

Long Noncoding RNAs as a Key Player in Hepatocellular Carcinoma

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ABSTRACT: Hepatocellular carcinoma (HCC) is a major malignancy in the liver and has emerged as one of the main cancers in the world with a high mortality rate. However, the molecular mechanisms of HCC are still poorly understood. Long noncoding RNAs (lncRNAs) have recently come to the forefront as functional non-protein-coding RNAs that are involved in a variety of cellular processes ranging from maintaining the structural integrity of chromosomes to gene expression regulation in a spatiotemporal manner. Many recent studies have reported the involvement of lncRNAs in HCC which has led to a better understanding of the underlying molecular mechanisms operating in HCC. Long noncoding RNAs have been shown to regulate development and progression of HCC, and thus, lncRNAs have both diagnostic and therapeutic potentials. In this review, we present an overview of the lncRNAs involved in different stages of HCC and their potential in clinical applications which have been studied so far.

KEYWORDS: Long noncoding RNAs, microRNAs, hepatocellular carcinoma, cancer

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Introduction

Hepatocellular carcinoma (HCC) is the primary cancer of the liver. It has become one of the most frequent cancer conditions accounting for the third leading cause of deaths caused by cancer.¹ Hepatocellular carcinoma-caused mortality is higher especially in developing countries, such as Asian and African region countries,² where hepatitis B virus (HBV) and hepatitis C virus (HCV) have been related to HCC.³ Hepatocellular carcinoma is associated with a poor prognosis rate and various risk factors that are not limited to chronic infections with HBV, HCV, alcohol-induced liver disease, cirrhosis, and diabetes mellitus, to name just a few.^{4–6} It has been a common observation that patients with a history of liver cirrhosis and chronic liver diseases have a predisposition toward developing HCC. An additional risk is posed by environmental factors, nutritional factors, endocrine factors, obesity, and certain hereditary conditions such as hemochromatosis.⁷ Moreover, there is a multitude of causal factors which influence the onset of HCC, and they include nonalcoholic steatohepatitis, alcohol abuse, and chronic HBV/HCV.⁸

The complexity of HCC pathology has made it challenging to identify the main causative agents for its onset and development.² Different protein-coding genes have been shown to have roles in development and progression of the disease. These genes include genes involved in regulation of cell cycle, apoptosis, DNA damage response, and cell signaling.⁹ However, there is a discordance between the expression patterns of these genes, which has made it difficult to generate a model for genes involved in HCC.⁹ Due to the limited knowledge and nonuniformity in

HCC symptoms, many different treatment strategies have been developed in the past with limited success. The most commonly used clinical therapy for HCC treatment is liver transplantation and resection of the liver. However, these treatments have their own limitations and the risk of recurrence of cancer. Therefore, other clinical therapies have also been developed including radio frequency ablation¹⁰ and chemotherapy,¹¹ however, with limited success. Currently, there are no suitable treatment options because many of the target genes of these treatments are part of different pathways critical to cell function. Therefore, it can be stated that there are many knowledge gaps in our understanding and the molecular processes involved in HCC. In the recent years, increasing amounts of research have focused on the causative noncoding RNAs (ncRNAs) involved in HCC that may shed some light in understanding the still unclear molecular processes and agents involved in HCC.^{12–14}

Advances in techniques used for studying transcriptome have led to the emergence of ncRNAs as a vital regulatory essence of the genome. Recently, a study based on the Encyclopedia of DNA Elements (ENCODE) revealed that more than 90% of the genome is transcribed, and therefore, there is an enormous number of RNA transcripts generated apart from messenger RNAs (mRNAs).¹⁵ These transcribed ncRNAs are non-protein-coding RNAs which act as their own functional entity. Examples of ncRNAs include transfer RNAs, small nucleolar RNA, ribosomal RNAs, small interfering RNAs, small nuclear RNAs, microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), and long non coding RNAs (lncRNAs). These ncRNAs



form a vast and overlapping network of transcripts which have largely unknown functions. Although much of the research on HCC has focused on the gene expression patterns of traditional protein-coding genes, many research groups have recently attempted the expression-based identification of ncRNAs.¹⁶ These studies have discovered several ncRNAs which show differential expression patterns and regulatory effects on initiation, progression, and aggressiveness of HCC.¹⁶ MicroRNAs such as *miR-21*, *miR-122*, *miR-221/222*, *miR-520*, and *miR-657* have been shown to be promising biomarkers and therapeutic targets for HCC.^{17–20} The piRNAs also hold some therapeutic potential according to a recent study by Rizzo et al²¹ who identified 125 piRNAs deregulated in HCC and cirrhotic liver cells. They identified many important regulatory genes targeted by these piRNAs such as cell cycle regulators, tumor suppressor genes, and genes involved in apoptosis.²¹ PIWI proteins have also been found dysregulated in HCC. For example, although increased levels of PIWIL1 protein was observed in HCC cell lines, decreased expression of PIWIL1 led to arrest of metastasis and invasion.^{22,23}

Long noncoding RNAs have also been shown as important regulators of HCC. Long noncoding RNAs, as the name implies, are 200 bp (base pairs) to several kilobases (kb) long and are generally transcribed by RNA polymerase II,^{24,25} spliced, polyadenylated, and sometimes localized in the nucleus or cytoplasm. They hold a significant place in the HCC development, and these have recently emerged as one of the major players in regulating genome dynamics. This is in part achieved by their highly specialized expression patterns in response to signals and different cell types²⁶ which are highly regulated in a spatiotemporal manner.²⁷ Due to their varied nature, the functions of lncRNAs have not been fully unraveled, and many lncRNAs are still uncharacterized functionally.²⁵ Their involvement in the regulation of different stages of HCC is an exciting and rapidly evolving area of research. In this review, we bring forward a few significant studies and highlight the roles of different lncRNAs in HCC.

HCC: Complexity and Known Mechanisms

As stated in the “Introduction” section, HCC is the primary cancer in the liver. Its incidence and mortality rates mostly correlate with increasing age, whereas it is 3 times prevalent in men than women.²⁸ As alcohol use and chronic liver diseases are known risk factors for this cancer, ~70% to 90% of the HCC cases derive from chronic liver disease and cirrhosis.²⁹ Liver hepatocytes undergo extensive proliferation, development of fibrous tissue, formation of cancerous nodules, and apoptosis during HCC development.²⁹ The ultimate liver failure leads to the poor prognosis and high mortality rates associated with HCC.

