Are MicroRNAs Reliable Prognostic Biomarkers in Liver Hepatocellular Carcinoma Development?

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Abstract

Hepatocellular Carcinoma (HCC) is a complex multi-step process which involves genetic and epigenetic alterations. This disease is often not detected until late in development. It is necessary to discover and validate new sensitive, non-invasive, diagnostic and prognostic biomarkers able to highlight changes in disease status and liver function. Deregulation of microRNAs activity plays an important role in pathogenesis of chronic Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) infections, Non-alcoholic Fatty Liver Disease (NAFLD) and in the progression to HCC. The assessment of serum microRNAs profiles may provide insights related to the chronic viral hepatitis molecular mechanism which could lead to HCC. MicroRNAs are involved in controlling the metabolic pathways of infected cells and also the immune-metabolic response to viral infection in the liver. Also, microRNAs are crucial regulators of metabolic-related disorders, such as non-alcoholic fatty liver disease. At the moment, in chronic viral hepatitis B, C and NAFLD the reliable diagnosis is liver biopsy with its disadvantages. Therefore, microRNAs detection may improve early identification of individuals with high risk for HCC development. Identification of microRNAs aberrant expression and their oncogenic or tumor suppressor molecular targets, it is new useful for potential biomarkers characterization and for clinical development of new therapies for HCC.

Keywords: MicroRNA; HBV; HCV; HCC; NAFLD; Biomarkers; Viral replication

Introduction

HCC is among the most common solid malignant tumours of the liver with an incidence in Romania between 10-20 cases to 100,000 inhabitants per year. HCC is more likely to develop in man than in women [1]. Chronically infected patients with HBV and HCV have an increased risk for development of cirrhosis and hepatocellular carcinoma. Others major risk factors for HCC include chronic alcohol consumption, non-alcoholic fatty liver disease and dietary aflatoxin exposure [2]. In 2013 the prevalence for HBV in Romania was 7.9% and for HCV was 5.6% [3]. The prevalence of NAFLD is also high, about 20% [4]. The development of tumors, including HCC, is a complex multi-step process which involves the deregulation of multiple intracellular and extracellular signalling pathways [5]. In HCC development and progression have been found genetic and epigenetic alterations of the Retinoblastoma (RB), p53, Rat Sarcoma Virus oncogene (RAS), wingless-type (WNT) and transforming growth factor (TGF)-β pathways [5].

In our experience, many patients do not respond to current therapy against hepatitis viruses. Therefore, there is a need for identification and validation of new, specific minimally-invasive biomarkers for early detection of changes in the liver tissue. In this context direct role of microRNAs in controlling cellular pathways is relevant in liver tumorigenesis. The assessment of circulating microRNAs expression in serum of patients could evaluate disease severity and the likelihood of disease progression. Cell-specific microRNA expressions which are involved in chronic liver pathogenesis and the correlation with chronic HBV and HCV infections are an emerging area of research. Many studies showed the role of microRNAs alterations in cancer biology, inclusive in HCC, facilitating tumor growth, invasiveness, angiogenesis and avoiding the immune response by controlling targeted messenger RNA expression which is involved in these processes [6-8]. MicroRNAs act as molecules which triggers a receptor-mediated response. These can be released in extracellular environment in exosomes from biological fluids, acting in this way as "hormones" [9]. Discovery of circulating microRNAs offer new tools for non-invasive exploration of different pathologies with essential characteristics of reliable biomarkers: stability, resistance to RNase digestion, preserved among species [10]. MicroRNAs from the liver may enter the serum passively through apoptosis and necrosis or actively through secretion of exosomes and viral particles [11]. According to recent researches, microRNAs have increased potential for clinical use. Identification of microRNAs aberrant expression and their oncogenic or tumor suppressor molecular targets, it is necessary for potential biomarkers characterization and for clinical development of new therapies [12]. The ability of microRNAs to regulate key cellular processes and numerous metabolic pathways by simultaneously directing many targets shows their therapeutic potential [13]. The liver is the main organ responsible for metabolism, detoxification and drug metabolism [14]. Liver injury or inflammation could be reflected by microRNAs expression profiles [15]. The role of miRNAs in chronic HBV and HCV infection has been reported in many studies adding other dimensions for understanding virus pathogenesis [16-23].
Both viruses (HBV and HCV) cause systematic metabolic alteration in hepatocyte by affecting different cellular factors and pathways, in order to establish a more permissive microenvironment for viral replication and pathogenic effects [7]. MicroRNAs are involved in controlling metabolic state of infected cells and also the immune-metabolic response to liver viral infection [24]. MicroRNAs are involved in regulation of numerous virus-host interactions affecting hepatitis-viruses could be regulated by host microRNAs which target viral genomes or cellular factors [25]. Some miRNAs facilitate viral replication, while, some others may even serve as potential anti-viral agents [8].

