lncRNAs) (4). Although the vast majority of lncRNAs have yet to be further characterized, many of these transcripts are unlikely to represent transcriptional 'noise' as a significant number have been shown to exhibit cell type-specific expression, localization to subcellular compartments and association with human diseases, especially cancers (5,6). Recently, roles for lncRNAs as drivers of tumor suppressive and oncogenic functions have appeared in diverse cancer types (7). Through various mechanisms (epigenetic silencing, splicing regulation, lncRNA–micro RNA interaction, lncRNA–protein interaction and genetic variation), HCC-related lncRNAs play critical regulatory roles in diverse biological processes involved in HCC progression, including cell proliferation, apoptosis, invasion and metastasis and angiogenesis (Table 1) (8,9). Nowadays, lncRNAs are found to be present in body fluid, like plasma and urine, and can be detected by PCR (10,11). It makes lncRNA to be a potential biomarker for various diseases. Similarly, some HCC-related lncRNAs are suggested to be useful as novel potential biomarkers for HCC diagnosis, prognosis and prediction of response to therapy because of their presences in body fluid and good specificity and accessibility (12). Here, we highlight the emerging impacts of lncRNAs in HCC, with a particular focus on the latest insights on advances made in understanding the mechanisms and functions of HCC-related lncRNAs.

**Abbreviations:** CREB, cAMP response element binding protein; Dreh, down-regulated expression by HBx; HBV, hepatitis B virus; HBx, HBV X protein; HCC, hepatocellular carcinoma; HULC, highly upregulated in liver cancer; LALR, lncRNA associated with liver regeneration; LET, low expression in tumor; lncRNA, long non-coding RNA; LSD1, lysine-specific demethylase 1; MALAT-1, metastasis associated in lung adenocarcinoma transcript 1; MEG3, maternally expressed gene 3; miRNA, micro RNA; mRNA, messenger RNA; MVIH, microvascular invasion in HCC; PGK1, phosphoglycerate kinase 1; PRC2, polycomb repressive complex 2; RERT, a lncRNA whose sequence overlaps with Ras-related GTP-binding protein 4b and prolyl hydroxylase 1; TNM, tumor node metastasis; TSA, trichostatin A.

### *Classification of lncRNAs*

The mammalian genome encodes many thousands of non-coding transcripts including both short transcripts (<200 nucleotides in length) and lncRNAs (>200 nucleotides in length) (13). As for lncRNAs, they can be divided into five broad categories according to the location relative to nearby protein-coding genes: (i) intergenic, (ii) intronic, (iii) bidirectional, (iv) antisense and (v) sense (3,14,15) (Figure 1). Several recent studies indicated that lncRNA classification may reflect functional characterization. Clark *et al.* (16) determined the half-lives of ~800 lncRNAs in the mouse Neuro-2a cell line and revealed that intergenic and antisense RNAs are more stable than those derived from introns. Additionally, the classification of lncRNAs can also provide some crucial information for studying the potential mechanism of lncRNAs. Khalil *et al.* (17) found that many human large intergenic non-coding RNAs could associate with chromatin-modifying complexes and affect gene expression. Therefore, according to the lncRNA classification, it would be easy for us to look for lncRNA's cellular localization and get relative information about the nearby genes. However, these different classes are not mutually exclusive, and it is not yet clear how the classification reflects biological function.

### *Structure of lncRNAs*

Although lncRNAs constitute a large fraction of the transcriptome, only a few lncRNAs have been structurally annotated. Works on structure of lncRNAs showed that functional lncRNAs share some primary sequence features. By analyzing 204 functional lncRNAs and their splicing variants, Niazi *et al.* (18) obtained sequence features in functional lncRNAs, including paucity of introns, low GC content, poor start codon and open reading frame contexts. In addition, the presence of motifs embedded in the lncRNA primary sequence enables lncRNAs to specifically associate with DNA, RNA and/or protein. Many lncRNAs provide alternative micro RNA (miRNA) binding sites or half-STAU1-binding sites to regulate expression levels of protein-coding genes (19). Emerging studies have also revealed that large- and small-scale mutations in the lncRNA primary sequence are highly correlated with diseases (20).

Apart from the primary sequence features, recent studies have developed genome-scale approaches to measure RNA secondary structures, resulting in a better structural understanding of several lncRNAs. Much like miRNAs, many lncRNAs have a significant secondary structure, which is critical for specific binding and function (21). For example, the lincRNA HOTAIR can form multiple double stem-loop structures for binding to the lysine-specific demethylase 1 and polycomb repressive complex 2 (PRC2) histone modification complexes (22). LncRNA SRA has a complex structural

**Table I.** LncRNAs that have been or might be linked to HCC

| LncRNA | Species | Classification | Characteristics | Molecular mechanism | Expression | Emerging roles in HCC | Genome position | Reference |
|---|---|---|---|---|---|---|---|---|
| Dreh | Mouse | Sense | 728 bp, 2 exons, downregulated by HBx | LncRNA–protein interaction | Down | Promote metastasis | chr17:64,767,111-64,767,931 | (9) |
| H19 | Human | LincRNA | ~2.3 kb, imprinted at the Igf2 locus. | LncRNA–protein interaction | Down | Suppress metastasis | chr11:2,016,406-2,019,065 | (43–46) |
| HEIH | Human | LincRNA | Highly expressed in HCC. | Epigenetic regulation | Up | Promote proliferation | chr5:180,256,954-180,258,618 | (27) |
| HOTAIR | Human | Antisense | ~2.2 kb; spliced, polyadenylated | Epigenetic regulation | Up | Promote metastasis | chr12:54,356,092-54,368,740 | (22,47,48) |
| HOTTIP | Human | Bidirectional | HOXA distal transcript | LncRNA–protein interaction | Up | Promote proliferation | Chr7:27,238,194-27,246,878 | (35,36) |
| HULC | Human | LincRNA | ~500 nt, spliced, polyadenylated, 2 exons | LncRNA–miRNA interaction<br>LncRNA–protein interaction | Up | Promote proliferation | chr6:8,652,369-8,654,079 | (31,32,37,40,49) |
| LALR1 | Mouse | Antisense | ~480 bp, associated with liver regeneration | LncRNA–protein interaction | Up | Promote proliferation | Chr17:24,155,833-24,156,739 | (8) |
| LET | Human | Intronic | ~2.3 kb, NPTN intronic transcript 1 | Epigenetic regulation<br>LncRNA–protein interaction | Down | Suppress metastasis | chr15:73,859,365-73,861,635 | (50) |
| MALAT-1 | Human | LincRNA | ~7 kb single exon transcript | Splicing regulation<br>RNA–protein interaction | Up | Promote metastasis | chr11:65,265,233-65,273,939 | (28,29)<br>(51,52) |
| MEG3 | Human | LincRNA | ~1.6 kb, imprinted at the Dlk1-Gtl2 locus | Not clear | Down | Induce apoptosis | chr14:101,292,445-101,327,368 | (53–55) |
| MVIH | Human | Sense | ~2.1 kb, microvascular invasion related lncRNA | LncRNA–protein interaction | Up | Promote angiogenesis | chr10:79,798,541-79,800,686 | (56) |
| RERT | Human | Sense | ~3 kb, RAB4B-EGLN2 readthrough | Genetic variation | UP | Not clear | chr19:41,284,124-41,314,346 | (39) |
| uc002mbe.2 | Human | LincRNA | ~3.5 kb , highly increased upon TSA treatment | Not clear | TSA induced | Induce apoptosis | chr19:4,769,117-4,772,568 | (57) |

