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TOPIC HIGHLIGHT

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

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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 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.

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 hepatitis C virus (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 housekeeping 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 ncRNAs 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]. With the development of next generation sequencing (NGS) techniques, a growing number of lncRNAs have been identified, characterized and functionally annotated[5,6]. 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"[5,6]. 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[7,8]. 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 hepatitis B virus (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 NCRNAS IN HCV-INDUCED HCC

microRNAs

miRNAs regulate gene expression primarily through post-transcriptional repression[3,4,9,10]. 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]. miRNAs are likely to play significant roles in the development and progression of cancers, including HCC[12,13] and HCV replication[9,14-16]. 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], colorectal cancer[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 on-

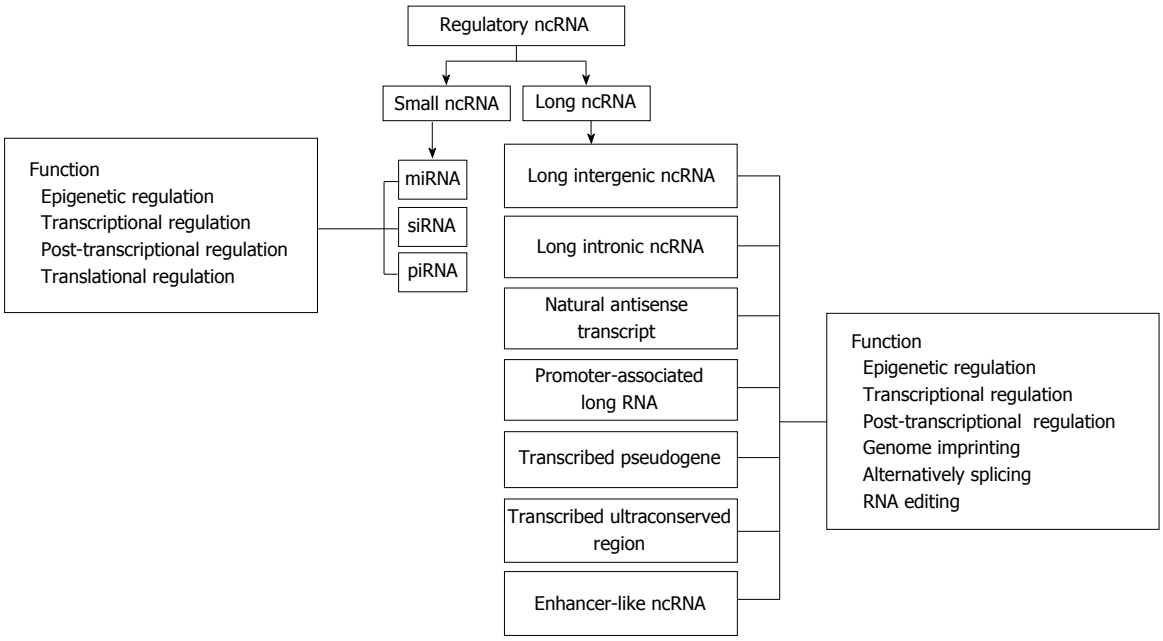

Figure 1 Classification of regulatory non-coding RNA and function in gene regulation. Regulatory non-coding RNA (ncRNAs) are divided into two major groups based on transcript size, small ncRNAs such as microRNA, small interfering RNA and piwi-interacting RNA (piRNA), as well as long ncRNAs with size greater than 200 nt. Both small ncRNA and long ncRNA have important regulatory function in gene expression. miRNA: microRNA; siRNA: small interfering RNA; piRNA: piwi-interacting RNA.

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

ncRNAs	Chromosomal location	Differential expression level	Clinical relevance	Ref.
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]
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]
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]
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]
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]
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]
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]
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]
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]
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]
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]

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]
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]
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]
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]
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]
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]
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]
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]
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]
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]
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]
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]
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]

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

cogenic 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]. 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*[20] identified and validated the apoptosis inhibitor 5 (API-5) as a specific target gene for miR-224. 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]. 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*.[20,22,23].

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[24,25]. Unexpectedly, miR-122 was also shown to be required for HCV replication[14-16]. 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]. miR-122 exerts several functions in the HCV life cycle[27,28]. 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 exonuclease Xrn1[27,28]. 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[12,29,30]. 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]. In parallel with these observations, overexpression of miR-122 inhibits anchorage-independent growth, migration, invasion and tumor formation in nude mice[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]. miR-199a/b-3p is the third most highly expressed miRNA in the liver[34], and was also found to be consistently decreased in HCC patients with HBV infection[34] and alcohol consumption[35]. Its decrement significantly correlates with poor survival of HCC patients[34]. Down-regulation of miR-199a-3p results in a pronounced increase in cell proliferation while overexpression miR-199a-3p inhibits cell proliferation by imposing G_1 cell cycle arrest[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].

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]. The down-regulation of miR-214 has been reported in HCC[20,38,39] and other cancers such as cervical cancer, whereas increase in miR-214 was found to significantly reduce growth of Hela cells[40]. In addition, reduced level of miR-214 is associated with invasion, stem-like traits and early recurrence of HCC[41]. Re-expression of miR-214 significantly suppressed the growth of HCC cells *in vitro* and reduced the tumorigenicity *in vivo*[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.

LncRNAs

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]. 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], 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], HEIH[47], MVIH[48], MALAT-1)[49], HULC[50-52], H19[53-55], CUDR[56], YIYA[57], lncRNA-Dreh[58], lncRNA-LET[59] and MEG3[60,61] 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]. Moreover, introduction of HOTAIR into human liver cancer cells promoted more rapid proliferation compared to controls[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]. Lai *et al*[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]. The expression level of HEIH in HBV-induced HCCs is significantly associated with recurrence and is an important independent prognostic factor for survival[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]. 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 diag-

nostic 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[63,64]. 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*[33] 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 biofluids 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 *et al*[65] 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[66,67], 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*[68] has recently demonstrated a new therapeutic approach to transthyretin amyloidosis by RNA interference (RNAi). 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].

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 overexpression 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] 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 re-

cent 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[42-48,53-55,57,59-62]. 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]. 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?

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