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Review

The hepatitis B virus ribonuclease H as a drug target

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ABSTRACT

Chronic hepatitis B virus (HBV) infection is a leading cause of hepatitis, liver failure, and hepatocellular carcinoma. An outstanding vaccine is available; however, the number of infections remains high. Current anti-HBV treatments with interferon α and nucleos(t)ide analogs clear the infection in only a small minority of patients, and either induce serious side-effects or are of very long duration. HBV is a small, enveloped DNA virus that replicates by reverse transcription via an RNA intermediate. The HBV ribonuclease H (RNaseH) is essential for viral replication, but it has not been exploited as a drug target. Recent low-throughput screening of compound classes with anti-Human Immunodeficiency Virus RNaseH activity led to identification of HBV RNaseH inhibitors in three different chemical families that block HBV replication. These inhibitors are promising candidates for development into new anti-HBV drugs. The RNaseH inhibitors may help improve treatment efficacy enough to clear the virus from the liver when used in combination with existing anti-HBV drugs and/or with other novel inhibitors under development. This article forms part of a symposium in *Antiviral Research* on "An unfinished story: from the discovery of the Australia antigen to the development of new curative therapies for hepatitis B."

Contents

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1. HBV: disease and genomic replication

Hepatitis B virus (HBV) is an enveloped DNA virus that replicates in the liver. It is a member of the *Hepadnaviridae* family that includes the animal viruses Duck Hepatitis B Virus (DHBV) and Woodchuck Hepatitis Virus (WHV) (Dandri et al., 2005). HBV chronically infects up to 350 million people world-wide (Seeger et al., 2013). The infection causes hepatitis, fibrosis, cirrhosis, liver failure and over half of all cases of hepatocellular carcinoma (Lavanchy, 2005). Together, this leads to an annual death toll of over 500,000 (Sorrell et al., 2009).

HBV replicates its genome by reverse transcription of a viral pregenomic RNA within cytoplasmic capsid particles (Summers and Mason, 1982; Tavis and Badtke, 2009). Reverse transcription is catalyzed by two enzymatic activities located on different domains of the viral polymerase protein (Chang et al., 1990; Radziwill et al., 1990). The reverse transcriptase copies the pregenomic RNA into minus-polarity DNA, and the ribonuclease H (RNaseH) destroys the viral RNA after it has been copied so that the plus-polarity DNA strand can be made. The direct product of HBV replication is a partially double-stranded DNA molecule within cytoplasmic capsid particles. These capsids may be enveloped and secreted from the cell as mature virions, or they may be transported to the nucleus where the DNA is converted to an episomal covalently-closed circular molecule (cccDNA) (Fig. 1). The cccDNA is key to HBV biology because it is the transcriptional template for all HBV RNAs (it is functionally equivalent to an integrated retroviral provirus).

2. Limitations to current anti-HBV therapy

The nucleos(t)ide analog drugs that dominate HBV therapy have transformed management of HBV chronic infections. The best drugs, tenofovir and entecavir, suppress HBV replication by 4–5 $\log_{10}$ or more in up to 70–90% of patients, often to below the common detection limit of ~200–400 copies/ml (Cox and Tillmann, 2011; Kwon and Lok, 2011; van Bommel et al., 2010; Woo et al., 2010) with little to no drug resistance even after prolonged treatment (Zoulim, 2011). This remarkable success for a monotherapy has made HBV infection controllable for those able to afford its high costs (Block et al., 2013; Lui et al., 2010), with major health benefits for the treated individuals (Dienstag, 2009; Liaw, 2013; Marcellin and Asselah, 2014).

Despite the profound suppression of HBV titers induced by nucleos(t)ide analogs, treatment reduces cccDNA levels by only about 1 $\log_{10}$ even after years of continuous drug exposure (Cheng et al., 2011; Werle-Lapostolle et al., 2004; Wong et al., 2006). Consequently, HBV infections are cleared in only 2–8% of patients after many years of treatment (Liaw, 2013). This persistence of the cccDNA causes viral titers to resurge if the nucleos(t)ide analogs are withdrawn, and hence treatment is essentially life-long. cccDNA persistence is in part due to its long apparent halflife, which is estimated to be 10 days in non-dividing tissue culture cells (Cai et al., 2012) and may be up to 30–60 days in the liver based on inhibitor studies in animal models (Addison et al., 2002; Moraleda et al., 1997; Zhu et al., 2001). However, maintenance of the cccDNA is also due to ongoing viral HBV replication during nucleos(t)ide analog therapy. This is revealed by sequential accumulation of resistance mutations to the nucleos(t)ide analogs (Ghany and Liang, 2007; Monto et al., 2010; Zoulim and Locarnini, 2009), and is confirmed by genetic analyses of viral DNA in the liver during therapy that explicitly demonstrate replenishment of the cccDNA even in the absence of clinically detectable viremia (Coffin et al., 2011). This opens an opportunity for improving antiviral therapy by suppressing HBV replication further than is currently possible.