architecture, organized into four distinct domains, with a variety of secondary structure elements and interacts with a variety of proteins (23). Additionally, non-canonical end maturation of the metastasis associated in lung adenocarcinoma transcript 1 (MALAT-1) ncRNA involves a cloverleaf secondary element at its 3′-end (24). Taken together, structural architecture of lncRNAs may contribute to a better understanding of the mechanisms responsible for functional lncRNAs.

### *Functional mechanisms of lncRNAs in HCC*

Recent studies have shown that lncRNAs are involved in various human solid tumors including HCC through various means (25). Moreover, the underlying mechanisms of lncRNAs in HCC are diverse, including epigenetic silencing, splicing regulation, lncRNA–miRNA interaction, lncRNA–protein interaction and genetic variation (Figure 2). Specifically, almost every step in the life cycle of genes—from transcription to messenger RNA (mRNA) splicing, RNA decay and translation—can be influenced by lncRNAs (26).

### *Epigenetic silencing*

A general model for lncRNA-dependent gene-silencing mechanism is presented: lncRNAs interact with chromatin, recruit histone-modifying enzymes, then induce chromatin modification and DNA methylation, finally contribute to the epigenetic silencing of target genes. In 2011, an lncRNA high expression in HCC (lncRNA-HEIH) is found to interact with enhancer of zeste homolog 2 (EZH2), a key component of PRC2, then recruit PRC2 to p16 promoter and repress the expression of p16 gene, thereby contributing to cell cycle arrest (27). This model indicates that lncRNAs may be regulators of epigenetic mechanisms while actively participating in gene regulation. Despite these exciting developments, the basic mechanism of how these lncRNAs work is still mostly unknown.

### *Splicing regulation*

Alternative splicing of pre-mRNAs can produce complex protein isoforms from a single mRNA in cell. Evidence showed that lncRNAs can influence this important process. In terms of HCC, a best-characterized lncRNA involved in splicing regulation is MALAT-1. Recent studies showed that the upregulation of MALAT-1 is a frequent event in human HCC (28,29). MALAT-1 regulated alternative splicing of endogenous target genes through interaction with the serine/arginine-rich family of nuclear phosphoproteins (SR proteins). Subsequently MALAT-1 can interact with SR proteins, influence the distribution of these and other splicing factors such as SF2/ASF and CC3 antigen, change the cellular levels of phosphorylated forms of SR proteins and finally modulate alternative splicing of various pre-mRNAs, such as oncogenic transcription factor B, CTHRC1 and several other motility-related genes. However, to date, whether MALAT-1 can target certain critical genes through splicing regulation in HCC is still not clear. And the mechanism for upregulation of MALAT-1 in HCC remains unknown.

### *LncRNA–miRNA interaction*

LncRNA can also function as a competing endogenous RNA and ‘sponges’ miRNAs, thus regulating the expression of target mRNAs. This lncRNA–miRNA interaction was firstly put forward in 2007, a study in *Arabidopsis thaliana* that found an lncRNA IPS1 to bind to the miRNA miR-399 and inhibit its ability to regulate PHO2 mRNA (30). Recently, evidences came to light suggesting that several HCC-related lncRNAs may also regulate gene expression by binding to miRNAs and consequently preventing specific miRNAs from binding to their target mRNAs. A typical example is HULC, a highly up-regulated lncRNA in liver cancer, transcribed from human chromosome 6p24.3. The HULC gene consists of two exons and a single intron, whereas HULC contains a polyA tail and particularly a conserved target site of miR-372 (31). In 2011, Wang *et al.* (32) discovered the autoregulatory loop of HULC through inhibition miRNA-372. A binding site