**Hepatitis C virus and microRNAs**

HCV is a hepatotropic virus with positive-sense single-stranded RNA genome, belonging in the Flaviviridae family [21,25]. HCV is the only human virus that is able to cause two different cancers: HCC and, rarely, Non-Hodgkin’s Lymphoma (NHL) [26]. HCV viral proteins interact with host cellular factors and regulate various signalling pathways to facilitate virus mediated persistent infection [27]. Recent studies have shown that virus replication and pathogenesis during HCV infection are modulated by several miRNAs which are key players in virus-host interactions. Also, HCV modulates the expression of miRNAs in order to regulate critical gene networks [27]. Interaction between miRNAs and RNA viruses leads to increased stability of the viral RNA [28]. MicroRNAs activity is one of the main regulatory pathways involved in hepatic lipid and glucose metabolism which is closely linked to hepatitis virus replication [15].

Singaravelu et al. showed that miR-185 and miR-130b are antiviral hepatocellular factors that regulate immuno-metabolism in the infected hepatocytes. HCV-mediated inhibition of these microRNAs expression has significant effects in liver lipid metabolism. Cellular lipidic environment is crucial to HCV life cycle. HCV-induced lipid accumulation in hepatocytes causes down regulation of miR-185 and miR-130b expression indicating that the virus actively counteracts the host defense [24]. Li et al. have investigated the functions and underlying mechanisms of miR-25, let-7, and miR-130 miRNAs families in modulating chronic HCV infection. The authors demonstrated that these miRNAs could repress multiple essential cellular co-factors of HCV, thus interfering with various concurrent signal pathways that are essential for HCV life cycle. The expression of these HCV-restricting miRNAs was significantly down regulated revealing the roles of these RNA molecules in mediating HCV infection and HCV-related pathogenesis [25].

Let-7 family microRNA suppresses expression of RAS gene. The Ras proteins play critical roles in the development of many types of common human cancers [29]. Studies conducted by Bruni et al. in cell lines infected with HCV clones showed that miR-128a, miR-196a and miR-142-3p may be differentially regulated by HCV to evade immunity and could be mediators of antiviral response to β-interferon [30]. MicroRNA-122 is one of the first microRNAs to be identified as a “tissue specific”, representing 70% of all hepatic microRNAs. MicroRNA-122 is involved in a complex signalling network in the liver biological processes of development and differentiation, in hepatic lipid metabolism, responses to stress, and HCC [31,32]. Many studies have demonstrated that the most abundant liver-specific miR-122, is associated with lipid and cholesterol metabolism during HCV infection and can increase the abundance of HCV RNA through direct binding to the 5’UTR of viral genome [20,33,34]. MicroRNA-122 is up regulated in chronic HCV-infected sera and may serve as an interesting biomarker for early liver inflammation responses in HCV patients [35]. MicroRNA-122 has several target mRNAs that encode proteins involved in the development of HCC; such proteins include prolyl 4-hydroxylase subunit a1 (P4HA1), pyruvate kinase PKM and mannan-binding lectin serine protease 1 (MASP1) [36]. Involvement of miR-122 in HCV replication has led to the development of an experimental anti-HCV drug (Miravirsen) targeting this microRNA [26].

