



REVIEW ARTICLE

Non-coding RNAs: New therapeutic targets and opportunities for hepatocellular carcinoma

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Abstract: Non-coding RNAs (ncRNA) are RNA molecules without protein coding functions owing to the lack of an open reading frame (ORF). Based on the length, ncRNAs can be divided into long and short ncRNAs; short ncRNAs include miRNAs and piRNAs. Hepatocellular carcinoma (HCC) is among the most frequent forms of cancer worldwide and its incidence is increasing rapidly. Studies have found that ncRNAs are likely to play a crucial role in a variety of biological processes including the pathogenesis and progression of HCC. In this review, we summarized the regulation mechanism and biological functions of ncRNAs in HCC with respect to its application in HCC diagnosis, therapy and prognosis.

Keywords: hepatocellular carcinoma; ncRNA; miRNA; lncRNA; piRNA

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer, which is ranked fifth in the incidence of malignant tumors and is the third most common cause of cancer-related mortality^[1,2]. Different etiologies may be associated with different molecular carcinogenic pathways^[3]. The occurrence of liver cancer is closely related to viral hepatitis, alcohol abuse and fatty liver disease in non-alcoholic patients. In China, chronic hepatitis and liver cirrhosis are the principal causes of HCC and disease progression is mainly related to the chronic inflammation of the liver caused by a repeated injury of liver cells and proliferation of such condition^[4-8]. It is also closely linked to molecular mechanisms including oncogene activation in liver cells, tumor suppressor gene inactivation and HCC-associated signaling pathway activation^[9,10].

Understandably, HCC poses a serious threat to human health. However, its pathogenesis remains unclear. Curative treatments, which are mostly surgical in nature (e.g., resection and liver transplantation), are suitable only during the early tumor stages. In the intermediate or advanced stages, curative options are often lacking^[11,12]. Therefore, identifying effective biomarkers are very important for the diagnosis of HCC. Disease prognosis depends on the aggressiveness of HCC and the residual liver function. Thus, an accurate prediction of disease prognosis and stage is crucial in optimizing a personalized treatment regime. New biomarkers, particularly those reflecting tumor aggressiveness, are important for improving the prognostic assessment of HCC patients^[13,14]. In recent years, a large number of studies have found that changes in the expression of non-coding RNAs (ncRNA) have a pivotal role in the development and progress of HCC. De facto, ncRNAs associated with

cancer have become a new research focus for the diagnosis and treatment of HCC.

ncRNAs are transcribed RNA molecules with little or no protein coding capacity and represent approximately 97% of RNAs in higher eukaryotic organisms. ncRNAs also include structural or housekeeping ncRNAs such as transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA), as well as regulatory ncRNAs that function as gene expression regulators^[15,16]. Based on their length, ncRNAs can be divided into long and short ncRNAs; short ncRNAs include, but are not limited to, miRNAs and piRNAs^[17]. ncRNAs are not only involved in life sustaining activities but are also closely related to tumor cell differentiation, proliferation, migration, invasion and infiltration. Previous research suggested that ncRNAs play an important role in the pathogenesis and development of HCC^[18].

Micro RNAs and HCC

Micro RNAs (miRNA) are a family of small (18–25 nucleotides in length) ncRNAs that control the stability and translation of protein-coding messenger RNAs (mRNA)^[19]. The discovery of miRNAs, which are involved in the regulation of virtually all cell functions, has opened new avenues for cancer diagnosis, prognosis and prediction of treatment response.

Massively parallel signature sequencing (MPSS) of miRNAs can identify miRNomes accurately. Hou et al. used MPSS to carry out an in-depth analysis of the miRNomes in normal liver tissues (distal normal liver tissue of liver hemangioma), hepatitis B virus (HBV)-infected liver, severe chronic hepatitis B liver,

HBV-related HCC cells, hepatitis C virus (HCV)-related HCC cells and HCC cells without HBV or HCV infection. It was discovered that nine miRNAs accounted for 88.2% of the miRNome in human liver (*Figure 1*). The three most abundantly expressed miRNAs are miR-122, miR-192 and miR-199a/b-3p, accounting for 52.0%, 16.9% and 4.9% of miRNome, respectively^[20]. It is obvious that miRNAs play a very important role in normal liver physiology, and the deregulation of miRNAs leads to the development and progress of HCC. These miRNAs, either abundantly or lowly expressed, play an important role in hepatocarcinogenesis. In recent years, scientists have studied the deregulated expression mechanism of miRNAs, along with the mechanism of occurrence and development of HCC. Some miRNAs have been proven to act as new potential targets of HCC therapeutic intervention (*Table 1*).