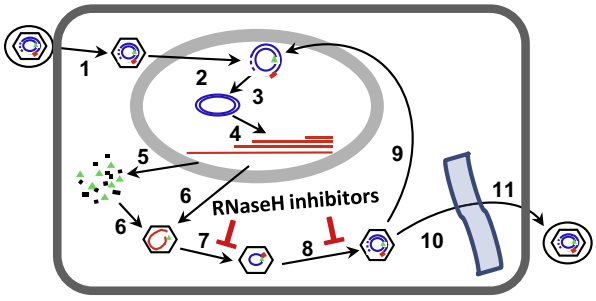

Fig. 1. HBV replication cycle. Binding of HBV virions to hepatocytes followed by fusion of the viral envelope with the plasma membrane releases core particles into the cytoplasm **(1)**. Core particles are transported to the nucleus, where they release the partially double-stranded viral DNA **(2)**, and the DNA is converted into cccDNA inside the nucleus **(3)**. Viral RNAs are transcribed **(4)** and translated to produce the viral proteins **(5)**. The viral pregenomic RNA is encapsidated into core particles as a complex with viral polymerase protein **(6)**. The minus-polarity DNA strand is synthesized by the reverse transcriptase activity of the polymerase with concomitant degradation of the pregenomic RNA by the RNaseH activity **(7)**. The plus-polarity DNA strand is synthesized by the reverse transcriptase **(8)**. Mature core particles are then either transported back into the nucleus to maintain the cccDNA pool **(9)** or are enveloped by budding into the endoplasmic reticulum **(10)** and are non-cytolytically secreted as mature virions **(11)**. RNaseH inhibitors block steps 7 and 8. Modified from (Hu et al., 2013).

3. RNaseH enzymes

3.1. General features of RNaseH enzymes

RNaseHs (Hostomsky et al., 1993) cleave RNA when the RNA is bound to DNA in a heteroduplex. They help destroy failed transcription products, remove RNA primers during DNA synthesis, and contribute to reverse transcription of viral and retrotransposon genomes. They belong to the nucleotidyl transferase enzyme superfamily whose members share a similar protein fold and enzymatic mechanisms (Nowotny, 2009; Yang and Steitz, 1995). This family includes *Escherichia coli* RNaseH I and II (Katayanagi et al., 1990; Lai et al., 2000), human RNaseH 1 and 2 (Lima et al., 2001), retroviral RNaseH and integrases (Dyda et al., 1994), and many other nucleases and transposases. The canonical RNaseH structure contains about 100 amino acids with four conserved carboxylates (the "DEDD" motif) that coordinate two divalent cations (Nowotny et al., 2005). Integrases have a similar protein fold, but they employ only three carboxylates to coordinate the essential divalent cations (the "DDE" motif) (Nowotny, 2009). The nucleic acid cleavage mechanism is believed to require both divalent cations to promote a hydroxyl-mediated nucleophilic scission reaction (Klumpp et al., 2003; Nowotny and Yang, 2006; Yang and Steitz, 1995).

3.2. The HBV RNaseH

The HBV RNaseH was originally identified by sequence homology to known RNaseH enzymes (Khudyakov and Makhov, 1989; Schodel et al., 1988), and its existence was confirmed by evaluating the effects on viral replication of mutating the predicted RNaseH motifs (Chang et al., 1990; Chen and Marion, 1996; Gerelsaikhan et al., 1996). Ablating HBV RNaseH activity causes accumulation of long RNA:DNA heteroduplexes, truncates most minus-polarity DNA strands, and blocks production of the plus-polarity DNA strand. Virions can still be secreted when the RNaseH activity is blocked, but they contain defective RNA:DNA heteroduplex genomes (Gerelsaikhan et al., 1996; Wei et al., 1996). These genomes are biologically inert due to the severe damage to the viral DNA,