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**Non-coding RNAs in hepatitis C-induced hepatocellular carcinoma: Dysregulation and implications for early detection, diagnosis and therapy**

Hou W *et al*. ncRNAs in hepatitis C-induced HCC

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**Abstract**

Hepatitis C virus (HCV) infection is one of main causes of hepatocellular carcinoma (HCC) and the prevalence of HCV-associated HCC is on the rise worldwide. It is particularly important and helpful to identify potential markers for screening and early diagnosis of HCC among high-risk individuals with chronic hepatitis C, and to identify target molecules for the prevention and treatment of HCV-associated-HCC. Small non-coding RNAs, mainly microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) with size greater than 200 nucleotides, are likely to play important roles in a variety of biological processes, including development and progression of HCC. For the most part their underlying mechanisms of action remain largely unknown. In recent years, with the advance of high-resolution of microarray and application of next generation sequencing (NGS) techniques, a significant number of non-coding RNAs (ncRNAs) associated with HCC, particularly caused by HCV infection, have been found to be differentially expressed and to be involved in pathogenesis of HCV-associated HCC. In this review, we focus on recent studies of ncRNAs, especially miRNAs and lncRNAs related to HCV-induced HCC. We summarize those ncRNAs aberrantly expressed in HCV-associated HCC and highlight the potential uses of ncRNAs in early detection, diagnosis and therapy of HCV-associated HCC. We also discuss the limitations of recent studies, and suggest future directions for research in the field. miRNAs, lncRNAs and their target genes may represent new candidate molecules for the prevention, diagnosis and treatment of HCC in patients with HCV infection. Studies of the potential uses of miRNAs and lncRNAs as diagnostic tools or therapies are still in their infancy.

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**Key words:** MicroRNA; Long non-coding RNAs; Non-coding RNAs; Hepatitis C virus; Hepatocellular carcinoma

**Core tip:** Regulatory non-coding RNAs (ncRNAs), mainly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are likely to play important roles in a variety of biological processes, including development and progression of hepatitis C-induced hepatocellular carcinoma (HCC). In this review, we focus on recent studies of ncRNAs, especially miRNAs and lncRNAs associated with HCV-induced HCC. We summarize those ncRNAs aberrantly expressed in HCV-induced HCC and highlight the potential of these ncRNAs to aid in early detection, diagnosis and therapy of HCV-induced HCC. Further, we discuss the limitations of current studies, and suggest future directions for research in the field.

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**INTRODUCTION**

Non-coding RNAs (ncRNAs) are transcribed RNA molecules with little or non-protein coding capacity; they represent approximately 97% of RNAs in higher eukaryotic organisms. ncRNAs include structural or house-keeping ncRNAs such as transfer RNA, ribosomal RNA, small nuclear RNA and small nucleolar RNA, as well as regulatory ncRNAs, which function to regulate gene expression. Based on transcript size, regulatory ncRNAs are classified into two major groups, small non-coding RNAs such as microRNAs (miRNAs), approximately 22 nucleotides (nt) in length, and long non-coding RNAs (lncRNAs) with sizes longer than 200 nt (Figure 1). Based upon a large number of experimental studies carried out over the past decades or two, it is now generally well-accepted that miRNAs play an important role in the regulation of gene expression primarily through post-transcriptional destabilization, translational repression of target mRNAs which bear complementary sites, or a combination of these two mechanisms[[1-4](#_ENREF_1)]. With the development of next generation sequencing (NGS) techniques, a growing number of lncRNAs have been identified, characterized and functionally annotated[[5](#_ENREF_5),[6](#_ENREF_6)]. lncRNAs are still among the least well-understood of transcripts. Several lines of evidence have suggested that lncRNAs are biologically functional rather than transcriptional “noise”[[5](#_ENREF_5),[6](#_ENREF_6)]. Thus, lncRNAs have recently enjoyed increased and deserved attention, although the underlying mechanisms by which they function remain largely unexplored and unifying theories regarding their actions are still vague. ncRNAs including miRNAs and lncRNAs have been reported to be associated with cancer, including hepatocellular carcinoma (HCC), a highly prevalent and deadly cancer because of its frequent recurrence and/or metastasis.

HCC is among the most frequent forms of cancer worldwide, and its incidence is increasing rapidly. This increase is related to several factors. Chief among these are chronic hepatitis B and C (CHC) infections, and fatty liver disease. Indeed, hepatitis C virus (HCV) infection is one of the leading underlying causes of HCC, increasing the risk for HCC development by nearly 17-fold compared to healthy individuals[[7](#_ENREF_7),[8](#_ENREF_8)]. In recent decades and especially in recent years, HCC incidence has increased sharply, and has been attributed largely to HCV infection. HCV-induced HCC typically develops in the setting of cirrhosis (advanced chronic liver diseases), although it does also occur in the absence of cirrhosis. Similarly, the development of HCC has been observed in mice expressing HCV transgenes in the absence of appreciable hepatic inflammation and fibrosis, suggesting that HCV infection is likely to have direct and unique cancer-promoting effects, which may be different from other carcinogenic factors such as those due to HBV and fatty liver disease. Understanding and insight into unique ncRNAs involved in HCV-induced HCC may suggest new approaches for diagnosis, prevention and treatment of HCV-induced HCC. To date, there have been few reports on differentially expressed lncRNAs in HCV-induced HCC. In this review, we will summarize recent studies regarding ncRNAs related to HCV-induced HCC. We will then address the potential utility of these ncRNAs in early detection, diagnosis and therapy of HCV-associated HCC. Finally, we will discuss the limitations of current knowledge, and suggest future directions for research in this field.

**DYSREGULATED NON-CODING RNAS IN HCV-INDUCED HCC**

***microRNAs***

miRNAs regulate gene expression primarily through post-transcriptional repression[[3](#_ENREF_3),[4](#_ENREF_4),[9](#_ENREF_9),[10](#_ENREF_10)]. Sequence complementarity in the 6-8 base pair “seed regions” at the end of miRNA-mRNA heteroduplexes seem to determine the specificity of miRNA-target RNA interactions[[11](#_ENREF_11)]. miRNAs are likely to play significant roles in the development and progression of cancers, including HCC[[12](#_ENREF_12),[13](#_ENREF_13)] and HCV replication[[9](#_ENREF_9),[14-16](#_ENREF_14)]. Identification and characterization of dysregulated miRNAs specific to HCV-induced HCC in tissue- and biofluid-based studies are important and helpful to reveal therapeutic targets or diagnostic markers, in particular, molecular signatures for the detection and early diagnosis of HCC among HCV patients in high-risk groups. The miRNAs reported differentially expressed in HCV-induced HCC are summarized in Tables 1 and 2, and we now summarize their known biological functions, and the molecular mechanisms and pathways in which they might be involved.

