# Noncoding RNA as Therapeutic Targets for Hepatocellular Carcinoma

Joseph George, PhD[1] Tushar Patel, MBChB[2]

[1] Department of Cancer Biology, Mayo Clinic, Jacksonville, Florida
[2] Departments of Transplantation and Cancer Biology, Mayo Clinic, Jacksonville, Florida

**Address for correspondence** Tushar Patel, MBChB, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224 (e-mail: patel.tushar@mayo.edu).

Semin Liver Dis 2015;35:63–74.

## Abstract

Recent studies have suggested that noncoding RNAs (ncRNAs) contribute to the pathogenesis and progression of hepatocellular carcinoma (HCC). These RNA genes may be involved in various pathobiological processes such as cell proliferation, apoptosis, angiogenesis, invasion, and metastasis. Aberrant expression of ncRNA resulting from deregulated epigenetic, transcriptional, or posttranscriptional activity has been described in several studies. ncRNAs are comprised of a highly diverse group of transcripts that include microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) as well as several other types of RNA genes. Understanding the molecular mechanisms by which ncRNA contribute to hepatocarcinogenesis may enable the design of ncRNA-based therapeutics for HCC. In this review, the authors provide a perspective on therapeutic applications based on the emerging evidence of a contributory role of miRNAs and lncRNAs to the pathogenesis and progression of HCC. In addition, ncRNAs that are deregulated in expression in HCC may have utility as potential prognostic or diagnostic markers.

**Keywords**
- hepatocellular carcinoma
- noncoding RNA
- microRNA
- long noncoding RNA
- biomarker

Hepatocellular carcinoma (HCC) is a global health problem: It is the third leading cause of cancer mortality and the sixth most common cancer worldwide.[1] At an advanced stage, this cancer is associated with a dismal prognosis due to lack of curative treatment.[2] Like many other cancers, HCC is characterized by dysregulation of multiple gene networks and signaling pathways that are normally involved in tissue homeostasis. These genetic effects can involve both protein-coding genes as well as noncoding RNA (ncRNA) genes. Although the former have been the focus of intense investigation, the latter, with the exception of microRNAs (miRNAs), are only now gaining recognition as contributors to HCC. ncRNAs are functional RNAs that are not transcribed into a protein. A significant proportion of the human genome is actively transcribed into ncRNAs, whereas only less than 2% of genome sequences encodes for protein coding genes.[3]

Transcribed ncRNAs include functionally important RNAs such as transfer RNA (tRNA) and ribosomal RNA (rRNA), as well as small nucleolar RNAs (snoRNAs) that guide chemical modification of RNA molecules, small interfering RNAs (siRNAs) that interfere with translation of proteins, small nuclear ribonucleic acids (snRNAs) that process pre-messenger RNAs (mRNAs), piwi-interacting RNAs (piRNAs) that are linked to transcriptional gene silencing of retrotransposons, microRNAs (miRNAs) that modulate mRNA expression, and long noncoding RNAs (lncRNAs) with mostly unknown functions.[4,5] Indeed, there are several different types of ncRNA, and the transcriptional landscape is extremely heterogeneous. Although the number of ncRNAs encoded within the human genome is unknown,[6] thousands of pervasively transcribed ncRNAs have been identified, and the numbers of such transcripts are greater than those of protein-coding mRNA. Furthermore, some ncRNAs also show clear evolutionary conservation that indirectly supports a functional role. Several ncRNAs, such as miRNA and some recently identified long ncRNAs, have been shown to play regulatory roles in diverse biological processes as well as in pathological processes such as tumorigenesis.[6,7] Data regarding

**Issue Theme** miRNA in Liver Pathobiology, Diagnosis, and Therapy; Guest Editor, Gregory J. Gores, MD

Copyright © 2015 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel: +1(212) 584-4662.

**DOI** http://dx.doi.org/10.1055/s-0034-1397350.
**ISSN** 0272-8087.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

involvement of most types of ncRNA in HCC are currently lacking; herein we will focus on miRNAs and lncRNAs that have been implicated in the pathogenesis of HCC.

## MicroRNAs in Hepatocellular Carcinoma

MicroRNAs are small ncRNA molecules of around 22 nucleotides in length that may regulate gene expression, either by inhibiting target mRNA translation or by inducing its degradation through pairing with complementary sequences within the 3′-untranslated regions (UTRs) of targeted transcripts at the posttranscriptional and/or translational level.[8,9] To date, around 2,000 miRNAs have been identified in humans using advanced sequencing technology.[10,11] Many of these have been shown to play critical roles in normal cellular functions such as proliferation, apoptosis, and invasion.[12] Deregulated expression of several miRNAs has been reported in many different human diseases, and in particular has been extensively investigated in many human cancers, including HCC. It is estimated that approximately 2,000 miRNAs regulate or control expression of approximately 30,000 genes, tuning their protein synthetic machinery.[13] Widespread alterations of miRNAs occur across the human genome in a broad array of human cancers, and miRNA expression has been implicated in the pathogenesis and progression of various cancers.[14] In fact, miRNAs may function either as tumor-suppressor genes or as oncogenes, by targeting and silencing mRNAs involved in carcinogenesis. Recent studies show that miRNA expression can be more useful than mRNA-based profiling for identifying tissue type of tumor origin.[15] MicroRNAs have been implicated in several processes that define and contribute to malignancy such as regulation of apoptosis and cell proliferation, angiogenesis, deregulated cell signaling, etc. Deregulation of miRNA has been postulated as a critical component of malignant transformation and tumor progression. Consequently, there is much interest in developing miRNA-targeted therapies for HCC.[14]

There are extensive data from several publications that describe the involvement of various miRNAs and their differential expression in HCC as well as in other types of cancers. Deregulated expression of miRNA could contribute to the pathogenesis of HCC through the downregulation of miRNAs that modulate oncogenes, or the upregulation of miRNA that can target tumor suppressor genes.[16,17] Among the most prominent deregulated miRNAs in HCC are miR-221, miR-21, and miR-18 that are upregulated in HCC and miR-122a, miR-199a, and miR-200 that are downregulated in HCC. ►**Table 1** summarizes selected differentially expressed miRNAs, their target genes, and potential involvement in HCC or other cancers.

Chronic viral infections such as hepatitis B (HBV) and hepatitis C (HCV) account for the majority of all cases of HCC. Integration of the HBV DNA into the host genome deregulates the cellular transcription program and sensitizes liver cells to carcinogenesis.[70] miRNA can regulate HBV infection through modulation of viral gene expression or binding to viral gene transcripts.[71] miR-18a targets estrogen receptor-α (ER-α). Overexpression of miR-18a occurs in HCC in females and provides a mechanism whereby ER-α mediated suppression of HBV transcription can be blocked.[72] Modulation of DNA methyltransferases by miR-152 or miR-148, or the modulation of HDAC4 by miR-1 can inhibit HBV replication.[73] Conversely, the hepatitis B virus X protein (HBx) can modulate expression of several miRNA including members of the let-7 family that can target several different oncogenic proteins that are commonly downregulated in HCC. Persistent infection with HCV leads to the development of cirrhosis through repeated epithelial injury, tissue repair, and regeneration,[74,75] providing a milieu that facilitates mutations and genomic aberrations, for example, promoter methylation and deregulated expression of tumor suppressors such as p16 and p53 that can lead to carcinogenesis.[76,77] Conversely, miRNA-196 can direct inhibit HCV transcription, and thereby may reduce risk of HCC.

Hepatocellular carcinoma can also occur in the setting of nonviral-induced cirrhosis as in alcoholic liver disease. Alcohol administration has been associated with reduced expression of miRNAs such as miR-199, miR-200, and miR126, which have also been described to be deregulated in HCC. Another risk factor for HCC is nonalcoholic steatohepatitis (NASH); HCC can arise in the setting of fibrosis and cirrhosis. In a dietary model of steatohepatitis, we reported deregulated expression of several miRNAs including upregulation of miR-155.[78] miR-155 can target C/EBP β, a tumor suppressor gene that interestingly has also been shown to downregulate HBV transcription.