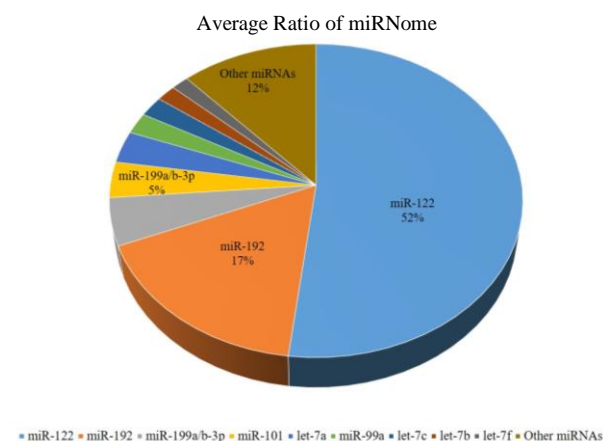


Figure 1 miRNAs expressed in a healthy human liver

Table 1 miRNAs linked to HCC

MicroRNA	Target	Expression in HCC	Roles in HCC	References
miR-122	Cyclin-G1, IGF-1R, Wnt1, AKT3, CUTL1, ADAM10, ADAM17	↓	Promote growth, cell cycle progression and inhibit apoptosis, EMT	22, 24–26, 30
miR-192	Zeb2	↓	Promote apoptosis	95
miR-199	PAK4, MTOR, c-Met, F2D7, β-catenia, CyclinD1, Myc	↓	Inhibit cell proliferation	20, 31–33
miR-101	Ezh2	↓	Promote apoptosis	34–36
Let-7	TRIB2	↓	Promote cell proliferation	37–40
miR-99a	IGF-1R, mToR, Ago2	↓	Inhibit cell proliferation	41–42
miR-195	BCL-w	↓	Inhibit apoptosis	46
miR-221	ODIT4, P27, P57	↑	Promote cell cycle progression	43, 51–52
miR-1	API-5, endothelin-1	↓	Promote apoptosis and inhibit cell proliferation	48, 49
miR-224	HOXD10, PPP2R1B	↑	Promote migration and invasion	44, 56–59
miR-21	MAP2K3, PPCD4	↑	Promote cell proliferation	53–55

miR-122

miR-122, which is a liver-specific miRNA, is the most abundant miRNA in the liver. Although it is expressed abundantly in mouse and human liver cells, it has a very low level of expression in most tissues^[21]. miR-122 plays a critical role in regulating hepatocyte development and differentiation, lipid metabolism, as well as stress response^[22]. Tsai et al. used a computational approach and identified multiple miR-122 candidate target genes from two independent expression microarray datasets in an orthotopic HCC model *in vivo* with tumorigenesis, angiogenesis and intrahepatic metastasis, proving that liver-specific miR-122 is significantly down-regulated in HCC with intrahepatic metastasis, and negatively regulates tumorigenesis by targeting ADAM17^[23]. miR-122 directly down-regulates cyclin G1 expression, and an inverse correlation between miR-122 and cyclin G1 expression exists in HCC tissues^[24]. Since cyclin G1 negatively regulates the p53 protein stability by acting on the B' sub-unit of phosphatase 2A, miR-122 can increase the expression of p53 and its transcriptional activities by acting on cyclin G1^[25]. Supplementary research have proven that miR-122 can inhibit hepatocarcinogenesis, epithelial-mesenchymal transition (EMT) and angiogenesis by targeting BCL-w, IGF-1R, Wnt1, CUTL1, AKT3 and ADAM10, among other things^[25-28]. Moreover, a number of conditions such as spontaneous hepatocarcinogenesis, a series of abnormal expression of genes including cell growth and apoptosis, EMT, as well as inflammation and tumors, could be observed in miR-122-deficient mice^[29].

