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# Aurora kinase A promotes hepatitis B virus replication and expression

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ABSTRACT

Cellular protein kinases play critical roles in various steps of the hepatitis B virus life cycle. We found that viral replication in infected or transfected hepatoma cell was markedly inhibited by treatment with A-443654, a specific inhibitor of Akt. The antiviral mechanism of the drug mainly depended on the downregulation of Aurora A, a protein kinase that plays an essential role in mitosis but has not been implicated in the viral life cycle. Our data indicated that Aurora kinase A enhances viral replication and expression independently of its kinase activity required for mitotic function. Our findings suggest that mitotic kinases, considered to be an attractive target of antitumor agents, also provide a novel target for the development of antiviral therapy.

## 1. Introduction

Chronic hepatitis B virus (HBV) infection, affecting an estimated ~350 million individuals worldwide, poses a major risk of developing hepatocellular carcinoma and hepatitis. The currently approved treatments for chronic hepatitis, including nucleotide analogs and pegylated interferon-alpha, are suboptimal and require more effective new therapies (reviewed in Trepo et al., 2014).

HBV is an enveloped DNA virus that belongs to the *Hepadnaviridae* family. After entry into hepatocytes, the ~3.2 kb-long, partially double-stranded, relaxed circular (rc) DNA is transported to the nucleus and converted into a covalently closed circular (ccc) DNA, which persists in the nucleus as a minichromosome and directs the cellular transcription machinery to synthesize viral RNAs. One of the transcripts, which also codes for the viral core (HBc) and the polymerase (Pol) proteins is enclosed in the capsid and serves as the pregenomic (pg) RNA template for the Pol-catalyzed, reverse transcriptional synthesis of viral DNA. The newly formed rcDNA nucleocapsid is then either enveloped and secreted out of cells or translocated to the nucleus to amplify cccDNA (reviewed in Cabalerro et al., 2018).

Earlier studies have identified several cellular protein kinases involved in the HBV life cycle. Cyclin-dependent kinase 2 (CDK2) phosphorylates serine/threonine residues at the C-terminus of core protein (Ludgate et al., 2012). In addition, the core protein can be phosphorylated by serine/arginine-rich protein kinase 1 (SRPK1) (Daub et al., 2002) and Polo-like kinase 1 (PLK1) (Diab et al., 2017). Phosphorylation of the core protein by these kinases is essential or beneficial to viral replication, and provides a target for developing antiviral therapy.

Aurora kinases are serine/threonine protein kinases that play a primary role in centrosome organization and mitotic spindle assembly in the cellular G2/M transition (Marumoto et al., 2005). Gene amplification and elevated expression of Aurora kinases are often found in multiple tumor tissues, associated with increased numbers of centrosomes and spindles, which makes these kinases popular targets for the development of anticancer agents (Kollareddy et al., 2012).

In the current study, chemical inhibitors of broad kinase specificity were investigated to determine their inhibitory effects on HBV replication. The inhibitor assay and kinase expression data indicated that Aurora kinase A promotes HBV replication independently of its mitotic function.

## 2. Materials and methods

### 2.1. Cell culture, plasmids and chemical reagents

HepG2 (ATCC HB-8065) cells were maintained in DMEM supplemented with 10% FBS (HyClone) and 50 μg/ml gentamicin (Gibco) at 37 °C in 5% $CO_2$. HBV1.3, HBV1.3P$^-$ and HBV1.3X$^-$ DNA constructs were previously described (Wang et al., 2009; Ko et al., 2014). The pCMV-SPORT6-AURKA plasmid was provided by the Korea Human Gene Bank of the Medical Genomics Research Center. Aurora kinase A mutant constructs T288D, K162R and T288A were generated by site-directed mutagenesis. The small-molecule inhibitors used are listed in Supplementary Table 1. A-443654 and ML-9 were purchased from Cayman. MLN4924 was purchased from Millipore. C646, Kenpaullone, PP1, SU6656, SB202190, BIRB0796, BI-D1870, LY294002, VX680, MLN8237 and IC261 were purchased from Selleckchem. 3 TC and

