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Expression of MicroRNA-155 in Hepatitis B Virus-Related to Hepatocellular Carcinoma

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ABSTRACT
Hepatitis B virus (HBV) infection is still a global health problem and a major precipitation factor for hepatocellular carcinoma (HCC). MicroRNAs (miRNAs) consist of small non-coding RNAs that regulate gene expression at the post-transcriptional level, thus involving in primary biological processes, including proliferation, differentiation, and apoptosis of cells. MicroRNA-155 is known as an oncogene in some cancer cells and has a relationship with the severity of cancer cells. Here, we investigated the level of microRNA-155 expression in hepatocellular carcinoma patients associated with HBV infection. It was found that the expression of microRNA-155 in the blood plasma of HBV-related to HCC patients was higher and up-regulated by 2.33-fold compared to the blood plasma of HCC patients without HBV infection. This result suggests that microRNA-155 may have a major regulatory role in hepatocarcinogenesis associated with hepatitis B infection and potentially be a therapeutic target for HBV patients developing HCC treatment.

Keywords: *MicroRNA-155, HBV Infection, Hepatocellular carcinoma*

1. INTRODUCTION

Hepatitis B virus (HBV) infection is a world health problem which is still a common infection that is often found with around 2 million people having a history of contact with HBV. More than 350 million people in the global population are chronic HBV carriers making the virus one of the most common human pathogens. HBV is a member of the small enveloped DNA family of viruses called hepadnaviruses, which infect several mammals and birds. HBV is a member of the DNA-encased virus family called hepadnavirus, which can infect mammals and birds. Many epidemiological studies have shown that there is a strong correlation between chronic HBV infection and the development or incidence of hepatocellular carcinoma (HCC). Chronic infection due to HBV can progress to cirrhosis and subsequently change the structure and the function of the liver cells to become HCC if not treated properly [1].

HBV can lead to the formation of liver cancer in several ways. Many sources describe the various pathways involved in this process, including the accumulation of genetic changes due to immune-mediated liver inflammation, oxidative stress induction, and several specific mechanisms by viruses involving viral proteins such as HBx and HBs. These viral proteins cause mutagenesis by integrating HBV DNA into the host genome, which will interfere with endogenous gene expression or cause chromosomal instability, epigenetic changes through modification of genomic methylation status, and also regulation by microRNA [2].

HCC is one of the major malignant liver tumors that significantly threaten global health. HCC was counted for 70% -80% as a primary liver cancer which is rare to be detected at an early stage and the number of survival of HCC patients continues to decline. HCC ranks 5th as malignant cancer commonly occurs in males and the 7th in women with mortality rates of male HCC patients ranks 2nd and female patients at 6th in the world. About ¾ of the total cases occur in Asian countries due to the high prevalence of chronic HBV infection [3,4].

HCC developments allegedly involve the alteration of essential gene regulation for cellular processes such as control of cell cycle, cell growth, apoptosis, and migration, and cell deployment. Many studies have studied the genes and proteins that underlie the development of HCC. One of the regulators that regulate the expression of a protein is microRNA. MicroRNAs are also often associated with a process of carcinogenesis due to able to control the expression of key proteins involved in cancer-associated

pathways [5]. MicroRNA is a short non-coding RNA consisting of ~ 22 nucleotides. MicroRNA is a post-transcriptional regulation key of gene expression. MicroRNAs interact with 3 'untranslated regions (UTRs) of mRNAs by forming a complex with RNA-induced silencing factor (RISC) that causes translational suppression and/or degradation of the target [6,7].

MicroRNA-155 is expressed in B cells, T cells, monocytes, and macrophages that are active during the processing of B-Cell Integration Clusters (BIC). This BIC gene is located on chromosome 21q21. MicroRNA-155 is one of the microRNAs that have increased regulation in some solid cancer and hematological malignancies. MicroRNA-155 has been widely reported to have associations with the progression of leukemia, breast cancer, lung cancer, and stomach tumors and is often a target in diagnosis, prognosis, and therapy [8,9].

The previous study identified that microRNA-155 is reported as an oncomir in HCC because of increased expression in cancer cells and targets many tumor suppressor genes in HCC. MicroRNA-155 has significant elevation expression levels in HCC tissue 1.5-6 times compared to normal liver tissue [10]. A study conducted by Guan et al. showed a significantly increased regulation of microRNA-155 in HCC tissue and had a positive correlation to HCC cell invasion(11). Some studies also suggest that increased expression of microRNA-155 in HCC shows a tumorigenic role in accelerating cell proliferation, and regulating cancer stem cells, and self-renewal in HCC cells [9].

