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TOPIC HIGHLIGHT

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Diagnostic and therapeutic application of noncoding RNAs for hepatocellular carcinoma

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Abstract

MicroRNAs (miRNAs) are small, noncoding RNA molecules that regulate gene expression posttranscriptionally, targeting thousands of messenger RNAs. Long noncoding RNAs (lncRNAs), another class of noncoding RNAs, have been determined to be also involved in transcription regulation and translation of target genes. Since deregulated expression levels or functions of miRNAs and lncRNAs in hepatocellular carcinoma (HCC) are frequently observed, clinical use of noncoding RNAs for novel diagnostic and therapeutic applications in the management of HCCs is highly and emergently expected. Here, we summarize recent findings regarding deregulated miRNAs and lncRNAs for their potential clinical use as diagnostic and prognostic biomarkers of HCC. Specifically, we emphasize the deregulated expression levels of such noncoding RNAs in patients' sera as noninvasive biomarkers, a field that requires urgent improvement in the clinical surveillance of HCC. Since nucleotide-based strategies are being applied to clinical therapeutics, we further summarize clinical and preclinical trials using oligonucleotides involving the use of miRNAs and small interfering RNAs against HCC as novel therapeutics. Finally, we discuss current open questions, which must be clarified in the near future for realistic clinical applications of these new strategies.

Key words: MicroRNA; Long noncoding RNA; Hepatocellular carcinoma; Clinical trials; Biomarker

Core tip: In this review, we summarize the latest findings on deregulated microRNAs (miRNAs) and long noncoding RNAs in hepatocellular carcinomas (HCCs) with a focus on their clinical use as novel diagnostic and prognostic

biomarkers. In addition, we summarize the current status of clinical and preclinical oligonucleotide therapies including miRNAs and small interfering RNAs as novel HCC therapeutics. This review will enable the readers to understand the current status of clinical applications and knowledge of noncoding RNAs in HCC management.

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INTRODUCTION

Noncoding RNAs contain multiple classes of RNAs that are not transcribed into proteins. While most noncoding RNAs studied to date are microRNAs (miRNAs), many noncoding RNAs with various lengths have also been reported.

MiRNAs are short, single-stranded RNAs that are expressed in most organisms[1-3]. Through gene expression regulation at a posttranscriptional level, miRNAs are involved in various physiological and pathological processes[4,5]. Since the discovery of miRNA lin-4 in Caenorhabditis elegans[6,7], as of August 2014, 1881 miRNA precursors and 2588 mature miRNA sequences in humans are deposited in miRBase, a miRNA database by the Sanger Institute[8]. MiRNAs are dysregulated in nearly all types of cancer[9,10], and specific signatures of aberrantly expressed miRNAs in specific cancers may have diagnostic and therapeutic implications[11,12].

Long noncoding RNAs (lncRNAs) also play crucial roles in transcription and translation[13,14]. Similar to miRNAs, their dysregulation is also associated with human cancers[15]. One of the most well-studied lncRNAs is the *HOX* transcript antisense intergenic RNA (HOTAIR). Class I homeobox genes (*HOX* in humans) encode 39 transcriptional factors initially described as master regulators of embryonic development[16] and display a unique gene network organization. HOTAIR, a 2.2-kb-long RNA residing within the *HOXC* locus, was initially described in breast cancer tissues, where it is highly expressed[17]. In addition to HOTAIR, many other lncRNAs are dysregulated in cancer tissues. Thus, lncRNAs may also be candidates for biomarker discovery and therapeutic applications in hepatocellular carcinomas (HCCs)[18].

In contrast to miRNAs and lncRNAs, short interfering RNAs (siRNAs) are double-stranded RNAs that degrade mRNAs through perfect matches with their target sequences. Although human telomerase reverse transcriptase was recently found to function as an RNA-dependent RNA polymerase and contribute to RNA silencing[19], its activities are not dominant in mammals. Additionally, endogenously produced siRNAs may play functional roles under limited circumstances in humans[20]. However, the exogenous application of synthesized siRNAs is an attractive method that could be used to intervene in crucial gene expression under pathological conditions, including cancers[21].

HCC is the third leading cause of cancer-related mortality worldwide[22]. Although advances have been made in early detection and interventional therapies, a continuing need exists to develop novel approaches for the management of advanced HCC[23]. While many reports have described deregulated expression levels or functions of miRNAs and lncRNAs in HCCs, we will focus on the potential clinical use of noncoding RNAs in the very near future for novel diagnostic and therapeutic applications in the management of HCCs.