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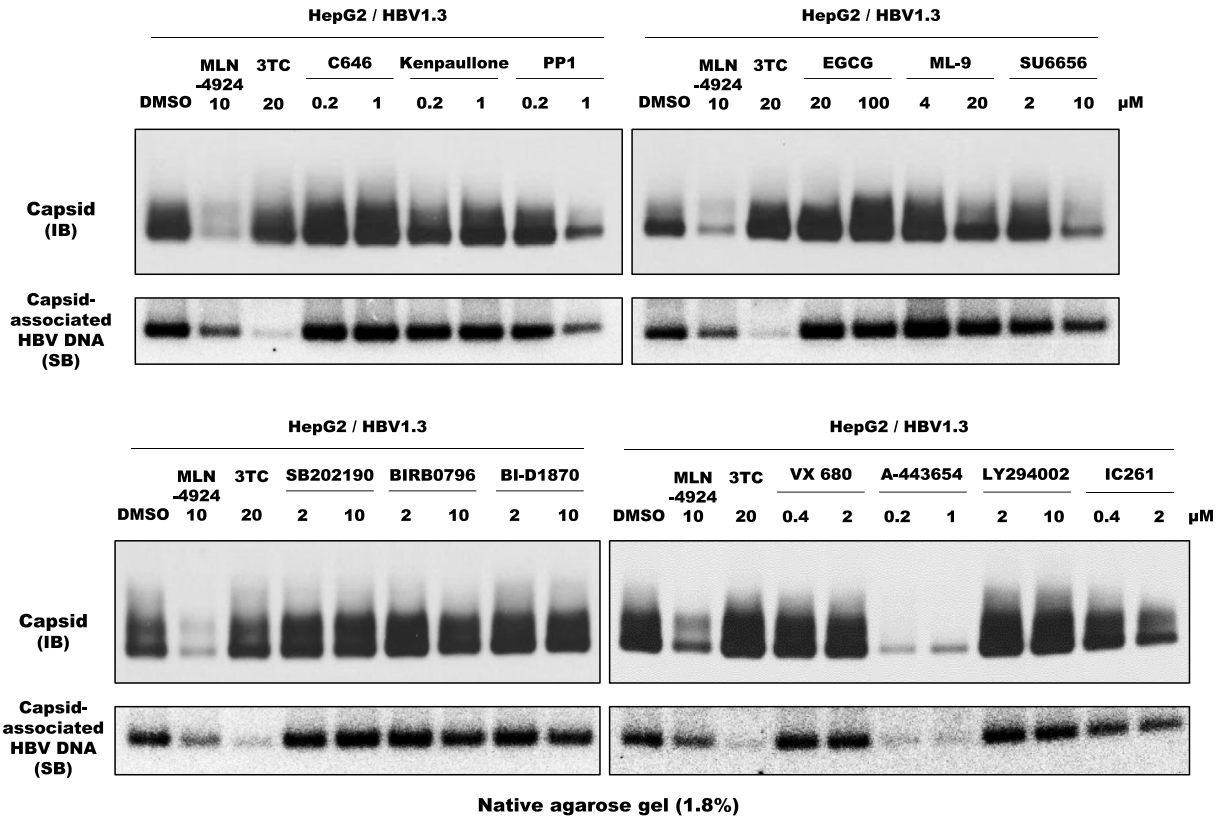


**Fig. 1. Effect of small-molecule kinase inhibitors on HBV**. HepG2 cells transfected with HBV1.3 DNA (2 μg/well) were treated with the indicated chemicals at two different concentrations or DMSO for 24 h (Upper) Viral capsids in the cell lysate were separated in a 1.8% non-denaturing agarose gel, transferred onto a PVDF membrane and immunoblotted (IB) with anti-HBc antibody. (Lower) Capsid-associated viral DNA on the membrane was denatured and detected by Southern blotting (SB). The cells treated with 10 μM MLN4924 or 20 μM 3 TC were analyzed for comparative purposes. Among the 13 compounds tested, the most notable inhibition was observed with A-443654, a potent and selective inhibitor of the Akt kinases.

EGCG were purchased from Sigma-Aldrich. JetPRIME polyethylenimine (from Polyplus-transfection) was used for DNA transfection. Lipofectamine 2000 (from Invitrogen) was used to transfect cells in suspension with Aurora kinase siRNAs (Supplementary Table 2) purchased from GenePharma.

### 2.2. Virus stock and infection

HBV stock and HepG2-NTCP cells that express the human receptor protein NTCP were prepared as described (Jeong et al., 2016). The cells were inoculated with the HBV stock in DMEM containing 10% FBS, 4% PEG 8000 and 2.5% DMSO for 16 h, followed by the removal of viral inoculum and replacement with fresh media containing 2.5% DMSO but no PEG.

### 2.3. Southern and Northern blotting

Viral DNA was isolated from transfected or infected cells as described with a minor modification (Jeong et al., 2016). Briefly, the cells were resuspended in 0.2% NP-40 lysis buffer and nuclei were removed by centrifugation at 12,000 g for 5 min. The cytoplasmic fraction was treated with 0.1 mg/ml DNase I (Roche) for 1 h and the viral capsids were precipitated with 10% PEG 8000. The capsid-associated DNA was extracted with proteinase K (ThermoFisher) treatment and analyzed by Southern blotting with $^{32}$P-labeled HBV-specific DNA probe. Signals were quantified with FLA-7000 (Fujifilm) and Multi Gauge v3.0. In some experiments, capsid in the cytoplasmic fraction was directly resolved in a nondenaturing agarose gel and capillary-transferred to an Immobilon-P transfer PVDF membrane (Millipore) in 20X SSC buffer. The capsid DNA on the membrane was denatured in 0.2 N NaOH and 0.15 M NaCl for 5 min, neutralized in 0.2 M Tris-HCl (pH 7.5) and 1.5 M NaCl for 5 min, cross-linked in a UV cross-linker (CL-1000, UVP) and hybridized with HBV-specific probe. Viral cccDNA was extracted from the nuclear fraction by the Hirt extraction protocol, treated with T5 Exonuclease (NEB) to remove non-supercoil DNA and detected by Southern blotting. An aliquot of cccDNA sample was digested with EcoRI to give rise to a 3.2 kb linear DNA. To assess viral RNA, total RNA was extracted from the cells with RiboEx (GeneAll) followed by separation on a 1.5% agarose gel containing 2.2 M formaldehyde. Viral RNA was analyzed by Northern blot analysis. The signal of the blots was quantified and shown in a bar graph with the mean and standard deviation of three independent experiments. P-values were calculated by two-tailed Student's t-test using Excel software. P-value $< 0.05$ was considered statistically significant.