***Up-regulated miRNAs***

miRNAs profiling studies in HCV-induced HCCs compared with paired controls have found that a number of miRNAs are significantly elevated in HCV-induced HCCs, compared with normal controls. miR-1269 is the most increased in HCV-associated HCCs in contrast to normal livers, HCV-associated cirrhosis, or HBV-associated liver failure. Up-regulation of miR-1269 has also been found in other cancers such as breast cancer[[17](#_ENREF_17)], colorectal cancer[[18](#_ENREF_18)] and laryngeal squamous cell carcinoma (LSCC), one of the most common head and neck malignancies with no significant difference between tumors with and without lymphatic metastasis, suggesting that miR-1269 did not affect metastasis of LSCC. Thereto date there have been few, if any, reports on function and role of miR-1269 in HCC. Nevertheless, the increased expression of miR-1269 in HCV-induced HCC when compared with controls suggests that this miRNA may have an oncogenic role in HCV-induced HCC.

Interestingly, miR-224, miR-224-3p and their precursor are significantly up-regulated in HCV-associated HCCs compared to normal livers, HCV-associated cirrhosis and HBV-associated liver failure. miR-452, also significantly up-regulated in HCV-induced HCC, was recently shown to be coordinately expressed with its neighboring miR-224 in HCC through epigenetic mechanisms[[19](#_ENREF_19)]. The DNA that encodes miR-224 is located on the X-Chromosome. miR-224 has been reported to be a cancer-related miRNA, including in HCC. Wang et al identified and validated the apoptosis inhibitor 5 (API-5) as a specific target gene for miR-224[[20](#_ENREF_20)]. Additionally, SMAD family member 4 (SMAD4) has been identified as another target gene for miR-224. Over-expression of miR-224 increases the concentration of SMAD4 protein in murine granulosa cells, while SMAD4 RNA levels remain unchanged, suggesting a post-transcriptional role for miRNA-224[[21](#_ENREF_21)]. It is likely that miR-224 plays a role in cell proliferation, migration, invasion, and anti-apoptosis in HCC, and is involved in hepatocarcinogenesis by directly binding to its validated gene targets such as API-5, SMAD4, *etc.*[[20](#_ENREF_20),[22](#_ENREF_22),[23](#_ENREF_23)].

miR-122, a liver specific miRNA, is the most abundant miRNA expressed in hepatocytes (accounting for approximately 70% of total miRNA’s) and the most extensively studied miRNA in liver diseases. miR-122 has major effects on several enzymes of cholesterol metabolism[[24](#_ENREF_24),[25](#_ENREF_25)]. Unexpectedly, miR-122 was also shown to be required for HCV replication[[14-16](#_ENREF_14)]. The effects of miR-122 depend upon the context and location of its cognate seed sequence binding sites. The sites in the 5’ region are mostly associated with up-regulation of expression, whereas those in the 3’ untranslated region are mostly associated with repression of expression[[26](#_ENREF_26)]. miR-122 exerts several functions in the HCV life cycle[[27](#_ENREF_27),[28](#_ENREF_28)]. Recent studies have shown that miR-122 acts to protect HCV genome from degradation, and therefore stabilizes HCV RNA by decreasing activity of the cytosolic exonulease Xrn1[[27](#_ENREF_27),[28](#_ENREF_28)]. The role of miR-122 in HCC has been controversial. Some but not all studies suggest that miRNA-122 is preserved and increased specifically in HCV-associated HCC[[12](#_ENREF_12),[29](#_ENREF_29),[30](#_ENREF_30)]. Nevertheless, a decrease in expression of miR-122 or undetectable miRNA-122 in human hepatoma cell lines such as HepG2 and Hep3B cell has been observed[[31](#_ENREF_31)]. In parallel with these observations, over-expression of miR-122 inhibits anchorage-independent growth, migration, invasion and tumor formation in nude mice[[31](#_ENREF_31)]. This needs to be further studied in the future.

***Down-regulated miRNAs***

The miR-199 family members including miR-199a-5p, miR-199a-3p and miR-199b are the most down-regulated miRNAs in HCV-induced HCC compared to normal livers, HCV-associated cirrhosis and HBV-associated liver failure[[32](#_ENREF_32)]. miR-199a/b-3p is the third most highly expressed miRNA in the liver[[34](#_ENREF_34)], and was also found to be consistently decreased in HCC patients with HBV infection[[34](#_ENREF_34)] and alcohol consumption[[35](#_ENREF_35)]. Its decrement significantly correlates with poor survival of HCC patients[[34](#_ENREF_34)]. Down-regulation of miR-199a-3p results in a pronounced increase in cell proliferation while over-expression miR-199a-3p inhibits cell proliferation by imposing G1 cell cycle arrest[[36](#_ENREF_36)]. The target mRNAs for the miR-199a-3p have been predicted using bioinformatic approaches and validated experimentally. For example, mammalian target of rapamycin (mTOR) has been identified as one of important targets for miR-199a-3p binding. Through negative regulation of oncogenic mTOR, miR-199a-3p inhibits tumor proliferation[[37](#_ENREF_37)].

miR-214 has been reported to be down-regulated by 5.5-fold in HCV-induced HCC compared to normal livers, HCV-associated cirrhosis and HBV-associated liver failure[[32](#_ENREF_32)]. The down-regulation of miR-214 has been reported in HCC[[20](#_ENREF_20),[38](#_ENREF_38),[39](#_ENREF_39)] and other cancers such as cervical cancer, whereas increase in miR-214 was found to significantly reduce growth of Hela cells[[40](#_ENREF_40)]. In addition, reduced level of miR-214 is associated with invasion, stem-like traits and early recurrence of HCC[[41](#_ENREF_41)]. Re-expression of miR-214 significantly suppressed the growth of HCC cells *in vitro* and reduced the tumorigenicity *in vivo*[[41](#_ENREF_41)]. In the same study, the enhancer of zeste homologue (EZH2) and β-catenin (CTNNB1) were identified and validated as two functional target mRNAs of miR-214. Silencing miR-214 increased stem-like cells through activation of CTNNB1. Furthermore, the up-regulation of EZH2, CTNNB1 and the down-regulation of E-cadherin (CDH1), known to inhibit cell invasion and metastasis in HCC patients, correlated with earlier recurrent HCC and were independent predictors of poor survival.