miR-199

miR-199 is the third most abundantly expressed miRNA in the liver and it is also a tissue-specific, lowly expressed miRNA in HCC^[20]. miR-199a/b-3p can target tumor promoting PAK4 in order to suppress HCC growth by inhibiting PAK4/Raf/MEK/ERK pathways, both *in vitro* and *in vivo*^[20]. Moreover, miR-199a-3p can act on the mammalian target of rapamycin (mTOR) and c-Met in HCC cells. Restoring attenuated levels of miR-199a-3p in HCC cells leads to a G1-phase cell cycle arrest, reduces invasive capability, enhances susceptibility to hypoxia, and increases sensitivity to doxorubicin-induced apoptosis^[30]. Recent research also revealed that the overexpression of miR-199a could significantly down-regulate the expression of genes downstream of the Frizzled type 7 receptor (FZD7), which include β -catenin, Jun, Cyclin D1 and

Myc. FZD7 is the most important Wnt receptor involved in cancer development and progression. In other words, miR-199a can target FZD7 and its downstream genes to inhibit the development and progression of HCC by inhibiting the Wnt signaling pathway^[31,32]. Furthermore, the expression of miR-199 is significantly increased in HBV-infected tissue, which demonstrates that stimulating the expression of miR-199 could inhibit HBV replication and introduce antiviral activity into the hepatic cells^[32].

miR-101

miR-101, another miRNA that is found abundantly in the liver, is also a tissue-specific miRNA expressed in HCC^[33]. Methyltransferase zeste homolog 2 (EZH2), an enzyme that is highly expressed in HCCs, can promote the invasion and metastasis of HCC through epigenetic modifications and the silencing of a series of miRNAs, including miR-101^[34]. However, recent research discovered that miR-101 can also directly target EZH2 to repress proliferation, colony formation, cell cycle progression, invasion of HCC in all tested cell lines (HepG2, Hep3B, Huh7, PLC/PRF5, SNU182, HepaRG and BEL-7402) and to enhance the sensitivity of chemotherapy drugs such as doxorubicin^[35]. Therefore, miR-101 and the regulation of EZH2 display a mutually inhibiting relationship in a stable and normal liver. This balance is broken by HCC, leading to the deregulation of expression for both components. Furthermore, the hepatitis B virus X protein (HBx) can inhibit the expression of miR-101, leading to the up-regulation of miR-101 target proteins such as RAB5A and DNMT3A. RAB5A could promote cancer cell proliferation and migration, while DNMT3A promotes the development of HCC by changing the levels of DNA methylation in liver cells, which could provide a new molecular mechanism for HBV-related HCC^[36,37].

let-7 family

The let-7 family plays a vital role in normal cellular activity of liver cells and the carcinogenesis of HCC. It is the earliest discovered human miRNA, and let-7a/b/c/f has a certain level of expression in normal liver^[38]. HBx protein reduces the expression of let-7a, leading to a high expression of STAT3, a target molecule of let-7a, and promotes cell proliferation and tumorigenesis^[39]. The let-7b suppresses HCV replicon activity and down-regulates HCV accumulation, leading to reduced

infectivity of cell culture-derived HCV (HCVcc). A mutational analysis identified let-7b binding sites at the coding sequences of NS5B and 5'-UTR of HCV genome that were conserved among various HCV genotypes^[40].

miRNA-99

miR-99a is found to be the sixth most abundant microRNA in the miRNome of normal human liver but is markedly down-regulated in HCC^[41]. miR-99a dramatically suppresses HCC cell growth *in vitro* by inducing the G1 phase cell cycle arrest. Furthermore, protein levels of IGF-1R and mTOR are found to be inversely correlated to miR-99a expression in HCC tissues. miR-99a inhibits IGF-1R and mTOR pathways, and subsequently suppresses the expression of cell cycle-related proteins including cyclin D1 in HCC cells^[41]. Recent research suggested that miR-99a can target Argonaute-2 (Ago2) to inhibit tumor growth and obstruct the function of miR-21, thus relieving the inhibition of the phosphatase and tensin homolog (*PTEN*) gene imposed by miR-21^[42].