There have been many studies showing the formation of hepatocellular carcinoma cells has various mechanisms. One of the main mechanisms is hepatocarcinogenesis by chronic hepatitis B virus infection. Moreover, it is also known that microRNA has an important regulatory role in hepato-carcinogenesis, including microRNA-155. Therefore, this study would like to investigate microRNA-155 expression in HCC patients with the association of HBV infection.

2. PATIENTS AND METHODS

2.1. Patients and Samples

This research was an analytical, observational study with a cross-sectional design conducted in 31 blood plasma of HCC patients. Subjects were recruited from General Hospitals at Yogyakarta, Indonesia. The inclusion criteria were: aged 18-65 years old, primary HCC diagnosis was confirmed by histology examination, and two imaging (Doppler ultrasound and 3 phase MSCT-scan abdomen). The exclusion criteria were: severe infection diseases, cardiovascular disease, and cancers originated from other organs. All subjects who meet the criteria of the study read and signed the informed consent form before the study began.

2.2. MicroRNA Analysis

The RNA isolation was prepared from blood plasma (6 mL blood venous was fixed by EDTA, centrifuged at 15,000g roe 10 minutes). The total RNA isolation was performed from 200 µl of blood plasma using miRCURY RNA Isolation Kit-Biofluid (Cat No.300112, Exiqon). Total RNA isolation for microRNA was performed using miRCURY RNA isolation Kit-Biofluid, followed by cDNA synthesis for miR using Universal cDNA synthesis kit ll, 8-64 rxns, and thermal cycler PCR (Biorad c 1000). qRT-PCR for microRNA was performed using Real-time qPCR (Biorad CFX 96). The sequence-specific primers were used for miR target: miR-155-5p. Relative quantification of microRNA-155 expression in HBV related HCC versus HCC without HBV was calculated using $2^{-\Delta\Delta Cq}$ method, ΔΔCq = ΔCq (experimental group) - ΔCq (control group). The calculation of expression levels of regulation used Livak's method.

2.3. Data Analysis and Statistics

The expression of miR 155-5p was analyzed by Biorad CFX Manager 96 Software to obtain Cq (cycle of quantification), melting curve, and melt peak curve values. The difference in the expression of microRNA between HBV related HCC patients versus HCC patients without HBV was tested using the Mann-Whitney test with $p<0.05$ as a significant value.

3. RESULT

3.1. MicroRNA-155 Expression was Up-regulated in Hepatitis B Virus-Related to Hepatocellular Carcinoma Patients

A total of 31 HCC subjects who enrolled in the study were examined for the expression of microRNA-155 in blood plasma samples. There were 20 subjects with HBV infection (HBsAg positive) and 11 subjects without HBV infection (HBsAg negative). MicroRNA-155 expression was up-regulated in 70% (14/20) of HBV-related to HCC blood plasma samples. While in HCC patients without HBV infection blood plasma samples, there were only 18% (2/11) had up-regulated microRNA-155 expression (Table 1).

Based on Livak's method, the expression of microRNA-155 in HBV related HCC subjects had an increasing regulation as much as 2.33 fold compared to HCC subjects without HBV infection. This was also demonstrated through the Mann-Whitney test that microRNA-155 was significantly up-regulated in HBV-related HCC blood plasma compared to HCC patients without HBV infection blood plasma (p-value=0.006). Based on this regulation, microRNA-155 may be acting as oncomir in HBV related to HCC subjects.