Telomere shortening and dysfunction in hepatocytes have been shown to contribute to the chromosome instability in HCC. Moreover, studies across multiple countries have identified allelic loss from chromosome arms, mutations in protein-coding genes such as the tumor suppressor gene *p53*, the oncogene β catenin (*CTNNB1*) and Janus kinase 1 (*JAK1*),

telomerase reverse transcriptase (*TERT*), cyclin-dependent kinase inhibitor 2A (*CDKN2A*), phosphatase and tensin homolog (*PTEN*), myeloid-lymphoid leukemia (*MLL*), fibroblast growth factor 19 (*FGF19*), and *AXIN1* were found to be associated with patients with HCC.^{30–33} For this reason, Wnt/ β -catenin and JAK/STAT pathways are considered to be the most affected cellular signaling pathways in HCC.³¹ The presence of mutations these genes have been shown to be elevated by infections with HBV or HCV.^{30,34} More sophisticated molecular analyses (Figure 1) such as exome sequencing have revealed that the gene groups that are the most affected in HCC include leucine-rich repeat-containing family, histone methyltransferases, nucleotide-binding domain, calcium channel subunits, chromatin remodelers, and oxidative stress-associated genes.^{33,35} Although these molecular targets have provided many critical insights regarding HCC pathogenesis and potential treatment targets, there is still a need for finding more efficient and druggable molecular targets. The studies summarized in this review regarding the role of lncRNAs in HCC provide hope for this goal.

LncRNAs: Functions and Regulation

Functions of lncRNAs

Widespread use of high-throughput sequencing technology has enabled identifications of new functional aspects of lncRNAs. Their function is known to be dependent on their origin within the genome and their structure. Long noncoding RNAs can originate from different genomic locations where they can be intragenic (long intronic RNAs, sense lncRNAs, antisense lncRNAs), intergenic (long intergenic ncRNAs), enhancer-associated, promoter-associated, and telomere repeat-containing RNAs^{36,37} (Figure 2). Once transcribed, they can function through interactions with DNA, RNA, as well as protein components of a genome, due to which they can have such a wide impact on gene expression by modulating transcription, translation, and other regulatory processes.^{26,36,38} There is a vast heterogeneity in known functions of lncRNAs. There is no single categorization of their functions, and they can be broadly classified as a major gene regulator. It appears that they are involved in almost all regulatory pathways.

Protein-lncRNA interactions. For instance, most lncRNAs form associations with different proteins assembling into ribonucleoprotein complexes which are the main effector molecules. Long noncoding RNAs possess the ability to form different 3-dimensional structures which provide them the capacity to bind and regulate different protein components.³⁹ According to Rinn and Chang,⁴⁰ lncRNAs can serve as guides where they aid proper localization of various protein complexes. For instance, the *lincRNA-p21* helps in the localization of hnRNP-K to specific promoters.⁴¹ Some lncRNAs such as *PANDA* may also serve as decoys, which prevents p53-mediated apoptosis by associating with NF- κ B transcription

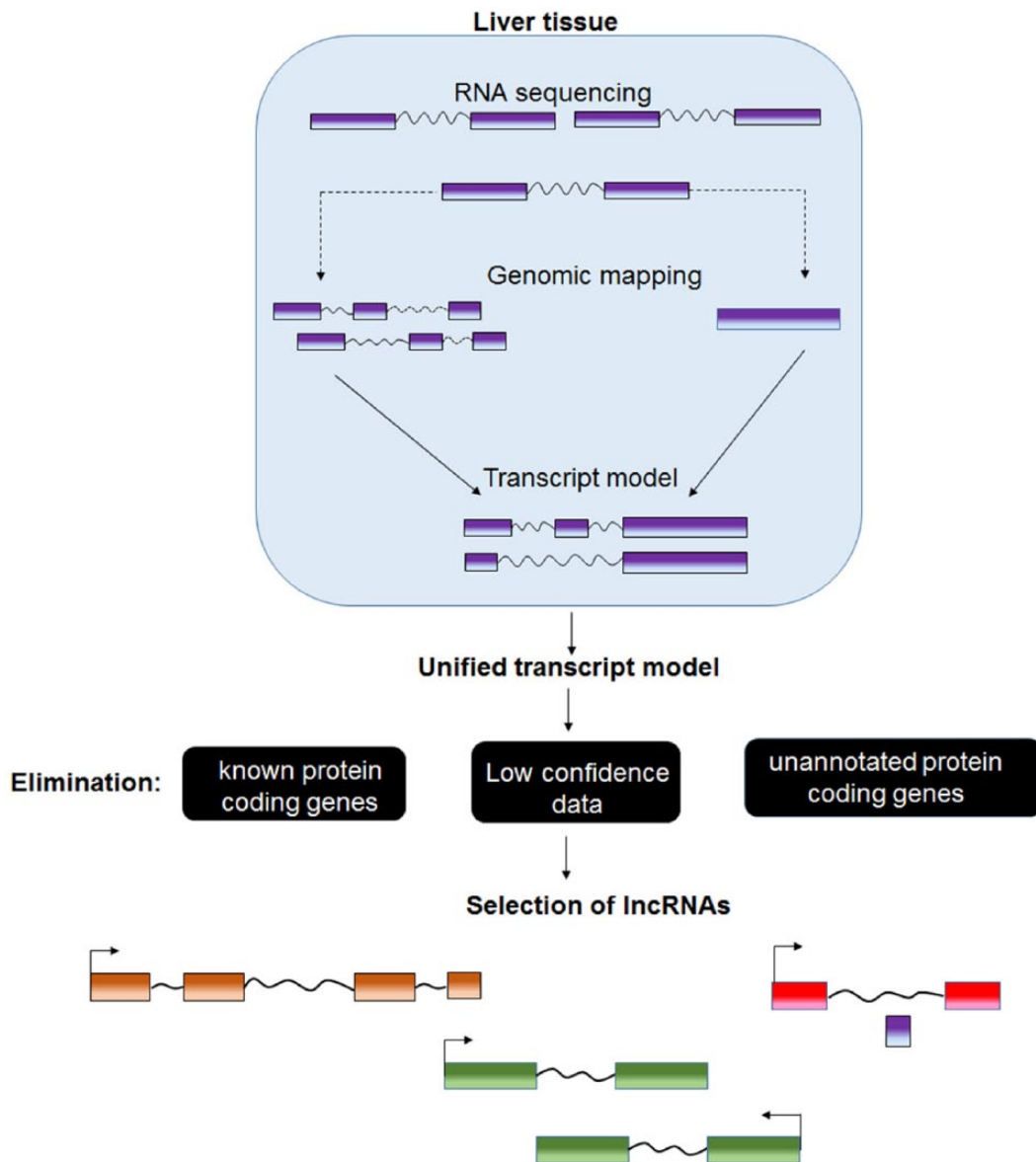


Figure 1. The different genomic localizations and biogenesis of long noncoding RNAs.