Other vital miRNAs

Some miRNAs such as miR-1, miR-21, miR-195, miR-224 and miR-221 that are not abundantly expressed in normal liver have abnormal expression levels in HCC. These miRNAs are closely related to the development and progression of HCC (*Table 1*)^[43-46]. miR-1 has been reported as a down-regulated miRNA in various human malignancies and has a tumor suppression function^[47]. Some potential target genes such as gap junction protein (GJA1), tankyrase (TNKS2) and monocyte to macrophage differentiation-associated 2 (MMD2) have been found in miR-1 by the Target Scan software. Luciferase activity assay or Northern blot were used to identify these genes^[48]. Some research have demonstrated that miR-1 promoted the apoptosis of HCC cells by targeting apoptosis inhibitor-5 (API-5), and inhibited its proliferation by targeting endothelin-1^[48,49]. However, a recent study suggests that miR-1 might be a potential tumor activator. Inhibiting the expression of miR-1 could decrease proliferation, induce apoptosis, and inhibit the migration and invasion of tumor endothelial cells (TEC) of human HCC^[50].

Though miR-221 is not a highly expressed miRNA in normal liver tissues, it has been found to be highly expressed in a variety of malignant solid tumors including HCC^[51]. As reported, miR-221 could increase cell synthesis and cell cycle progress, and promote HCC by targeting the cell cycle kinase inhibitory protein p27 and p57^[52]. Meanwhile, miR-221 could promote tumor de-

velopment by inhibiting another target, DNA-damage-inducible transcript 4 (DDIT4), and interfering with mTOR signaling^[43,52].

The abnormal expression of miR-21 was first found in glioma and later confirmed to play a key role in the occurrence and development of cancer (including HCC), with a high specific expression in many tumors^[53]. In HCC HepG2 cells, miR-21 promoted cell proliferation targeting MAP2K3^[54]. Moreover, miR-21 can target programmed cell death 4 (PDCD4) to activate the expression of downstream c-Jun, MMP-2, MMP-9 and AP-1. AP-1 has a positive feedback loop of transcription. This feedback loop would increase the risk of invasion and the metastasis of HCC^[53]. A study of HBV infection reported for the first time that HBx down-regulated the expression of PDCD4 and up-regulated miR-21, and the HBx protein induced the activation of the IL-6- STAT3 signaling pathway. The overexpression of PDCD4 can suppress tumorigenicity. The deregulation of PDCD4 by HBx through miR-21 represents a potential novel mechanism for the down-regulation of PDCD4 in HBV-related HCCs and provides new insights into the development of HCC^[55].

miR-224 is also a highly expressed miRNA in HCC tissue. miR-224 can target homeobox D10 (HOXD10) to enhance the phosphorylation of PAK4, and promotes the invasion and metastasis of HCC cells via the expression of MMP-9^[56]. *In vitro* and *in vivo* models confirmed that miR-224 promotes cell proliferation by targeting SMAD4^[57]. It was also reported that miR-224 could increase the risk of HCC by targeting PPP2R1B molecules, thus activating the AKT signaling pathway^[58]. As for the mechanism of miR-224, histone acetylation is directly associated with the expression of miR-224^[59]. On the other hand, inflammatory signals (such as PS, LT α and TNF- α) enhance the transcription and expression of miR-224 through the NF- κ B signaling pathway^[44]. In conclusion, miRNAs can influence pathogenesis and the development of HCCs in many ways and these include participating in cell growth, apoptosis, EMT and angiogenesis (*Figure 2*).

Long non-coding RNA and HCC

Commonly defined as non-protein-coding RNA molecules longer than 200 nucleotides, long non-coding RNAs (lncRNA) can control gene expression in several ways including exogenous silencing, cut regulation, miRNA interactions, protein-protein interactions and genetic variations^[60-62]. In recent years, the functions of lncRNAs in cancer have been broadly investigated. In general, lncRNAs play an important role in a wide range