***Long non-coding RNAs***

The discovery of lncRNAs ushered in a new and exciting area of study, although at the time lncRNAs were first found, they were considered to be merely transcriptional “noise”[[5](#_ENREF_5)]. Recently, with fast development and application of NGS techniques, the numbers of lncRNAs continues to grow at a rapid pace, and it is increasingly clear that lncRNAs are a new class of regulators of gene expression, being involved in diverse biological processes and human diseases such as cancer. The association of lncRNAs with HCC has been studied and summarized[[42](#_ENREF_42)], although the mechanisms whereby effects of the LncRNAs are realized are largely unknown. Analysis of the differentially expressed lncRNAs in HCCs (underlying etiology not specified) have revealed that a number of lncRNAs such as HOTAIR[[43-46](#_ENREF_43)], HEIH[[47](#_ENREF_47)], MVIH [[48](#_ENREF_48)], MALAT-1)[[49](#_ENREF_49)], HULC[[50-52](#_ENREF_50)], H19[[53-55](#_ENREF_53)], CUDR[[56](#_ENREF_56)], YIYA[[57](#_ENREF_57)], lncRNA-Dreh[[58](#_ENREF_58)], LncRNA-LET[[59](#_ENREF_59)] and MEG3[[60](#_ENREF_60),[61](#_ENREF_61)] are associated with HCC. Most of these lncRNAs are up-regulated in HCCs, but less expressed or undetectable in normal controls. HCC patients with HOTAIR expression had significantly poorer prognoses and larger primary tumor sizes than those without HOTAIR expression[[46](#_ENREF_46)]. Moreover, introduction of HOTAIR into human liver cancer cells promoted more rapid proliferation compared to controls[[46](#_ENREF_46)]. Functional gene annotation analysis of TUC338 indicated predominant effect on genes involved in cell growth in both human and murine cells, suggesting that TUC338 plays a critical role in regulation of transformed cell growth and in the pathobiology of HCC [[62](#_ENREF_62)]. Lai *et al*[[49](#_ENREF_49)] found up-regulation of MALAT-1 in both liver cancer cell lines and HCC patient samples. HCC patients with high level of MALAT1 had a significantly increased risk of tumor recurrence after liver transplantation. MVIH was identified to be related to frequent microvascular invasion and higher tumor-node-metastasis stages as well as to decreased overall survival. In addition, in mouse models it promoted tumor growth and intrahepatic metastasis by activating angiogenesis[[48](#_ENREF_48)]. The expression level of HEIH in HBV-induced HCCs is significantly associated with recurrence and is an important independent prognostic factor for survival[[47](#_ENREF_47)]. Further studies indicated that HEIH plays a key role in the regulation of zeste homolog 2 (EZH2) and that this association was required for the repression of EZH2 target genes, suggesting that HEIH is an oncogenic lncRNA that promotes tumor progression[[47](#_ENREF_47)]. Thus far, few studies have been focused on lncRNAs specific to HCV-induced HCC although HCV infection is one of the major causes of HCC, and HBV and HCV cause hepatocarcinogenesis by different mechanisms.

**CLINICAL IMPLICATIONS FOR DIAGNOSIS AND THERAPY**

miRNAs, lncRNAs and their target genes comprise a large and still growing number of candidate molecules for the prevention, diagnosis and treatment of HCC in patients with HCV infection, and studies of the potential use of miRNAs and lncRNAs as therapeutic or diagnostic approaches is still in its infancy. In this section, we mainly discuss clinical potentials of miRNAs and lncRNAs for HCV-induced HCC diagnosis and therapy.

***miRNAs and lncRNAs to aid in diagnosis of HCV-induced HCC***

The biomarkers currently available for screening and early diagnosis of HCC, including serum alpha-fetoprotein (AFP), des-gamma-carboxyprothrombin, and AFP-L3 fraction or assaying cells from tissue biopsy by needle aspiration and surgical resection, suffer from numerous limitations[[63-65](#_ENREF_63)]. Most patients chronically infected with HCV are asymptomatic for many years, and the average time to develop HCC after onset of HCV infection is about 28 years. The long latency period between initial HCV infection and development of HCC provides an important time window of opportunity for individuals to be monitored for disease progression and intervention. Therefore, the development of more reliable markers for diagnosis of HCC at an early stage and better approaches for HCC screening and early detection are urgently needed. The recent study from Abdalla et al to identify urinary miRNAs as biomarkers specific for early detection of HCV-induced HCC, appears to be attractive and promising. The significantly up-regulated and down-regulated urinary miRNAs as listed in Tables 1 and 2 can be considered as promising candidate miRNA urinary markers for the early detection and diagnosis of HCV-induced HCC among high-risk HCV patients (Genotype 4). Of the identified miRNAs, miR-618 was found to have a sensitivity of 64% and a specificity of 68% for detecting HCC among HCV-positive individuals, whereas the sensitivity and specificity of urinary miR-650 were 72% and 58%, respectively. Also worthy of note, miR-618/650 in tandem improved the specificity to 75%, greater than the traditional methods based on serum levels of AFP. The urinary miRNAs signatures found in this study may be of great value and applied for the early diagnosis of HCC, before the onset of disease in high-risk patients infected with HCV. However, it is noted that this study was carried out in patients infected with HCV genotype 4, the most prevalent HCV genotype in Egypt. The potential for their use in the early diagnosis of HCC caused by different HCV genotypes other than genotype 4 needs further investigation and independent confirmation. So far, few studies have been reported regarding on lncRNAs signatures in biofluilds specific to HCV-induced HCC, which may represent an exciting area for future exploration.

***miRNAs and lncRNAs for HCV-induced HCC therapy***

Recent studies have suggested the exciting possibility that ncRNAs may represent a novel therapeutic strategy for human diseases. The miR-122 antagonist, miravirsen, a locked nucleic acid-modified DNA phosphorothioate antisense oligonucleotide that sequesters mature miR-122 in a highly stable heteroduplex, already has shown promising results in phase 2a clinical trials at seven international sites. In this clinical study, Janssen and colleagues evaluated the safety and efficacy of miravirsen in 36 patients with chronic HCV genotype 1 infection. This landmark study demonstrated that the use of miravirsen produced prolonged dose-dependent reductions in HCV RNA with no evidence of development of viral resistance. Meanwhile, targeting a number of other miRNAs such as miR-33a/b for the treatment of atherosclerosis[[66](#_ENREF_66),[67](#_ENREF_67)], miR-208/449 for chronic heart failure, miR-34 and the let-7 miRNA for cancer, await entering clinical trials. In addition, Coelho et al has recently demonstrated a new therapeutic approach to transthyretin amyloidosis by RNA interference (RNAi)[[68](#_ENREF_68)]. In this phase I clinical trial, a potent antitransthyretin small interfering RNA was encapsulated in lipid nanoparticles, delivered to human hepatocytes, and resulted in a significant reduction of transthyretin, establishing proof of concept for RNAi therapy[[68](#_ENREF_68)].