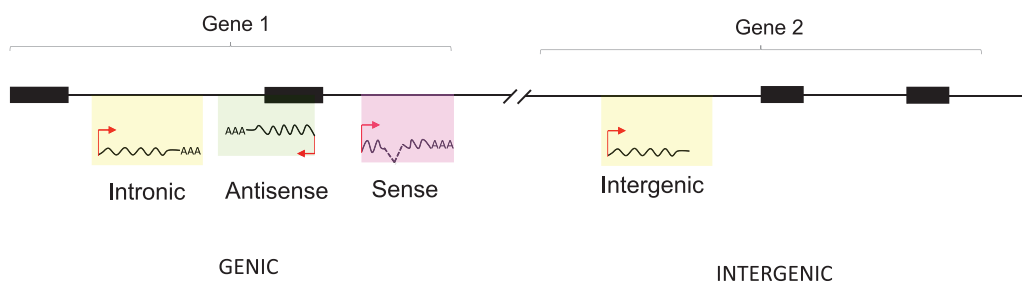


Figure 2. Sequencing methods used in identification of long noncoding RNAs.

factor.⁴² Many other lncRNAs can serve as scaffolds such as the telomerase RNA *TERC*.⁴³ Long noncoding RNAs regulate alternative splicing machinery through protein interactions. The alternatively spliced lncRNA transcripts are tissue and developmental stage specific which helps in achieving the delicate balance of gene expression in a spatiotemporal manner.^{44–46} Notable examples include metastasis-associated lung

adenocarcinoma transcript 1 (*MALAT1*) and nuclear-enriched abundant transcript 1 (*NEAT1*). *MALAT1* regulates serine-arginine-rich proteins involved in alternative splicing. *MALAT1* relocates these proteins to splicing speckles, influencing the alternative splicing process.⁴⁵ The function of *NEAT1* has not been fully elucidated. However, it was found to localize in the paraspeckles adjacent to the nuclear speckles of

MALAT1 and has been proposed to play a role in regulating alternative splicing during adipocytes differentiation.⁴⁶

Many lncRNAs also influence the expression of genes present in their vicinity by transcriptional interference. These lncRNAs have been observed to be transcribed beyond the promoter region of the genes in their vicinity and therefore prevent the gene expression either by interfering with the binding of different transcription factors to their binding sites or by causing changes in the epigenetic marks leading to transcriptional silencing of the gene.^{47,48} Therefore, it can be stated that transcription of lncRNAs regulates expression of genes in their vicinity or the neighbor genes, mainly functioning as antisense lncRNAs in *cis* or in *trans*⁴⁹ (Figure 2). Antisense transcription of lncRNAs has been known to regulate neighboring genes at multiple levels including transcription, posttranscription, translation, and post-translation.⁴⁹ Positive regulation of genes or transcriptional activation due to transcription of the nearby lncRNAs has also been reported⁵⁰ where certain lncRNAs have been found associated with promoters (promoter-associated RNAs) and enhancers (enhancer-associated RNAs). These lncRNAs can directly influence the expression of genes leading to transcriptional activation as well as repression.^{51,52} Another example of lncRNA having regulatory impacts on protein-coding genes is the β -APP-cleaving enzyme 1 antisense (*BACE1AS*) which regulates translation of β -APP-cleaving enzyme 1 (BACE1).⁵³ This enzyme is a major player in the Alzheimer disease. The interaction between *BACE1AS* and BACE1 stabilizes this protein resulting in increased abundance of BACE1.⁵³

Chromatin-lncRNA interactions. At the genomic level, lncRNAs interact with DNA and function as scaffold molecules which regulate the chromatin structure and function. Scaffold lncRNAs have also been shown to have influence genome epigenetically.^{43,54} They are also able to cause chromatin structural changes and gene expression changes through altering epigenetic mechanisms which include DNA methylation, histone posttranslational modifications, and ncRNAs such as miRNAs.⁵⁵ As stated in the previous section, lncRNAs mediate transcriptional silencing or activation through modulating epigenetic changes at specific gene loci. Long noncoding RNAs also have an impact on the epigenetic modifications, by aiding in altering the epigenetic landscape in different conditions. These alterations occur primarily due to the recruitment of different epigenetic regulatory proteins to specific gene loci. Many lncRNAs such as *HOTAIR*, *Rep A*, and *ANRIL* have been known to recruit epigenetic proteins which then create alternative epigenetic marks.^{56,57} *ANRIL* which interacts with polycomb repressive complex (PRC) proteins (I and II) and *HOTAIR* which also interacts with PRC complex II and regulate chromatin remodeling.⁵⁶ Other than causing epigenetic modifications, many lncRNAs also regulate genetic imprinting. These lncRNAs influence the expression of a gene in a parent-specific manner.⁵⁸

RNA-lncRNA interactions. Long noncoding RNAs are also capable of interacting with RNA molecules either as target-mimetic or sponge/decoy manner. One of the best examples of lncRNA interactions with RNA in both of these ways can be found with miRNAs where miRNA-lncRNA interactions lead to reduced activity of miRNAs.^{59,60} Long noncoding RNAs have also been shown to regulate small RNAs and thereby regulate expression of their target genes. Many lncRNAs such as *Xist* and *Tsix* serve as precursor molecules for small RNAs where the small RNAs are transcribed from these lncRNA molecules.^{61,62} As opposed to their function as progenitors or giving rise to miRNAs, lncRNAs also have the capability to inhibit the actions of miRNAs by interfering miRNA binding to their target genes, which is usually referred to as miRNA sponges.^{63–66} As this requires lncRNAs to compete with miRNAs, they are also called competing endogenous RNAs.⁶⁷ Examples include *Linc-MD1* which acts as a sponge for *miR-133* and *miR-135* in gastric cancer and many examples from HCC that are not limited to *H19:let-7*, *linc-RoR: miR-145*, and *lncRNA-ATB: miR-200*.⁶⁸

Many lncRNAs play important roles in embryonic stem cell development thereby increasing their potential regulatory manifolds.⁶⁹ Also, many lncRNAs are involved in the regulation of various aspects of differentiation of cells where these have been shown to regulate cellular identity development by changing the patterns of gene expression.⁷⁰ The cellular differentiation-associated functions performed by lncRNAs include programming and reprogramming of cell fate (eg, *Eyf2* lncRNA) and self-renewal (eg, *ANCR* and *TINCR*).⁷¹ Moreover, pluripotency of cells is regulated by lncRNAs *AKO28326* and *AK141205* which regulate transcription factors Oct and Nanog and aid in maintaining the pluripotent state of stem cells.⁷² This whole plethora of functions performed by lncRNAs seems to be just the edge of a horizon which needs to be further explored.

Collectively, lncRNAs have shown a vast variability, not only in their biogenesis but also in their functionality. Long noncoding RNAs have proven to be the silent key of genome and are increasingly being linked to many different types of functions. These studies discussed above provide convincing proof of the regulatory potential of lncRNAs reinforcing the currently developing notion that “protein-coding genes are slaves of their regulatory ncRNAs.” Therefore, in the future, many new aspects of lncRNAs will be unraveled which will help to further the knowledge not only of the lncRNAs but also about different regulations imposed by these elements. This knowledge will empower us with new ways to fight various human diseases with a focus on cancer.