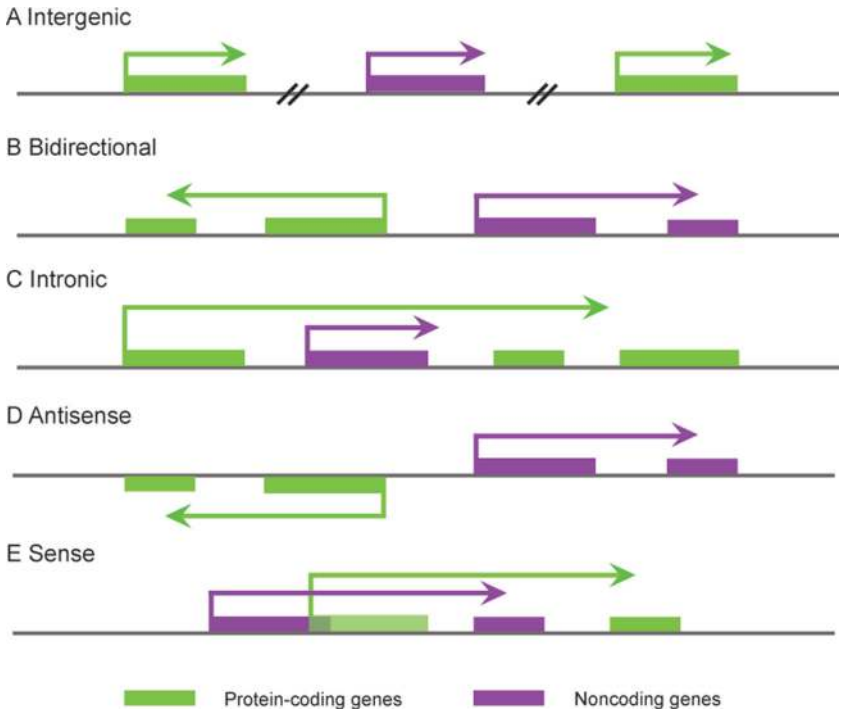


**Fig. 1.** LncRNAs can be divided into five categories according to their location relative to nearby protein-coding genes. (**A**) Intergenic lncRNAs (also termed large intervening non-coding RNAs or lincRNAs) are lncRNAs with separate transcriptional units from protein-coding genes. One definition required lincRNAs to be 5 kb away from protein-coding genes. (**B**) Bidirectional lncRNAs are transcripts that initiate in a divergent fashion from the promoter of a protein-coding gene; the precise distance cutoff that constitutes bidirectionality is not defined but is generally within a few hundred base pairs. (**C**) Intronic lncRNAs are lncRNAs that initiate inside of an intron of a protein-coding gene in either direction and terminate without overlapping exons. (**D**) Antisense lncRNAs initiate inside or 3′ of a protein-coding gene and are transcribed in the opposite direction of protein-coding genes and overlap at least one coding exon. (**E**) Sense lncRNAs sequence overlaps with the sense strand of a protein-coding gene.

of cAMP response element binding protein (CREB) is found in the promoter region of HULC. Phospho-CREB through protein kinase A pathway binds to CREB binding site and induces upregulation of HULC. And then HULC directly binds to miR-372 and represses its expression and activity. The reduction of miR-372 induces increased level of Prkacb (cAMP-dependent protein kinase catalytic subunit beta), a target mRNA of miR-372. Interestingly, Prkacb can facilitate phosphorylation of CERB in protein kinase A pathway. Finally, an autoregulatory loop (CREB-HULC-miR372-Prkacb) is formed (Figure 3B). Nowadays, to facilitate the study of lncRNA–miRNA interactions, researchers have developed different databases, such as miRcode (http://www.mircode.org) and CHIPBase (http://deepbase.sysu.edu.cn/chipbase/) (33,34). This interaction can contribute to the better understanding of lncRNAs and miRNAs.

*LncRNA–protein interaction*

LncRNAs can participate in global cellular behaviors by binding to specific proteins. Through lncRNA–protein interactions, lncRNAs can serve as structural components or modulate protein activity or alter protein localization. As for HCC, lncRNA HOTTIP, upregulated in HCC, is able to directly coordinate and control the activation of several 5′ HOXA genes including HOXA13 and HOXA11 via interacting with the WDR5/MLL complex (35,36). Interestingly, certain protein binding with lncRNAs can also regulate the expression of lncRNA. Take HULC as an example. HULC can bind with the IGF2 mRNA-binding protein 1 (IGF2BP1) in hepatocytes. Then IGF2BP1 recruits CNOT1, a component of the CCR4-NOT deadenylase complex and brings HULC into close proximity to the CCR4-NOT deadenylase complex, which initiates RNA degradation from the 3′-end, eventually specifically distablizing HULC (Figure 3B) (37). Thus, it is possible for one lncRNA to bind to different proteins. Therefore, the functional mechanism of lncRNA may depend on its target protein.

*Genetic variation*

Surprisingly, emerging studies also revealed the presence of large- and small-scale mutations in the lncRNA primary sequence that is highly correlated with cancer. Cheetham *et al.* (38) found that of 301 single nucleotide polymorphisms currently linked to cancer, only 12 (3.3%) change the protein amino-acid sequence, whereas most are located in the introns of protein-coding genes (40%) or intergenic regions (44%). Hence, mutations potentially affected a large number of lncRNAs. Recently, variations in lncRNA genes, either germline or somatic, are demonstrated to contribute to HCC. In an independent case–control study consisting of 444 HCC cases and 450 controls, a 4 bp insertion/deletion polymorphism (rs10680577) within RERT-lncRNA (a lncRNA whose sequence overlaps with Ras-related GTP-binding protein 4b and prolyl hydroxylase 1) is found to contribute to hepatocarcinogenesis and modulate HCC risk, possibly by affecting RERT-lncRNA structure (39). Additionally, Liu *et al.* (40) investigated two single nucleotide polymorphisms, rs7763881 in HULC and rs619586 in MALAT-1, in 1300 hepatitis B virus (HBV) positive HCC patients, 1344 HBV persistent carriers and 1344 subjects with HBV natural clearance. Finally, the variant genotypes of rs7763881 in HULC are significantly associated with decreased HCC risk, whereas the variant genotypes of rs619586 in MALAT-1 are associated with decreased HCC risk with a borderline significance. Compared with variations in protein-coding genes, where certain single nucleotide mutation can completely change protein structure or function, the effects of variations in lncRNAs may be more difficult to detect experimentally. Better annotation of lncRNA primary and secondary structures should improve the detection of genome aberrations that affect lncRNAs.