As summarized and discussed earlier in this review, many of the significantly dysregulated cellular miRNAs as listed in Tables 1 and 2, and currently found to be involved in the modulation of cell growth, apoptosis and invasiveness, can be considered as potential therapeutic targets for HCV-induced HCC therapy. The over-expression of these specific mature miRNAs can be achieved by synthetic miRNAs mimics or expression vectors. When inhibition of the selected miRNAs is desirable, antagomir or antisense oligos complementary to the specific miRNAs can be used. However, the introduction of the miRNA-based agents into clinical trials and the development of new therapeutic agents are hampered by a number of factors. The major road block is still the big challenge of developing a small animal model used in biomedical research to understand roles of ncRNAs in the pathogenesis of HCV-induced HCC. Among nonhuman species, only chimpanzees have thus far been capable of being infected with HCV, and disease in them is generally relatively mild. Most recently, Dorner *et al*[[69](#_ENREF_69)] has reported a breakthrough and milestone in development of a genetically humanized mouse model for HCV research, in which the entire HCV life cycle can be completed and immune system is fully functioning. This genetically humanized mouse model will allow us to gain new insights into not only an important biology of HCV but also carcinogenesis of HCC caused by HCV. Additional challenging factors remain which slow the progress of the miRNA-based agents into clinical trials and new drugs. The dysregulated miRNAs in HCV-induced HCC as discussed earlier in this review were identified in individual studies, lacking consensus among the different reports, having been attributed to the differences among miRNA probe, staging and grade of malignancy of the tumor and different HCV genotypes. Therefore, there is still a long way to go before the miRNA-based therapy can be used in the clinic in the prevention and treatment of HCV-induced HCC.

**CONCLUSION**

As we summarized and discussed in this review, the recent findings of roles of ncRNAs in not only regulating HCV life cycle but also their contribution to pathogenesis of HCV-induced HCC have been remarkable, despite the fact that we may have unveiled only a small portion of the very large number of ncRNAs; this is probably especially true for lncRNAs. We are only beginning to understand the nature and extent of the involvement of lncRNAs in disease. Recently, a number of LncRNAs have been found to be aberrantly regulated in cancer, including HCC[[42-48](#_ENREF_43),[53-55](#_ENREF_53),[57](#_ENREF_57),59-62]. However, at the present time there are no reports on aberrantly lncRNAs exclusively associated with HCV-induced HCC, and thus remain a large unexplored and undefined area, which may allow us to better understand the role of lncRNAs in the pathogenesis of HCV infections and HCC and to identify better therapeutic targets and more sensitive diagnostic markers.

The intracellular ncRNAs that were significantly altered in HCV-induced HCC have been proposed to be potential molecular targets for therapy to combat HCV and HCV-induced HCC. Furthermore, over the past years, extracellular ncRNAs, particularly ncRNAs in exosomes, have risen to be promising as biomarkers with diagnostic or prognostic value. Exosomes (30-100 nm in diameter) are one of the class of microvesicles found in biofluids, including blood, ascites fluid, urine, culture media of cell cultures, *etc.*. Exosomes carry with them various nucleic acids, including ncRNAs (*e.g.,* miRNAs and lncRNAs) and proteins from their cells of origin, which allow us to achieve access to molecular information about their cell-of-origin without biopsying or destroying the actual cells themselves[[70-80](#_ENREF_70)]. This is of particular importance because direct cellular biopsy may be difficult or otherwise unattainable for screening high-risk populations, such as screening for HCC in CHC patients. It is highly anticipated that future studies on exosomal ncRNAs in different stages of HCC in HCV patients, which reflect the stepwise carcinogenic process from preneoplastic lesions to HCC may unveil better and reliable markers to aid in HCC early diagnosis among CHC patients and tracking of disease progression, which may directly benefit patients affected with HCC. Furthermore, there is a possibility that ncRNAs in exosomes may be taken by hepatocytes as a part of the cell-to-cell communication to spread HCV and promote carcinogenic signal transduction among hepatocytes, and therefore studies on exosomal ncRNAs associated with HCV-induced HCC may suggest novel molecular targets to help prevent and treat HCV-induced HCC patients.

In conclusion, the recent discovery of ncRNAs ushered in exciting and novel area to explore. Meanwhile, challenges to investigators are obvious: With respect to ncRNAs in HCV-induced HCC, in-depth knowledge on functional roles for ncRNAs in HCV-induced HCC through well-designed studies are required to shed light on the molecular pathways of carcinogenesis and to aid in truly exploiting the potential of ncRNAs to serve as molecular targets or markers with a real value. Would it be possible in the future to use exosomal ncRNAs from body fluids such as blood, ascites or urine, for screening and early diagnosis of HCC among HCV patients at high-risk? Could we successfully slow or prevent the development of HCC in HCV patients using selected ncRNA antagomirs or mimics as novel approaches?

**REFERENCES**

1 **Bartel DP**. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; **136**: 215-233 [PMID: 19167326 DOI: 10.1016/j.cell.2009.01.002]

2 **Ghildiyal M**, Zamore PD. Small silencing RNAs: an expanding universe. *Nat Rev Genet* 2009; **10**: 94-108 [PMID: 19148191 DOI: 10.1038/nrg2504]

3 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438]

4 **Ambros V**. The functions of animal microRNAs. *Nature* 2004; **431**: 350-355 [PMID: 15372042]

5 **Ponting CP**, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell* 2009; **136**: 629-641 [PMID: 19239885 DOI: 10.1016/j.cell.2009.02.006]

6 **Wilusz JE**, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev* 2009; **23**: 1494-1504 [PMID: 19571179 DOI: 10.1101/gad.1800909]

7 **Chisari FV**. Unscrambling hepatitis C virus-host interactions. *Nature* 2005; **436**: 930-932 [PMID: 16107831]