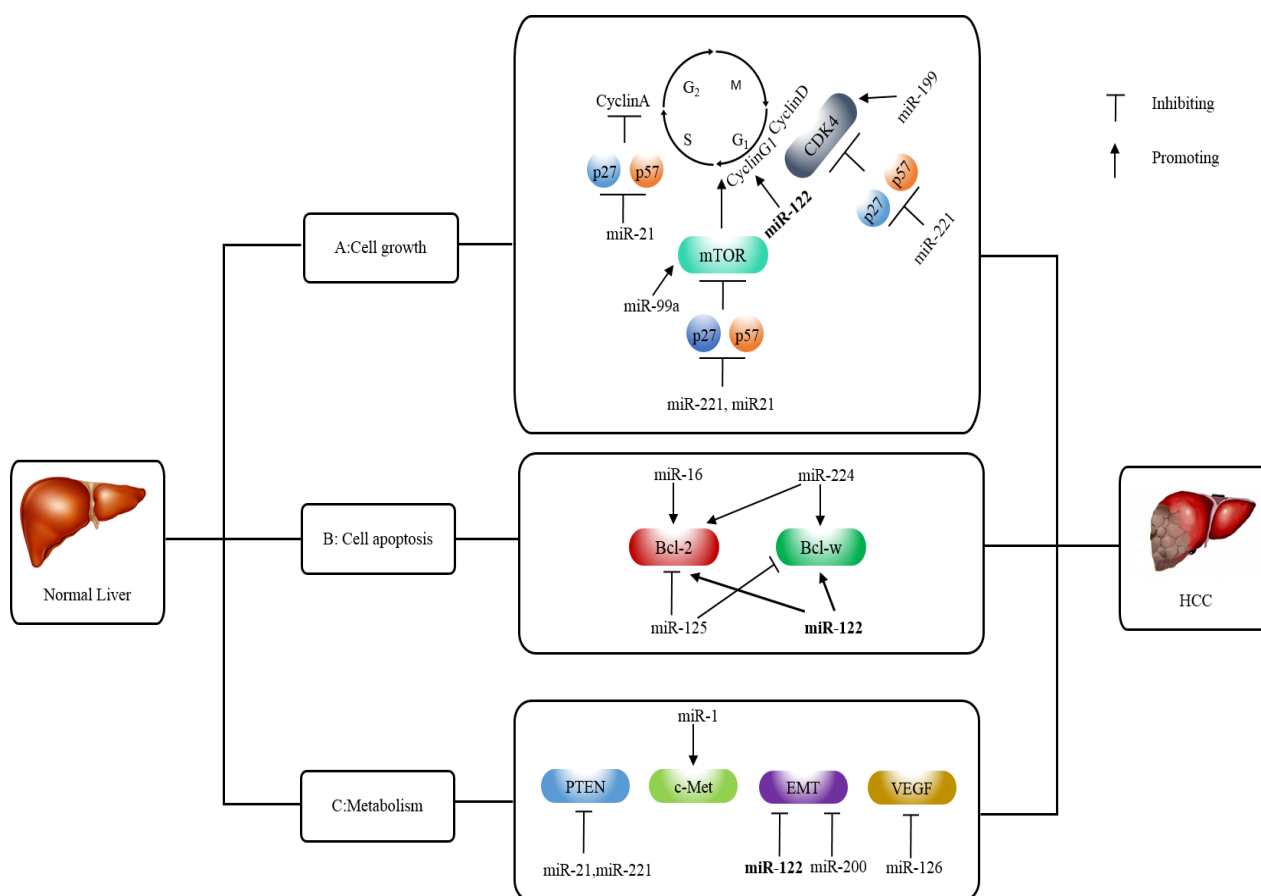


Figure 2 Regulation pathways of miRNAs in the liver

of biological processes including cell proliferation, cell cycle, apoptosis, cell differentiation and invasion^[60]. They act as important regulators in various biological processes of different diseases, including HCC^[61]. based on previous reports, HCC-related lncRNAs can influence HCC initiation, progression and treatment^[61]. Currently, only a fraction of HCC-related lncRNAs has been studied, thus providing an opportunity for further discovery that could potentially afford new strategies in the diagnosis and treatment of HCC. Systematic identification of lncRNAs and an adept understanding of their mechanisms should facilitate the development of new therapeutics for HCC (Table 2).

Highly up-regulated in liver cancer

Highly up-regulated in liver cancer (HULC) is the first lncRNA to be found that is highly expressed in HCCs. It is located on chromosome 6p24.3 and is conserved in primates. HULC transcription yields about 500 nt in length^[63]. It is a spliced and polyadenylated ncRNA that localizes to the cytoplasm, where it is reportedly associ-

ated with ribosomes^[64]. It has also been identified that HULC is highly up-regulated in HCCs and is closely related to liver metastasis^[64]. HBx could up-regulate the expression of HULC by targeting P18 and promoting HCC cell proliferation^[65]. Liu et al. have shown that HULC rs7763881 mutation in the promoter region of genes contributed to the reduced susceptibility of HCC HBV carriers. This also suggested that HULC single nucleotide diversity (single nucleotide polymorphisms or SNPs) would lead to chronic HBV infections and an increased risk of HCC occurrence^[66].