## Long Noncoding RNAs in Hepatocellular Carcinoma

Long noncoding RNAs (lncRNAs) are ncRNAs with a size of more than 200 nucleotides in length.[79,80] Their biological effects are not well understood compared with miRNA, but they are increasingly being implicated in the regulation of gene expression through diverse mechanisms. LncRNAs can be transcribed by RNA polymerase II, and subsequently undergo cotranscriptional modifications such as polyadenylation and pre-RNA splicing.[81] Although lncRNAs can vary considerably in length, their postulated median length is approximately 592 nucleotides, which is much shorter compared with a median length of approximately 2,453 nucleotides for mRNA.[82] Most lncRNAs are confined to the nucleus and involved in the epigenetic regulation of gene expression.[82] They can be identified through bioinformatics analyses or a high-throughput analysis such as microarrays and transcriptome analysis.[83] Recently, lncRNAs have been recognized to have crucial roles in the regulation of gene expression and modulation of signaling pathways.[84] Several lncRNAs, such as H19, highly upregulated in liver cancer, TUC338, maternally expressed 3, and metastasis-associated lung adenocarcinoma transcript 1 are aberrantly expressed in human HCC (►**Table 2**). Similar to protein-coding genes, lncRNA show tissue-specific expression, chromatin marks, independent gene promoters, regulation by transcription factors, and splicing of multiple exons into a mature transcript.[113] Large-scale genome-wide sequencing and next-



This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

**Table 1** Differential expression of selected microRNAs (miRNAs) in hepatocellular carcinoma

| miRNA | Gene locus | Upregulated or downregulated | Target gene | Associated functions | References |
|---|---|---|---|---|---|
| miR-1 | 20q13.33 | Down | c-Met, ET-1 | Metastasis, proliferation | 18 |
| miR-7–1 | 9q21.32 | Down | PIK3CD, mTOR, p70S6K | Tumorigenesis, metastasis | 19 |
| miR-15a | 13q14 | Down | Bcl-2, cyclin D1, AKT3 | Proliferation, apoptosis | 20,21 |
| miR-16–1 | 13q14 | Down | Bcl-2, cyclin D1, AKT3 | Proliferation, apoptosis | 20,21 |
| miR-17 | 13q31–32 | Up | c-Myc, E2F | Angiogenesis | 22 |
| miR-18 | 13q31 | Up | c-Myc, E2F | Angiogenesis | 23 |
| miR-19a | 13q31.3 | Up | c-Myc, E2F | Angiogenesis | 24,25 |
| miR-20a | 13q31.3 | Up | c-Myc, E2F | Angiogenesis | 23 |
| miR-21 | 17q23 | Up | PTEN | Metastasis | 26,27 |
| miR-25 | 7q22.1 | Up | Bim | Apoptosis | 28 |
| miR-26a | 3p22.2 | Down | CDK6, cyclin D1 | Cell Cycle, angiogenesis | 29,30 |
| miR-29 | 1q32.2 | Down | Bcl-2, Bcl-w, Ras | Apoptosis | 31,32 |
| miR-34a | 1p36.22 | Down | Cyclin D1, CDK4, and CDK2, c-Met | Cell cycle, proliferation, metastasis | 33,34 |
| miR-92–1 | 13q31.3 | Up | c-Myc, E2F | Angiogenesis | 35 |
| miR-93 | 7q22.1 | Up | Bim | Apoptosis | 36 |
| miR-106b | 7q22.1 | Up | Bim | Apoptosis | 37 |
| miR-122a | 18q21 | Down | Cyclin G1, Bcl-w | Cell cycle, apoptosis | 38,39 |
| miR-124 | 8q12.2 | Down | ROCK2, EZH2 | Metastasis | 40,41 |
| miR-125b | 11q24 | Down | Bcl-2, Bcl-w | Apoptosis | 42,43 |
| miR-126 | 9q34.3 | Down | VEGF, VCAM-1 | Angiogenesis, metastasis | 44 |
| miR-132 | 17p13.3 | Down | VEGF | Angiogenesis | 45 |
| miR-136 | 14q32 | Down | Bcl-2 | Apoptosis | 46 |
| miR-141 | 12p13 | Down | EMT, Tiam1 | Metastasis | 47 |
| miR-145 | 5q32–33 | Down | IRS1 | Metastasis | 48,49 |
| miR-146a | 5q34 | Down | TRAF6, IRAK1 | Angiogenesis, metastasis | 50 |
| miR-148a | 7p15.2 | Down | DNMT-1, c-Met | Metastasis | 51,52 |
| miR-155 | 21q21 | Up | RhoA, TLR | Metastasis | 53 |
| miR-195 | 17p13 | Down | CDK6, cyclin D1 | Cell cycle | 54,55 |
| miR-198 | 3q13.33 | Down | c-Met | Metastasis | 56 |
| miR-199a | 19p13.2 | Down | mTOR, PAK4 | Proliferation, apoptosis | 57,58 |
| miR-200 | 1p36.3 | Down | EMT | Metastasis | 59 |
| miR-216a | 2p16.1 | Up | PTEN | Metastasis | 60 |
| miR-221 | Xp11.3 | Up | Bmf; CDKN1B/p27/Kip1; CDKN1C/p57/Kip2, PTEN | Apoptosis, proliferation, angiogenesis, metastasis | 61,62 |
| miR-222 | Xp11.3 | Up | AKT, PTEN | Angiogenesis, metastasis | 63,64 |
| miR-223 | Xq12–13.3 | Down | Cyclin E, RhoB | Cell cycle, Apoptosis | 65 |
| miR-224 | Xq28 | Up | Bcl-2, Bcl-w | Apoptosis | 66,67 |
| miR-449a | 5q11.2 | Down | c-Met | Metastasis | 68 |
| miR-519d | 19q13.42 | Up | PTEN | Metastasis | 69 |

Abbreviations: EMT, epithelial–mesenchymal transition; TLR, toll-like receptors.

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

**Table 2** Selected long noncoding RNAs (lncRNA) implicated in hepatocellular carcinoma

| lncRNA | Gene locus | Size (kb) | Potential role in liver cancer | References |
|---|---|---|---|---|
| HULC | 6p24.3 | 0.56 | Upregulated in HCC. Increased expression is associated with histological grade or HBV. | 85–89 |
| TUC338 | 12q13.13 | 0.59 | Increased in cirrhosis and HCC. Modulates cell growth. | 5 |
| HEIH | 5q35.3 | 1.7 | Associated with HBV-HCC. | 90,91 |
| MEG3 | 14q32.3 | 1.8 | Deregulated in HCC, associated with methylation. Predictive biomarker for monitoring epigenetic therapy. | 90,92,93 |
| MVIH | 10q22-q23 | 2.1 | Microvascular invasion in HCC. Predicts recurrence-free survival, overall survival. | 90,94,95 |
| UCA1/CUDR | 19p13.12 | 2.3 | Involved in chemotherapeutic resistance. | 96,97 |
| H19 | 11p15.5 | 2.3 | Increased in HCC or in peritumor areas Low peritumor/tumor expression correlates with prognosis. Suppression of tumor metastasis through miR-220-dependent inhibition of EMT, drug resistance. | 59,97–102 |
| HOTAIR | 12q13.13 | 2.3 | Inhibition reduces invasion and increases chemosensitivity. | 90,103–106 |
| HOTTIP | 7p15.2 | 7.9 | Upregulated in HCC. Predicts disease outcomes and tumor progression. | 107 |
| MALAT-1 | 11q13.1 | 8.7 | Upregulated in HCC. Associated with tumor metastasis and recurrence. | 90,108,109 |
| LINC-ROR | 18q21.31 | 22.8 | Tumor cell survival during hypoxia. | 84,110,111 |
| lncRNA-ATB | | 2.4 | Activated by TGF-β, promotes EMT, and triggers STAT3 signaling. | 112 |

Abbreviations: EMT, epithelial–mesenchymal transition; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TGF, tumor growth factor.

generation sequencing first identified several thousands of lncRNA transcripts within the mouse transcriptome, and subsequently also in humans.[114] More recently, tiling microarrays and RNA-sequencing have increased the numbers of known lncRNA.[115] Similar to other RNA, lncRNA that are involved in specific diseases may have clinical or pathological roles as markers of disease or clinical behavior.

