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Virology

Brief Communication

microRNA expression in hepatitis B virus infected primary treeshrew hepatocytes and the independence of intracellular miR-122 level for *de novo* HBV infection in culture

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ABSTRACT

Infection of Hepatitis B virus (HBV) in hepatocytes has been known to be controlled by multiple cellular factors, while the relationship of the infection and liver microRNAs remains obscure. In this study, a miRNA database, containing 168 unique mature miRNA members from primary hepatocytes of a primate-like animal, northern treeshrew (*Tupaia belangeri*) that is the only species susceptible for HBV infection other than human and chimpanzee, was established. The relative level of a liver predominant microRNA, miR-122, was markedly increased upon HBV infection of the primary tupaia hepatocyte (PTH). However, introducing neither miR-122 nor its antagonist anti-miR-122 into PTHs, or, HepG2–NTCP that is HepG2 cells with the newly identified receptor sodium taurocholate cotransporting polypeptide (NTCP) did not alter the viral infection on these cells. These data suggest that *de novo* HBV infection of cultured hepatocytes does not depend on the expression level of intracellular miR-122 of the target cells.

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Introduction

Hepatitis B Virus (HBV) is a small DNA virus replicating via a unique reverse transcription step that is accurately regulated by viral and host factors (Dandri and Locarnini, 2012; Hirschman, 1979; Seeger and Mason, 2000). It infects liver parenchyma hepatocytes and causes acute or chronic hepatitis. About 2 billion people have been infected by HBV and 240 million of them are chronically infected worldwide. HBV infection frequently leads to more sever diseases such as cirrhosis and liver cancer and about 600,000 people die of HBV and its associated diseases annually (Ott et al., 2012; Wright, 1980). Available treatments for HBV, including interferon and nucleotide/nucleoside analogs, may reduce viral load but hardly lead to a cure for hepatitis B (Lai and Yuen, 2008). Novel interventions targeting to other steps of the viral life cycle with new action mechanisms are needed.

MicroRNAs (miRNAs) are small non-coding RNA molecules found in plants and animals. Biogenesis of miRNA is from a long primary transcripts, it functions in transcriptional and post-transcriptional regulation levels by targeting a diverse set of cellular genes (Murchison and Hannon, 2004). There are increasing evidences indicating that miRNAs also play important roles in virus infection. It has been shown that miR-122 is critical for hepatitis C virus (HCV) infection (Jopling et al., 2005). By interacting with the 5′ non-coding region of the HCV genome, miR-122 greatly facilitates HCV viral RNA replication; while sequestration of miR-122 in the liver resulted in marked loss of HCV viral RNAs *in vitro* and *in vivo* (Jopling et al., 2005). Recently, inhibiting miR-122 was shown to be an effective strategy in treating HCV infected primates or patients with high efficacy, prolonged virological response and low risk of resistance (Lanford et al., 2010; Janssen et al., 2013). Modulating miRNA thus presents a new and promising strategy for treatment against viral infections.

Studies of the infection of HBV, including those on the interplays of the virus and cellular miRNAs, has been impeded by the lack of an effective culture system *in vitro*. Indeed, only primary

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human hepatocyte (PHH), primary *Tupaia belangeri* hepatocyte (PTH) and HepaRG, a human hepatic progenitor cell line, were shown to be able to support viral infection of HBV *in vitro* (Glebe and Urban, 2007). Therefore, previous investigations regarding miRNA for HBV infection were mainly carried out by using transfection of viral DNA into hepatocarcinoma cell line HepG2 or Huh7 (Liu et al., 2011; Wang et al., 2012). Among various miRNAs, miR-122 was of particular interest to HBV infection due to its high liver specificity and abundance as well as its important role in liver homeostasis (Chen et al., 2011; Li et al., 2013; Tsai et al., 2012). However, the physiological relevance of these studies remains uncertain since they were not from *bona fade* HBV infection. Using the primary hepatocytes from tupaia, we recently showed that sodium taurocholate cotransporting polypeptide (NTCP), a liver bile acids transporter responsible for most Na$^+$-dependent bile acids uptake in hepatocytes, is a functional receptor for HBV, its satellite Hepatitis D virus (HDV) (Yan et al., 2012) and infection of woolly monkey HBV (WMHBV) on PTH (Zhong et al., 2013). HepG2 cells complemented with human or tupaia-NTCP could be infected by HBV at a similar efficiency of that on primary human hepatocytes. In this study, we profiled the expression of microRNAs in PTH through RNA deep sequencing, we further utilized PTH as well as HepG2–NTCP culture systems to investigate the role of miRNA-122 in the *de novo* HBV infection of the target cells.