H19

H19 is the first ncRNA gene to be found. It is an imprinted oncofetal gene, located on chromosome 11p15.5 and lies within 200 kb downstream of the *IGF-2* gene. The loss of imprinting at the H19 locus typically results in an overexpression of H19 in liver cancer^[67,68]. *IGF-2* and H19 alleles are selectively expressed as these two genes are imprinted in the opposite directions^[67]. The

Table 2 lncRNAs that have been or might be linked to HCC

lncRNA	Expression in HCC	Roles in HCC	References
HULC	↑	Mediate HBV-induced cell proliferation and anchorage-independent growth	64–66
H19	↑	Promote growth after hypoxia recovery, cell cycle progression and inhibit apoptosis	68, 69, 96
MALAT1	↑	Promote cell proliferation, migration and invasion	77
MEG3	↓	Promote apoptosis and inhibit proliferation	72
HOTAIR	↑	Suppress the expression of various tumor suppressor genes through the induction of histone methylation	79, 80, 97, 98
Uc002mbe.2	↓	Promote apoptosis	99
lncRNA MVIH	↑	Promote migration	100, 101
lncRNA-LET	↓	Inhibit invasion	16
lncRNA HEIH	↑	Promote growth	62
TUC338	↓	Knockdown of TUC338 decreases anchorage dependent and independent growth of hepatocellular carcinoma cells	100
lncRNA dreh	↓	Inhibit cell proliferation, migration and invasion	62

overexpression of an ectopic *H19* gene enhances the tumorigenic properties of breast cancer cells according to previous investigations. Moreover, the expression of *H19* is positively controlled by E2F1 but suppressed by p53^[69]. Meanwhile, *H19* has been implicated as having both oncogenic and tumor suppression properties. in HCC's HepG2 cells. Evidently, knocking down *E2F1* could down-regulate the expression of *H19* whilst suppressing cell growth and invasion in HCC's HepG2 cells. Conversely, the overexpression of *E2F1* could up-regulate *H19* and promote cell growth as well as invasion in HepG2 cells. These data suggest that AFB1 could regulate HepG2 cells' growth and invasion via association with *E2F1* and *H19*. This is the first known study of the relationship among mRNA-like ncRNA, *H19* and AFB1^[68].

Maternally Expressed Gene 3

Maternally expressed gene 3 (*MEG3*) is an imprinted gene belonging to the imprinted DLK1-MEG3 locus, located at chromosome 14q32.3 in humans^[71]. *MEG3* is the first lncRNA found to be expressed in a variety of normal tissues. As a tumor suppressor, *MEG3* is reportedly linked to the pathogenesis of malignancies (including HCCs)^[72]. Zhuo et al. have shown that *MEG3* plays a crucial role in regulating gene expression by recruiting DNMT1, and promoting the cell growth of HCC cells^[72]. *MEG3* is minimally expressed in HCC cells compared to normal liver cell lines. It has been discovered that *MEG3* expressed in HCC could inhibit cell proliferation and

induce apoptosis^[73]. Additionally, the tumor-suppressive effect of *MEG3* has been confirmed *in vivo* and *in vitro*, and analyzing the accumulation of p53 using Kaplan-Meier analyses and Cox proportional regression deduced that *MEG3* promoted HCC cell proliferation and apoptosis. It has been suggested that *MEG3* may be a potential biomarker for predicting the survival rate of HCC patients^[74].

Metastasis-associated lung adenocarcinoma transcript 1

The novel metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an ncRNA of more than 8000 nt that is expressed on chromosome 11q13. For HCC, the most extensively studied lncRNA that is involved in splicing regulation is MALAT1^[75]. MALAT1 regulates alternative splicing of endogenous target genes by interacting with the serine/arginine-rich family of nuclear phosphoproteins (SR proteins). It could interact with SR proteins, alter the cellular levels of phosphorylated SR proteins, influence the distribution of these and other splicing factors (e.g., SF2/ASF and CC3 antigen), and regulate the alternative splicing of various pre-mRNAs (e.g., oncogenic transcription factor B, collagen triple helix repeat containing 1 (*CTHRC1*) and a few other motility-related genes)^[76]. Nevertheless, the role of MALAT1 in targeting certain critical genes via the regulation of gene splicing in HCC, along with its up-regulation mechanism, remains unconfirmed even though recent investigations have shown that MALAT1