Recent studies have indicated that lncRNAs are important regulators of development and involved in various pathological processes. The lncRNAs MALAT1 (metastasis associated lung adenocarcinoma transcript 1), HOTAIR (HOX transcript antisense intergenic RNA), H19, HULC (highly upregulated in liver cancer), and PRNCR1 (prostate cancer non-coding RNA1) are aberrantly expressed in a variety of human cancers, especially in HCC.[90,116] MALAT1 promotes tumor cell viability, invasion, and metastasis and is markedly upregulated in human and experimentally induced murine HCC.[108,109] It was observed that inhibition of MALAT1 in HepG2 cells could effectively reduce cell viability, motility, invasiveness, and increase sensitivity to apoptosis.[108] The lncRNA, HOTAIR is significantly increased in HCC tissues from patients as well as in liver cancer cell lines and the expression is correlated with poor prognosis.[103–105] Furthermore, HOTAIR can downregulate RNA binding motif protein-38 and promote cell migration and invasion in HCC cell lines.[106] The H19 gene encodes a 2.3-kb lncRNA located at 11p15.5 that is exclusively expressed from the maternal allele, and involved in genomic imprinting during growth and development.[100] H19 expression is upregulated in HBV-associated HCC.[101] The HULC gene is a 556-bp nucleotide located on chromosome 6p24.3 and was first described as a lncRNA with highly specific upregulation in HCC.[117] Elevation of HULC by HBx promotes hepatoma cell proliferation through suppression of p18 and may play a significant roles in hepatocarcinogenesis.[88] Despite the pervasive effects of lncRNA in the regulation of gene expression and development, the function of lncRNAs in the molecular pathogenesis of HCC and other cancers is not yet clear.

Ultraconserved RNAs (ucRNAs) are a group of lncRNAs transcribed from ultraconserved elements (UCEs), genomic sequences greater than 200 bases that are 100% identical across the genomes of human, mouse, and rat.[118] A total of 481 UCEs have been identified ranging in size from 200 to



This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

approximately 800 base pairs.[118] Some UCEs are located at genomic regions and fragile sites implicated with cancers. Deregulated expression of transcribed ucRNA has been observed in several cancers including leukemia and colon cancer.[119] A correlation of some ucRNA clinical prognostic factors, such as Myc expression, has been reported in neuroblastoma.[120] We have reported the involvement of ucRNAs in HCC and have identified and cloned the ucRNA TUC338 that is overexpressed in HCC, which can modulate cell cycle progression and proliferation.[5] Despite the emerging evidence implicating a role of ucRNA in tumor growth and progression, the precise role of ucRNAs remains unknown. A novel role of a transcribed ultraconserved RNA TUC339 as an intercellular signaling mediator of growth was suggested in a recent study showing enrichment of this ucRNA in extracellular vesicles released from HCC cells.[121]

These observations indicate the potential involvement of diverse lncRNA in tumor growth, tumor spread, and in intercellular signaling in HCC.

## ncRNAs for the Diagnosis of Hepatocellular Carcinoma

Several ncRNAs including both miRNA and lncRNA have been demonstrated in the circulation, and in other body fluids in HCC and other cancers.[122–125] These observations offer the potential that miRNA may be useful for clinical applications as diagnostic or prognostic biomarkers of disease. MicroRNA can be detected in the circulation enclosed within extracellular vesicles such as exosomes or microvesicles, associated with high-density lipoprotein or in association with proteins such as Argonaute 2.[126,127] Given the potential for miRNA to be transported to various sites within the circulation, there is a possibility for miRNA to have effects in different tissues. However, the biological effects of these circulating miRNA are not well known. MicroRNA enclosed within extracellular vesicles can be taken up by cells with the subsequent modulation of intercellular signaling in HCC cells.[128] In contrast, the functional effects of nonvesicular miRNA have not been elucidated or described. RNA molecules are not generally taken up by passive mechanisms, and are prone to degradation by endogenous nucleases. Thus, the presence of miRNA within extracellular vesicles may also provide stability to ensure their functionality. Irrespective of the functional effects, the ability to detect circulating miRNA that are selectively released under certain conditions provides an opportunity for their use as potential biomarkers of disease.

Chronic infection of HCV is a major cause for HCC, and a search for biomarkers of HCC in persons with chronic HCV would be clinically useful. It was reported that miR-100, miR-122, miR-221, and miR-224 are highly increased specifically in HCV-associated HCC.[129,130] Urinary miRNA has also been suggested as a biomarker of HCC by providing a noninvasive approach for the early diagnosis of HCC, before the onset of disease in HCV-positive patients.[131] In addition, circulating miR-101 is identified as a potential biomarker for HBV-related HCC and is correlated with the clinicopathological features and prognosis of HCC patients.[132]

## ncRNAs as Prognostic Biomarkers for Hepatocellular Carcinoma

Detection of the expression of specific miRNAs in tissues or in the circulation may provide useful prognostic information about the course of the disease. Deregulated expression of several miRNAs such as miR-199a, miR-199b-3p, miR-26, and miR-29 in HCC tissues has been shown to correlate with patient survival.[133] Furthermore, it was observed that the decreased expression of miR-148b in HCC patients is associated with poor survival prognosis.[52] Karakatsanis et al reported that miR-21, miR-31, miR-122, miR-221, and miR-222 were significantly upregulated in HCC tissues and the expression of miR-21, miR-31, miR-122, and miR-221 in HCC correlated with cirrhosis, while miR-21 and miR-221 associated with tumor stage and poor prognosis.[134] The interpretation of studies is complicated by the heterogeneity and diverse etiologies of HCC.

Like miRNA, the expression of certain lncRNAs can occur in a cancer-specific manner, raising the potential for their use in diagnosis, prognosis, or therapy. High expression of HOTAIR has been shown to predict tumor recurrence in patients with HCC.[103–105] HOTAIR has also been shown to correlate with a poor outcome in other cancers such as breast cancer.

The risk of HCC has been associated with single nucleotide polymorphisms (SNPs) in several different miRNAs. These are summarized in ►**Table 3**.

Similar data for polymorphisms at genomic sites for lncRNA implicated in HCC have not been reported. However, the majority of cancer-related single-nucleotide polymorphisms identified are located in noncoding regions of the genome that could represent sites of lncRNA transcripts.[156] Evaluation of genetic variations has not yet been incorporated into clinical practice, and further validation of their utility for this purpose is needed. In particular, data from some studies for miRNA-associated SNPs have not been concordant, likely due to differences in ethnic background.

## Therapeutic Modulation of ncRNA Expression for Hepatocellular Carcinoma

Although surgical resection or transplantation may offer the prospect of cure, many patients with HCC are too advanced for these procedures at the time of diagnosis. For these patients, locoregional or systemic therapies are of varying efficacy at slowing tumor progression. Targeting ncRNAs that are deregulated in HCC and contribute to the tumor phenotype or tumor chemosensitivity may offer potential new therapeutic strategies. miRNAs can potentially suppress expression of several genes and thereby could modulate multiple signaling pathways in cancer growth. Consequently, there is increasing interest in therapeutic strategies to modulate expression of miRNAs.[80,157–159] Therapeutic use of miRNA-based strategies requires the ability to deliver to the desired sites of action, acceptable safety with minimal toxicity, and a tolerable off-target impact of targeting miRNA in other tissues. Knowledge of essential miRNAs that are specifically involved in HCC pathogenesis or progression can suggest specific targets for therapeutic intervention. One of the most prominently expressed miRNAs in many human cancers,

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

**Table 3** Genetic single nucleotide polymorphisms in selected noncoding RNAs associated with risk of hepatocellular carcinoma

| Association | MicroRNA | Single nucleotide polymorphism | References |
|---|---|---|---|
| Positive | miR-let-7 | rs10877887 | 135 |
| Positive | miR-34b/c | rs4938723 | 136–139 |
| Positive | miR-101–1 | rs7536540 | 140 |
| Positive | miR-101–2 | rs12375841 | 140 |
| Positive | miR-106b-25 | rs999885 | 141,142 |
| Inconsistent | miR-146a | rs2910164 | 143–145 |
| Inconsistent | miR-149 | rs2292832 | 146–148 |
| Inconsistent | miR-196a2 | rs11614913 | 145,147,149–152 |
| Negative | miR-371–373 | rs3859501 | 153 |
| Inconsistent | miR-499 | rs3746444 | 144,146–148,154 |
| Positive | HULC | rs7763881 | 155 |
| Negative | MALAT1 | rs619586 | 155 |