8 **Bartosch B**, Thimme R, Blum HE, Zoulim F. Hepatitis C virus-induced hepatocarcinogenesis. *J Hepatol* 2009; **51**: 810-820 [PMID: 19545926 DOI: 10.1016/j.jhep.2009.05.008]

9 **Hou W**, Tian Q, Zheng J, Bonkovsky HL. MicroRNA-196 represses Bach1 protein and hepatitis C virus gene expression in human hepatoma cells expressing hepatitis C viral proteins. *Hepatology* 2010; **51**: 1494-1504 [PMID: 20127796 DOI: 10.1002/hep.23401]

10 **Hou W**, Tian Q, Steuerwald NM, Schrum LW, Bonkovsky HL. The let-7 microRNA enhances heme oxygenase-1 by suppressing Bach1 and attenuates oxidant injury in human hepatocytes. *Biochim Biophys Acta* 2012; **1819**: 1113-1122 [PMID: 22698995]

11 **Lewis BP**, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; **120**: 15-20 [PMID: 15652477]

12 **Coulouarn C**, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009; **28**: 3526-3536 [PMID: 19617899 DOI: 10.1038/onc.2009.211]

13 **Pineau P**, Volinia S, McJunkin K, Marchio A, Battiston C, Terris B, Mazzaferro V, Lowe SW, Croce CM, Dejean A. miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci U S A* 2010; **107**: 264-269 [PMID: 20018759 DOI: 10.1073/pnas.0907904107]

14 **Jopling CL**, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005; **309**: 1577-1581 [PMID: 16141076]

15 **Jopling CL**. Regulation of hepatitis C virus by microRNA-122. *Biochem Soc Trans* 2008; **36**: 1220-1223 [PMID: 19021529 DOI: 10.1042/BST0361220]

16 **Shan Y**, Zheng J, Lambrecht RW, Bonkovsky HL. Reciprocal effects of micro-RNA-122 on expression of heme oxygenase-1 and hepatitis C virus genes in human hepatocytes. *Gastroenterology* 2007; **133**: 1166-1174 [PMID: 17919492]

17 **Persson H**, Kvist A, Rego N, Staaf J, Vallon-Christersson J, Luts L, Loman N, Jonsson G, Naya H, Hoglund M, Borg A, Rovira C. Identification of new microRNAs in paired normal and tumor breast tissue suggests a dual role for the ERBB2/Her2 gene. *Cancer Res* 2011; **71**: 78-86 [PMID: 21199797 DOI: 10.1158/0008-5472]

18 **Hamfjord J**, Stangeland AM, Hughes T, Skrede ML, Tveit KM, Ikdahl T, Kure EH. Differential expression of miRNAs in colorectal cancer: comparison of paired tumor tissue and adjacent normal mucosa using high-throughput sequencing. *PLoS One* 2012; **7**: e34150 [PMID: 22529906 DOI: 10.1371/journal.pone.0034150]

19 **Wang Y**, Toh HC, Chow P, Chung AY, Meyers DJ, Cole PA, Ooi LL, Lee CG. MicroRNA-224 is up-regulated in hepatocellular carcinoma through epigenetic mechanisms. *FASEB J* 2012; **26**: 3032-3041 [PMID: 22459148 DOI: 10.1096/fj.11-201855]

20 **Wang Y**, Lee AT, Ma JZ, Wang J, Ren J, Yang Y, Tantoso E, Li KB, Ooi LL, Tan P, Lee CG. Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-224-specific target. *J Biol Chem* 2008; **283**: 13205-13215 [PMID: 18319255 DOI: 10.1074/jbc.M707629200]

21 **Yao G**, Yin M, Lian J, Tian H, Liu L, Li X, Sun F. MicroRNA-224 is involved in transforming growth factor-beta-mediated mouse granulosa cell proliferation and granulosa cell function by targeting Smad4. *Mol Endocrinol* 2010; **24**: 540-551 [PMID: 20118412 DOI: 10.1210/me.2009-0432]

22 **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**: 565-575 [PMID: 22989374 DOI: 10.1111/j.1440-1746.2012.07271.x]

23 **Li Q**, Wang G, Shan JL, Yang ZX, Wang HZ, Feng J, Zhen JJ, Chen C, Zhang ZM, Xu W, Luo XZ, Wang D. MicroRNA-224 is upregulated in HepG2 cells and involved in cellular migration and invasion. *J Gastroenterol Hepatol* 2010; **25**: 164-171 [PMID: 19793168 DOI: 10.1111/j.1440-1746.2009.05971.x]

24 **Esau C**, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP. miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. *Cell Metab* 2006; **3**: 87-98 [PMID: 16459310]

25 **Girard M**, Jacquemin E, Munnich A, Lyonnet S, Henrion-Caude A. miR-122, a paradigm for the role of microRNAs in the liver. *J Hepatol* 2008; **48**: 648-656 [PMID: 18291553 DOI: 10.1016/j.jhep.2008.01.019]

26 **Jopling CL**, Schütz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 2008; **4**: 77-85 [PMID: 18621012 DOI: 10.1016/j.chom.2008.05.013]

27 **Shimakami T**, Yamane D, Jangra RK, Kempf BJ, Spaniel C, Barton DJ, Lemon SM. Stabilization of hepatitis C virus RNA by an Ago2-miR-122 complex. *Proc Natl Acad Sci U S A* 2012; **109**: 941-946 [PMID: 22215596 DOI: 10.1073/pnas.1112263109]

28 **Li Y**, Masaki T, Yamane D, McGivern DR, Lemon SM. Competing and noncompeting activities of miR-122 and the 5' exonuclease Xrn1 in regulation of hepatitis C virus replication. *Proc Natl Acad Sci U S A* 2013; **110**: 1881-1886 [PMID: 23248316 DOI: 10.1073/pnas.1213515110]

29 **Ura S**, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Sunakozaka H, Sakai Y, Horimoto K, Kaneko S. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology* 2009; **49**: 1098-1112 [PMID: 19173277 DOI: 10.1002/hep.22749]

30 **Varnholt H**, Drebber U, Schulze F, Wedemeyer I, Schirmacher P, Dienes HP, Odenthal M. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. *Hepatology* 2008; **47**: 1223-1232 [PMID: 18307259 DOI: 10.1002/hep.22158]

31 **Bai S**, Nasser MW, Wang B, Hsu SH, Datta J, Kutay H, Yadav A, Nuovo G, Kumar P, Ghoshal K. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem* 2009; **284**: 32015-32027 [PMID: 19726678 DOI: 10.1074/jbc.M109.016774]