*Emerging roles of lncRNAs in HCC*

Currently, lncRNAs were found to be deregulated in several human cancers and show tissue-specific expression. Importantly, some were

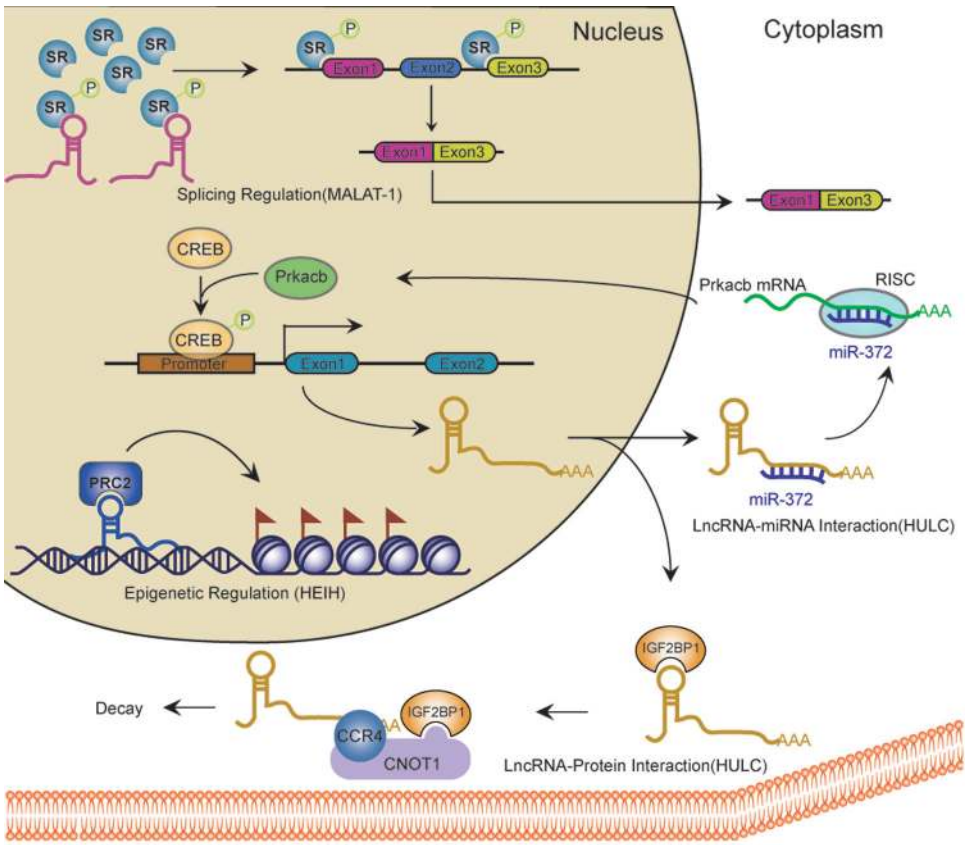


**Fig. 2.** Functional mechanisms of lncRNAs in HCC. HCC-related lncRNAs can participate in diverse biological processes by various means including epigenetic regulation, splicing regulation, lncRNA–miRNA interaction and lncRNA–protein regulation. HEIH can recruit PRC2 complex, then induce chromatin modification and DNA methylation, finally contribute to the epigenetic silencing of target genes (epigenetic regulation). MALAT-1 can change the cellular levels of phosphorylated forms of SR proteins, finally modulating alternative splicing of various pre-mRNAs (splicing regulation). Additionally, HULC can interact with miR-372 and finally form an autoregulatory loop through inhibition of miRNA-372 (lncRNA–miRNA interaction). Moreover, IGF2BP1 can bind to HULC and guide it to CCR4-NOT complex, eventually initiating HULC degradation (lncRNA–protein interaction). HEIH, high expression in HCC; P, phosphorylation; RISC, RNA-induced silencing complex; SR, serine/arginine-rich family of nuclear phosphoproteins.

elucidated to influence the hallmarks of cancer, including proliferation, apoptosis, invasion and metastasis and angiogenesis (41). Similarly, evidences showed that the lncRNA expression profiles between HCC tumor tissues and normal liver samples or peritumoral tissues are quite different (42) and various lncRNAs have been or might be linked to HCC (Table I). Moreover, misexpression of lncRNAs may also affect these fundamental biological capacities of HCC.

*Proliferation*

One of the most prominent characteristics of a cancer cell is its ability to proliferate constantly and in the absence of external stimuli. To get independent of proliferation signals and achieve unlimited growth, cancer cells will change the production or function of growth promoting or inhibiting factors in multiple ways (58). In several types of cancers, as well as in HCC, the analysis of lncRNA expression has led to the identification of lncRNAs promoting or repressing cell proliferation. Here we focus on two best-characterized lncRNAs-HULC and LALR, which can impact proliferation through targeting various key regulators in different pathways.

Du *et al.* (49) found that HULC can be upregulated by HBV X protein (HBx) and then promotes hepatocyte proliferation via downregulating p18 (hyphantria cunea nucleopolyhedrovirus) (Figure 3B). In their study, HBx is demonstrated to activate the HULC promoter via CREB. Importantly, silencing HULC resulted in a significant decrease of cell proliferation of HBx stably transfected cell lines in a time-dependent manner, whereas overexpression of HULC was able to enhance the proliferation of L-O2 cells, a derivative of parental HepG2 cells. The same consequences were conformed in xenografts in nude mice of the same cells. And then, p18, a tumor suppressor by translocating to the nucleus to activate p53 pathway (59), is thought to be a target gene of HULC. Further analysis showed that HULC promotes proliferation of hepatocytes through downregulating p18 *in vitro* and *in vivo*. However, whether HULC downregulates p18 through direct binding or indirect interaction remains unclear in this essay. Further validation is needed.

Another example of lncRNA involved in proliferation is lncRNA-LALR1 (lncRNA associated with liver regeneration), a ~480 bp non-coding RNA in mouse. In Xu's study, LALR1 is specifically upregulated in hepatocytes after two-thirds partial hepatectomy in mouse and can reduce the $G_0/G_1$ population in hepatocytes, finally accelerating cell proliferation. For further mechanism investigation, LALR1 can activate the Wnt/β-catenin pathway in mouse hepatocytes by suppressing AXIN1. Specifically, LALR1 could associate with transcription factor CTCF, recruiting CTCF to the AXIN1 promoter region to inhibit its expression. With the decreased expression of Axin1, the stability of the β-catenin destruction complex recede and meanwhile active β-catenin is released and binds to proteins such as T cell factor-4 and lymphoid enhancement factor and translocates to the nucleus to control the transcription of target genes, including c-myc and cyclin D1. Finally, LALR1 facilitates mouse cell cycle progression and hepatocyte proliferation. Importantly, a human ortholog RNA of LALR1 (hLALR1) is found to be expressed in human liver tissues. But whether it functions like mouse LALR1 needs further investigation (Figure 3A) (8).