Regulation of lncRNA expression

To target lncRNAs in therapy for associated diseases, a thorough understanding of regulatory mechanisms of lncRNA expression is necessary. Long noncoding RNAs appear to follow regulatory processes similar to mRNA, such as transcription by RNA Pol

II, and RNA modifications, such as polyadenylation, 5' capping, and splicing.^{73,74} Protein-coding genes and lncRNAs seem to share many of the common transcription factors such as SP1, which is involved in transcription of lncRNAs with bidirectional promoters.⁷⁵ In addition, lncRNA expression is regulated by epigenetic mechanisms such as DNA methylation and miRNAs. For instance, the maternally expressed gene 3 (*MEG3*) lncRNA promoter is regulated by DNA methylation, and hypermethylation of the *MEG3* promoter is associated with reduced expression in HCC.⁷⁶ Moreover, the same gene is regulated by *miR-29* in HCC in which *miR-29* prevents DNMT1 methylating *MEG3* promoter.⁷⁶ MicroRNAs can cause cleavage and decay of lncRNAs such as *miRNA-let-7b* and *lincRNA-p21* and *miRNA-141* and *HOTAIR* in cancer conditions.⁶⁰ Posttranscriptional regulation and lncRNA decay have been important functions carried out mainly by miRNA-lncRNA interactions.⁷³

Involvement of LncRNAs in Human Diseases and Cancer

Long noncoding RNAs form an important layer in the regulatory circuit of a genome, and their dysregulation has been associated with different diseases and malignancies. Whenever there is a dysregulation of lncRNA expression, its target genes are affected which in turn leads to various diseases. In different cancer types, mutations and expression deficits of lncRNAs have been detected in, but are not limited to, prostate cancer, lung cancer, colon cancer, bladder cancer, and breast cancer.^{77,78} Long noncoding RNAs have also been linked to neurodegenerative diseases such as Alzheimer diseases, cardiovascular diseases, diabetes, and HIV/AIDS, to name a few.⁷⁸

Aberrant expression of lncRNAs with important roles in cell cycle regulation and other regulatory pathways has been associated with different diseases including cancer.^{79,80} Examples of cell cycle regulating lncRNAs are growth-arrested DNA damage-inducible gene 7 (*Gadd7*) which regulates TAR DNA-binding protein (TDP-43) and cyclin-dependent kinase 6 (Cdc6), in turn regulating the progression of the cell cycle.⁸¹ In cancer conditions, many lncRNAs possess both oncogenic and tumor suppressor activities depending on their localization.²³ Steroid receptor RNA activator (*SRA*) is a lncRNA which regulates steroid-responsive genes. *SRA* was found to be dysregulated and showing high-expression patterns in different types of cancers including breast, uterus, and ovarian cancers.^{82,83} A lncRNA that acts as oncogenes and aids in tumor development and progression is prostate cancer gene expression marker 1 (*PCGEM1*) and was found upregulated in prostate cancer, contributing to initiation and progression of prostate cancer. Furthermore, *PCGEM1* is a target of *miR-145*, an miRNA that plays a tumor suppressive role in prostate cancer.^{84,85} Certain lncRNAs function as tumor suppressor genes. Growth arrest-specific 5 (*GAS5*) is one such tumor suppressor, which regulates the cellular inhibitor of apoptosis 2 (*cIAP2*) gene in response to starvation and it has been found to be downregulated in

prostate and breast cancers.^{86,87} Many previous studies have shown that any change in the epigenetic landscape of the genome may predispose the cells toward cancer, and lncRNAs have been shown to create varying epigenetic marks.⁸⁸ Many lncRNAs cause changes in promoter DNA methylation of tumor suppressor genes by directing DNA methyltransferases (DNMTs) to these genes and in turn silencing them.⁸⁹ Long noncoding RNAs also aid in chromatin remodeling and nucleosome reorganization of tumor suppression genes silencing them.⁹⁰ This is carried out by interaction of lncRNAs and recruitment of chromatin remodeling complexes such as SWI/SNF and NuRD to target gene promoters which subsequently alter histone modifications and positioning of nucleosomes at the promoter regions. Increased nucleosome occupancy and rendering a repressive chromatin structure lead to silencing of genes.⁹¹ In addition, many lncRNAs such as *MALAT1* are involved in metastasis and progression of cancer.

Gene regulatory activities of lncRNAs at the epigenomic, transcriptional, posttranscriptional, and posttranslational level have also contributed to diseases other than cancer. The Alzheimer disease is one of the most common irreversible neurodegenerative diseases which occurs due to the degeneration of synapse and neurons in the brain leading to memory loss and decline in cognitive abilities.⁹² Recent studies have suggested the possible involvement lncRNA *BACE1AS* to influence the accumulation of amyloid precursor protein in patients with Alzheimer disease.⁹³ Although lncRNAs have been shown to influence gene expression in *cis*, in Prader-Willi syndrome, *IPW* lncRNA was found to be upregulating the *DLK1-DIO3* region in *trans*. This association caused the downregulation of maternally expressed genes in the paternally imprinted region.⁹⁴ Furthermore, the authors observed that *IPW* created chromatin modifications to achieve the upregulated gene expression.⁹⁴ Moreover, deregulation of *Fendrr*, *Trpm3*, and *Scarb2* lncRNAs has been seen in heart failure.⁹⁵ In facioscapulohumeral muscular dystrophy, *DBE-T* is deregulated.^{96,97} All these studies point toward the fact that there are many aspects that need to be explored about lncRNAs.

Roles of LncRNAs in HCC

Hepatocellular carcinoma is one of the most prevalent forms of cancer, and its incidence is increasing at a very high rate. However, its pathophysiology is less understood, and the underlying causes are even less understood. Recently, lncRNAs have been shown to have a high stake in HCC, and many lncRNAs have been associated with HCC which include *MVIH*, *H19*, *HEIH*, *HULC*, *TUC338*, and *MEG3*.⁹⁸⁻¹⁰⁴ In this review, we have discussed the lncRNAs whose functions have been deduced and their involvement in onset, progression, and apoptosis of liver cancer cells (Table 1). There is a large number of lncRNAs which have been found to be important regulators of cancer development, progression, and metastasis. Many lncRNAs have been identified to be involved in HCC and will be discussed in the following sections.

Table 1. Expression and functions of known lncRNAs which have regulatory roles in HCC.

