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Reactive oxygen species promote heat shock protein 90-mediated HBV capsid assembly

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ABSTRACT

Hepatitis B virus (HBV) infection induces reactive oxygen species (ROS) production and has been associated with the development of hepatocellular carcinoma (HCC). ROS are also an important factor in HCC because the accumulated ROS leads to abnormal cell proliferation and chromosome mutation. In oxidative stress, heat shock protein 90 (Hsp90) and glutathione (GSH) function as part of the defense mechanism. Hsp90 prevents cellular component from oxidative stress, and GSH acts as antioxidants scavenging ROS in the cell. However, it is not known whether molecules regulated by oxidative stress are involved in HBV capsid assembly. Based on the previous study that Hsp90 facilitates HBV capsid assembly, which is an important step for the packing of viral particles, here, we show that ROS enrich Hsp90-driven HBV capsid formation. In cell-free system, HBV capsid assembly was facilitated by ROS with Hsp90, whereas it was decreased without Hsp90. In addition, GSH inhibited the function of Hsp90 to decrease HBV capsid assembly. Consistent with the result of cell-free system, ROS and buthionine sulfoximine (BS), an inhibitor of GSH synthesis, increased HBV capsid formation in HepG2.2.15 cells. Thus, our study uncovers the interplay between ROS and Hsp90 during HBV capsid assembly.

1. Introduction

Hepatitis B virus (HBV), a member of the hepadnaviridae family, has infected over two billion people worldwide [1]. Approximately 240 million HBsAg-positive individuals remain chronically infected and chronic HBV infection is associated with liver disease, including the development of hepatocellular carcinoma (HCC) and liver cirrhosis [1,2]. HBV generates reactive oxygen species (ROS) through altering mitochondrial function, and ROS can affect viruses by changing the redox state of the cell and by activating transcription factors such as NF-kB, which elevates the level of viral replication [3–5].

HBV has a partially double-stranded DNA genome consisting of four open reading frames (ORF), denoted Cp (core protein), Sp (surface protein), Pol (polymerase), and HBx (X protein) [6]. Following the infection of hepatocytes, the HBV genome is converted into covalently closed circular DNA (cccDNA) by a DNA repair system in the nucleus [7]. Subsequently, pregenomic RNA (pgRNA) is produced from host RNA polymerase, and it is packaged from the core protein with polymerase and other components such as host factors in the cytoplasm.

One of the host factors for HBV capsid formation is heat shock protein 90 (Hsp90). Hsp90 is activated by p23 and ATP to form the Hsp90 complex facilitating maturation of client protein [8], and the activated Hsp90 facilitates HBV capsid assembly in encapsidation [9]. Moreover, Hsp90 is associated with oxidative stress. In oxidative stress, ROS influences Hsp90 protecting activated 20S proteasomes to promote degradation of oxidized substrates [10].

Cells continuously produce ROS, which induce oxidative stress and are neutralized by antioxidant systems, as part of the metabolic process [11]. A low level of ROS is essential in several physiologic processes of the cell including proliferation, apoptosis, cell cycle arrest, etc [12]. At high ROS level, however, ROS causes oxidative stress and a toxic environment to the cells [13]. This stressful condition is known to play a major role in HCC mainly by enhancing DNA damage and by modifying some key cellular process for development [13].

Virus-induced ROS have an effect not only on infected cells but also on the virus itself. Based on the previous study that HBV-induced ROS can cause HCC [3,4,14] and the expression level of

Abbreviations: NAC, N-acetyl-L-cysteine; qPCR, quantitative PCR; CD, circular dichroism; H_2O_2, hydrogen peroxide; GSH, reduced glutathione.

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Hsp90 is elevated in HCC tumor tissue [15], we hypothesized that ROS might improve Hsp90-driven HBV production. In this research, we exploited cell-free system and HepG2.2.15 cells to test the functional significance of ROS in Hsp90-driven HBV capsid formation. We aimed to determine the effect of oxidative stress on HBV capsid assembly. Our results showed that HBV capsid formation was increased with ROS-induced changes in the conformation of Hsp90 but was decreased by ROS without the Hsp90. Meanwhile, we also found repressive effect of an antioxidant, glutathione (GSH), on HBV capsid formation. Overall, we discovered a previously uncharacterized relation between ROS and Hsp90 and a function of GSH for the Hsp90-drived HBV capsid assembly.

2. Materials and methods

2.1. Expression and purification of Cp149, p23, B23, and Hsp90

Cp149, p23, nucleophosmin (B23), and Hsp90 were cloned directly using a pET28b vector for Cp149, p23, and Hsp90 (Novagen) and pET21a vector for B23 (Novagen) respectively. All constructs were transformed into BL21 (DE3) + pLysS *E. coli* (Novagen), and purified and stored with 10% glycerol at −20 °C as previously described [9,16].