Notably, although MALAT-1 is suggested to control cell cycle progression in many solid tumors and to be upregulated in HCC, loss

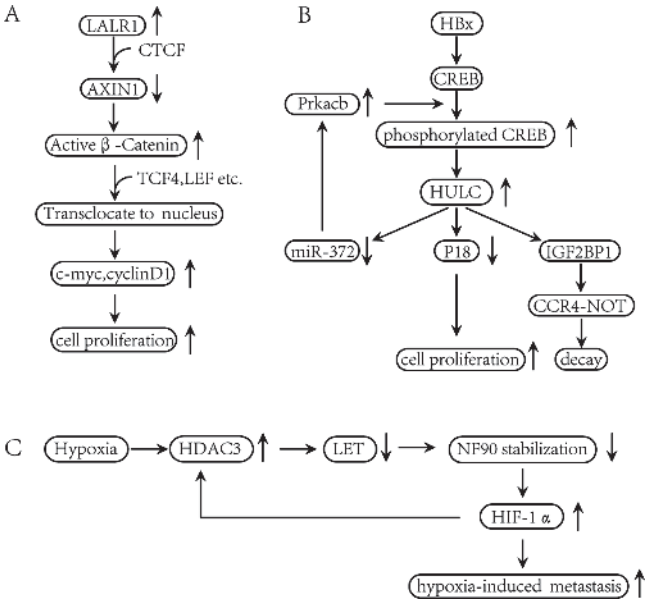


**Fig. 3.** The pathways in which typical HCC-related lncRNA are implicated. (**A**)LncRNA-LALR1 accelerates hepatocyte proliferation during liver regeneration by activating Wnt/beta-Catenin signaling. (**B**) The pathways in which HULC are involved. HBx protein upregulates HULC via CREB. The elevation of HULC promotes hepatoma cell proliferation via down-regulating p18. Moreover, HULC can be upregulated through an auto-regulatory loop (CREB-HULC-miR372-Prkacb). Additionally, HULC can bind to IGF2BP1 protein. Then IGF2BP1 recruits the CCR4-NOT deadenylase complex, finally leading to HULC decay. (**C**) LncRNA LET inhibits the metastasis of HCC through a hypoxia-inducible factor 1, alpha subunit (HIF-1α) / histone deacetylase 3 (HDAC3)/ LET/NF90 pathway.

of MALAT-1 does not affect proliferation, cell cycle progression or nuclear architecture in human liver cancer cells (51,60). The corresponding mechanism remains to be further studied.

*Apoptosis*

Programmed cell death by apoptosis serves as a natural barrier to cancer development. It can be induced by various external as well as internal stimuli. Various cancers were reported to attenuate apoptosis via different pathways (caspases pathway, p53 pathway, etc.) and eventually become therapy resistant (52). This notion holds true also for HCC.

Braconi *et al.* (61) found that the expression of maternally expressed gene 3 (MEG3) is markedly reduced in four human HCC cell lines compared with normal hepatocytes and enforced expression of MEG3 in HCC cells significantly induce apoptosis. MEG3 gene is an imprinted gene belonging to the imprinted DLK1–MEG3 locus located at chromosome 14q32.3 in human. It consists of 10 exons and can yield multiple MEG3 transcript isoforms due to alternative RNA splicing. MEG3 RNA is predicted by *Mfold* (a computer program widely used to predict the secondary structures of nucleic acids based on their thermostability) to fold into three major motifs, M1, M2 and M3, which are related to its p53-activating function (53). Evidences elucidated that MEG3 is expressed in normal tissues, whereas its expression is lost in an expanding list of primary human tumors and tumor cell lines. In terms of HCC, MEG3 is lost or significantly reduced in >80% cases (53). In Braconi's study, apoptotic HCC cells are significantly increased after transfection with MEG3. Interestingly, an increase of p53 is also conformed in these cells. This result consists with the previous finding that MEG3 can functionally interact with p53 and selectively activate expression of p53 target genes (62). Hence, enhanced expression of MEG3 in hepatocytes can induce apoptosis through p53-dependent transcription (61). However, the p53 expression varies in different liver cancer cell lines, whereas MEG3 expression is significantly reduced. So, it is possible that the apoptotic effect of MEG3 is only partially mediated by p53 in HCC. Alternative mechanisms may be function in place.

Besides, another study showed that liver cancer-downregulated lncRNA uc002mbe.2 could be induced by trichostatin A (TSA) treatment and its expression is positively correlated with the apoptotic effect of TSA in HCC cells. Knockdown the expression of uc002mbe.2 significantly reduced TSA-induced apoptosis of Huh7 cells (54). But the underlying mechanism of how uc002mbe.2 promotes TSA-induced apoptosis has not yet been discussed.

*Invasion and metastasis*

Accumulating evidences have demonstrated that many patients die from metastases and not from the primary tumor. Invasion and metastasis comprise various biological processes, beginning with local invasion, then intravasation into nearby blood and lymphatic vessels, transit through the lymphatic and hematogenous systems, extravasation, micrometastases and finally colonization (41,58). Recently many HCC-related lncRNAs are found to play important roles in cell metastasis and invasion.

A typical example is lncRNA-Dreh (an lncRNA downregulated expression by HBx). In 2013, Dreh is first characterized to be significantly downregulated in HBx-transgenic mice and mouse liver cells expressing HBx. Suppression of cellular Dreh promotes the migration and invasion activity of liver cancer cells *in vitro* and *in vivo*. Further experiments showed that Dreh can specifically bind to vimentin, a type III intermediate filament and the major cytoskeletal component of mesenchymal cells and then inhibit HCC metastasis by modifying the expression and reorganization of vimentin. Vimentin is significantly downregulated and present as helical filament structures extending from the nuclear membrane to the cellular membrane in the Dreh-transfected cells, whereas the filament structures are retracted to the nuclear membrane in the control cells. Moreover, extensive filamentous aggregation and many irregular fragmented aggregated structures can also be found in the cytoplasm of Dreh-transfected cells. All these changes about vimentin would result in instability of the cells and finally promote cell migration. Moreover, a human ortholog RNA of Dreh (hDREH) is identified to be frequently downregulated in HBV-related HCC tissues and its decrement significantly correlated with poor survival of HCC patients. Therefore, it would be of great clinical significance to further study the hDREH (9).