### 2.4. Immunoblotting

Cells were lysed in RIPA buffer, and proteins in the lysate were separated in a denaturing polyacrylamide gel and transferred to a PVDF membrane. The membrane was incubated with 5% skim milk in TBST buffer and the following primary antibodies: anti-AURKA, anti-phospho-Akt1 (S473) (Cell signaling), anti-Akt1, anti-GAPDH, anti-Hsp70, anti-phospho-GSK3β (S9) (Santa Cruz), anti-phospho-histone H3 (S10) (Epitomics), anti-histone H3 (Abcam) and anti-HBc (Dako) antibodies. HRP-conjugated secondary antibodies (from Bio-Rad) and ECL reagents (Visual Protein) were used for protein detection. In some experiments, the viral capsid in cell lysate was separated in a non-denaturing agarose gel, capillary-transferred to a PVDF membrane in 20X SSC buffer and probed with an anti-core antibody (kindly provided by Professor K. Kim of Ajou University).

### 2.5. Cell viability measurements

Viability was measured with WST-8 reagents of Cell Counting Kit-8 (Dojindo Molecular Tech.) according to the provided protocol.

## 3. Results

### 3.1. HBV replication is impaired by Akt inhibition

To seek cellular protein kinases involved in the HBV life cycle, we treated HBV1.3 DNA-transfected HepG2 hepatoma cells with several small-molecule inhibitors of broad kinase specificity. For each compound, the lower concentration used was set based on the lowest effective concentration reported in literatures and the higher concentration was set 5- or 10-fold that of the lower concentration (Ref. Supplementary Table 1). The cells treated for 24 h with the indicated drugs were harvested and the viral capsid in the cytoplasmic fraction was separated in a non-denaturing agarose gel. The capsid was capillary-transferred to PVDF membrane and probed for the viral core protein and DNA by immunoblotting and Southern hybridization, respectively (Fig. 1). PP1, ML-9, SU6656, A-443654 and IC261 were chosen based on the primary result and tested further in multiple assays. The most notable and reproducible inhibition was observed with the compound A-443654, a potent and selective inhibitor of the Akt kinase (Luo et al., 2005). Capsid-associated core protein and DNA levels were markedly decreased at micromole concentrations of the compound. As comparatively shown in the experiment, reduction in the capsid and viral DNA levels by A-443654 treatment was different from the inhibition by the polymerase inhibitor 3 TC but appeared more similar to the pattern of the compound MLN4924, a protein modification inhibitor that interferes with the HBx-mediated transactivation of viral gene expression (Decorsiere et al., 2016). A separate HBV transfection experiment also showed significant, and drug-dose dependent inhibition on viral capsid, core protein, DNA and RNA (Fig. 2). Cell viability was not affected at 0.2 or 1 μM A-443654, while some toxicity was observed at 5 μM in 24 h treatment. However, toxicity became evident in 48 h treatment even at 0.2 μM. A similar antiviral effect of A-443654 was observed in HepG2-NTCP cells infected with culture-derived virus (Fig. 3).

The strong antiviral effect of A-443654 that we observed suggested a virus supporting role of Akt, a serine/threonine kinase that is activated by the phosphatidylinositol 3-kinase (PI3K) in growth-stimulating conditions. PI3K and Akt, constituting an important proliferative pathway, are frequently found to be elevated in multiple cancers (reviewed in Engelman, 2009) and can be activated by virus infection (reviewed in Buchkovich et al., 2008). Our result, pointing to a supportive role of Akt for HBV, a DNA virus that replicates in close association with cell cycle and thus seems likely to be facilitated by the growth proliferating and antiapoptotic functions of the kinase, however, is not in keeping with previous data that indicated the virus suppressive effect of the PI3K/Akt pathway (Guo et al., 2007; Ondracek and McLachlan, 2011; Rawat and Bouchard, 2015; Xiang and Wang, 2018). According to these studies, PI3K/Akt signaling can be activated in HBV infection but exerts, in turn, in a rather restrictive effect on viral replication. These authors showed that viral replication was inhibited by overexpression of a constitutively active form of Akt, whereas treatment with PI3K/Akt inhibitors enhanced viral replication. Such a repressive mechanism on the virus was suggested to reduce the metabolic burden on the host cell, and in combination with the antiapoptotic function of the pathway, it might contribute to promoting cell survival and thus prolong persistent infection. We assumed that A-443654 works differently from the other inhibitors and sought an alternative mechanism.