32 **Diaz G**, Melis M, Tice A, Kleiner DE, Mishra L, Zamboni F, Farci P. Identification of microRNAs specifically expressed in hepatitis C virus-associated hepatocellular carcinoma. *Int J Cancer* 2013; **133**: 816-824 [PMID: 23390000 DOI: 10.1002/ijc.28075]

33 **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 [PMID: 22211142]

34 **Hou J**, Lin L, Zhou W, Wang Z, Ding G, Dong Q, Qin L, Wu X, Zheng Y, Yang Y, Tian W, Zhang Q, Wang C, Zhang Q, Zhuang SM, Zheng L, Liang A, Tao W, Cao X. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011; **19**: 232-243 [PMID: 21316602 DOI: 10.1016/j.ccr.2011.01.001]

35 **Borel F**, Han R, Visser A, Petry H, van Deventer SJ, Jansen PL, Konstantinova P, Reseau Centre de Ressources Biologiques Foie F. Adenosine triphosphate-binding cassette transporter genes up-regulation in untreated hepatocellular carcinoma is mediated by cellular microRNAs. *Hepatology* 2012; **55**: 821-832 [PMID: 21932399 DOI: 10.1002/hep.24682]

36 **Wang J**, He Q, Han C, Gu H, Jin L, Li Q, Mei Y, Wu M. p53-facilitated miR-199a-3p regulates somatic cell reprogramming. *Stem Cells* 2012; **30**: 1405-1413 [PMID: 22553189 DOI: 10.1002/stem.1121]

37 **Wu D**, Huang HJ, He CN, Wang KY. MicroRNA-199a-3p regulates endometrial cancer cell proliferation by targeting mammalian target of rapamycin (mTOR). *Int J Gynecol Cancer* 2013; **23**: 1191-1197 [PMID: 23851675 DOI: 10.1097/IGC.0b013e31829ea779]

38 **Jiang J**, Gusev Y, Aderca I, Mettler TA, Nagorney DM, Brackett DJ, Roberts LR, Schmittgen TD. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clin Cancer Res* 2008; **14**: 419-427 [PMID: 18223217 DOI: 10.1158/1078-0432.CCR-07-0523]

39 **Wong CC**, Wong CM, Tung EK, Au SL, Lee JM, Poon RT, Man K, Ng IO. The microRNA miR-139 suppresses metastasis and progression of hepatocellular carcinoma by down-regulating Rho-kinase 2. *Gastroenterology* 2011; **140**: 322-331 [PMID: 20951699 DOI: 10.1053/j.gastro.2010.10.006]

40 **Yang Z**, Chen S, Luan X, Li Y, Liu M, Li X, Liu T, Tang H. MicroRNA-214 is aberrantly expressed in cervical cancers and inhibits the growth of HeLa cells. *IUBMB Life* 2009; **61**: 1075-1082 [PMID: 19859982 DOI: 10.1002/iub.252]

41 **Xia H**, Ooi LL, Hui KM. Correction: MiR-214 Targets β-Catenin Pathway to Suppress Invasion, Stem-Like Traits and Recurrence of Human Hepatocellular Carcinoma. *PLoS One* 2012; **7** [PMID: 23094111 DOI: 10.1371/annotation/1be2a62e-45a1-4c13-9a8d-f265005a21e0]

42 **Zhang Q**, Pu R, Du Y, Han Y, Su T, Wang H, Cao G. Non-coding RNAs in hepatitis B or C-associated hepatocellular carcinoma: potential diagnostic and prognostic markers and therapeutic targets. *Cancer Lett* 2012; **321**: 1-12 [PMID: 22425745 DOI: 10.1016/j.canlet.2012.03.011]

43 **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**: 2119-2128 [PMID: 22289527]

44 **Tsai MC**, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. *Science* 2010; **329**: 689-693 [PMID: 20616235 DOI: 10.1126/science.1192002]

45 **Gupta RA**, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010; **464**: 1071-1076 [PMID: 20393566 DOI: 10.1038/nature08975]

46 **Ishibashi M**, Kogo R, Shibata K, Sawada G, Takahashi Y, Kurashige J, Akiyoshi S, Sasaki S, Iwaya T, Sudo T, Sugimachi K, Mimori K, Wakabayashi G, Mori M. Clinical significance of the expression of long non-coding RNA HOTAIR in primary hepatocellular carcinoma. *Oncol Rep* 2013; **29**: 946-950 [PMID: 23292722 DOI: 10.3892/or.2012.2219]

47 **Yang F**, Zhang L, Huo XS, Yuan JH, Xu D, Yuan SX, Zhu N, Zhou WP, Yang GS, Wang YZ, Shang JL, Gao CF, Zhang FR, Wang F, Sun SH. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. *Hepatology* 2011; **54**: 1679-1689 [PMID: 21769904 DOI: 10.1002/hep.24563]

48 **Yuan SX**, Yang F, Yang Y, Tao QF, Zhang J, Huang G, Yang Y, Wang RY, Yang S, Huo XS, Zhang L, Wang F, Sun SH, Zhou WP. 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**: 2231-2241 [PMID: 22706893 DOI: 10.1002/hep.25895]

49 **Lai MC**, Yang Z, Zhou L, Zhu QQ, Xie HY, Zhang F, Wu LM, Chen LM, Zheng SS. Long non-coding RNA MALAT-1 overexpression predicts tumor recurrence of hepatocellular carcinoma after liver transplantation. *Med Oncol* 2012; **29**: 1810-1816 [PMID: 21678027 DOI: 10.1007/s12032-011-0004-z]

50 **Liu Y**, Pan S, Liu L, Zhai X, Liu J, Wen J, Zhang Y, Chen J, Shen H, Hu Z. 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**: e35145 [PMID: 22493738 DOI: 10.1371/journal.pone.0035145]

51 **Du Y**, Kong G, You X, Zhang S, Zhang T, Gao Y, Ye L, Zhang X. 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**: 26302-26311 [PMID: 22685290 DOI: 10.1074/jbc.M112.342113]

52 **Hammerle M**, Gutschner T, Uckelmann H, Ozgur S, Fiskin E, Gross M, Skawran B, Geffers R, Longerich T, Breuhahn K, Schirmacher P, Stoecklin G, Diederichs S. Posttranscriptional destabilization of the liver-specific long noncoding RNA HULC by the IGF2 mRNA-binding protein 1 (IGF2BP1). *Hepatology* 2013; Epub ahead of print [PMID: 23728852 DOI: 10.1002/hep.26537]

