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Mini-review

Non-coding RNAs in hepatitis B or C-associated hepatocellular carcinoma: Potential diagnostic and prognostic markers and therapeutic targets

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ABSTRACT

Non-coding RNA (ncRNA), a class of RNAs that do not code protein but have regulatory functions, can regulate gene expression and replication of hepatitis B virus or hepatitis C virus and play an important role in the virus–host interaction and the development of hepatocellular carcinoma (HCC). Deregulated ncRNAs in surgically removed hepatic tissues and circulation can be prognostic and diagnostic markers, respectively. ncRNAs functioning as either tumor suppressors or oncogenes can be therapeutic options. Here, we summarize the deregulated ncRNAs associated with the infections and HCC and focus on their roles on early diagnosis, prognosis prediction and therapeutic option of HCC.

1. Introduction

The human genome contains about 20,000 protein-coding genes, representing <2% of the total genome sequence [1]. Moreover, up to 90% of the genome is actively transcribed, generating an extraordinary amount of RNAs without coding capacity [2]. Currently, only a small proportion of non-coding RNAs (ncRNAs) have experimentally proven functions. Some ncRNAs play increasingly important roles in biological processes that are essential to both fundamental life activities such as embryonic development and pathogenic processes of complex diseases, such as cancer [3,4]. According to the length, ncRNAs are roughly classified into small ncRNA ranging from 18 nucleotides (nt) to 200 nt and long ncRNA (lncRNA) ranging from 200 nt to ~100 kb [4]. Small ncRNAs are represented by a broad range of RNA species including microRNAs (miRNAs or miRs), small interfering RNAs (siRNAs), piwi interacting RNAs, small nucleolar RNAs, and small nuclear RNAs. miRNAs, a type of highly conserved ncRNAs with an average length of 22 nt, can regulate gene expression *via* binding to the 3′-untranslated region (UTR), coding region or 5′UTR of the target mRNA. They can serve as major regulators of gene expression and as intricate components of the cellular gene expression networks [5,6]. It is estimated that up to 60% of the human genome may be regulated by miRNAs [7]. As miRNA-targeted genes are frequently involved in cell proliferation, differentiation, and apoptosis, the deregulation of miRNAs can cause disorders of these important processes and eventually lead to carcinogenesis. miRNAs can act as either oncogenes or tumor suppressors and deregulated miRNAs can be found in almost all human cancers [8]. The classes of lncRNAs include mRNA-like transcript lacking significant open reading frames (ORF), long intergenic ncRNA, antisense and intron-encoded transcript, pseudogene, transcribed ultraconserved region, and promoter-associated long RNA [9]. lncRNAs whose expression is spatially and temporally regulated may exert their gene-regulatory roles *via* epigenetic silencing, splicing regulation, translational control, and regulating apoptosis and cell cycle [10,11]. Some miRNAs such as miR-29a and transcription factors such as p53 can regulate the expression of lncRNAs [12,13]. Alterations in the primary and secondary structures and expression levels of lncRNAs and their cognate RNA-binding proteins may be related to human diseases. Their aberrant expression in cancers can influence the cell growth, invasion and metastasis [9,14]. Although the precise mechanism by which ncRNAs contribute to the pathogenesis of malignant diseases remains unclear, it is well accepted that ncRNAs participate in a wide-repertoire of biological processes involving in carcinogenesis and metastasis. Some ncRNAs have diagnostic and prognostic potentials and may also serve as therapeutic targets for cancers.

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Hepatocellular carcinoma (HCC) is the third leading cause of worldwide cancer-related death. The major risk factor for HCC is chronic hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infection [15]. There are few effective biomarkers for early diagnosis and prognosis prediction of HCC. The curative treatment strategies are limited for those diagnosed at the late stage. Increasing evidence has shown that miRNAs play important roles in chronic HBV or HCV infection and the development of HCC. Furthermore, miRNA profiling may aid in molecular classification of HCC patients for prognosis and therapeutic decision making [16]. lncRNAs have not been studied as much as miRNAs, but recent progress has indicated that the role of lncRNAs in HCC development is as important as miRNAs. In this review, we summarize the major deregulated miRNAs and lncRNAs involved in the virus infection and the development and prognosis of HCC, and focus on their roles as potential diagnostic and prognostic markers and therapeutic targets.

2. Effect of HBV or HCV infection on host miRNAs expression

Some oncogenic viruses such as Epstein–Barr virus can encode miRNAs. However, no solid evidences have shown that HBV or HCV can encode miRNAs except that a candidate HBV-encoded pre-miRNA is predicted using *in silico* computational approaches [17]. HBV, HCV, and their functional proteins are likely to exhibit their pathogenic roles *via* regulating the expression of host-derived miRNAs in the affected liver. The deregulated miRNAs serve as mediators of virus–host interaction, playing an important role in persistent infection, carcinogenesis, and HCC treatment *via* regulating host gene expression.