### 3.2. HBV replication is supported by Aurora kinase A

Among the other potential targets of A-443654, we confirmed the expression of protein kinase A (PKA), protein kinase C-related protein kinase 2 (PRK2), Down-syndrome related protein kinase 1 (DYRK1) and Aurora kinase A (AURKA) in HepG2 cells (data not shown). Treatment with siRNAs specific to these kinases produced no discernible effect on the virus, except for AURKA, a serine/threonine kinase that regulates

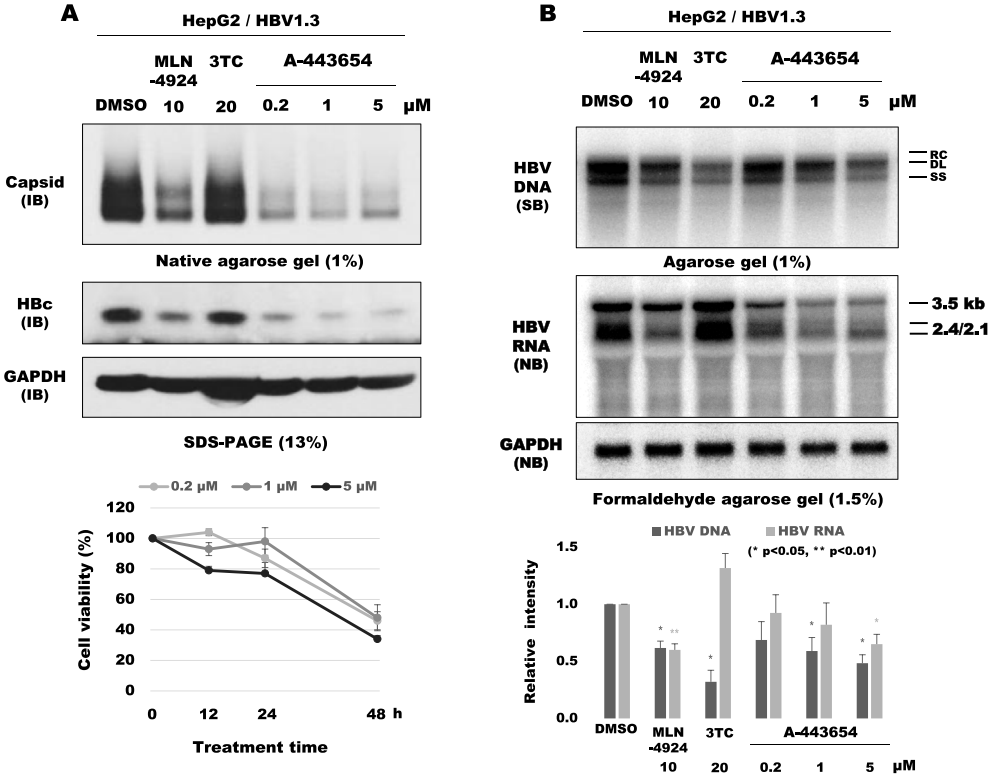


**Fig. 2. Inhibition of HBV replication and expression by A-443654.** HBV1.3-transfected HepG2 cells were treated with increasing doses of A-443654 (0.2, 1 or 5 μM) for 12 h. (A) Viral capsids in the cell lysate were separated in a 1% non-denaturing agarose gel (Upper) and in a denaturing polyacrylamide gel followed by immunoblotting with anti-HBc antibody (Middle). GAPDH was probed as a loading control. Viability of the cells treated with A-443654 (0.2, 1 or 5 μM) for 12, 24 and 48 h was measured with WST-8 reagents (Lower). (B) Viral DNA extracted from the HBV1.3-transfected HepG2 cells were analyzed by Southern blotting (Upper). Indicated on the right are the relaxed circular (RC), duplex linear (DL) and single-stranded (SS) viral DNAs. Viral RNA was analyzed by Northern blotting (Middle). The viral mRNAs (3.5-, 2.4- and 2.1 kb in length) are indicated. The smallest viral mRNA (expected size of ~0.7 kb) was not detectable in this assay. GAPDH mRNA served as a loading control. The signal intensity of DNA (in black) and GAPDH-normalized RNA (in gray) was shown with mean and SD (Lower). Statistical significance was calculated by Student's t-test. P-value < 0.05 was considered statistically significant.