Results

RNA deep sequencing of the small RNAs from hepatocytes of treeshrew and establishment of tupaia hepatocyte microRNA databases

We first generated a tupaia hepatocyte miRNA database by using whole RNA from PTH from tree shrew (*Tupaia belangeri chinensis*). Freshly isolated PTH were cultured on collagen coated plates and inoculated or not with HBV at 100 multiplicities of genome equivalents (m.g.e.). Total RNA was extracted on 5 days post inoculation (5 dpi), RNA with size between 18–28 nt was fractionated by 7 M urea denaturing PAGE Gel. These small RNAs were ligated with the adapters at both 3′ end and 5′ end, followed by reverse transcription and polymerase chain reaction (RT–PCR) to amplify the cDNA, the small DNA was then sequenced with the method of 36 cycle single end sequencing using a Genome Analyzer IIx instrument (Fig. 1A). After data collection and processing, about 10 million raw total reads of miRNA were obtained in
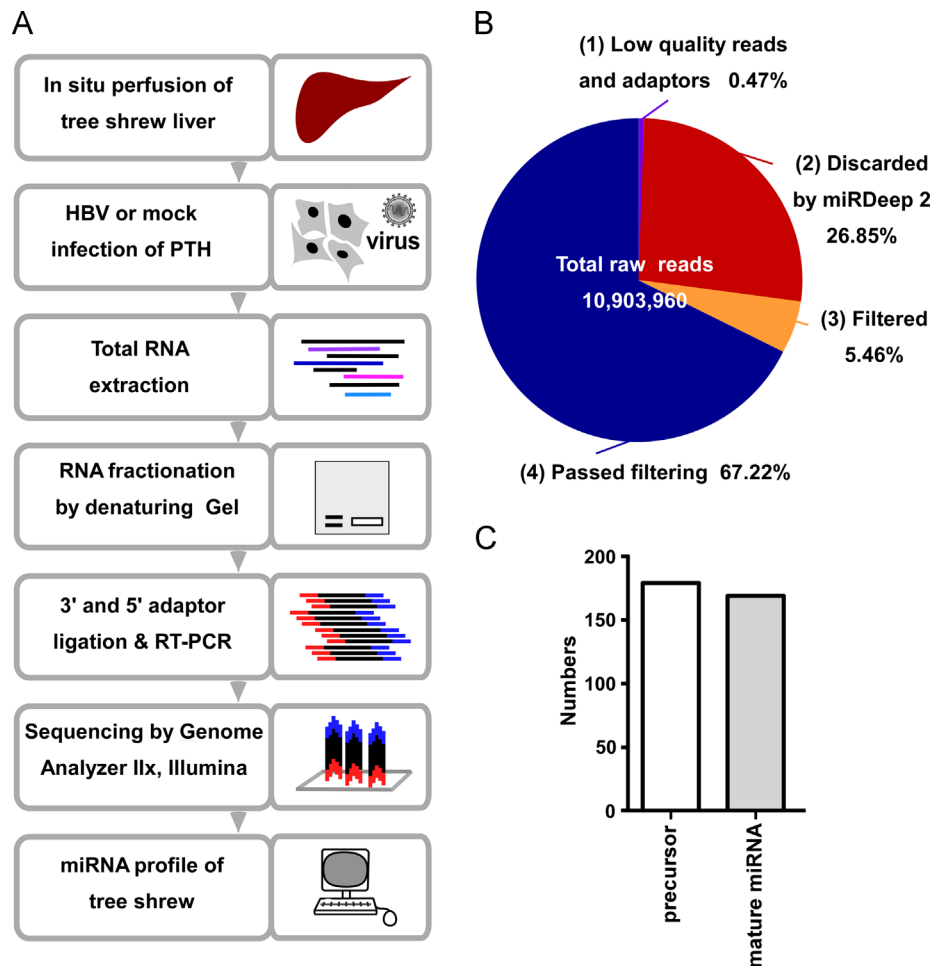
Fig. 1. *Profiling miRNAs of treeshrew hepatocyte by deep sequencing*: (A) Flow chart of miRNA data collection and processing. Fresh PTHs were isolated and plated, the cells were inoculated or not with HBV. At 5 dpi, total RNA were extracted, small RNAs with size of 18–28 nt were fractioned from 5–10 μg of total RNA using 7 M urea polyacrylamide gel. cDNA libraries was prepared by RT–PCR after adapter ligation at 3′ and 5′. The cDNAs were sequenced with a 36 cycle single end sequencing protocol on a Genome Analyzer IIx, Illumina instrument. Total raw reads were obtained, and subjected to data analysis, unique mature miRNA was presented. (B) Pie chart of microRNA raw reads of uninfected PTH. Total reads were 10,903,960. Low quality reads and adapter sequences were discarded (~0.47%). After prediction by miRDeep2, about ~26.85% of small RNA was filtered. The precursors and mature sequences passed filtering were further selected, and additional ~5.47% of total raw reads was discarded. About 67.22% of total raw reads passed the entire filtering and selection process. (C) The number of precursors and unique mature miRNAs from PTH. 179 precursors and 168 unique mature miRNAs was obtained.