**Hepatitis B virus and microRNAs**

HBV is a non-cytopathic enveloped hepatavirus with a circular, partially double-stranded DNA genome [8]. Chronic hepatitis B infection has many steps: the immune-tolerant phase, the immune-reactive Hepatitis B e Antigen (HBeAg)–positive phase, the inactive HBV carrier state, and HBBeAg-negative chronic hepatitis [22]. Host cellular microRNAs are able to promote or repress the HBV lifecycle, either by direct interaction with HBV transcripts or by indirect targeting cellular mediators, involved in the HBV pathogenesis [19]. HBV infection dysregulates cellular miRNAs controlling in this way, the host genes expression to promote its replication [21]. HBV encodes HBV-miR-3 which controls the process of self-replication by inhibiting the synthesis of viral proteins and HBV replication [37]. It has been shown that almost all HBV viral proteins modulate different host miRNAs (let7-family, miR-29a, miR-101, miR-145, miR-21, miR-222) which are involved in the modulation of host hepatocarcinogenesis and immune response [8]. Also, HBV replication is modulated by its own proteins [37]. HBx play an important role in HBV-related HCC by regulation of apoptosis mediated by p53, Fas and TGF-β [38]. Single-Nucleotide Polymorphisms (SNPs) in miRNA encoding genes could be associated with the susceptibility to persistent HBV and, consequently, HCC development [8]. It was shown that specific miRNAs are involved in several aspects of HBV biology. Zhang et al. have identified that miR-216b and miR-188-5p expressions were significantly lower in plasma of patients with HBV related-HCC, compared with the healthy controls, while miR-324-3p, miR-484 and miR-454 have increased expressions. These differentially expressed miRNAs in the plasma of patients with HCC could suggest molecular transformation within tumorigenesis process such as abnormal proliferation, invasion and Epithelial Mesenchymal Transition (EMT) [39]. Price et al. have identified that in HBeAg-positive patients plasma levels of miR-122-5p, miR-125b-5p, miR-192-5p, miR-193b-3p, and miR-194-5p are higher compared with HBeAg-negative patients. Thus, association of these microRNAs with HBV, DNA and HBsAg levels are correlated with viral replication [22]. It has been reported that in liver tissue miR-15a can down regulate Snail1 mRNA and enhance the transforming growth factor β1 (TGF-β1) signaling pathway [40]. TGF-β1 signaling is involved in cell proliferation and tumorigenesis [41]. HBV mRNA acts as endogenous competitor for down regulating of miR-15a leading to up regulate Snail1 expression. HBV viral strategy to exploit its compact genome for promoting tumorigenesis in involved in the development of HBV-related HCC [40]. HBV has been found to down regulate other miRNAs in order to control various signalling pathways. Increases in circulating miR-122 levels in plasma might be caused by HBV-induced upregulation of miR-122 expression [22]. In the study conducted by Akamatsu et al. miR-122 was independently related with HBV DNA level, whereas miR-125b was independently associated with levels of HBV DNA, HBsAg and HBeAg. MiR-22 and
miR-1275 were independently associated with serum g-glutamyl transpeptidase levels [16]. Wang et al. found that miR-150, miR-342-3p, miR-663, miR-20b, miR-92a-3p, miR-376c-3p, and miR-92b are specifically altered in HBV-related HCC. These miRNAs regulate various genes that encode for cell cycle and apoptosis regulators [38]. Singh et al. have analyzed differential expression of 17 microRNAs in liver biopsy samples from different stages of HBV infection and liver disease: immune-tolerant phase, acute viral hepatitis, no fibrosis, early or late fibrosis, and healthy controls. They showed that in the immune-tolerant group elevated levels of miR-199a-5p, miR-221-3p and let-7a-5p contribute at suppression of innate immune response allowing HBV replication and viral persistence. In the acute viral hepatitis patients, miR-125b-5p and miR-3613-3p were up-regulated, whereas miR-940 was down-regulated, which might affect cell proliferation through the signal transducer and activator of transcription 3 pathway. In early fibrosis, miR-34b-3p, miR-1224-3p, and miR-1227-3p were up-regulated, while miR-499a-5p was down-regulated. They possibly mediate chronic inflammation. In advanced fibrosis, miR-1, miR-10b-5p, miR-96-5p, miR-133b, and miR-671-5p were up-regulated, while miR-20b-5p and miR-455-3p were down-regulated, possibly allowing chronic disease progression [42]. Qiu et al. demonstrated a positive correlation between let-7a miRNA transcription and HBV replication at patients with HCC, as well as in HCC cell lines. Also, they suggest that let-7a miRNA inhibition could be used as an antiviral therapy to inhibit HBV replication and to prevent the development of HCC [43].