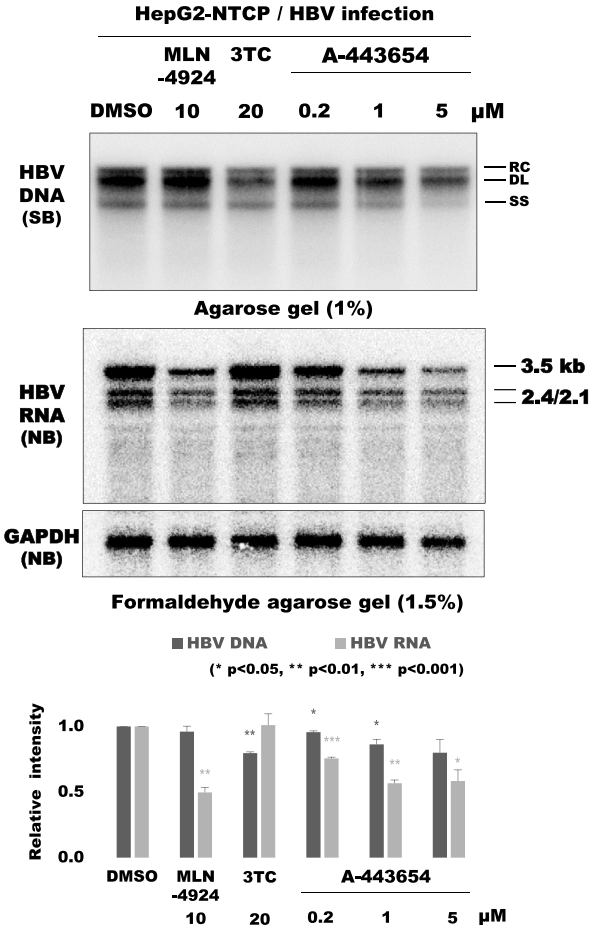


**Fig. 3. Inhibition of HBV infection by A-443654**. HepG2-NTCP cells were inoculated with HBV (at $5 \times 10^5$ Geq/cell) for 16 h in the presence of 4% PEG and 2.5% DMSO. The cells were treated with the indicated chemicals for 12 h at 6 days post inoculation. Viral DNA and RNA were analyzed as described above. GAPDH mRNA served as a loading control. The relative intensity was shown in a bar graph.

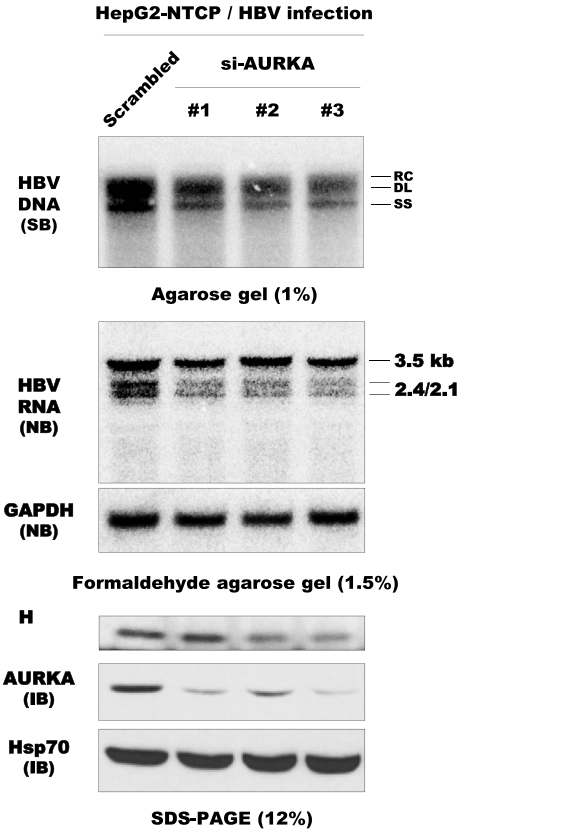


**Fig. 4. Effect of Aurora kinase A knockdown on HBV**. HepG2-NTCP cells were transfected in suspension with each of Aurora kinase A siRNAs (indicated as #1, #2 and #3) or scrambled control at 100 nM. The cells were plated and infected the next day as described above. Southern blot analysis of viral DNA was performed after 5 days (Upper). Northern blot analysis of viral RNA and GAPDH mRNA was performed after 5 days (Middle). HBc and Aurora kinase A were analyzed after 3 days by immunoblotting (Lower). Hsp70 was probed as a loading control.

centrosome maturation and spindle formation in mitosis (reviewed in Marumoto et al., 2005). Compared with the control siRNA, knockdown of the AURKA notably inhibited viral replication and expression in the infected cells (Fig. 4). The AURKA knockdown data suggested a virus supporting role of the kinase, although its depletion by siRNA was not sufficient to fully reproduce the inhibition by A-443654.