LONG NCRNA	FUNCTIONS	EXPRESSION	REFERENCES
<i>HULC</i>	Tumor growth and high proliferation rate	Upregulated	Geng et al, ¹⁰⁵ Kim and Lee ¹⁰⁶
<i>HOTAIR</i>	Proliferation of tumor cells	Upregulated	Lu et al ¹⁰⁷
<i>H19</i>	HCC development; metastasis and invasion of HCC through AKT/GSK-3 β /Cdc25A signaling pathway	Upregulated	Kim et al, ¹⁰⁸ Lv et al ¹⁰⁹
<i>HEIH</i>	Recurrence in HBV-HCC	Upregulated	Wu et al ¹¹⁰
<i>MALAT1</i>	Increased HCC cell migration; tumor metastasis and recurrence through Wnt/TCF/ β -catenin and Hippo/yes-associated protein (YAP) signaling pathways	Upregulated	Nordin et al, ¹¹¹ Wang et al ¹¹²
<i>MEG3</i>	Decrease the anchorage-dependent and anchorage-independent cell growth and the introduction of apoptosis; regulation of HCC progression by UHRF1/DNMT1/MEG3/p53 axis signaling pathway	Downregulated	Gabory et al, ¹¹³ Zhou et al ¹¹⁴
<i>HOTTIP</i>	Cell proliferation and viability	Upregulated	Yap et al ¹¹⁵
<i>TUG1</i>	Regulate the cell growth	Upregulated	Wu et al ¹¹⁶
<i>DILC</i>	Suppress liver cancer by inhibiting the autocrine signaling pathway of IL-6/STAT3	Downregulated	Jones and Baylin ¹¹⁷
<i>CCAT1</i>	Proliferation of cancerous cells	Upregulated	Nakagawa and Kageyama ¹¹⁸
<i>URHC</i>	Facilitate cell proliferation	Upregulated	Saxena and Carninci ¹¹⁹
<i>ANRIL</i>	Cell proliferation, invasion, and migration of HCC cells	Upregulated	Hayashi et al ¹²⁰
<i>CUDR</i>	Inhibits H3K27me3, increases HULC expression	Upregulated	Gui et al ¹²¹
<i>LncRNA-ATB</i>	Promotes cell invasion and metastasis	Upregulated	Yuan et al ¹²²
<i>BANCR</i>	Cell proliferation, migration, and invasion	Upregulated	Zhou and Gao ¹²³
<i>CAMTA1</i>	Promotes proliferation, stem cell-like properties, and tumorigenesis	Upregulated	Ding et al ¹²⁴
<i>FTX</i>	Acts as sponge for miR-374a, inhibits HCC cell epithelial-mesenchymal transition, and invasion	Downregulated	Liu et al ¹²⁵
<i>GIHCG</i>	Promotes HCC cells' proliferation, migration, and invasion	Upregulated	Sui et al ¹²⁶
<i>GPC3-AS1</i>	Promotes cell proliferation and migration and xenograft tumor growth	Upregulated	Zhu et al ¹²⁷
<i>PCAT1</i>	Higher level PCAT1 is associated with poor prognosis and survival	Upregulated	Yan et al ¹²⁸
<i>PlncRNA-1</i>	Promotes cell proliferation, migration, and invasion	Upregulated	Dong et al ¹²⁹
<i>linc-ROR</i>	Activates cellular stress pathways and modulation of cellular responses to chemotherapy	Upregulated	Takahashi et al ¹³⁰
<i>SNHG15</i>	Proposed to be a tumor promoter	Upregulated	Zhang et al ¹³¹
<i>SOX2OT</i>	Promotes HCC cell migration	Upregulated	Shi and Teng ¹³²
<i>LncSox4</i>	Initiates liver TIC self-renewal through Stat3-Sox4 pathway	Upregulated	Chen et al ¹³³
<i>SRHC</i>	Tumor suppressor	Downregulated	Zheng et al ¹³⁴
<i>CPS1-IT1</i>	Reduced cell proliferation, migration, and invasion capacities	Downregulated	Wang et al ¹³⁵
<i>TCF7</i>	Induces liver self-renewal and tumor propagation by activating Wnt pathway	Upregulated	Wang et al ¹³⁶
<i>BRM</i>	Tumor initiation through YAP signaling	Upregulated	Zhu et al ¹³⁷
<i>ZEB2-AS1</i>	Regulates tumor growth and metastasis	Upregulated	Lan et al ¹³⁸
<i>LINC00152</i>	Promotes cell proliferation and tumor growth	Upregulated	Ji et al ¹³⁹
<i>LINC01225</i>	Invasion and cell proliferation by regulating EGFR/Ras/Raf-1/MEK/MAPK signaling pathway	Upregulated	Wang et al ¹⁴⁰

Table 1. (Continued)

LONG NCRNA	FUNCTIONS	EXPRESSION	REFERENCES
<i>AFAP1-AS1</i>	Promotes cell proliferation, migration, and invasion	Upregulated	Lu et al ¹⁴¹
<i>uc.338</i>	Promotes cell proliferation and induces cell cycle progression	Upregulated	Bo et al ¹⁴²
<i>CCHE1</i>	Regulates the ERK/MAPK pathway and serves as an oncogene	Upregulated	Peng and Fan ¹⁴³
<i>GAS5</i>	Binds to glucocorticoid receptor and modulates mTOR signaling	Downregulated	Tu et al ¹⁴⁴
<i>lnc-β-Catm</i>	Promotes methylation of β-catenin in HCC cancer stem cells	Upregulated	Zhu et al ¹⁴⁵
<i>lncRNA-LALR1</i>	Facilitates cyclin D1 expression and activating Wnt-catenin pathway	Upregulated	Xu et al ¹⁴⁶
<i>HBx-LINE1</i>	Activates the Wnt β-catenin signaling in HBV-HCC	Inducer	Liang et al, ¹⁴⁷ Chauhan et al, ¹⁴⁸ Mulrooney-Cousins et al ¹⁴⁹
<i>DANCR</i>	Associated with CTNNB1 and β-catenin	Upregulated	Yuan et al ¹⁵⁰
<i>ZNF674-AS1</i>	Drives self-renewal of liver cancer stem cells	Downregulated	Zhang et al ¹⁵¹
<i>lncRNA-HAND2-AS1</i>	Hypermethylated in portal vein tumor thrombosis and liver metastasis	Downregulated	Yang et al ¹⁵²
<i>Linc00974</i>	Invasion and proliferation of HCC through TGF-β and Notch signaling pathways	Deregulated biomarker	Tang et al ¹⁵³
<i>hPVT1</i>	Promotes cell cycle and stem cell-like properties in HCC	Upregulated	Ding et al ¹⁵⁴
<i>PTENP1</i>	Represses tumorigenic properties of HCC	Downregulated	Chen et al ¹⁵⁵
<i>ZNRD1</i>	DNA damage and repair via ERCC	Upregulated	Wen et al ¹⁵⁶
<i>UCF1</i>	Promotes HCC by inhibition of miR216b and activation of FGFR1/ERK signaling pathway	Upregulated	Cao et al ¹⁵⁷
<i>MT1D</i>	Proliferates HCC via accumulation of p53	Upregulated	Yu et al ¹⁵⁸

Abbreviations: HCC, hepatocellular carcinoma; lncRNAs, long noncoding RNAs; ncRNA; noncoding RNA; TGF-β, transforming growth factor β.