Abbreviations: HULC, highly upregulated liver cancer; MALAT1, metastasis associated lung adenocarcinoma transcript 1.

including HCC, is miR-21, overexpression of which is associated with tumor stage and poor prognosis.[160] Several other miRNAs such as miR-151 and miR-221/222 are also markedly increased in expression in HCC patients, have defined oncogenic targets, and are viable potential targets for therapeutic intervention.[122] Similarly, therapeutic approaches to modulate downregulated tumor-suppressing miRNAs in HCC are also an attractive strategy to arrest tumor progression. We have observed that silencing of upregulated miR-221 using 2'-O-methyl phosphorothioate-modified anti-miR-221 oligonucleotide can prevent orthotopic tumor progression in experimental animals and increase survival rate.[61] Furthermore, silencing of miR-221 reduced tumor cell proliferation, increased markers of apoptosis, and resulted in cell cycle arrest.[61]

Of particular relevance to HCC occurring in the setting of viral infection, therapeutic modulation of expression of ncRNAs associated with viral hepatitis and HCC may have the potential to reduce disease progression as well as hepatocarcinogenesis. In one study, 18 miRNAs were exclusively expressed in HCV-associated HCC, with high specificity and selectivity compared with all other liver diseases and normal tissue.[129] miR-122 is a hepatocyte-specific miRNA that is implicated in cholesterol, lipid, and iron metabolism. miR-122 stimulates HCV replication through a unique and unusual interaction with two binding sites in the 5′-UTR of HCV genome to mediate the stability of the viral RNA.[161] Thus, therapeutic targeting miR-122 for the treatment of chronic HCV is attractive. Indeed, miR-122 silencing in chronic HCV infected chimpanzees using a locked nucleic acid- (LNA-) modified phosphorothioate oligonucleotide complementary to the 5′ end of miR-122 resulted in a potent and sustained inhibition of HCV replication.[162] Phase I and II clinical trials using anti-miR-122 oligonucleotides that target miR-122 have shown both the safety and efficacy of this approach in humans. An alternate approach is the use of small molecules to regulate miR-122 for HCV. However, the use of these strategies is confounded by reports that miR-122 knockout in mice enhances liver tumor formation.

## Strategies to Manipulate miRNA

There are several techniques that can be used to therapeutically manipulate the expression of miRNAs. ►**Fig. 1** illustrates various strategies to modulate miRNA expression, through either inhibition of miRNA or through replacement of miRNAs, and thereby modulate downstream gene expression. Replacement of miRNA may be accomplished using miRNA mimetics, whereas inhibition of miRNA can be accomplished by siRNAs or small hairpin RNAs. The delivery of a specific anti-miRNA into cells prevents the miRNA from binding to their cognate target genes thereby silencing miRNA function. In another strategy, an expression vector carrying multiple binding sites to a targeted miRNA is introduced into cells, which serves as a "sponge" and results in competitive inhibition of the target miRNA.[163] The delivery of miRNAs for replacement or constructs that can modulate the expression of miRNA in tumor tissues can be accomplished using diverse techniques such as lentiviral or adeno-associated viral vectors, cationic lipid nanoparticles, or direct local administration. We have demonstrated the therapeutic efficacy of chemically modified, cholesterol conjugated antisense oligonucleotides for the treatment of intrahepatic HCC xenografts in mice.[61] Moreover, modulation of miR-26a resulted in a reduction of tumor formation and lentiviral vectors carrying miRNA mimics against osteopontin successfully prevented the metastasis of HCC into lungs in a mouse model.[164]

Long noncoding RNA may also represent potential therapeutic targets. H19 is a lncRNA that is highly expressed in HCC and has oncogenic effects. Therapeutic targeting of H19 has been evaluated in patients with pancreatic, bladder, and ovarian cancer; studies in HCC are also warranted. Strategies to modulate expression of HCC-associated lncRNA are similar to those described for miRNA.

Hepatic uptake occurs rapidly following systemic administration of antisense oligonucleotides; thus, hepatic tumors are appropriate indications for miRNA or lncRNA-based therapeutic



This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

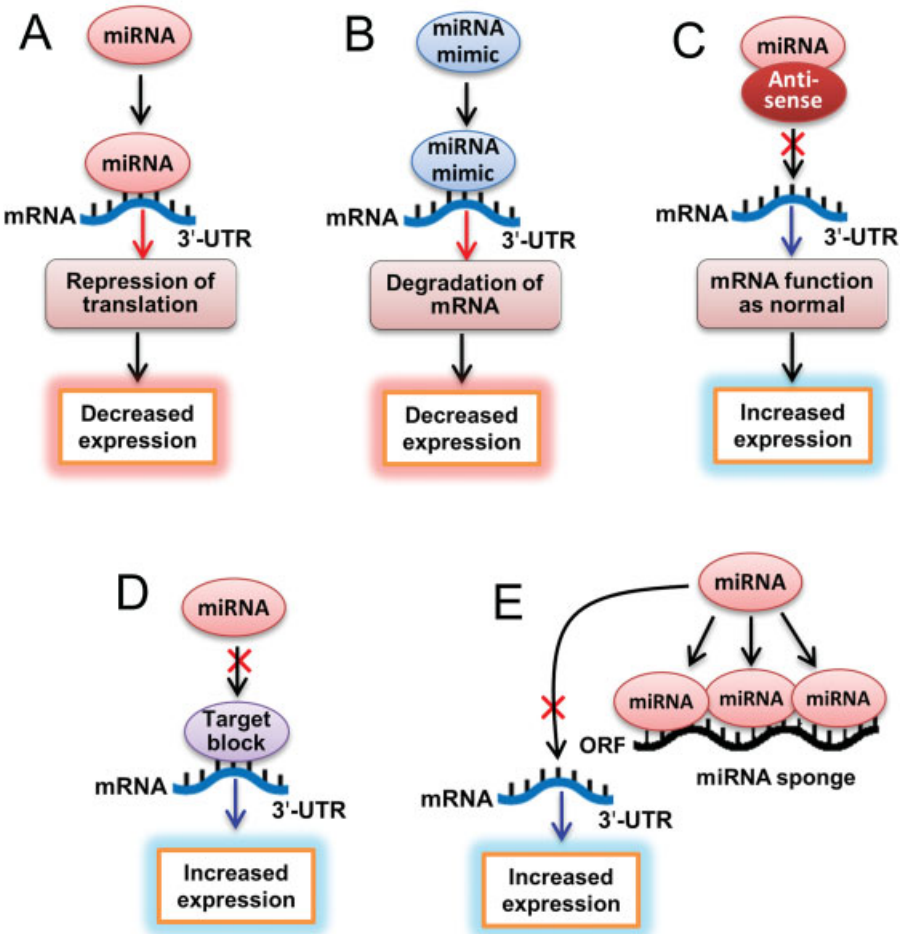


**Fig. 1** Strategies to manipulate microRNA (miRNA) expression in hepatocellular carcinoma. (**A**) Schematic of functional effect of miRNA in suppressing a target messenger RNA (mRNA) that is involved in maintaining the tumor phenotype. Endogenous miRNA binds to complementary sequences in three prime untranslated region (3′-UTR) of the target gene resulting in translational repression of mRNA. (**B**) miRNA mimics comprise the exact oligonucleotide sequence of the endogenous miRNA that binds to the same target gene and degrade the mRNA. (**C**) An antisense oligonucleotide strongly binds to a specific miRNA, silences its activity, and prevents binding to the target mRNA. (**D**) A target block is an oligonucleotide designed to bind to a portion of miRNA target on a specific mRNA without inhibition of translation thereby prevents miRNA-mediated repression. (**E**) A miRNA sponge consists of an open reading frame (ORF) with a 3′-UTR that contains several binding sites for a particular miRNA and serves as a competitive inhibitor.

approaches compared with most other types of cancers. Chemical modifications reduce degradation of native oligonucleotides. Encapsulation within nanoparticles may offer similar advantages, but are limited by immune cell activation and nonspecific uptake. Finally, it is not clear how many lncRNA regulate target gene expression and control multiple signaling pathways. A better understanding of the role of ncRNAs and the mechanism of regulation of target gene transcripts as well as molecular signaling events would accelerate the development of novel therapeutic strategies for the arrest of primary hepatic tumors.