NONCODING RNAS AS BIOMARKERS FOR HCC

Deregulated expression levels of noncoding RNAs in HCC tissues

Although several published reports have described deregulated expression levels of miRNAs and lncRNAs in HCC tissues[18,24,25], the data thus far vary greatly. The differences may be because of several reasons, including the use of different techniques or samples as controls, normal liver tissues *vs* nonneoplastic tissues around tumors, background livers with various fibrosis staging, inflammation activities, or etiologies, such as hepatitis B, hepatitis C, or steatohepatitis, as well as the age or sex of the tissue-derived patients; any of these factors may cause the differential expression status of miRNAs. Regardless of these limitations, the plenty data about dysregulated miRNAs in HCCs suggests that noncoding RNAs play crucial roles in hepatocarcinogenesis[24].

Deregulated expression of noncoding RNAs in HCC as prognostic/diagnostic markers

Deregulated expression levels of noncoding RNAs in HCC tissues that may be clinically useful as prognostic/diagnostic markers will be described herein. The landmark paper that initially addressed this issue focused on *miR26* expression levels in HCC tissues and was published in the New England Journal of Medicine[26]. In this study, HCC showed frequently reduced levels of *miR26*, and patients exhibited low *miR26* expression with a shorter overall survival but a better response to interferon therapy, indicating that miRNA expression status is associated with survival and response to therapy.

Expression levels of miRNAs have tissue specificities. In the liver, *miR122*, *miR192*, and *miR199a/b-3p* are highly expressed miRNAs of all mRNAs in the liver[27]. The role of *miR122* loss in hepatocarcinogenesis was confirmed in a mouse model[28,29], and its expression is decreased in HCCs, especially non-viral HCCs[27]. Decreased expression of *miR122* is also linked with poor prognosis of HCC[30]. Although *miR192* was not deregulated in HCCs in previous studies, *miR199a/b-3p*

Table 1 Representative noncoding RNAs in sera for Hepatocellular carcinoma diagnosis

MiRNA	Expression levels in HCC	Possible targets	Ref.
MiR21	Upregulated	PTEN, AKT, C/EBPb	[32,39,58]
MiR222	Upregulated	PP2A, p27, DDIT4	[42,43,59]
MiR223	Upregulated	Stathmin	[44]
HULC	Upregulated	IGF2BP1	[45-47]

HCC: Hepatocellular carcinoma; HULC: Highly up-regulated in liver cancer; PTEN: Phosphatase and tensin-like protein; AKT: V-akt murine thymoma viral oncogene homolog; C/EBPb: CCAAT/enhancer-binding protein beta; PP2A: Protein phosphatase 2A; IGF2BP1: Insulin-like growth factor 2 MRNA binding protein 1.

is frequently decreased in HCCs[27]. In contrast, *miR21*, whose expression is increased when rat hepatectomy[31], is upregulated as an onco-miRNA, resulting in the promotion of HCC[32]. *MiR21* expression in HCC tissues confers resistance to the antitumor effect of interferon-α and 5FU combination therapy[33].

Similar to miRNAs, expression levels of lncRNAs are also dysregulated in HCC tissues[18]. Among them, HOTAIR is overexpressed in HCC tissues and may confer chemoresistance[34]. Metastasis-associated lung adenocarcinoma transcript 1, which was initially discovered as an lncRNA associated with metastasis[35], is also upregulated in HCC tissues and may be useful as a biomarker for tumor recurrence. Recently, *HOXA* transcript at the distal tip (HOTTIP) was discovered to be located in physical contiguity with the *HOXA13* gene and upregulated in HCC tissues, and this was also associated with metastasis formation and poor patient survival[36]. These results show the functional importance of lncRNA dysregulation in HCC tissues and indicate their possible use as novel prognostic and diagnostic biomarkers.

Noncoding RNAs in the sera of patients with HCC as diagnostic markers

Although α-fetoprotein (AFP), AFP-L3, and des-gamma-carboxy prothrombin are useful noninvasive biomarkers for HCC surveillance[37], novel and sensitive biomarkers that can detect early HCC are needed. The identification of tumor-specific alterations in circulating nucleic acids of patients with cancer as noninvasive methods of cancer diagnosis is encouraging[38]. Although RNAs are generally considered unstable, they are actually quite stable and readily detected in patient serum and plasma. Microarrays, polymerase chain reaction methods, and next-generation sequencing technologies are generally utilized to detect circulating noncoding RNAs.

Although many reports have described circulating miRNA levels in patients with HCC, only a few tests have been reproducible. For example, data regarding upregulation of circulating *miR21*, *miR222*, and *miR223* in patients with HCC are inconsistent[32,33,38-44]. Highly upregulated in liver cancer, a 1.6-kb lncRNA, is also upregulated in HCC tissues[45-47] and is detected in the plasma of patients with HCC[18,48]. Although these results are encouraging, more work is needed to make the usability of circulating noncoding RNAs as novel biomarkers more reliable (Table 1). Specificity and sensitivity, as well as methods to quantitate small amounts of RNAs in sera with high reproducibility and the universal control to adjust the obtained data from differing times and samples, need to be urgently determined[49].