HULC

HULC is a lncRNA ~1.6 kb in length and contains 2 exons. It has been found to be upregulated in HCC and colorectal cancers which metastasize to the liver^{101,159} (Figure 3). It has been reported that cyclic adenosine monophosphate response element binding protein (CREB) transcription factor upregulates the expression of HULC, especially in Hep3B cells¹⁶⁰ (Figure 3). Furthermore, the expression of HULC in HepG2 cells was also reported to be upregulated by the interaction of HBV HBx protein with CREB.¹⁶¹ In a study by Hammerle et al,¹⁶² another regulator of expression of HULC was discovered to be insulinlike growth factor 2 mRNA-binding protein (IGF2BP). The upregulation of HULC has been associated with tumor growth and a higher proliferation rate which is caused due to downregulation of the tumor suppressor gene *p18*. In addition, HULC has also been reported to influence HCC cells by regulating the expression of peroxisome proliferator-activated receptor α (PPARA) transcriptional factor by creating methylation marks at the CpG islands in the promoter of *miR-9*.¹⁶³ Thereby, it elevates the expression of PPARA which in turn increases expression of *ACSL1* gene. This leads to the high recurrence rates of HCC.¹⁶³

It has been proposed that HULC acts as a sponge for *miR-372* and thereby causing upregulated expression of its target genes.¹⁶⁰ Patients with HCC have shown high levels of HULC in plasma which can be developed as a biomarker for prognosis of HCC.^{164,165} Although HULC was the first lncRNA found to have upregulated expression in HCC, the mechanisms of its action have not yet been fully determined.¹⁶⁶

HOTAIR

HOTAIR is a ~2.2-kb-long lncRNA, transcribed from the *HOXC* locus in antisense manner and hence named as HOX antisense intergenic RNA.¹⁶⁷ It was first observed in fibroblast causing the silencing of *HOXD* locus via establishing trimethylation on H3K27.⁵⁶ HOTAIR recruits PRC2 and lysine specific demethylase 1 (LSD1) protein complexes to the *HOXD* gene cluster and changes its histone methylation status and chromatin configuration.¹⁶⁸ Thereby, it causes silencing of tumor suppressor genes.¹⁶⁷ It has been implicated in breast, colorectal, gastrointestinal, and pancreatic cancers apart from HCC.^{108,168,169} HOTAIR interacts with PRC2 through an 89-mer domain at the 5' end of the sequence.¹¹⁰ This region is

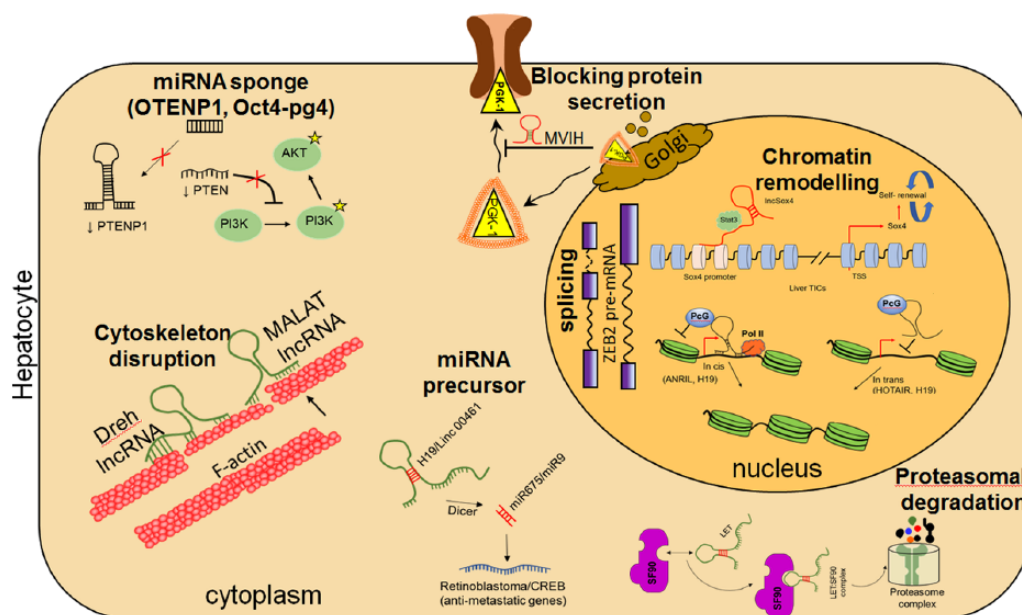


Figure 3. A general overview of the 3 basic mechanisms opted by lncRNAs to regulate different aspects of hepatocellular carcinoma. (A) cyclic adenosine monophosphate response element binding protein prevents the expression of *HULC* by binding to it and in turn preventing the growth and/or recurrence of hepatocellular carcinoma. (B) *H19* and many other lncRNAs have been shown to act as miRNA sponges; here, *H19* encodes *miR-675* in its first exon and thereby it can prevent the function of *miR-657*. Furthermore, knockdown of this lncRNA was shown to prevent hepatocellular carcinoma. (C) Another lncRNA *ANRIL* binds to the PRC complex II (polycomb repressive complex II) forming a complex which causes the epigenetic silencing of *KLF2* (Krüppel-like factor 2). lncRNA indicates long noncoding RNA.

also known as the minHOTAIR, whereas LSD1 protein interacts with *HOTAIR* via the 646-mer at the 3' end.¹⁶⁷ It has been reported that *HOTAIR* is overexpressed in HCC, and knockdown of *HOTAIR* reduces HCC proliferation.¹⁰⁵ Furthermore, the cells having lower expression of *HOTAIR* become more responsive to chemotherapy.¹⁶⁹

H19

H19 is expressed from a ~2.3-kb-long, maternally imprinted gene. This lncRNA acts as both an oncogene and a tumor suppressor gene. However, its exact mechanism of action is still uncertain.¹¹¹ *H19* is expressed during embryogenesis, whereas transcriptionally silent in adult tissues.¹¹³ It was also shown that this lncRNA was expressed in response to maternal undernutrition. In relation to cancer pathogenesis, it was found to be involved in metastasis and angiogenesis.^{113,170} *H19* is coexpressed with another maternally imprinted gene, namely, insulinlike growth factor 2 (*IGF-2*) which has been proposed to play a role in cancer onset via different epigenetic modifications.¹⁰⁶ This observation is further strengthened by epigenetic abnormalities observed at *IGF-2* and *H19* loci in HCC.¹⁷¹ However, many other studies have reported that many different mechanisms are operating at this locus, and no single process can be attributed to cancer onset and progression with certainty.^{172–175} The association of *H19* with the development of HCC was further validated by the observation that knockdown of *H19* prevented the development of HCC.⁹⁹ Another interesting characteristic of the *H19* lncRNA is that it encodes

miR-675 in its first exon, and therefore, it can act as an miRNA sponge as well as a reservoir of *miR-675*.^{176,177} *H19* has been shown to have versatile functions which entail epigenetic domains as well. It was reported that *H19* interacts with enhancer of zeste homolog 2 (*EZH2*) which is a part of a protein complex called as PRC2¹⁷⁸ (Figure 3).