2.1. Effect of HBV on host miRNAs

Acute and chronic HBV infections lead to distinct profiling of deregulated miRNAs in hepatocytes. Zhang et al. [18] compared miRNA profiling of HBV-negative HepG2 cells, HepG2 cells transiently transfected with a 1.3-fold full-length HBV genome as an acute infection model, and HepG2.2.15 cells with stable HBV expression as a chronic infection model, and found that 77 and 48 miRNAs were deregulated in acute HBV infection and chronic HBV infection compared to HBV negative cells, respectively. Of the 25 miRNAs deregulated in common, 14 changed coherently and 11 changed inversely between the two phases of infection. Comparative analysis of miRNA expression among the infection models and HCC (mostly HBV carriers) showed that perturbation of miRNA expression in the chronic infection model was closer to that in HCC patients than that in the acute infection model and expression of miR-221 gradient increased in the three phases from acute infection to HCC. Similarly, 11 up-regulated (miR-23a, -99b, -125a, -146a, -181a, -181c, -181d, -183, -196b, -200b, -429) and 7 down-regulated (miR-15a, -16, -17, -202, -338, -378, -422a) miRNAs have been found in HepG2.2.15 cells as compared with parental HepG2 cells using microarray assays [19]. The mechanism by which HBV regulates cellular miRNAs is largely unknown. One possible way is through HBV x protein (HBx). HBx plays a key role in regulating HBV replication and hepatocarcinogenesis [20]. It up-regulates 7 (miR-30c, -99b, -125a, -191, -193b, -199a, -342) and down-regulates 11 (let-7a, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, miR-20a, -98, -106a, -196a) miRNAs in the HBx-transfected HepG2 cells in a microarray assay [21]. Among the down-regulated miRNAs, let-7a, the most highly expressed let-7 family member, acts as a tumor suppressor and negatively regulates cellular proliferation partially *via* targeting signal transducer and activator of transcription 3 (STAT3). HBx up-regulates CD59 by let-7i at the post-transcriptional level, contributing to escape of HCC cells from complement-dependent cytotoxicity [22]. HBx also down-regulates the miR-16 family in malignant hepatocytes; furthermore the HBx-induced repression of miR-15a/16 in HepG2 cells is mediated by c-Myc [23]. miR-29a is dramatically up-regulated in p21-HBx transgenic mice, HBx-transfected HCC cells and HepG2.2.15 cells; and is responsible for cell migration *via* targeting gene-phosphatase and tensin homolog (PTEN) [24]. Carboxyl-terminal truncated HBx (Ct-HBx) is frequently evident in HBV-associated HCC tissues, either in cytoplasm or in the integrated genome, and plays a critical role in hepatocarcinogenesis *via* activating cell proliferation [25,26]. Compared to hepatocytes expressing the full-length HBx, those expressing Ct-HBx grow much faster. Both HBx forms can up-regulate miR-23a and miR-125a, and down-regulate miR-19a/b; however, more than 80% of the miRNAs up-regulated by full-length HBx are either down-regulated or unaffected by Ct-HBx. Ct-HBx down-regulates while full-length HBx up-regulates the expression of a set of miRNAs (miR-146a, -193b, -29c, -365, -190, -210 and -26a/b) with growth-suppressive functions. The Ct-HBx suppresses the expression of certain miRNAs *via* binding to their promoters to inhibit the transcriptional activity [27]. So we assume that Ct-HBx may have a stronger pro-carcinogenesis function than full-length HBx *via* regulating the expression of different miRNAs. In addition, the difference in miRNA profiling between HepG2 cells transfected with a 1.3-fold full-length HBV genome and those transfected with HBx indicates that other HBV functional proteins are involved in the miRNA regulation.

2.2. Effect of HCV on host miRNAs

HCV infection or transfection can greatly alter miRNA profiling in human hepatoma cells. In Huh7.5.1 hepatoma cells, 108 deregulated miRNAs are documented whose expression levels alter with >2.0-fold in response to HCV infection, as compared with those infected with UV-inactivated HCV *in vitro*. The majority of the differentially expressed miRNAs are up-regulated, including miR-149*, -373*, -638, -675, -887, -888, -940, -1181, -1234, and -1469; a fraction of miRNAs are down-regulated, including miR-24, -181a, -210, -221, -455-3p, -455-5p, -664, and -923. Gain of miR-221, -455-3p and loss of miR-149*, -373* expression suppress HCV genotype 1b RNA abundance [28]. Braconi et al. [29] evaluated miRNAs profiling in HepG2 cells stably transfected with full-length HCV genome compared to those transfected with empty vector, and found that changes in miRNA expression occurred with 23 miRNAs >2-fold up-regulated (including miR-130a, -99b, -181b, -768-3p, -565, -126*, -146a, and -193b) and 10 miRNAs >2.0-fold down-regulated (including miR-206, -627, and -196a). Of those, miR-193b whose expression was up-regulated by 5-fold in HCV-transfected HepG2 cells was predicted to target Mcl-1, an anti-apoptotic protein affecting the response of HCC to sorafenib. Thus, HCV might exhibit its function *via* modulating miRNA expression.

3. Regulatory role of host-derived miRNAs on the replication and gene expression of HBV and HCV

Host miRNAs cannot only be regulated by virus infection but also affect virus replication and viral protein expression *via* specifically binding to the viral sequences or targeting some related host genes, playing an important role in the virus-induced hepatocarcinogenesis.

3.1. Regulatory role of miRNAs on HBV

A 995-bp conserved region among the most common HBV subtypes within the viral polymerase ORF and the overlapping surface