Importantly, another lncRNA-LET (lncRNA low expression in tumor) is found to play a critical role in hypoxia-induced metastasis in HCC (Figure 3C). LET is generally downregulated in various cancer types, including HCC, colorectal cancer and squamous cell lung carcinoma. Gain and loss of function of LET in orthotopic tumor models of nude mouse showed that LET reduces hepatic invasion and abdominal metastases. Yang *et al.* (55) finally determined a hypoxia-inducible factor 1, alpha subunit (HIF-1a)/histone deacetylase 3 (HDAC3)/LET/NF90 pathway. Precisely, HDAC3, which has been recently demonstrated to be regulated by HIF-1a, can repress LET by reducing the histone acetylation-mediated modulation of the LET promoter region under hypoxic conditions. Subsequently, the downregulation of LET reduces the direct interactions between LET and NF90, then enhance the stabilization of NF90 and finally increases the expression of HIF-1a, a target mRNA of NF90 involved in hypoxia-induced metastasis. Hence, LET inhibit the metastasis of HCC through this positive feedback loop. This feedback loop really adds the complexity of gene regulation network.

Besides, other HCC-related lncRNAs have been found to be involved in metastasis. For instance, lncRNA H19 can associate with the protein complex hnRNP U/PCAF/RNAPol II, activating miR-200 family through histone acetylation, thus suppressing HCC progression metastasis (57). In addition, another well-known lncRNA, HOTAIR, is upregulated in HCC. Knocking down of HOTAIR in HepG2 cells leads to significantly reduced invasive capability, thus contributing to a less invasive and malignant phenotype of the HCC

cells. Although HOTAIR have been demonstrated to regulate a variety of genes through epigenetic slicing, its exact molecular mechanism in HCC mostly remains unknown (43,50).

*Angiogenesis*

As the tumor mass and size increases, the formation of new blood vessels is required to not only provide nutrients and oxygen but also allow tumors to dispose their metabolic wastes and enter the hematogenous metastatic process, as well. Often, tumor cells induce proangiogenic factors or block antiangiogenic signals to turn on an 'angiogenic switch'. Increasing evidences showed that lncRNAs also function in regulating the angiogenic process (41).

One such lncRNA in HCC is lncRNA-MVIH (lncRNA associated with microvascular invasion in HCC). In 2012, Yuan *et al.* (47) found that MVIH is generally overexpressed and negatively correlates with recurrence-free survival and overall survival in HCC. Furthermore, MVIH is elucidated to be specifically associated with phosphoglycerate kinase 1 (PGK1), an enzyme of glycolysis and a factor that can be secreted by tumor cells and inhibit angiogenesis, as well. Finally, MVIH weakens the function of PGK1 in angiogenesis inhibition by reducing the secretion of PGK1 but could not regulate the function of PGK1 in glycolysis. The similar results are also found in clinical samples. MVIH expression level inversely correlates with the serum level of PGK1 and positively correlates with the microvessel density. However, the mechanism for the upregulation of MVIH in HCC is still not well investigated.

*Others*

There are also some HCC-related lncRNAs participate in the progression of HCC by other means. Some certain lncRNAs are suggested to involve in drug resistance. Take H19 lncRNA as an example. H19 gene belongs to a highly conserved imprinted *H19-IGF2* locus, encoding a ~2.3 kb long, capped, spliced, and polyadenylated RNA. Notably, the structure of the H19 gene corresponds to a typical intergenic miRNA primary transcript (63). It contains a microRNA (miR-675) in exon 1, which may be responsible for the oncogenic activity of H19 (64). H19 is upregulated in doxorubicin-resistant R-HepG2 cells and is believed to induce P-glycoprotein expression and MDR1-associated drug resistance in HepG2 cells through regulation of MDR1 promoter methylation (48).

Although many lncRNAs were found to be associated with HCC by different means, the mechanism of functional HCC-related lncRNA is still not entirely elucidated and needs further investigation. But notably, all these biological processes mentioned above might provide new avenues for therapeutic intervention against HCC progression.

*Potential diagnostic and therapeutic applications of LncRNAs in HCC*

LncRNAs are emerging as biomarkers for HCC and other diseases due to their attractive features. Many lncRNAs have restricted tissue-specific and cancer-specific expression patterns. Often, a significant increase or decrease in lncRNA expression levels is found in tumors compared with normal tissues. Moreover, similar as circulating miRNAs, some types of lncRNAs are demonstrated to be present in body fluids, like urine and plasma, which can be easily obtained by the least invasive methods (10,56). But the exact mechanism for the release of lncRNAs into body fluids is still not well defined. Some studies suggested that the apoptosis and necrosis of cancer cells in the tumor microenvironment could make a difference. RNA may be packaged into microparticles, including exosomes, microvesicles, apoptotic bodies and apoptotic microparticles, thus secreted into body fluids (10,44). Given this specificity and good accessibility, lncRNAs may be superior biomarkers than many current protein-coding biomarkers.

As for HCC, some lncRNAs may be useful as novel potential biomarkers for diagnosis, prognosis and prediction of response to therapy. HULC can be detected with higher frequency in the plasma of HCC patients compared with healthy controls and may serve as a promising non-invasive novel biomarker for the detection and diagnosis of HCC (31,46). MVIH, for example, can serve as a predictor for HCC patients' poor recurrence-free survival after hepatectomy. In a cohort of 215 HCC patients, overexpressing MVIH is related to a higher tumor node metastasis stage as well as decreased recurrence-free survival and overall survival (47). Additionally, Yang *et al.* (50) examined the expression of HOTAIR in 110 HCC samples (among them, 60 were undergone liver transplantation) and finally found that overexpression of HOTAIR predicts tumor recurrence in HCC patients following liver transplantation. The similar phenomenon is also observed in mouse. LncRNAs originating from the Dlk1-Dio3 imprinted gene cluster showed progressive increases in phenobarbital-mediated liver tumor promotion and can be used as novel candidate biomarkers for liver tumor promotion (46).