HEIH

High expression in HCC (*HEIH*) is a lncRNA which was found to have elevated expression patterns in HCC as is evident from its name.¹⁰⁰ It is an oncogene and it interacts with *EZH2*. Furthermore, high expression of *HEIH* has been associated with recurrence of hepatitis B-related HCC (HBV-HCC). *HEIH* has been used as a biomarker for prognosis of HCC, and an elevated expression is an indication of advanced stage of HCC.¹⁰⁰

MALAT1

The lncRNA *MALAT1* stands for metastasis-associated lung adenocarcinoma transcript 1. In the cancerous state, this locus harbors chromosomal translocations and mutations.¹⁷⁹ This lncRNA was also found to have an upregulated expression in HCC cell lines.¹⁰⁵ Differential expression of *MALAT1* has been found in different cancer types found in lung, liver, pancreas, colon, breast, prostate, and ovaries. *MALAT1* expression can be used as a biomarker for detection of recurrence of liver cancer after liver transplantation, and therefore, it can be developed as a therapeutic target.¹⁰²

MEG3

MEG3 is a lncRNA ~1.7 kb in length and is a maternally imprinted gene.¹⁸⁰ This lncRNA has been observed to be downregulated in HCC cell lines, and its reintroduction has been reported to decrease the anchorage-dependent and anchorage-independent cell growth and apoptosis.¹⁰⁴ Braconi et al¹⁰⁴ reported that due to the absence of *MEG3*, the promoter regions of some genes involved in HCC show hypermethylation. *MEG3* expression has been observed to be downregulated in a number of cancer conditions including liver, lung, glioma, and cervical cancers.^{107,181–184} For instance, reduced levels of *MEG3* was found in cervical cancer tissues in contrast to the adjacent healthy tissues in 108 patients in association with increasing tumor size and metastasis.¹⁸⁵ In a study by Anwar et al¹⁸⁴ *MEG3* was observed to be the most commonly downregulated lncRNA in HCC. Demonstrating its connection with the epigenetic landscape, 60% of HCC cases reported by Anwar et al¹⁸⁴ correlated with increased DNA methylation. It was also reported that *MEG3* competes for *miR-181* family and thereby regulates the progression of gastric cancer.¹⁸⁶

HOTTIP

HOX A transcript at distal tip (*HOTTIP*) is a lncRNA generated from the distal tip of *HOXA13* gene, and *HOTTIP* expression was found to be upregulated in HCC and pancreatic cancer.^{187–189} *HOTTIP* interacts with WD-repeat-containing protein 5/mixed lineage leukemia (WDR5/MLL) complex regulating the expression of *HOXA* locus. It in turn under the regulation of *HOXA13* gene creates a feedback loop regulation.¹⁸⁸ In a recent study, Ge et al¹⁹⁰ demonstrated that *miR-192* and *miR-204* could suppress the expression of *HOTTIP* through the involvement of Argonaute 2 (AGO2)-mediated RNA interference in HCC cells. The suppression of *HOTTIP* by these miRNAs was shown to be reducing HCC cell viability, whereas induced *HOTTIP* expression by inhibiting these miRNAs increased cellular proliferation. These observations suggest that *HOTTIP* and its associated miRNAs could potentially be developed as a potential therapeutic strategy for HCC.

TUG1

Taurine upregulated gene 1 (*TUG1*) is a lncRNA that is ~7.1 kb in length. It has been shown to be involved in lung cancer, bladder cancer, and osteosarcoma apart from its involvement in liver cancer.^{191–193} It was initially identified in mouse retinal cells responding to taurine signals in a screen designed to identify upregulated genes.¹⁹⁴ *TUG1* shows a high expression in HCC. It also regulated cell growth by epigenetic silencing of Krüppel-like factor 2 (KLF2) transcription factor.¹⁹⁵ Furthermore, knockdown of *TUG1* induced apoptosis of HCC cells and in hepatoblastomas.^{195,196} Therefore, *TUG1* has potential therapeutic applications for treatment of HCC.^{195,196}

DILC

This lncRNA was observed to have reduced expression in all HCC cell lines as deduced from microarray expression data and was so named as lncRNA downregulated in liver cancer (*DILC*).¹⁹⁷ This lncRNA was reported to be able to suppress liver cancer by inhibiting the autocrine signaling pathway of IL-6/STAT3.¹⁹⁷

CCAT1

Colon cancer-associated transcript 1 (*CCAT1*) is a lncRNA that was initially found to be upregulated in gastric and colon cancer.^{198,199} It is ~2.6 kb in length and located in the vicinity of the *c-Myc* gene.¹⁹⁸ *CCAT1* was also found to be upregulated in HCC as compared with noncancerous liver tissue.²⁰⁰ It was associated with poor prognosis as well as aiding proliferation of cancerous cells in HCC. *CCAT1* influences proliferation of cancer cells as it acts as a sponge for *let-7* miRNA thereby increasing the expression of *HMG2* and *c-Myc*.²⁰⁰ Therefore, *CCAT1* holds potential therapeutic application as a molecular marker for HCC diagnosis as well as a target for HCC therapy.

URHC

This lncRNA was identified as the most frequently encountered lncRNA having an upregulated expression in HCC, and therefore, the name upregulated in HCC (*URHC*).²⁰¹ *URHC* was observed to downregulate *ZAK* and thereby facilitate cell proliferation.²⁰¹ As downregulation of *URHC* was shown to induce apoptosis and decrease proliferation of HCC cells, this can be potentially developed as a therapeutic target for therapy.²⁰¹

ANRIL

ANRIL stands for antisense ncRNA, a 3.8-kb lncRNA found within in the *INK4* locus. It is transcribed by RNA polymerase II and subsequently alternatively spliced.²⁰² It was initially identified in familial melanoma tissues.²⁰³ Since then, it has been observed to be involved in a number of cancer conditions including HCC, prostate cancer, gastric cancer, and non-small-cell lung cancer (NSCLC) development.^{202,204–207} It is a *trans*-lncRNA which recruits the chromobox 7 (CBX7) domain of PRC1 and PRC2 (Figure 3) to the *CDKN2A/CDKN2B* loci. This recruitment generates H3K27 methylation which in turn silences the genomic loci.^{115,208} As a result, the *p14*, *p15*, and *p16* genes get suppressed which are involved negative regulation of cell cycle, senescence, and apoptosis mechanisms.^{115,208} Moreover, an alternatively spliced transcript of *ANRIL*, namely, *p15* antisense (*p15AS*) has been shown to downregulate the expression of *p15*.²⁰⁹ *ANRIL* was overexpressed in HCC tissues.²⁰⁵ Increased expression and increased binding of *ANRIL*

to PRC2 caused epigenetic silencing of *KLF-2* in HCC.²⁰⁵ However, downregulation of *ANRIL* was shown to inhibit cell proliferation, invasion, and migration of HCC cells.²¹⁰ They also showed the positive regulatory role of the transcription factor SP1 in regulating *ANRIL* in HCC and the potential of SP1 knockdown in downregulating *ANRIL* expression in HCC cells. Thus, *ANRIL* can be potentially developed as a therapeutic target for HCC treatment.^{209,210}