## Summary

Currently, a large amount of literature and experimental data is available regarding the involvement of ncRNAs such as miRNAs in liver cancers. The data regarding other ncRNA such as lncRNA are also rapidly expanding. A characterization of their deregulated expression will provide potential markers for the diagnosis, prognosis, and therapy of HCC. A better understanding of the molecular mechanisms underlying ncRNA-mediated tumorigenesis may help to identify appropriate targets for intervention to control tumor formation or progression. The development of ncRNA-based therapeutics has major challenges. These include the need for tumor delivery, avoidance of immune response, limitation of toxicity of targeting constructs, and minimization of off-target effects. Improved approaches for directed therapy targeting noncoding RNA with the capability of safe and effective administration in patients with liver disease are needed. Until then, a more immediate clinical application of knowledge of deregulated miRNAs and lncRNAs would reflect their use as candidate markers for diagnosis or prognosis.

## Abbreviations

| | |
|---|---|
| ER-α | estrogen receptor-α |
| HBV | hepatitis B virus |
| HBx | hepatitis B virus X protein |



This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

| | |
|---|---|
| HCV | hepatitis C virus |
| HCC | hepatocellular carcinoma |
| HOTAIR | HOX transcript antisense intergenic RNA |
| HULC | highly upregulated in liver cancer |
| LNA | locked nucleic acid |
| lncRNAs | long noncoding RNAs |
| MALAT1 | metastasis associated lung adenocarcinoma transcript 1 |
| mRNAs | messenger RNAs |
| miRNAs | microRNAs |
| NASH | nonalcoholic steatohepatitis |
| ncRNAs | noncoding RNAs |
| piRNAs | piwi-interacting RNAs |
| PRNCR1 | prostate cancer noncoding RNA1 |
| rRNA | ribosomal RNA |
| SNPs | single nucleotide polymorphisms |
| siRNAS | small interfering RNAs |
| snRNAs | small nuclear ribonucleic acids |
| snoRNAs | small nucleolar RNAs |
| tRNA | transfer RNA |
| UCEs | ultraconserved elements |
| ucRNAs | ultraconserved RNAs |
| UTRs | untranslated regions |

Acknowledgments
This work was supported by National Institutes of Health grants DK069370 and TR000884. We thank Ms. Kimberly McCoy for excellent secretarial support.

## References

1 Chun JM, Kwon HJ, Sohn J, et al. Prognostic factors after early recurrence in patients who underwent curative resection for hepatocellular carcinoma. J Surg Oncol 2011;103(2): 148–151
2 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61(2):69–90
3 Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011;12(12):861–874
4 Mattick JS, Makunin IV. Non-coding RNA. Hum Mol Genet 2006; 15(Spec No 1):R17–R29
5 Braconi C, Valeri N, Kogure T, et al. Expression and functional role of a transcribed noncoding RNA with an ultraconserved element in hepatocellular carcinoma. Proc Natl Acad Sci U S A 2011;108(2): 786–791
6 Morris KV, Ed. Non-Coding RNAs and Epigenetic Regulation of Gene Expression: Drivers of Natural Selection. Norfolk, England: Caister Academic Press; 2012
7 Qu Z, Adelson DL. Evolutionary conservation and functional roles of ncRNA. Front Genet 2012;3:205
8 Shukla GC, Singh J, Barik S. MicroRNAs: processing, maturation, target recognition and regulatory functions. Mol Cell Pharmacol 2011;3(3):83–92
9 Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136(2):215–233
10 Sassen S, Miska EA, Caldas C. MicroRNA: implications for cancer. Virchows Arch 2008;452(1):1–10
11 Li C, Feng Y, Coukos G, Zhang L. Therapeutic microRNA strategies in human cancer. AAPS J 2009;11(4):747–757
12 Holland B, Wong J, Li M, Rasheed S. Identification of human microRNA-like sequences embedded within the protein-encoding genes of the human immunodeficiency virus. PLoS ONE 2013;8(3):e58586
13 Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. Nature 2008;455(7209):58–63
14 Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014;9:287–314
15 Braconi C, Henry JC, Kogure T, Schmittgen T, Patel T. The role of microRNAs in human liver cancers. Semin Oncol 2011;38(6): 752–763
16 Lujambio A, Lowe SW. The microcosmos of cancer. Nature 2012; 482(7385):347–355
17 Chu R, Mo G, Duan Z, et al. miRNAs affect the development of hepatocellular carcinoma via dysregulation of their biogenesis and expression. Cell Commun Signal 2014;12:45
18 Datta J, Kutay H, Nasser MW, et al. Methylation mediated silencing of microRNA-1 gene and its role in hepatocellular carcinogenesis. Cancer Res 2008;68(13):5049–5058
19 Fang Y, Xue JL, Shen Q, Chen J, Tian L. MicroRNA-7 inhibits tumor growth and metastasis by targeting the phosphoinositide 3-kinase/Akt pathway in hepatocellular carcinoma. Hepatology 2012;55(6):1852–1862
20 Wang Y, Jiang L, Ji X, Yang B, Zhang Y, Fu XD. Hepatitis B viral RNA directly mediates down-regulation of the tumor suppressor microRNA miR-15a/miR-16-1 in hepatocytes. J Biol Chem 2013;288(25):18484–18493
21 Bonci D, Coppola V, Musumeci M, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med 2008;14(11):1271–1277
22 Chen L, Jiang M, Yuan W, Tang H. miR-17-5p as a novel prognostic marker for hepatocellular carcinoma. J Invest Surg 2012;25(3): 156–161
23 Murakami Y, Yasuda T, Saigo K, et al. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 2006;25(17):2537–2545
24 Li Y, Tan W, Neo TW, et al. Role of the miR-106b-25 microRNA cluster in hepatocellular carcinoma. Cancer Sci 2009;100(7): 1234–1242
25 Tan W, Li Y, Lim SG, Tan TM. miR-106b-25/miR-17-92 clusters: polycistrons with oncogenic roles in hepatocellular carcinoma. World J Gastroenterol 2014;20(20):5962–5972
26 Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007; 133(2):647–658
27 Zhou L, Yang ZX, Song WJ, et al. MicroRNA-21 regulates the migration and invasion of a stem-like population in hepatocellular carcinoma. Int J Oncol 2013;43(2):661–669
28 Su ZX, Zhao J, Rong ZH, Geng WM, Wu YG, Qin CK. Upregulation of microRNA-25 associates with prognosis in hepatocellular carcinoma. Diagn Pathol 2014;9:47
29 Kota J, Chivukula RR, O'Donnell KA, et al. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell 2009;137(6):1005–1017
30 Yang X, Zhang XF, Lu X, et al. MicroRNA-26a suppresses angiogenesis in human hepatocellular carcinoma by targeting hepatocyte growth factor-cMet pathway. Hepatology 2014;59(5): 1874–1885
31 Xiong Y, Fang JH, Yun JP, et al. Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. Hepatology 2010;51(3):836–845
32 Parpart S, Roessler S, Dong F, et al. Modulation of miR-29 expression by α-fetoprotein is linked to the hepatocellular carcinoma epigenome. Hepatology 2014;60(3):872–883
33 Xie K, Liu J, Chen J, et al. Methylation-associated silencing of microRNA-34b in hepatocellular carcinoma cancer. Gene 2014; 543(1):101–107