is regularly up-regulated in HCC^[77]. Lai et al. have used quantitative real-time polymerase chain reaction (qRT-PCR) to assess the expression of MALAT1 in nine HCC cell lines and 112 HCC cases, which included 60 liver transplantation (LT) cases with complete follow-up data. MALAT1 was clearly up-regulated in both cell lines and clinical tissue samples, and patients with a high level of MALAT1 demonstrated a significantly increased risk of tumor recurrence after LT, especially those who exceeded the Milan criteria^[77]. MALAT1 has been confirmed as an independent prognostic factor for predicting HCC recurrence using multivariate analysis. The inhibition of MALAT1 in HepG2 cells could effectively reduce cell viability, motility, invasiveness and increase apoptotic sensitivity. Therefore, lncRNA MALAT1 plays a vital role in tumor progression and could be used as a novel biomarker to predict tumor recurrence post-LT, in addition to being a potential therapeutic target^[77].

HOX Antisense Intergenic RNA

The lncRNA HOX antisense intergenic RNA (*HOTAIR*) is expressed from the developmental HOXC locus located on chromosome 12q13.13^[78]. *HOTAIR* is highly expressed in HCC. Knocking *HOTAIR* would inhibit matrix metalloproteinase-9 (MMP9) and the vascular endothelial growth factor (VEGF) protein, thus significantly inhibiting proliferation of HCC cells Bel-7402^[79]. In order to explore the relationship between *HOTAIR* and the invasion and metastasis of HCC, Geng et al. measured the expression of the oncogenic *HOTAIR* gene in 63 patients with HCC following hepatic resection. They found that the *HOTAIR* gene was significantly overexpressed in HCC tissues compared to the adjacent non-tumor tissues. Moreover, patients with a high expression of *HOTAIR* gene in their tumors had an increased risk of recurrence after hepatectomy^[79]. Yang et al. examined the expression of *HOTAIR* in 110 HCC samples and compared it to the prognosis of 60 HCC patients that have undergone LT, proving that high expression of *HOTAIR* in HCC could be a candidate biomarker for predicting tumor recurrences in HCC patients who have undergone liver transplant therapy and might be a potential therapeutic target^[80].

Piwi-interacting RNAs and HCC

Piwi-interacting RNA (piRNA) is a class of single-chain small RNA that is 26–31 nt in length, typically 29–30 nt, similar to miRNAs and repeat-associated siRNAs (rasiRNA), which also has a bias for 5'uridine^[81,82]. It directly regulates Piwi-dependent transposon silencing,

heterochromatin modification and germ cell maintenance^[83]. piRNA is considered to be the most mysterious small regulation RNA, especially in the formation mechanism of tumors^[84]. The abnormal regulation of piRNA in some cell lines may be playing a crucial role in an unknown way during the formation of tumors^[83]. Using high-throughput sequencing, Law et al. discovered the presence of ncRNAs and the involvement of a new piRNA, piR-Hep1, in liver tumorigenesis. In comparison to a corresponding adjacent non-tumoral liver, piR-Hep1 was up-regulated in 46.6% of HCC tumors and the silencing this piRNA inhibited cell viability, motility and invasiveness with a simultaneous decline in the level of active AKT phosphorylation^[83]. The regulatory mechanism of piRNA is immature, and thus necessitates further research.