53 **Zhang L**, Yang F, Yuan JH, Yuan SX, Zhou WP, Huo XS, Xu D, Bi HS, Wang F, Sun SH. Epigenetic activation of the MiR-200 family contributes to H19-mediated metastasis suppression in hepatocellular carcinoma. *Carcinogenesis* 2013; **34**: 577-586 [PMID: 23222811 DOI: 10.1093/carcin/bgs381]

54 **Iizuka N**, Oka M, Yamada-Okabe H, Mori N, Tamesa T, Okada T, Takemoto N, Tangoku A, Hamada K, Nakayama H, Miyamoto T, Uchimura S, Hamamoto Y. 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**: 3939-3944 [PMID: 12124323]

55 **Matouk IJ**, DeGroot N, Mezan S, Ayesh S, Abu-lail R, Hochberg A, Galun E. The H19 non-coding RNA is essential for human tumor growth. *PLoS One* 2007; **2**: e845 [PMID: 17786216]

56 **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**: 890-898 [PMID: 17416635]

57 **Yang F**, Yi F, Zheng Z, Ling Z, Ding J, Guo J, Mao W, Wang X, Wang X, Ding X, Liang Z, Du Q. Characterization of a carcinogenesis-associated long non-coding RNA. *RNA Biol* 2012; **9**: 110-116 [PMID: 22258142 DOI: 10.4161/rna.9.1.18332]

58 **Huang JF**, Guo YJ, Zhao CX, Yuan SX, Wang Y, Tang GN, Zhou WP, Sun SH. Hepatitis B virus X protein (HBx)-related long noncoding RNA (lncRNA) down-regulated expression by HBx (Dreh) inhibits hepatocellular carcinoma metastasis by targeting the intermediate filament protein vimentin. *Hepatology* 2013; **57**: 1882-1892 [PMID: 23239537 DOI: 10.1002/hep.26195]

59 **Yang F**, Huo XS, Yuan SX, Zhang L, Zhou WP, Wang F, Sun SH. Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. *Mol Cell* 2013; **49**: 1083-1096 [PMID: 23395002 DOI: 10.1016/j.molcel.2013.01.010]

60 **Anwar SL**, Krech T, Hasemeier B, Schipper E, Schweitzer N, Vogel A, Kreipe H, Lehmann U. Loss of imprinting and allelic switching at the DLK1-MEG3 locus in human hepatocellular carcinoma. *PLoS One* 2012; **7**: e49462 [PMID: 23145177 DOI: 10.1371/journal.pone.0049462]

61 **Wurmbach E**, Chen YB, Khitrov G, Zhang W, Roayaie S, Schwartz M, Fiel I, Thung S, Mazzaferro V, Bruix J, Bottinger E, Friedman S, Waxman S, Llovet JM. Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. *Hepatology* 2007; **45**: 938-947 [PMID: 17393520]

62 **Braconi C**, Valeri N, Kogure T, Gasparini P, Huang N, Nuovo GJ, Terracciano L, Croce CM, Patel T. 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**: 786-791 [PMID: 21187392 DOI: 10.1073/pnas.1011098108]

63 **Forner A**, Bruix J. Biomarkers for early diagnosis of hepatocellular carcinoma. *Lancet Oncol* 2012; **13**: 750-751 [PMID: 22738800 DOI: 10.1016/S1470-2045(12)70271-1]

64 **Bruix J**, Sherman M, Practice Guidelines Committee AAftSoLD. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051]

65 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607]

66 **Rayner KJ**, Esau CC, Hussain FN, McDaniel AL, Marshall SM, van Gils JM, Ray TD, Sheedy FJ, Goedeke L, Liu X, Khatsenko OG, Kaimal V, Lees CJ, Fernandez-Hernando C, Fisher EA, Temel RE, Moore KJ. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. *Nature* 2011; **478**: 404-407 [PMID: 22012398 DOI: 10.1038/nature10486]

67 **Rayner KJ**, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, van Gils JM, Rayner AJ, Chang AN, Suarez Y, Fernandez-Hernando C, Fisher EA, Moore KJ. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J Clin Invest* 2011; **121**: 2921-2931 [PMID: 21646721 DOI: 10.1172/JCI57275]

68 **Coelho T**, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, Perez J, Chiesa J, Warrington S, Tranter E, Munisamy M, Falzone R, Harrop J, Cehelsky J, Bettencourt BR, Geissler M, Butler JS, Sehgal A, Meyers RE, Chen Q, Borland T, Hutabarat RM, Clausen VA, Alvarez R, Fitzgerald K, Gamba-Vitalo C, Nochur SV, Vaishnaw AK, Sah DW, Gollob JA, Suhr OB. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N Engl J Med* 2013; **369**: 819-829 [PMID: 23984729 DOI: 10.1056/NEJMoa1208760]

69 **Dorner M**, Horwitz JA, Donovan BM, Labitt RN, Budell WC, Friling T, Vogt A, Catanese MT, Satoh T, Kawai T, Akira S, Law M, Rice CM, Ploss A. Completion of the entire hepatitis C virus life cycle in genetically humanized mice. *Nature* 2013; **501**: 237-241 [PMID: 23903655 DOI: 10.1038/nature12427]

70 **Théry C**, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002; **2**: 569-579 [PMID: 12154376]

71 **Xiao D**, Ohlendorf J, Chen Y, Taylor DD, Rai SN, Waigel S, Zacharias W, Hao H, McMasters KM. Identifying mRNA, microRNA and protein profiles of melanoma exosomes. *PLoS One* 2012; **7**: e46874 [PMID: 23056502 DOI: 10.1371/journal.pone.0046874]

72 **Rabinowits G**, Gerçel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin Lung Cancer* 2009; **10**: 42-46 [PMID: 19289371 DOI: 10.3816/CLC.2009.n.006]

73 **Pisitkun T**, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci U S A* 2004; **101**: 13368-13373 [PMID: 15326289]

74 **Michael A**, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG, Alevizos I. Exosomes from human saliva as a source of microRNA biomarkers. *Oral Dis* 2010; **16**: 34-38 [PMID: 19627513 DOI: 10.1111/j.1601-0825.2009.01604.x]

75 **Keller S**, Rupp C, Stoeck A, Runz S, Fogel M, Lugert S, Hager HD, Abdel-Bakky MS, Gutwein P, Altevogt P. CD24 is a marker of exosomes secreted into urine and amniotic fluid. *Kidney Int* 2007; **72**: 1095-1102 [PMID: 17700640]