NAFLD and microRNAs

NAFLD is a multifactorial disease that involves a variety of liver injury which may end up as HCC. This disease is defined as evidence of hepatic steatosis and is closely associated with metabolic syndrome, obesity, and cardiovascular diseases [14,44,45]. Progression of NAFLD to fibrosis is a result of multiple factors interaction, such as: insulin resistance, lipotoxicity, dietary, fatty acids, lipopolysaccharide generated by gut microbiota, inflammation, oxidative stress, genetic and epigenetic [44]. Liver biopsy still represents the only reliable method to establish nonalcoholic fatty liver disease severity and HCC [46]. Ideal biomarkers for follow-up the evolution of NAFLD to HCC should be minimally invasive, accurate, specific and sensitive. They need to be clinically validated. The discovery of important role of miRNAs in glucose and lipid metabolisms regulation reveals a connection between miRNAs and metabolic liver disorders. NAFLD is strongly associated with metabolic syndrome [47]. Several microRNAs have been extensively investigated and accepted to be potential regulators in the pathogenesis of NAFLD. Zhu et al. showed that the expression levels of miR-33, miR-33-5p and miR-33-3p were significantly decreased in high-fat-diet-induced NAFLD. These microRNAs interact with apelin (apln) and versican (vcan) genes which have been reported to be associated with NAFLD [44]. Also, in this study the expression levels of miR-200b-3p, miR-200b-5p and miR-200c-3p were increased in the liver of NAFLD rats. These microRNAs and their target genes involved in cholesterol biosynthetic processes might be crucial regulators in the pathogenesis of NAFLD [44]. MicroRNA-34a has a crucial role in regulation of liver inflammation and it is associated with significantly increased severity in NAFLD. Up-regulation of miR-34a in liver steatosis is an important part of a protective negative regulatory feedback mechanism intended to limit disease progression by preventing excessive lipid accumulation in the liver [48]. MicroRNA-34a could be used as a biomarker for disease severity in patients with NAFLD [45]. Serum miR-122 and miR-192 have a dual behaviour in NAFLD: increases in mild fibrosis and decreases in the most severe stages [46]. Feng et al. showed inflammatory factors are crucial mediators of the aberrant expression of hepatic miRNAs associated with NAFLD. Thus, they found that miR-200a, miR-200b, miR-200c, miR-146a, miR-146b and miR-152 were up-regulated both in fatty liver tissues and in cultured cells with FFAs and proinflammatory factors [49]. Nie et al. showed that the miR-182, miR-29b-3p and miR-741-3p are up-regulated in rats with NAFLD suggesting that these differentially expressed microRNAs may be involved in the pathogenesis of NAFLD [14].

Conclusion

Promising research centers around miRNAs expressions in chronic liver diseases progress has been made in an attempt to use the valuable biomarkers as predictors for liver injury prognosis and transition to HCC. Correlation of microRNAs pattern expression with clinical and pathological parameters indicates that microRNAs could become useful molecular biomarkers for hepatocarcinogenesis with different etiology. The assessment of aberrantly expressed microRNA in chronic liver diseases could reveal novel mechanisms of tumorigenesis. Virus encoded miRNA (HBV) may provide new potential biomarkers for chronic HBV infection suggesting a new insight of HBV replication mechanisms and regulation. Serum microRNA levels can reflect differences in the etiology and stage of chronic viral hepatitis. The complex miRNA-mRNA regulatory systems will advance our understanding of viral (HBV, HCV) – host interactions and disease mechanisms for developing innovative, preventive diagnostic approaches.

References