Application of ncRNAs in HCC diagnosis, treatment and prognosis

Serum miRNAs diagnosis and prognosis in HCC

Similar to their expression in tissues, serum miRNAs were aberrantly expressed in cancers including HCC. Even though the miRNA fragment is very small, it is stable to some extent. Thus, more sensitive detection methods such as qRT-PCR can be used to detect its presence in serum or other body fluid specimens for disease diagnosis^[11]. lncRNAs could be characterized by qRT-PCR, Northern blot analysis or *in situ* hybridization^[63]. In recent years, studies have shown that there are a large number of abnormally expressed miRNA in the serum of patients with HCC; some with low-level expressions of miR-16, let-7f, miR-21, miR-139, miR-101, miR-122 and miR-1, and some with high-level expressions of miR-17-5p, which probably indicated the recurrence of HBV-related HCC prognosis^[85]. Tan et al. also identified that the miRNAs are differentially expressed in cirrhosis that evolved into HBV-related HCC by analyzing miRNAs that exhibit differential expressions such as miR-122-5p, miR-199a-5p, miR-486-5p, miR-193b-5p, miR-206, miR-141-3p, miR-192-5p and miR-26a-5p^[86]. In the serum of patients with HCV-related HCC, low specific expressions of miR-30c-5p, miR-223-3p, miR-302c-5p and miR-17-5p, as well as high specific expression of miR-221, indicated the recurrence of HCC^[87,88].

miRNA-related intervention and therapy of HCC

In recent years, research on miRNA targeted therapy based on *in vitro* and *in vivo* HCC models using on-

colytic adenovirus vector has reported that the over-expressions of miR-34a and IL-24 would induce antitumor activity^[89]. It has also been discovered that transfecting miR-122 mimics into HCC mice using cationic lipid nanoparticles as carriers could highly target HCC cells, inhibiting proliferation and angiogenesis. The use of natural macromolecular nanoparticles as vectors to deliver both ncRNAs and antineoplastic agents in HCC therapy has been studied previously^[90]. Clinical data have shown that miR-21 in HCC cell lines and clinical HCC samples was a significant modulator of the anti-tumor effect of interferon-alpha (IFN- α) and 5-fluorouracil (5-FU). This suggested that miR-21 is a potentially suitable marker for the prediction of clinical response to the IFN- α /5-FU combination therapy^[91]. In addition, the abnormal expression of miRNA could be used as a therapeutic target, as well as a biomarker, for individualized treatment in patients with HCC. Meanwhile, researchers have learned that the prognosis is poor for HCC patients whose miR-26 is lowly expressed, but it can be improved via IFN therapy^[92].

lncRNA-related intervention and therapy in HCC

Highly expressed HULC greatly improved Edmondson grades and more importantly, HULC can be detected in the plasma of patients with HCC^[93]. This indicated that HULC could be utilized for diagnosis and could serve as a marker to indicate new non-invasive prognosis of HCC^[64]. Studies have discovered that *Linc00974* was stably expressed in the plasma. These joint indicators predict tumor growth and metastasis in HCC patients via the combined analysis of *Linc00974F-1* and *CYFRA21-1*. The combination of *Linc00974* and keratin 19 (*KRT19*) may be novel indices for the clinical diagnosis of HCC tumor growth and metastasis, while *Linc00974* may become a potential therapeutic target for the prevention of HCC progression^[94]. In addition, targeting RNA for HCC treatment offers new potential therapeutic strategies since *H19* has already been used in clinical trials. The effects of overexpression of *H19* or other lncRNAs on HCC therapy would require further research^[95].

Conclusion and perspectives

ncRNA is closely related to the pathogenesis and development of HCC. The abnormal expression of ncRNA plays a key role in this process. The main function of ncRNA is in reducing the expression of target genes. However, ncRNAs may also have other unknown abili-

ties that promote the transcription of target genes. The discovery of a new type and function of ncRNA would provide important leads in elucidating the molecular mechanisms or pathogenesis of HCC. ncRNA is not just a target gene but also a signal molecule that could influence the transduction signaling pathway and diagnosis of HCC. Meanwhile, the regulatory network of its internal numbers is still unclear. Therefore, a further study on ncRNA regulation of molecular networks will help clarify the pathogenesis of HCC for early disease diagnosis and aid the development of better therapeutic methods. With regard to the regulatory mechanism, ncRNA interactions (e.g., between miRNAs and lncRNAs) should be studied not as a type or a family. As for clinical therapy, the detection of ncRNAs in serum could provide new markers for the diagnosis of HCC. Furthermore, the detection of ncRNA expression profiles in HCC tissues will also provide fresh ideas for the study of disease pathology. An anticipated research focus is on the use natural macromolecular nanoparticles as the vector to deliver both ncRNAs and antineoplastic agents for HCC therapy. It may provide a new treatment method for HCC and potentially improve current understanding of ncRNA regulatory mechanisms, thus improving HCC-related drug discovery efforts.

Conflict of interest

The author declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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