4. DISCUSSION

HCC development is a multilevel process involving many factors. The various ways in which we can understand the principles underlying this malignancy have begun to focus on the molecular changes that occur in cell signaling and changes in the genome. To date, only a few publications have disclosed the role of microRNA-155 in viral infection. In this study, we focused on investigating microRNA-155 expression as a key post-transcription regulator of HBV-related HCC development. It has been widely reported that these microRNAs have correlation with immunity and immune-related inflammatory diseases. [12]. Moreover, It was also mentioned that microRNA-155 involved on the pathogenesis of several malignancies, including breast cancer [13,14], colon cancer [15], pancreatic cancer [16], and other malignancies. In addition, Wang et al. reported a significant increase in microRNA-155 expression in primary human HCC tissue [17]. Up-regulation of microRNA-155 leads to increased activity of tumorigenesis of HCC by targeting multiple tumor suppressors. Increased regulation of microRNA-155 is known to inhibit the expression of SOX6 (SOX family of transcription factor) which has a role in suppressing the proliferation of hepatocellular carcinoma cells. MicroRNA-155 also regulates the expression of AT-rich interaction domain 2 (ARID2) that plays a role in the phosphorylation of Akt signaling pathway, apoptosis regulation, and cell cycle. These regulations had the effect of increasing the tumor growth in cancer cells17. Another study also reported that microRNA-155 can trigger HCC by targeting the sex-determining region Y box 6 (SOX6), a transcriptional regulator that prevents cell growth through stimulation of the p21 growth regulator in a p53-dependent manner [18].

In the current study, elevated microRNA expression was observed in HBV-related HCC patient's blood plasma samples compared to HCC patients without HBV infection. This elevation was statistically significant and calculated as 2.33 fold, meaning that the relative expression of microRNA-155 in blood plasma samples of HCC patients with HBV infection was 2.33 times higher than in HCC patients without HBV infection. Research conducted by Wang et.al. also supported this finding by showing the expression of microRNA-155 was higher in adjacent non-tumor tissue of HBV-positive patients compared to adjacent non-tumor tissues of HBV-negative patients [22].

This result is mainly connected to the persistent level of HBx viral protein in HBV infection. Some studies showed that HBx protein mediated the upregulation of microRNA-155 [9,19]. In the current finding, it was consistent with research conducted by Su et al. mentioned that the expression of microRNA-155 was up-regulated by HBx viral protein in HBV-related HCC results in the downregulation of suppressor of cytokine signaling-1 (SOCS1) expression contributing to the elevation of Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway, leading to the suppression of HBV infection by augmenting interferon (IFN) signaling [21]. Alternatively, HBx also upregulates microRNA-155 which can attenuate HBV replication by closing the binding CCAAT / enhancer-binding protein (C / EBP) and activating the HBV enhancer (Enh) 11 / core promoter [17]. Furthermore, a recent study by Fu et al. found upregulation of microRNA-155 expression by dysregulation of HBx can promote proliferation cell, invasion and migration, but prevent apoptosis in HCC cells through direct targeting at 3 UTR of phosphatase and tensin homolog (PTEN) [23].

Table 1. Quantification of microRNA-155 relative expression

Sample group[a]	Regulation[b]		ΔCq microRNA-155 (Mean±SD)	ΔΔCq microRNA-155	Fold Change ($2^{-\Delta\Delta Cq}$)[b]	p-value[c]
	Up-regulated (n/%)	Down-regulated (n/%)				
HBV-related HCC (n=20)	14 (70)	6 (30)	6,53 ± 1,44	-1,52	Increasing regulation 2.33 fold	0.006
HCC without HBV infection (n=11)	2 (18.1)	11 (81.9)	8,05 ± 1,22			

analyzed by Biorad CFX 96 ManagerTM; [b.] Livak's method; [c.] analyzed by Mann-Whitney test with significant value p<0.05

MicroRNA-155 is widely known to act as an enchancer of antiviral immunity against HBV[24]. Wang et al. have demonstrated study showing that increased microRNA-155 expression can lead to prolonged exposure of inflammatory responses that can increase liver injury and induce HCC cell formation. [17]. It means, the presence of abnormal expression of microRNA-155 had implications for modulating the immune response during HBV infection, which can lead to the development of immune-mediated liver damage, thereby precipitating HCC [20].

5. CONCLUSION

In conclusion, the current study has shown that microRNA-155 was significantly up-regulated in HBV-related HCC patients. MicroRNA-155 is considered as an oncogenic microRNA with concomitant suppression of

their tumor suppressor targets. The presence of HBx protein in HBV infection mediates the upregulation of microRNA-155 and leads to the persistence level of this microRNA in the hepatocarcinogenesis process. We hope that further investigations into possible definitive mechanisms of HBV-related HCC will help improve understanding of this global malignancy and hopefully lead to the development of molecular-based therapeutic strategies targeting microRNA-155.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study design and examinations of the research, to the result analysis and to the writing of the paper content.

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