Three closely related Aurora kinase isoforms identified in mammalian cells differ in their specialized functions and subcellular localization: AURKA and the closely related isoform Aurora kinase B (AURKB) are found in the nucleus and in the cytoplasm; Aurora kinase C (AURKC) expression is confined to the germ cells (Li et al., 2015). The expression and localization of these kinases are regulated in a cell cycle-dependent manner. Our siRNA data suggesting the virus-supporting role of AURKA prompted us to further investigate the mechanism of this role through ectopic expression of the kinase in HBV replicating cells. Viral DNA and RNA levels increased by up to ~50% in correlation with AURKA expression, while cellular genes were not affected (Fig. 5). Virus-promoting activity of AURKA was also confirmed by a silencing/rescue experiment with siRNA (#2) that targets the 3′-UTR of AURKA and thus does not interfere with transfected gene. We found no effect of AURKB (data not shown). AURKC was not investigated in this study.

### 3.3. Aurora kinase A enhances viral gene expression HBx-independently

HBV replication depends on the viral cccDNA, and gene expression is regulated through the epigenetic alteration of the cccDNA chromatin (Lucifora et al., 2011; Riviere et al., 2015; Tropberger et al., 2015). HBx, a viral nonstructural protein, plays critical roles in the transactivation of viral promoters on cccDNA. An activation mechanism depends on HBx for the proteolytic destabilization of the Smc5/6 protein complex that interferes with the gene expression from viral minichromosomes (Decorsiere et al., 2016). We found that the replication and expression of the HBx-null DNA construct HBV1.3X$^-$ was also enhanced by co-transfection of AURKA, although a longer exposure was needed to obtain signals comparable to that of HBV1.3 (Fig. 6). The data indicated that AURKA enhances viral replication independently of HBx function.

Because HBV DNA synthesis occurs through reverse transcription of the viral RNA pregenome, the increase of viral DNA by AURKA could be the consequence of enhanced viral gene expression. To address whether the primary role of AURKA is in the viral transcription, we examined the cells transfected with the replication-defective construct HBV1.3P$^-$, which does not encode a functional Pol protein. Southern and Northern blot analyses showed that AURKA co-expression enhanced viral RNA expression from the construct, despite the lack of DNA synthesis. We also observed enhanced expression from the wild-type viral construct, for which DNA synthesis was blocked with the RT inhibitor 3TC. Because no new DNA was produced in these conditions, the viral RNA produced must be transcribed from transfected DNA. These results thus demonstrate that AURKA enhances viral transcription and that increased viral DNA is likely the consequence of enhanced gene expression. In contrast to the significant impacts observed for the viral expression and DNA synthesis, viral cccDNA was not affected by AURKA

