Noncoding RNA as Therapeutic Targets for Hepatocellular Carcinoma

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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

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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-

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.

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 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,

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

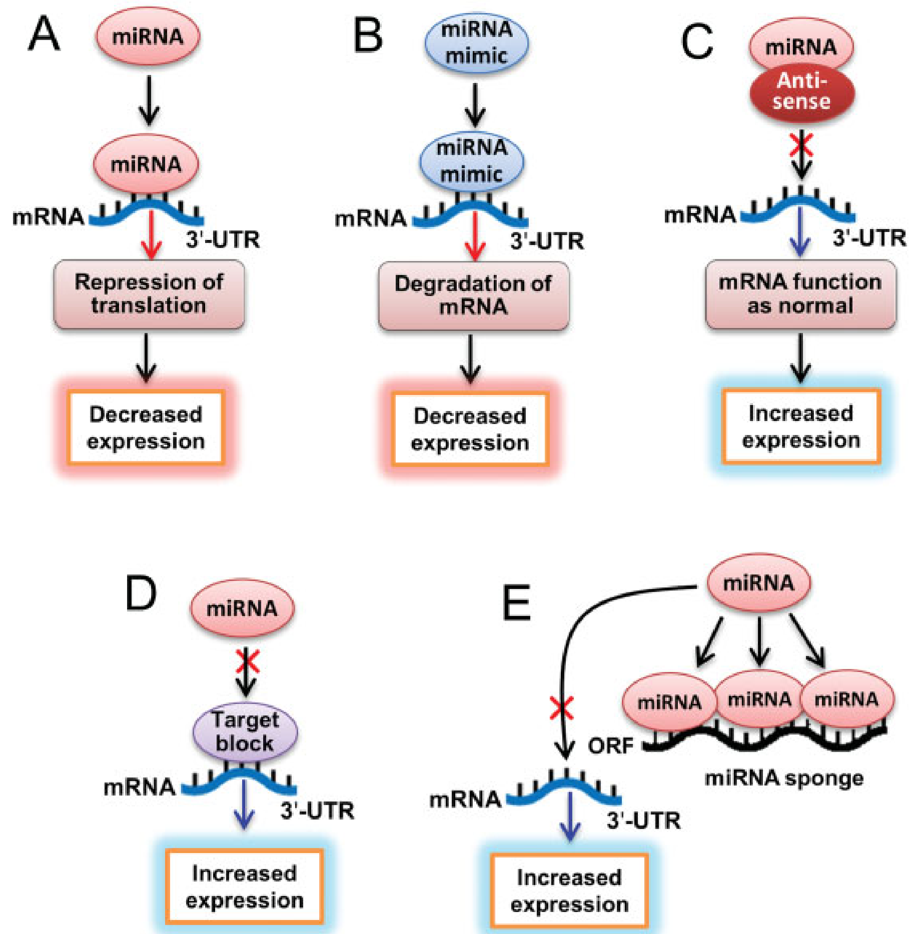

Fig. 1 Strategies to manipulate microRNA (miRNA) expression in hepatocellular carcinoma. (**A**) Schematic of functional effect of miRNA in suppressing a target messenger RNA (mRNA) that is involved in maintaining the tumor phenotype. Endogenous miRNA binds to complementary sequences in three prime untranslated 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

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

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