This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

34 Li N, Fu H, Tie Y, et al. miR-34a inhibits migration and invasion by down-regulation of c-Met expression in human hepatocellular carcinoma cells. Cancer Lett 2009;275(1):44–53
35 Aguda BD, Kim Y, Piper-Hunter MG, Friedman A, Marsh CB. MicroRNA regulation of a cancer network: consequences of the feedback loops involving miR-17-92, E2F, and Myc. Proc Natl Acad Sci U S A 2008;105(50):19678–19683
36 Xu D, He XX, Chang Y, Sun SZ, Xu CR, Lin JS. Downregulation of MiR-93 expression reduces cell proliferation and clonogenicity of HepG2 cells. Hepatogastroenterology 2012;59(120):2367–2373
37 Shen G, Jia H, Tai Q, Li Y, Chen D. miR-106b downregulates adenomatous polyposis coli and promotes cell proliferation in human hepatocellular carcinoma. Carcinogenesis 2013;34(1): 211–219
38 Gramantieri L, Ferracin M, Fornari F, et al. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Res 2007;67(13):6092–6099
39 Tsai WC, Hsu SD, Hsu CS, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest 2012; 122(8):2884–2897
40 Zheng F, Liao YJ, Cai MY, et al. The putative tumour suppressor microRNA-124 modulates hepatocellular carcinoma cell aggressiveness by repressing ROCK2 and EZH2. Gut 2012;61(2): 278–289
41 Lu Y, Yue X, Cui Y, Zhang J, Wang K. MicroRNA-124 suppresses growth of human hepatocellular carcinoma by targeting STAT3. Biochem Biophys Res Commun 2013;441(4):873–879
42 Kim JK, Noh JH, Jung KH, et al. Sirtuin7 oncogenic potential in human hepatocellular carcinoma and its regulation by the tumor suppressors MiR-125a-5p and MiR-125b. Hepatology 2013; 57(3):1055–1067
43 Gong J, Zhang JP, Li B, et al. MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R. Oncogene 2013;32(25):3071–3079
44 Chen H, Miao R, Fan J, et al. Decreased expression of miR-126 correlates with metastatic recurrence of hepatocellular carcinoma. Clin Exp Metastasis 2013;30(5):651–658
45 Wei X, Tan C, Tang C, et al. Epigenetic repression of miR-132 expression by the hepatitis B virus x protein in hepatitis B virus-related hepatocellular carcinoma. Cell Signal 2013;25(5):1037–1043
46 Zhao J, Wang W, Huang Y, et al. HBx elevates oncoprotein AEG-1 expression to promote cell migration by downregulating miR-375 and miR-136 in malignant hepatocytes. DNA Cell Biol 2014; 33(10):715–722
47 Liu Y, Ding Y, Huang J, et al. MiR-141 suppresses the migration and invasion of HCC cells by targeting Tiam1. PLoS ONE 2014; 9(2):e88393
48 Wang Y, Hu C, Cheng J, et al. MicroRNA-145 suppresses hepatocellular carcinoma by targeting IRS1 and its downstream Akt signaling. Biochem Biophys Res Commun 2014;446(4):1255–1260
49 Yang XW, Zhang LJ, Huang XH, et al. miR-145 suppresses cell invasion in hepatocellular carcinoma cells: miR-145 targets ADAM17. Hepatol Res 2014;44(5):551–559
50 Zhu K, Pan Q, Zhang X, et al. MiR-146a enhances angiogenic activity of endothelial cells in hepatocellular carcinoma by promoting PDGFRA expression. Carcinogenesis 2013;34(9): 2071–2079
51 Gailhouste L, Gomez-Santos L, Hagiwara K, et al. miR-148a plays a pivotal role in the liver by promoting the hepatospecific phenotype and suppressing the invasiveness of transformed cells. Hepatology 2013;58(3):1153–1165
52 Zhang Z, Zheng W, Hai J. MicroRNA-148b expression is decreased in hepatocellular carcinoma and associated with prognosis. Med Oncol 2014;31(6):984
53 Zhang Y, Wei W, Cheng N, et al. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. Hepatology 2012;56(5):1631–1640
54 Wang R, Zhao N, Li S, et al. MicroRNA-195 suppresses angiogenesis and metastasis of hepatocellular carcinoma by inhibiting the expression of VEGF, VAV2, and CDC42. Hepatology 2013;58(2): 642–653
55 Xu T, Zhu Y, Xiong Y, Ge YY, Yun JP, Zhuang SM. MicroRNA-195 suppresses tumorigenicity and regulates G1/S transition of human hepatocellular carcinoma cells. Hepatology 2009;50(1): 113–121
56 Tan S, Li R, Ding K, Lobie PE, Zhu T. miR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/c-MET pathway. FEBS Lett 2011;585(14):2229–2234
57 Jiang J, Gusev Y, Aderca I, et al. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. Clin Cancer Res 2008;14(2):419–427
58 Fornari F, Milazzo M, Chieco P, et al. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2010;70(12):5184–5193
59 Zhang L, Yang F, Yuan JH, et al. Epigenetic activation of the MiR-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. Carcinogenesis 2013;34(3): 577–586
60 Xia H, Ooi LL, Hui KM. MicroRNA-216a/217-induced epithelial-mesenchymal transition targets PTEN and SMAD7 to promote drug resistance and recurrence of liver cancer. Hepatology 2013; 58(2):629–641
61 Park JK, Kogure T, Nuovo GJ, et al. miR-221 silencing blocks hepatocellular carcinoma and promotes survival. Cancer Res 2011;71(24):7608–7616
62 Pineau P, Volinia S, McJunkin K, et al. miR-221 overexpression contributes to liver tumorigenesis. Proc Natl Acad Sci U S A 2010; 107(1):264–269
63 Wong QW, Ching AK, Chan AW, et al. MiR-222 overexpression confers cell migratory advantages in hepatocellular carcinoma through enhancing AKT signaling. Clin Cancer Res 2010;16(3): 867–875
64 Yang YF, Wang F, Xiao JJ, et al. MiR-222 overexpression promotes proliferation of human hepatocellular carcinoma HepG2 cells by downregulating p27. Int J Clin Exp Med 2014;7(4):893–902
65 Dong YW, Wang R, Cai QQ, et al. Sulfatide epigenetically regulates miR-223 and promotes the migration of human hepatocellular carcinoma cells. J Hepatol 2014;60(4):792–801
66 Zhang Y, Takahashi S, Tasaka A, Yoshima T, Ochi H, Chayama K. Involvement of microRNA-224 in cell proliferation, migration, invasion, and anti-apoptosis in hepatocellular carcinoma. J Gastroenterol Hepatol 2013;28(3):565–575
67 Yu L, Zhang J, Guo X, Li Z, Zhang P. MicroRNA-224 upregulation and AKT activation synergistically predict poor prognosis in patients with hepatocellular carcinoma. Cancer Epidemiol 2014;38(4):408–413
68 Buurman R, Gürlevik E, Schäffer V, et al. Histone deacetylases activate hepatocyte growth factor signaling by repressing microRNA-449 in hepatocellular carcinoma cells. Gastroenterology 2012;143(3):811–20.e1, 15
69 Fornari F, Milazzo M, Chieco P, et al. In hepatocellular carcinoma miR-519d is up-regulated by p53 and DNA hypomethylation and targets CDKN1A/p21, PTEN, AKT3 and TIMP2. J Pathol 2012; 227(3):275–285
70 Guerrieri F, Belloni L, Pediconi N, Levrero M. Molecular mechanisms of HBV-associated hepatocarcinogenesis. Semin Liver Dis 2013;33(2):147–156
71 Liu WH, Yeh SH, Chen PJ. Role of microRNAs in hepatitis B virus replication and pathogenesis. Biochim Biophys Acta 2011; 1809(11–12):678–685
72 Liu WH, Yeh SH, Lu CC, et al. MicroRNA-18a prevents estrogen receptor-alpha expression, promoting proliferation of hepatocellular carcinoma cells. Gastroenterology 2009;136(2): 683–693