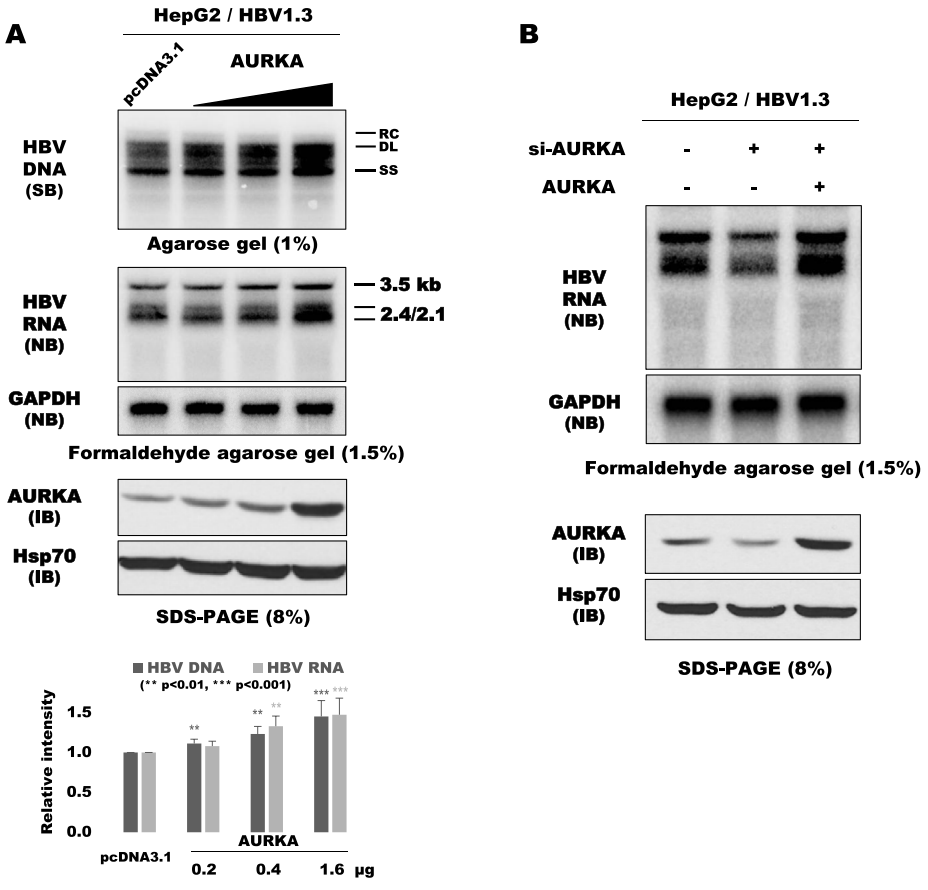


**Fig. 5. Augmentation of HBV replication by Aurora kinase A overexpression**. (A) HepG2 cells were co-transfected with HBV1.3 (0.4 μg/well) and Aurora kinase A expression plasmid (0.2, 0.4 or 1.6 μg/well). HBV DNA and RNA were analyzed after 4 days by Southern and Northern blotting, respectively (Upper). GAPDH mRNA served as a loading control. The Aurora kinase A expression was assessed by immunoblotting. Hsp70 was probed as a loading control (Middle). The relative intensity was shown in a bar graph (Lower). (B) HepG2 cells were transfected with siRNA #2 or scrambled control as described above, followed by transfection of 1 μg of Aurora kinase A expression plasmid or pcDNA3.1. Northern blot assay of viral RNA and GAPDH mRNA was performed after 4 days (Upper). The Aurora kinase A expression was analyzed by immunoblotting (Lower). Hsp70 was used as a loading control.

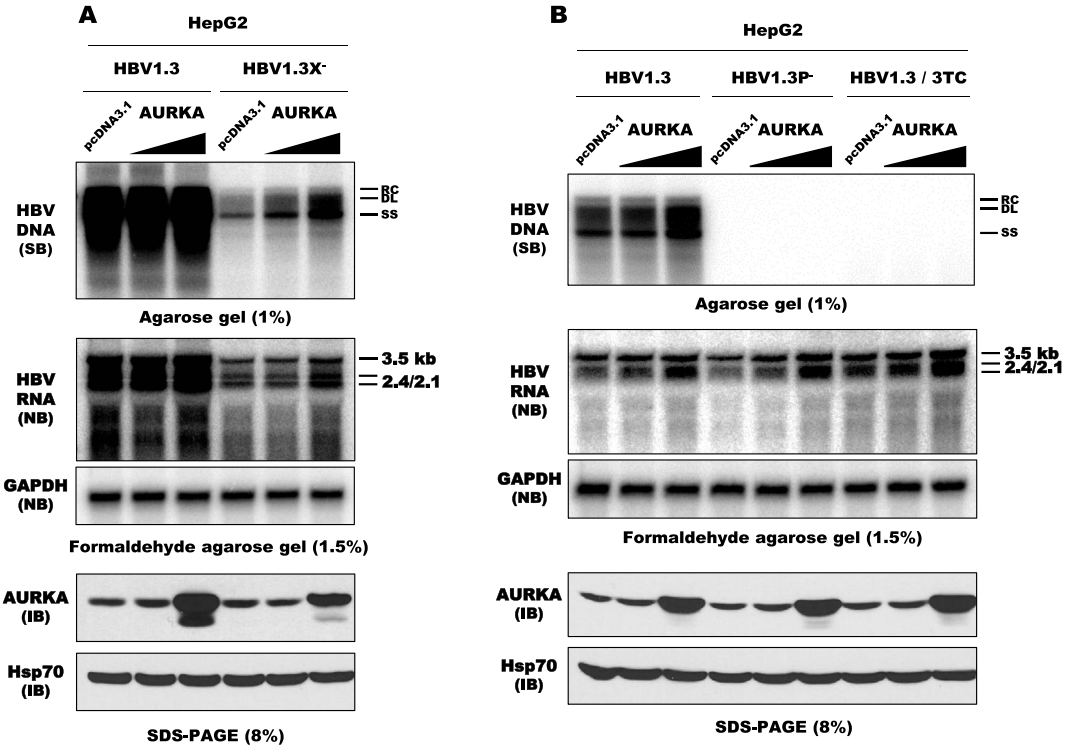


**Fig. 6. HBx-independent enhancement of HBV transcription by Aurora kinase A**. (A) HepG2 cells were co-transfected with HBV1.3 or HBx-null construct HBV1.3X$^-$ (each 0.4 μg/well) and Aurora kinase A plasmid (0.4 or 1.6 μg/well). Viral DNA and RNA were analyzed in 4 days by Southern blot and Northern blot analyses, respectively (Upper). Aurora kinase A was immunoblotted. Hsp70 was probed as a loading control (Lower). (B) HepG2 cells were co-transfected with HBV1.3 or P-null construct HBV1.3P$^-$ (each 0.4 μg/well) and Aurora kinase A plasmid (0.4 or 1.6 μg/well). Some samples were treated with 20 μM 3 TC. Viral DNA and RNA were analyzed in 4 days by Southern and Northern blot analyses, respectively (Upper). The Aurora kinase A expression was assessed by immunoblotting (Lower). Hsp70 was used as a loading control.