2.2. Analysis of HBV capsid assembly and sucrose density gradient analysis

To study the effect of capsid formation from 20 μM Cp149 dimer with several addictive including other proteins (20 μM of BSA, B23, Hsp90, p23) and chemicals, such as 0.5 mM ATP-r-S (Merck), 2 μM geldanamycin (GA, A.G. Scientific, Inc.), 50–200 μM hydrogen peroxide (H_2O_2, sigma), 200 μM *N*-acetyl-L-cysteine (NAC, sigma) and 1 mM GSH (sigma), assembly reaction was conducted in assembly buffer as a previous study [17]. Sucrose density gradient analysis was conducted by ultra-centrifugation as a previous study [9]. Fraction from 1 to 10 (10–50%) was detected by 15% SDS-PAGE using immunoblot analysis with rabbit polyclonal anti-HBV core antibody (Dako).

2.3. CD analysis

CD measurements were carried out with a J-815 (Jasco). Spectra were obtained using 1 nm bandwidth, a scan rate of 50 nm/min and

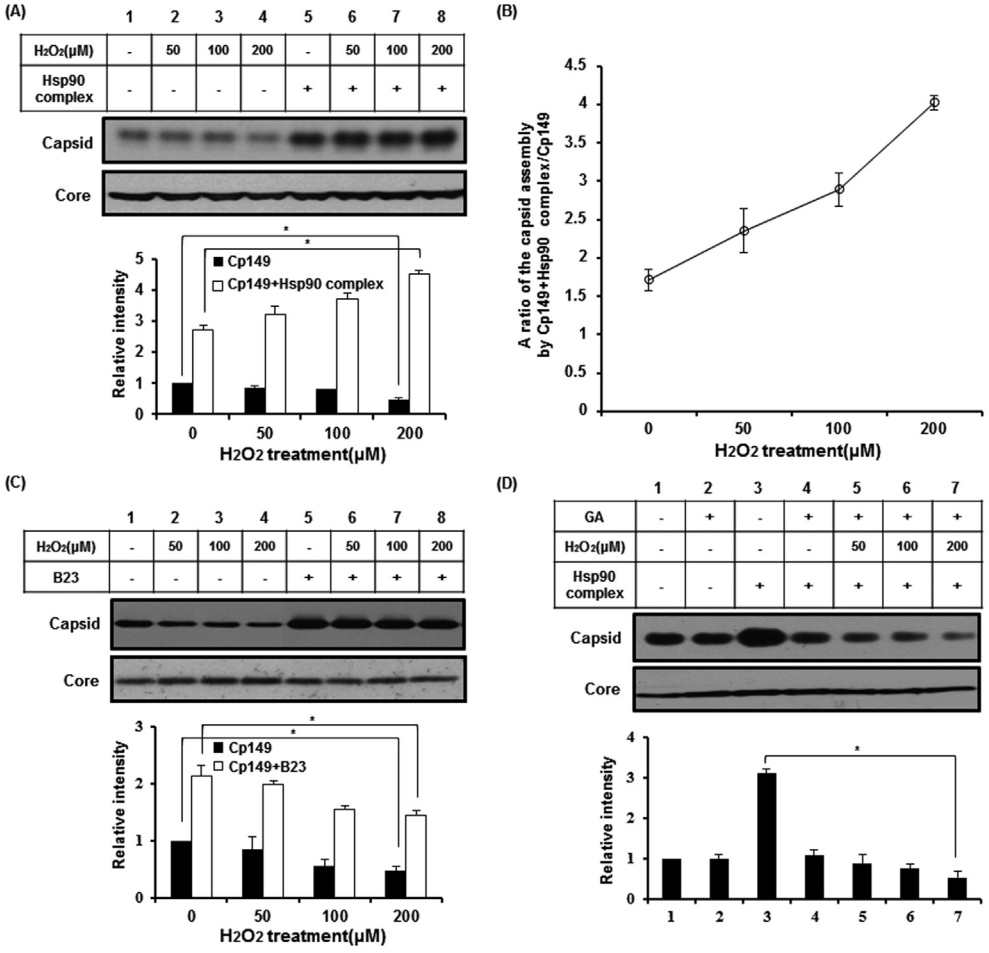

Fig. 1. ROS facilitate HBV capsid assembly in the presence of the Hsp90 complex. (A) For Hsp90 complex formation, 20 μM Hsp90 was incubated with 20 μM p23 and 0.5 mM ATP-r-S for 30 min at 30 °C and mixed with 20 μM Cp149 dimer in assembly buffer for 30 min at 30 °C with increasing concentrations of H_2O_2. (B) A ratio of capsid assembly dependent on the Hsp90 complex was analyzed in experiment for (A). (C) Increasing concentrations of H_2O_2 were incubated with 20 μM B23 and the 20 μM Cp149 dimer in same conditions as in (A). (D) 2 μM GA was added to Hsp90 with p23 and ATP-r-S for 30 min at 30 °C, and this mixture was incubated with Cp149 dimer for 30 min at 30 °C with increasing concentrations of H_2O_2. Samples in Fig. 1 were separated by 0.9% native agarose gel electrophoresis, and capsids were detected by immunoblot analysis with an anti-HBV core antibody. Core, the total amount of Cp149, was detected by 15% SDS-PAGE. The graph at the bottom of all gels represents the relative band intensity for each gel. Capsids without any additive were used as a standard (set to 1).