NONCODING RNAS AS NOVEL THERAPEUTICS AGAINST HCC

Ongoing clinical trials

Mounting evidence suggests that noncoding RNAs are frequently dysregulated in HCCs and possibly involved in oncogenesis and may therefore provide novel molecular targets as a therapeutic intervention. However, due to the complexity associated with pleiotropic miRNA functions and lncRNAs, the number of clinical trials is presently limited[50]. The leading nucleotide-targeting therapy, Miravirsen, an LNA-based *anti-miR122* against hepatitis C virus replication, has been successful in a Phase Ⅱ a study[51]. In addition, MRX34, a liposome-formulated *miR-34* mimic developed by Mirna Therapeutics, produced complete HCC regression in mouse models[52], and a Phase I study is currently recruiting patients with advanced liver cancer for HCC therapeutic intervention (NCT01829971).

While siRNAs are not endogenous noncoding RNAs, they can be described as noncoding RNAs that have been tried as novel therapeutics against HCC. ALN-VSP (Alnylam Pharmaceuticals), an RNAi therapeutic targeting vascular endothelial growth factor and kinesin spindle protein, has been shown to be well tolerated in Phase I studies (NCT008822180 and NCT01158079) for the treatment of primary and metastatic liver cancer. The results demonstrated disease control lasting more than 6 mo in the majority of patients, including a complete response in a patient with endometrial cancer who had multiple liver metastases. TMK-polo-like kinase 1 (PLK1) (Tekmira Pharmaceuticals), an RNAi targeting PLK1, is also under a Phase Ⅰ / Ⅱ trial (NCT01437007). Early results show that TKM-PLK is well tolerated and demonstrates clinical benefits. Although primary results from these potential therapeutics are encouraging, the benefits and unexpected side effects need to be determined, especially under long-term use.

Preclinical trials

Anti-miR21 and *anti-miR221* are under development for clinical use (Regulus Therapeutics). *MiR21* is one of the most validated microRNA targets, with numerous scientific publications suggesting that *miR21* plays an important role in the initiation and progression of cancers, including liver cancer[32,53,54]. Similarly, *miR221* has been identified to be upregulated in multiple cancers including liver cancer[54-56]. *Anti-miR21* and *anti-miR221* prolonged survival time in a preclinical mouse model

Table 2 Representative noncoding RNAs under clinical and preclinical trials for hepatocellular carcinoma therapeutics

Target	Name	Content	Vendor	Current status
MiR34	MRX34	Liposome-formulated miR-34 mimic	Mirna Therapeutics	Phase Ⅰ
VEGF/KSP	ALN-VSP	RNAi targeting VEGF/KSP	Alnylam Pharmaceuticals	Phase Ⅰ
PLK1	TMK-PLK1	RNAi targeting PLK1	Tekmira Pharmaceuticals	Phase Ⅰ/Ⅱ
MiR21	Anti-miR21	Antisense against miR21	Regulus Therapeutics	Preclinical
MiR221	Anti-miR221	Antisense against miR221	Regulus Therapeutics	Preclinical
MiR7	MiR7 mimic	MiR7 mimic	MiReven	Preclinical

VEGF: Vascular endothelial growth factor; KSP: Kidney-specific cadherin; PLK1: Polo-like kinase 1.

that genetically develops HCC. An *miR7* mimic is also under development (MiReven). Mir7 targets the phosphoinositide 3-kinase (PI3K) pathway and decreases tumor growth both *in vitro* and *in vivo*[57]. These results are summarized in Table 2.

CHALLENGES FOR BETTER CLINICAL TRANSLATION

Several other miRNAs, including lncRNAs, which are dysregulated in HCCs, can be attractive therapeutic targets by RNA mimics, antisense RNA, or siRNA. In fact, many publications have reported their efficacy. However, obstacles remain to be addressed[24]: (1) The more reproducibility of the results should be achieved to make the data more reliable; (2) Identification of driver miRNAs in oncogenesis is important to develop therapeutics targeting such miRNAs, although we may be able to use passive miRNAs as prognostic and diagnostic bio-markers; and (3) The delivery methods of oligonucleotides into specific tissues with improved oligonucleotide modification, and safety need to be seriously considered for utilizing miRNAs in clinical applications. Because miRNAs generally target multiple mRNAs, unexpected outcomes, "off-target effects," may occur, even when targeting a single miRNA.

More research to solve these issues is definitely needed for the improved translational application utilizing the data about miRNAs in HCCs.

CONCLUSION

The discovery of miRNAs and lncRNAs has opened up new possibilities for novel diagnostic and therapeutic tools against HCCs. However, several important issues remain to be resolved. We must conduct continuous research to develop innovative and useful applications of the miRNA data in the clinical management of HCCs.

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