Although lncRNAs are suggested to be biomarkers of HCC, some critical issues need to be resolved before their clinical appliance. Firstly, so far, a majority of studies are done in a small number of cases. Further validations in larger patient cohorts are needed to confirm the correlation and diagnosis sensitivity and specificity between certain lncRNAs and HCC. In the second place, another challenge relates to the low amount of total lncRNAs in plasma or serum or urine, which makes it necessary to use amplification to measure lncRNAs. To date, Q-PCR is the most widely used method. The concentration and quality of lncRNAs can be confounded by multiple technical factors in the preanalytical and analytical procedures. Therefore, normalization is an important aspect to measure lncRNAs. And it is very important for clinical laboratory to make a standard analytical protocol for sample preparing and operating process, thus minimize interprocedure bias and implement quality control. Finally, at the moment, most studies are investigating the utility of individual lncRNAs as biomarker for diseases, but a combination of multiple lncRNAs or lncRNAs and miRNAs may provide greater accuracy since different causes for the disease might result in different levels of plasma lncRNAs and miRNAs.

Apart from being biomarkers, lncRNAs may also provide new targets for HCC therapy. Some researchers suggest that lncRNAs might be directly applied as therapeutic agents. The use of RNAi for human therapy by *in vivo* administration has already been proved successful (65). However, it seems like a daunting task to use lncRNAs directly as therapeutic bullets because of the big size. To deliver lncRNAs into the cancer cells, gene therapy delivery systems (for example, viruses) would be required and meanwhile bring risk. On the other hand, lncRNAs might provide new therapeutic targets because of the lncRNA–miRNA interaction. It would also be possible to design synthetic small interfering RNAs, antisense oligonucleotide or miRNAs to target certain lncRNAs. Another indirect way to cancer-specific therapeutics would be targeting the lncRNA–protein interface and focusing on the cancer-specific transcript. Totally, continuous efforts should be made to achieve lncRNA-based cancer therapies.

## Conclusions

In conclusion, although plenty of key questions remain unanswered, many studies all suggest that lncRNAs act as important regulators in various biological processes in diverse diseases, including HCC. According to the latest studies, HCC-related lncRNAs can influence HCC initiation, progression and treatment. However, to date, lncRNA research still remains in its infancy. Only a small part of HCC-related lncRNAs have been well characterized and a large portion surely remains to be further discovered. They may provide new methods for the diagnosis and treatment of HCC. Systematic identification of lncRNAs and well understanding of their mechanisms should pave the way for designing therapeutics for HCC.