This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

73 Zhang X, Zhang E, Ma Z, et al. Modulation of hepatitis B virus replication and hepatocyte differentiation by MicroRNA-1. Hepatology 2011;53(5):1476–1485
74 Vezali E, Aghemo A, Colombo M. A review of the treatment of chronic hepatitis C virus infection in cirrhosis. Clin Ther 2010; 32(13):2117–2138
75 Rehm J, Taylor B, Mohapatra S, et al. Alcohol as a risk factor for liver cirrhosis: a systematic review and meta-analysis. Drug Alcohol Rev 2010;29(4):437–445
76 Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 2002;31(4):339–346
77 Ozen C, Yildiz G, Dagcan AT, et al. Genetics and epigenetics of liver cancer. New Biotechnol 2013;30(4):381–384
78 Wang B, Majumder S, Nuovo G, et al. Role of microRNA-155 at early stages of hepatocarcinogenesis induced by choline-deficient and amino acid-defined diet in C57BL/6 mice. Hepatology 2009;50(4):1152–1161
79 Perkel JM. Visiting "noncodarnia". Biotechniques 2013;54(6): 301–304, 303–304
80 Li CH, Chen Y. Targeting long non-coding RNAs in cancers: progress and prospects. Int J Biochem Cell Biol 2013;45(8): 1895–1910
81 Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009;10(3):155–159
82 Derrien T, Johnson R, Bussotti G, et al. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012;22(9):1775–1789
83 Yan B, Wang ZH, Guo JT. The research strategies for probing the function of long noncoding RNAs. Genomics 2012;99(2):76–80
84 Takahashi K, Yan IK, Haga H, Patel T. Modulation of hypoxia-signaling pathways by extracellular linc-RoR. J Cell Sci 2014;127 (Pt 7):1585–1594
85 Zhao Y, Guo Q, Chen J, Hu J, Wang S, Sun Y. Role of long non-coding RNA HULC in cell proliferation, apoptosis and tumor metastasis of gastric cancer: a clinical and in vitro investigation. Oncol Rep 2014;31(1):358–364
86 Xie H, Ma H, Zhou D. Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. Biomed Res Int 2013;2013:136106
87 Hämmerle M, Gutschner T, Uckelmann H, et al. Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). Hepatology 2013;58(5):1703–1712
88 Du Y, Kong G, You X, et al. 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 2012; 287(31):26302–26311
89 Wang J, Liu X, Wu H, et al. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. Nucleic Acids Res 2010;38(16):5366–5383
90 He Y, Meng XM, Huang C, et al. Long noncoding RNAs: novel insights into hepatocellular carcinoma. Cancer Lett 2014;344(1): 20–27
91 Yang F, Zhang L, Huo XS, et al. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 2011;54(5):1679–1689
92 Anwar SL, Krech T, Hasemeier B, et al. Loss of imprinting and allelic switching at the DLK1-MEG3 locus in human hepatocellular carcinoma. PLoS ONE 2012;7(11):e49462
93 Braconi C, Kogure T, Valeri N, et al. microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. Oncogene 2011;30(47):4750–4756
94 Nie FQ, Zhu Q, Xu TP, et al. Long non-coding RNA MVIH indicates a poor prognosis for non-small cell lung cancer and promotes cell proliferation and invasion. Tumour Biol 2014;35(8):7587–7594
95 Yuan SX, Yang F, Yang Y, et al. 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 2012;56(6):2231–2241
96 Tsang WP, Wong TW, Cheung AH, Co CN, Kwok TT. Induction of drug resistance and transformation in human cancer cells by the noncoding RNA CUDR. RNA 2007;13(6):890–898
97 Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett 2013;339(2): 159–166
98 Lv J, Yu YQ, Li SQ, Luo L, Wang Q. Aflatoxin B1 promotes cell growth and invasion in hepatocellular carcinoma HepG2 cells through H19 and E2F1. Asian Pac J Cancer Prev 2014;15(6):2565–2570
99 Tsang WP, Kwok TT. Riboregulator H19 induction of MDR1-associated drug resistance in human hepatocellular carcinoma cells. Oncogene 2007;26(33):4877–4881
100 Gabory A, Jammes H, Dandolo L. The H19 locus: role of an imprinted non-coding RNA in growth and development. BioEssays 2010;32(6):473–480
101 Iizuka N, Oka M, Yamada-Okabe H, et al. Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method. Cancer Res 2002;62(14):3939–3944
102 Ariel I, Miao HQ, Ji XR, et al. Imprinted H19 oncofetal RNA is a candidate tumour marker for hepatocellular carcinoma. Mol Pathol 1998;51(1):21–25
103 Geng YJ, Xie SL, Li Q, Ma J, Wang GY. Large intervening non-coding RNA HOTAIR is associated with hepatocellular carcinoma progression. J Int Med Res 2011;39(6):2119–2128
104 Yang Z, Zhou L, Wu LM, et al. Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. Ann Surg Oncol 2011;18(5):1243–1250
105 Ishibashi M, Kogo R, Shibata K, et al. Clinical significance of the expression of long non-coding RNA HOTAIR in primary hepatocellular carcinoma. Oncol Rep 2013;29(3):946–950
106 Ding C, Cheng S, Yang Z, et al. Long non-coding RNA HOTAIR promotes cell migration and invasion via down-regulation of RNA binding motif protein 38 in hepatocellular carcinoma cells. Int J Mol Sci 2014;15(3):4060–4076
107 Quagliata L, Matter MS, Piscuoglio S, et al. Long noncoding RNA HOTTIP/HOXA13 expression is associated with disease progression and predicts outcome in hepatocellular carcinoma patients. Hepatology 2014;59(3):911–923
108 Lai MC, Yang Z, Zhou L, et al. Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. Med Oncol 2012;29(3): 1810–1816
109 Lin R, Maeda S, Liu C, Karin M, Edgington TS. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. Oncogene 2007;26(6):851–858
110 Takahashi K, Yan IK, Kogure T, Haga H, Patel T. Extracellular vesicle-mediated transfer of long non-coding RNA ROR modulates chemosensitivity in human hepatocellular cancer. FEBS Open Bio 2014;4:458–467
111 Hou P, Zhao Y, Li Z, et al. LincRNA-ROR induces epithelial-to-mesenchymal transition and contributes to breast cancer tumorigenesis and metastasis. Cell Death Dis 2014;5:e1287
112 Yuan JH, Yang F, Wang F, et al. A long noncoding RNA activated by TGF-β promotes the invasion-metastasis cascade in hepatocellular carcinoma. Cancer Cell 2014;25(5):666–681
113 Takahashi K, Yan I, Haga H, Patel T. Long noncoding RNA in liver diseases. Hepatology 2014;60(2):744–753
114 Carninci P, Kasukawa T, Katayama S, et al; FANTOM Consortium; RIKEN Genome Exploration Research Group and Genome Science Group (Genome Network Project Core Group). The transcriptional landscape of the mammalian genome. Science 2005; 309(5740):1559–1563