76 **Andre F**, Schartz NE, Movassagh M, Flament C, Pautier P, Morice P, Pomel C, Lhomme C, Escudier B, Le Chevalier T, Tursz T, Amigorena S, Raposo G, Angevin E, Zitvogel L. Malignant effusions and immunogenic tumour-derived exosomes. *Lancet* 2002; **360**: 295-305 [PMID: 12147373]

77 **Keller S**, Ridinger J, Rupp AK, Janssen JW, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. *J Transl Med* 2011; **9**: 86 [PMID: 21651777 DOI: 10.1186/1479-5876-9-86]

78 **Hu G**, Drescher KM, Chen XM. Exosomal miRNAs: Biological Properties and Therapeutic Potential. *Front Genet* 2012; **3**: 56 [PMID: 22529849 DOI: 10.3389/fgene.2012.00056]

79 **Gallo A**, Tandon M, Alevizos I, Illei GG. The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One* 2012; **7**: e30679 [PMID: 22427800 DOI: 10.1371/journal.pone.0030679]

80 **Tandon M**, Gallo A, Jang SI, Illei GG, Alevizos I. Deep sequencing of short RNAs reveals novel microRNAs in minor salivary glands of patients with Sjögren's syndrome. *Oral Dis* 2012; **18**: 127-131 [PMID: 21895886 DOI: 10.1111/j.1601-0825.2011.01849.x]

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**Table 1 Summary of microRNAs significantly up-regulated in hepatitis C virus-induced hepatocellular carcinoma**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ncRNAs** | **Chromosomal location** | **Differential expression level** | **Clinical relevance** | **References** |
| **Liver miRNAs** |  |  |  |  |
| miR-1269 | 4q13.2 | 15.7-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-224 | Xq28 | 10.7-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-452 | Xq28 | 10.1-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-224-3p | Xq28 | 8.1-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-221 | Xp11.3 | 3.7-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-122 | 18q21.31 | > 2-fold, HCV-associated HCC (*n* = 43) *vs* normal livers (*n* = 3), *P* < 0.01; HCV associated dysplastic nodules (*n* = 9) *vs* normal livers (*n* = 3), *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[30](#_ENREF_30)] |
| miR-100 | 11q24.1 | > 2-fold, HCV-associated HCC (*n* = 43) *vs* normal livers (*n* = 3); HCV associated dysplastic nodules (*n* = 9) *vs* normal livers (*n* = 3) | Potential therapeutic target molecule to prevent HCV-induced HCC | [[30](#_ENREF_30)] |
| miR-10a | 17q21.32 | > 2-fold, HCV-associated HCC (*n* = 43) *vs* normal livers (*n* = 3); HCV associated dysplastic nodules (*n* = 9) *vs* normal livers (*n* = 3) | Potential therapeutic target molecule to prevent HCV-induced HCC | [[30](#_ENREF_30)] |
| **Urinary miRNAs** | |  |  |  |
| miR-625 | 14q23.3 | > 3-fold, HCV-associated HCC (*n* = 32) *vs* normal urine samples (*n* = 12), *P* < 0.05 | Potential marker for the detection/early diagnosis of HCC among high-risk HCV patients | [[33](#_ENREF_33)] |
| miR-532 | Xp11.23 | > 3-fold, HCV-associated HCC (*n* = 32) *vs* normal urine samples(*n* = 12), *P* < 0.05 | Potential marker for the detection/early diagnosis of HCC among high-risk HCV patients | [[33](#_ENREF_33)] |
| miR-618 | 12q21.31 | > 3-fold, HCV-associated HCC (*n* = 32) *vs* normal urine samples(*n* = 12), *P* < 0.05 | Potential marker for the detection/early diagnosis of HCC among high-risk HCV patients | [[33](#_ENREF_33)] |

HCV: Hepatitis C virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; miRNAs: MicroRNAs; ncRNAs: Non-coding RNAs.

**Table 2 Summary of microRNAs down-regulated in hepatitis C virus-induced hepatocellular carcinoma**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ncRNAs** | **Chromosomal location** | **Differential expression level** | **Clinical relevance** | **References** |
| **Liver miRNAs** |  |  |  |  |
| miR-199a-5p | 19q13.3 | 7.2-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-199a-3p | 19q13.3 | 6.9-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-199b-3p | 19q13.3 | 6.2-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-214 | 1q24.3 | 5.5-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-198 | 19p13.3 | Approximately 5-fold, HCV-associated HCC (*n* = 43) *vs* normal livers(*n* = 3), *P* < 0.01; HCV-associated Dysplastic nodules (*n* = 9) *vs* normal livers (*n* = 3), *P* < 0.01 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[30](#_ENREF_30)] |
| miR-139-3p | 11q13.4 | 4.6-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-139-5p | 11q13.4 | 4.4-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-424-3p | Xq26.3 | 3.9-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-125a-5p | 19q13 | 3.7-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-130a | 11q12.1 | 2.9-fold, HCV-associated HCC (*n* = 9) *vs* normal livers (*n* = 12) and other liver diseases [HCV-associated cirrhosis (*n* = 10), HBV-associated accurate liver failure (*n* = 4)], *P* < 0.05 | Potential therapeutic target molecule to prevent HCV-induced HCC | [[32](#_ENREF_32)] |
| miR-145 | 5q32 | > 2-fold; HCV-associated HCC (*n* = 43) *vs* normal livers(*n* = 3); HCV associated dysplastic nodules (*n* = 9) *vs* normal livers (*n* = 3) | Potential therapeutic target molecule to prevent HCV-induced HCC | [[30](#_ENREF_30)] |
| **Urinary miRNAs** |  |  |  |  |
| miR-516-5p | 19q13.42 | > 3-fold, HCV-associated HCC (*n* = 32) *vs* normal urine samples(*n* = 12), *P* < 0.05 | Potential marker for early diagnosis of HCC among high-risk HCV patients | [[33](#_ENREF_33)] |
| miR-650 | 22q11.22 | > 3-fold, HCV-associated HCC (*n* = 32) *vs* normal urine samples(*n* = 12), *P* < 0.05 | Potential marker for early diagnosis of HCC among high-risk HCV patients | [[33](#_ENREF_33)] |

HCV: Hepatitis C virus; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; miRNAs: MicroRNAs; ncRNAs: Non-coding RNAs.