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## References

1. Cervello,M. *et al*. (2012) Targeted therapy for hepatocellular carcinoma: novel agents on the horizon. *Oncotarget*, **3**, 236–260.
2. El-Serag,H.B. *et al*. (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology*, **132**, 2557–2576.
3. Ponting,C.P. *et al*. (2009) Evolution and functions of long noncoding RNAs. *Cell*, **136**, 629–641.
4. Wang,K.C. *et al*. (2011) Molecular mechanisms of long noncoding RNAs. *Mol. Cell*, **43**, 904–914.
5. Wilusz,J.E. *et al*. (2009) Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev*., **23**, 1494–1504.
6. Qureshi,I.A. *et al*. (2010) Long non-coding RNAs in nervous system function and disease. *Brain Res*., **1338**, 20–35.
7. Prensner,J.R. *et al*. (2011) The emergence of lncRNAs in cancer biology. *Cancer Discov*., **1**, 391–407.
8. Xu,D. *et al.* (2013) Long noncoding RNAs associated with liver regeneration 1 accelerates hepatocyte proliferation during liver regeneration by activating Wnt/beta-catenin signaling. *Hepatology*, **58**, 739–751.
9. Huang,J.F. *et al*. (2013) Hepatitis B virus X protein (HBx)-related long noncoding RNA (lncRNA) down-regulated expression by HBx (Dreh) inhibits hepatocellular carcinoma metastasis by targeting the intermediate filament protein vimentin. *Hepatology*, **57**, 1882–1892.
10. Huang,X. *et al*. (2013) Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics*, **14**, 319.
11. Ren,S. *et al*. (2013) Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 derived miniRNA as a novel plasma-based biomarker for diagnosing prostate cancer. *Eur. J. Cancer*, **49**, 2949–2959.
12. Van Roosbroeck,K. *et al*. (2013) miRNAs and long noncoding RNAs as biomarkers in human diseases. *Expert Rev. Mol. Diagn*., **13**, 183–204.
13. Guttman,M. *et al*. (2012) Modular regulatory principles of large non-coding RNAs. *Nature*, **482**, 339–346.
14. Rinn,J.L. *et al*. (2012) Genome regulation by long noncoding RNAs. *Annu. Rev. Biochem*., **81**, 145–166.
15. Yan,B. *et al*. (2012) Long noncoding RNA: its physiological and pathological roles. *DNA Cell Biol*., **31** (suppl. 1), S34–S41.
16. Clark,M.B. *et al*. (2012) Genome-wide analysis of long noncoding RNA stability. *Genome Res*., **22**, 885–898.
17. Khalil,A.M. *et al*. (2009) Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc. Natl Acad. Sci. USA*, **106**, 11667–11672.
18. Niazi,F. *et al*. (2012) Computational analysis of functional long noncoding RNAs reveals lack of peptide-coding capacity and parallels with 3′ UTRs. *RNA*, **18**, 825–843.
19. Novikova,I.V. *et al*. (2012) Sizing up long non-coding RNAs: do lncRNAs have secondary and tertiary structure? *Bioarchitecture*, **2**, 189–199.
20. Halvorsen,M. *et al.* (2010) Disease-associated mutations that alter the RNA structural ensemble. *PLoS Genet*., **6**, e1001074.
21. Volders,P.J. *et al.* (2013) LNCipedia: a database for annotated human lncRNA transcript sequences and structures. *Nucleic Acids Res*., **41**, D246–D251.
22. Gupta,R.A. *et al*. (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature*, **464**, 1071–1076.
23. Novikova,I.V. *et al*. (2012) Structural architecture of the human long non-coding RNA, steroid receptor RNA activator. *Nucleic Acids Res*., **40**, 5034–5051.
24. Wilusz,J.E. *et al*. (2008) 3′ end processing of a long nuclear-retained non-coding RNA yields a tRNA-like cytoplasmic RNA. *Cell*, **135**, 919–932.
25. Li,C.H. *et al.* (2013) Targeting long non-coding RNAs in cancers: progress and prospects. *Int. J. Biochem. Cell. Biol.*, **45**, 1895–1910.
26. Wapinski,O. *et al*. (2011) Long noncoding RNAs and human disease. *Trends Cell Biol*., **21**, 354–361.
27. Yang,F. *et al*. (2011) Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. *Hepatology*, **54**, 1679–1689.
28. Lin,R. *et al*. (2007) A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. *Oncogene*, **26**, 851–858.
29. Lai,M.C. *et al*. (2012) Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. *Med. Oncol*., **29**, 1810–1816.
30. Franco-Zorrilla,J.M. *et al*. (2007) Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet*., **39**, 1033–1037.
31. Panzitt,K. *et al*. (2007) Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. *Gastroenterology*, **132**, 330–342.
32. Wang,J. *et al*. (2010) CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. *Nucleic Acids Res*., **38**, 5366–5383.
33. Yang,J.H. *et al.* (2013) ChIPBase: a database for decoding the transcriptional regulation of long non-coding RNA and microRNA genes from ChIP-Seq data. *Nucleic Acids Res*., **41**, D177–D187.
34. Jeggari,A. *et al*. (2012) miRcode: a map of putative microRNA target sites in the long non-coding transcriptome. *Bioinformatics*, **28**, 2062–2063.
35. Wang,K.C. *et al*. (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature*, **472**, 120–124.
36. Quagliata,L. *et al.* (2013) lncRNA HOTTIP/HOXA13 expression is associated with disease progression and predicts outcome in hepatocellular carcinoma patients. *Hepatology*. doi:10.1002/hep.26740.
37. Hammerle,M. *et al.* (2013) Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). *Hepatology*, **58**, 1703–1712.
38. Cheetham,S.W. *et al*. (2013) Long noncoding RNAs and the genetics of cancer. *Br. J. Cancer*, **108**, 2419–2425.
39. Zhu,Z. *et al*. (2012) An insertion/deletion polymorphism within RERT-lncRNA modulates hepatocellular carcinoma risk. *Cancer Res*., **72**, 6163–6172.
40. Liu,Y. *et al*. (2012) A genetic variant in long non-coding RNA HULC contributes to risk of HBV-related hepatocellular carcinoma in a Chinese population. *PLoS One*, **7**, e35145.
41. Gutschner,T. *et al*. (2012) The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol*., **9**, 703–719.
42. Pan,Y.F. *et al*. (2013) Expression profile of altered long non-coding RNAs in patients with HBV-associated hepatocellular carcinoma. *J. Huazhong Univ. Sci. Technolog. Med. Sci*., **33**, 96–101.
43. Geng,Y.J. *et al*. (2011) Large intervening non-coding RNA HOTAIR is associated with hepatocellular carcinoma progression. *J. Int. Med. Res*., **39**, 2119–2128.
44. Orozco,A.F. *et al*. (2010) Flow cytometric analysis of circulating microparticles in plasma. *Cytometry A*, **77**, 502–514.
45. Xie,H. *et al*. (2013) Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. *Biomed. Res. Int*., **2013**, 136106.
46. Lempiäinen,H. *et al*. (2013) Identification of Dlk1-Dio3 imprinted gene cluster noncoding RNAs as novel candidate biomarkers for liver tumor promotion. *Toxicol. Sci*., **131**, 375–386.
47. Yuan,S.X. *et al.* (2012) Long noncoding RNA associated with microvascular invasion in hepatocellular carcinoma promotes angiogenesis and serves as a predictor for hepatocellular carcinoma patients' poor recurrence-free survival after hepatectomy. *Hepatology*, **56**, 2231–2241.
48. Tsang,W.P. *et al*. (2007) Riboregulator H19 induction of MDR1-associated drug resistance in human hepatocellular carcinoma cells. *Oncogene*, **26**, 4877–4881.
49. Du,Y. *et al*. (2012) Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation via down-regulating p18. *J. Biol. Chem*., **287**, 26302–26311.
50. Yang,Z. *et al*. (2011) Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. *Ann. Surg. Oncol*., **18**, 1243–1250.
51. Eissmann,M. *et al.* (2012) Loss of the abundant nuclear non-coding RNA MALAT1 is compatible with life and development. *RNA Biol*., **9**, 1076–1087.
52. Hengartner,M.O. (2000) The biochemistry of apoptosis. *Nature*, **407**, 770–776.
53. Zhou,Y. *et al*. (2012) MEG3 noncoding RNA: a tumor suppressor. *J. Mol. Endocrinol*., **48**, R45–R53.
54. Yang,H. *et al.* (2013) Induction of the liver cancer-down-regulated long noncoding RNA uc002mbe.2 mediates trichostatin-induced apoptosis of liver cancer cells. *Biochem. Pharmacol.*, **85**, 1761–1769.

55. Yang,F. *et al.* (2013) Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. *Mol. Cell.*, **49**, 1083–1096.
56. Lee,G.L. *et al*. (2011) Prostate cancer: diagnostic performance of the PCA3 urine test. *Nat. Rev. Urol*., **8**, 123–124.
57. Zhang,L. *et al*. (2013) Epigenetic activation of the MiR-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. *Carcinogenesis*, **34**, 577–586.
58. Hanahan,D. *et al*. (2011) Hallmarks of cancer: the next generation. *Cell*, **144**, 646–674.
59. Park,B.J. *et al*. (2005) The haploinsufficient tumor suppressor p18 upregulates p53 via interactions with ATM/ATR. *Cell,* **120**, 209–221.
60. Tripathi,V. *et al*. (2013) Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. *PLoS Genet.,* **9**, e1003368.
61. Braconi,C. *et al*. (2011) microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. *Oncogene,* **30**, 4750–4756.
62. Zhou,Y. *et al*. (2007) Activation of p53 by MEG3 non-coding RNA. *J. Biol. Chem.,* **282**, 24731–24742.
63. Smits,G. *et al*. (2008) Conservation of the H19 noncoding RNA and H19-IGF2 imprinting mechanism in therians. *Nat. Genet.,* **40**, 971–976.
64. Tsang,W.P. *et al*. (2010) Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer. *Carcinogenesis,* **31**, 350–358.
65. Davis,M.E. *et al*. (2010) Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature,* **464**, 1067–1070.

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