This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

115 Guttman M, Amit I, Garber M, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009;458(7235):223–227
116 Wang Z, Li X. The role of noncoding RNA in hepatocellular carcinoma. Gland Surg 2013;2(1):25–29
117 Panzitt K, Tschernatsch MM, Guelly C, et al. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology 2007;132(1): 330–342
118 Bejerano G, Pheasant M, Makunin I, et al. Ultraconserved elements in the human genome. Science 2004;304(5675): 1321–1325
119 Calin GA, Liu CG, Ferracin M, et al. Ultraconserved regions encoding ncRNAs are altered in human leukemias and carcinomas. Cancer Cell 2007;12(3):215–229
120 Mestdagh P, Fredlund E, Pattyn F, et al. An integrative genomics screen uncovers ncRNA T-UCR functions in neuroblastoma tumours. Oncogene 2010;29(24):3583–3592
121 Kogure T, Yan IK, Lin WL, Patel T. Extracellular vesicle-mediated transfer of a novel long noncoding RNA TUC339: a mechanism of intercellular signaling in human hepatocellular cancer. Genes Cancer 2013;4(7–8):261–272
122 Li W, Xie L, He X, et al. Diagnostic and prognostic implications of microRNAs in human hepatocellular carcinoma. Int J Cancer 2008;123(7):1616–1622
123 Borel F, Konstantinova P, Jansen PL. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. J Hepatol 2012;56(6):1371–1383
124 Hou W, Bonkovsky HL. Non-coding RNAs in hepatitis C-induced hepatocellular carcinoma: dysregulation and implications for early detection, diagnosis and therapy. World J Gastroenterol 2013;19(44):7836–7845
125 Xiao F, Zhang W, Zhou L, et al. microRNA-200a is an independent prognostic factor of hepatocellular carcinoma and induces cell cycle arrest by targeting CDK6. Oncol Rep 2013;30(5):2203–2210
126 Lv Z, Wei Y, Wang D, Zhang CY, Zen K, Li L. Argonaute 2 in cell-secreted microvesicles guides the function of secreted miRNAs in recipient cells. PLoS ONE 2014;9(7):e103599
127 Cereghetti DM, Lee PP. Tumor-derived exosomes contain microRNAs with immunological function: implications for a novel immunosuppression mechanism. Microrna 2014;2(3): 194–204
128 Kogure T, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology 2011;54(4):1237–1248
129 Diaz G, Melis M, Tice A, et al. Identification of microRNAs specifically expressed in hepatitis C virus-associated hepatocellular carcinoma. Int J Cancer 2013;133(4):816–824
130 Varnholt H, Drebber U, Schulze F, et al. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. Hepatology 2008;47(4):1223–1232
131 Abdalla MA, Haj-Ahmad Y. Promising candidate urinary microrna biomarkers for the early detection of hepatocellular carcinoma among high-risk hepatitis C virus Egyptian patients. J Cancer 2012;3:19–31
132 Fu Y, Wei X, Tang C, et al. Circulating microRNA-101 as a potential biomarker for hepatitis B virus-related hepatocellular carcinoma. Oncol Lett 2013;6(6):1811–1815
133 Hou J, Lin L, Zhou W, et al. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. Cancer Cell 2011;19(2):232–243
134 Karakatsanis A, Papaconstantinou I, Gazouli M, Lyberopoulou A, Polymeneas G, Voros D. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. Mol Carcinog 2013;52(4):297–303
135 Xie K, Liu J, Zhu L, et al. A potentially functional polymorphism in the promoter region of let-7 family is associated with survival of hepatocellular carcinoma. Cancer Epidemiol 2013;37(6): 998–1002
136 Xu Y, Liu L, Liu J, et al. A potentially functional polymorphism in the promoter region of miR-34b/c is associated with an increased risk for primary hepatocellular carcinoma. Int J Cancer 2011; 128(2):412–417
137 Liang TJ, Liu HJ, Zhao XQ, Yu CH, Li CS. Lack of association of MiR-34b/c polymorphism (rs4938723) with hepatocellular carcinoma: a meta-analysis. PLoS ONE 2013;8(7):e68588
138 Son MS, Jang MJ, Jeon YJ, et al. Promoter polymorphisms of pri-miR-34b/c are associated with hepatocellular carcinoma. Gene 2013;524(2):156–160
139 Tao T, Chen S, Xu B, et al. Association between hsa-miR-34b/c rs4938723 T > C promoter polymorphism and cancer risk: a meta-analysis based on 6,036 cases and 6,204 controls. Chin J Cancer Res 2014;26(3):315–322
140 Bae JS, Kim JH, Pasaje CF, et al. Association study of genetic variations in microRNAs with the risk of hepatitis B-related liver diseases. Dig Liver Dis 2012;44(10):849–854
141 Liu Y, Zhang Y, Wen J, et al. A genetic variant in the promoter region of miR-106b-25 cluster and risk of HBV infection and hepatocellular carcinoma. PLoS ONE 2012;7(2):e32230
142 Qi F, Huang M, Pan Y, et al. A genetic variant in the promoter region of miR-106b-25 cluster predict clinical outcome of HBV-related hepatocellular carcinoma in Chinese. PLoS ONE 2014; 9(1):e85394
143 Akkız H, Bayram S, Bekar A, Akgöllü E, Usküdar O, Sandıkçı M. No association of pre-microRNA-146a rs2910164 polymorphism and risk of hepatocellular carcinoma development in Turkish population: a case-control study. Gene 2011;486(1–2):104–109
144 Xiang Y, Fan S, Cao J, Huang S, Zhang LP. Association of the microRNA-499 variants with susceptibility to hepatocellular carcinoma in a Chinese population. Mol Biol Rep 2012;39(6): 7019–7023
145 Zhou B, Dong LP, Jing XY, et al. Association between miR-146aG>C and miR-196a2C>T polymorphisms and the risk of hepatocellular carcinoma in a Chinese population. Tumour Biol 2014;35(8):7775–7780
146 Kim WH, Min KT, Jeon YJ, et al. Association study of microRNA polymorphisms with hepatocellular carcinoma in Korean population. Gene 2012;504(1):92–97
147 Kou JT, Fan H, Han D, et al. Association between four common microRNA polymorphisms and the risk of hepatocellular carcinoma and HBV infection. Oncol Lett 2014;8(3):1255–1260
148 Wang XH, Wang FR, Tang YF, Zou HZ, Zhao YQ. Association of miR-149C>T and miR-499A>G polymorphisms with the risk of hepatocellular carcinoma in the Chinese population. Genet Mol Res 2014;13(3):5048–5054
149 Li XD, Li ZG, Song XX, Liu CF. A variant in microRNA-196a2 is associated with susceptibility to hepatocellular carcinoma in Chinese patients with cirrhosis. Pathology 2010;42(7):669–673
150 Akkız H, Bayram S, Bekar A, Akgöllü E, Ulger Y. A functional polymorphism in pre-microRNA-196a-2 contributes to the susceptibility of hepatocellular carcinoma in a Turkish population: a case-control study. J Viral Hepat 2011;18(7):e399–e407
151 Guo J, Jin M, Zhang M, Chen K. A genetic variant in miR-196a2 increased digestive system cancer risks: a meta-analysis of 15 case-control studies. PLoS ONE 2012;7(1):e30585
152 Kim HY, Yoon JH, Lee HS, et al. MicroRNA-196A-2 polymorphisms and hepatocellular carcinoma in patients with chronic hepatitis B. J Med Virol 2014;86(3):446–453
153 Kwak MS, Lee DH, Cho Y, et al. Association of polymorphism in pri-microRNAs-371-372-373 with the occurrence of

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.

hepatocellular carcinoma in hepatitis B virus infected patients. PLoS ONE 2012;7(7):e41983
154 Akkiz H, Bayram S, Bekar A, Akgöllü E, Üsküdar O. Genetic variation in the microRNA-499 gene and hepatocellular carcinoma risk in a Turkish population: lack of any association in a case-control study. Asian Pac J Cancer Prev 2011;12(11): 3107–3112
155 Liu Y, Pan S, Liu L, et al. A genetic variant in long non-coding RNA HULC contributes to risk of HBV-related hepatocellular carcinoma in a Chinese population. PLoS ONE 2012;7(4):e35145
156 Cheetham SW, Gruhl F, Mattick JS, Dinger ME. Long noncoding RNAs and the genetics of cancer. Br J Cancer 2013;108(12): 2419–2425
157 Braconi C, Patel T. Non-coding RNAs as therapeutic targets in hepatocellular cancer. Curr Cancer Drug Targets 2012;12(9): 1073–1080
158 Chen Z, Ma T, Huang C, et al. MicroRNA-148a: a potential therapeutic target for cancer. Gene 2014;533(1):456–457
159 D'Anzeo M, Faloppi L, Scartozzi M, et al. The role of micro-RNAs in hepatocellular carcinoma: from molecular biology to treatment. Molecules 2014;19(5):6393–6406
160 Tomimaru Y, Eguchi H, Nagano H, et al. MicroRNA-21 induces resistance to the anti-tumour effect of interferon-α/5-fluorouracil in hepatocellular carcinoma cells. Br J Cancer 2010;103(10): 1617–1626
161 Henke JI, Goergen D, Zheng J, et al. microRNA-122 stimulates translation of hepatitis C virus RNA. EMBO J 2008;27(24):3300–3310
162 Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2010;327(5962):198–201
163 Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. Nat Methods 2007; 4(9):721–726
164 Sun BS, Dong QZ, Ye QH, et al. Lentiviral-mediated miRNA against osteopontin suppresses tumor growth and metastasis of human hepatocellular carcinoma. Hepatology 2008;48(6):1834–1842

This document was downloaded for personal use only. Unauthorized distribution is strictly prohibited.