Other lncRNAs involved in HCC

There are many other lncRNAs which have been shown to take part in different aspects of HCC, and much more will be identified in future experiments. Certain notable lncRNAs include microvascular invasion (*MVIH*) in HCC which has been reported to be involved in HBV-related HCC tissues.⁹⁸ *MVIH* interacts with protein phosphoglycerate kinase 1 (PGK1) which causes increased angiogenesis and tumor growth.⁹⁸ Another lncRNA which has been shown to be involved in HCC is extra-coding *CEBPA* (*ecCebpa*) which is a functional lncRNA derived from *CEBPA* gene. *EcCebpa* binds to the promoter of *CEBPA* gene and interacts with DNMT1, preventing the promoter methylation. This has led to the increased expression of *CEBPA*.²¹¹

Influence of LncRNA on Epigenetic Landscape of HCC

Changes to the epigenetic landscape of a cell are indications of deviations which are very frequently associated with diseases and cancer. Furthermore, many studies have shown that epigenetic modifications may also play important roles in regulation of lncRNA expression.¹¹⁶ One major epigenetic modification influenced by lncRNAs is DNA methylation. In support of this notion, global hypomethylation has also been observed in HCC.²¹² Hypermethylation at specific promoters leading to the silencing of tumor suppressor genes and activation of oncogenes have also been reported in HCC.^{117,213} Long noncoding RNAs are involved in many different epigenetic processes where they may help in the recruitment of various proteins to sites of modifications and in altering the specificities and action of different protein complexes. Many lncRNAs provide a platform by functioning as scaffold molecules in the chromatin and recruiting different regulatory proteins to their sites of action. Examples of such lncRNAs include *ANRIL* and *HOTAIR*. Many lncRNAs have been found to regulate HCC via epigenetic mechanisms in many recent reports.^{118,119,214} DNA methylation and histone modification patterns are changed in HCC leading to the onset and progression of cancer.²⁰³ However, hypermethylation of DNA at specific gene loci (eg, angiogenesis inactivation gene) has been shown to inactivate the tumor suppressor genes in HCC.²¹⁵ Methylation patterns have been used to develop epigenetic signatures in HCC and normal liver cells which possess diagnostic value and thus can be used for predetermination of HCC candidates.²¹⁶ Many different approaches have been

described previously to develop as therapeutic markers to identify and categorize HCC onset and progression. As mentioned earlier, different oncogenes and their methylation patterns have been studied in normal and cancer tissues.²¹⁶ A method using gene re-expression after epigenetic unmasking has also been proposed to identify differential methylation patterns of tumor suppressor genes.²¹⁷ Another approach has been developed to study cancer-related promoters, where differentially methylated promoter regions in HCC, other cancers, as well as normal tissues are studied.²¹⁸ Song et al²¹⁹ proposed the consideration of promoters as well as surrounding methylated regions to be developed as markers for epigenetic modifications.

Other than the direct influence on the expression of tumor suppression genes, epigenetic modifications can also be influenced via the proteins and enzymes involved in creating epigenetic marks. The most important player among these proteins is DNA DNMTs, which have been shown to have an elevated expression in HCC tissues. These elevated DNMT levels might be considered as a reason behind differential methylation states.²²⁰ DNA methyltransferases are also targets of miRNAs which negatively regulate DNMTs. One such DNMT-targeting miRNA is *miRNA-29* which can inhibit levels of DNMTs including DNMT3A and 3B enzymes in many cancer conditions such as lung cancer cell lines and primary NSCLC.²²¹ This further influences the abundance of miRNAs in HCC which is also decreased.²²²

Variations in histone modifications have also been found between normal and HCC tissues. Histone modifications have also been proposed to be used as potential biomarkers for detection and diagnostic purposes. It has been shown that acetylation and the trimethylation at the H3K27 (H3K27Ac, H3K27me3), hyperacetylation at H3K9 (H3K9Ac), hyperacetylation of H4K8 (H3K8Ac), and trimethylation at the H3K4 (H3K4me3) are more pronounced in HCC.^{120,223} Other than these modifications, HCC epigenetic states can also be altered by the differential expression patterns of the components of protein complexes such as PRC I and PRC II. There is accumulating evidence for the involvement of EZH2 subunit of PRC II which has been found to be a target of different miRNAs and lncRNAs.²²⁴ SuZ12 and Bmi1 components of PRC II have also been shown to have differential expression patterns in HCC and may thus be important regulatory components of HCC pathways.^{225,226}

Significance of LncRNAs in Clinical Applications

Long noncoding RNAs show tissue-specific expression patterns,²²⁷ and therefore, they have a high usability as biomarkers for various types of cancers. Moreover, they are expressed in low copy numbers and show high diversity in transcripts in different tissues. Due to these characteristics displayed by lncRNAs, they can be developed as biomarkers for various types of cancers and disease conditions. It is very likely that techniques based on lncRNAs will show high sensitivity and low costs thereby having significant clinical applications.

Development of combinatorial therapy targeting different long and small ncRNAs together may yield better results which have been shown for *H19*, *MEG3*, and *HULC*.^{104,160,228} *H19* can suppress HCC as mentioned previously and it also interacts with a protein complex hnRNP U/PCAF/RNA Pol II which in turn activates the *miR-200* family by increasing histone acetylation.²²⁸ *MiR-29* regulates the expression of *MEG3* lncRNA in HCC.¹⁰⁴ *HULC* inhibits *miR-372* indirectly regulating the expression of different genes in HCC.¹⁶⁰ As *HULC* has also been observed in plasma and the level of plasma *HULC* has also been correlated with a positive HBV status, *HULC* can be developed as a biomarker for early detection of HCC.¹⁸⁷

Conclusions

More than 600 000 people worldwide are affected by HCC, demanding urgent discoveries on therapeutic targets and early diagnostic markers. Unfortunately, our current knowledge about the molecular pathways influencing HCC is incomplete. Studies so far have demonstrated that HCC occurs due to genetic, epigenetic, transcriptional, and translational imbalances. The studies that revealed lncRNAs as a cause for HCC and their implications as diagnostic markers have created a shift in focus of HCC research from protein-coding genes to lncRNAs and other ncRNAs as key players in HCC pathogenesis. Due to their diverse functions as tumor suppressors, oncogenes, transcriptional regulators, and epigenetic modifiers, involvement in intricate cellular networks have made them a molecular tool that can potentially be developed into suitable therapeutic and clinical strategies for treatment of HCC. The availability of new and improved techniques has enabled the study of different factors involved in HCC and hold promise for the discovery of many lncRNAs influencing HCC in the future. There is an urgent need to explore expression, functions, and regulation of lncRNAs further to fully understand their role in different phases of HCC onset, progression, and development. Their role in modulating epigenetic landscape of HCC should also be taken into account when these lncRNAs are developed as diagnostic or therapeutic tools for HCC.

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Author Contributions

MM and RC reviewed and approved the final manuscript.

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