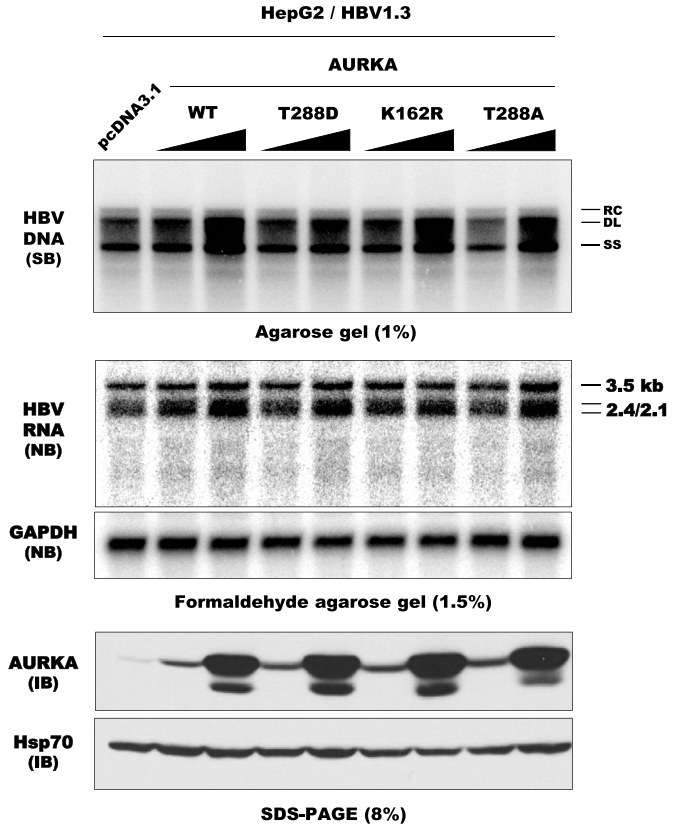


**Fig. 7. Kinase-independent role of Aurora kinase A for HBV replication.** HepG2 cells were cotransfected with HBV1.3 (0.4 μg/well) and the wild-type, phospho-mimetic (T288D), kinase-defective (K162R) or non-phosphorylatable (T288A) mutant constructs of Aurora kinase A plasmids (each 0.4 or 1.6 μg/well). Viral DNA was analyzed in 4 days by Southern blotting (Upper). Viral RNA extracted in 4 days was determined by Northern blotting (Middle). GAPDH mRNA served as a loading control. An aliquot of the cell lysate was immunoblotted with anti-Aurora A antibody (Lower). Hsp70 was probed as a loading control.

expression or any of the inhibitors A-443654, 3 TC or MLN4924 in the given period, reflecting the persistent nature of cccDNA (Supplementary Fig. 1).

### *3.4. Kinase activity is not essential for virus promoting function of Aurora kinase A*

The mitotic function of AURKA depends on phosphorylation at the threonine residue T288 in the kinase activation loop of AURKA (Tsia et al., 2003). We constructed a phosphorylation-mimicking, kinase-active mutant T288D and a kinase-dead mutant K162R that is incapable of ATP binding at the catalytic site. Viral replication was enhanced by the ectopic expression of T288D (Fig. 7). More importantly, kinase-dead mutant K162R showed a significant enhancement, contrary to the dominant negative effect expected for a kinase-defective mutant. Non-phosphorylatable mutant T288A also showed some virus-enhancing effect. These results indicated that the kinase activity essential for the mitotic function is not required for the virus stimulating function of AURKA.

### *3.5. Aurora kinase A expression is blocked by Akt inhibitor A-443654*

Kinase-independent role of AURKA in promoting HBV expression was further confirmed with AURKA-specific inhibitor MNL8237 (Zhang et al., 2018). No significant effect on the viral DNA and RNA was observed with this compound and also with the PI3K inhibitor LY294002 (Fig. 8). To determine a mechanism that would account for the kinase-independent role of AURKA and the antiviral effect of the kinase inhibitor A-443654, we noted data from Liu et al. showing that the compound inhibits AURKA expression but not its kinase activity at the concentrations used in our study, although its kinase activity can be inhibited at a considerably higher concentration of the drug (Liu et al., 2008). According to the authors, AURKA was among the most prominently downregulated genes identified from the expression array of cancer cell lines that had been arrested at G2/M, which was attributed to the inhibition of the AURKA promoter activity by the Akt inhibitor. A sequence element (Ets) in the AURKA gene promoter was found to be responsible for the transcriptional enhancement by AKT, but proteins that mediate the effect was not specified. Our immunoblot also showed that AURKA expression was abolished by A-443654 treatment, compared with a modest effect by PI3K inhibitor LY294002 and no effect by AURKA inhibitor MNL8237. Northern blot data for AURKA mRNA was also consistent with these results (Supplementary Fig. 2). We noted that AURKA expression level was reduced at 0.2 μM A-443654 in early times but rapidly recovered afterward, while it was kept reduced at higher drug concentrations (Data not shown). We consider this is why we see the drug effect on and off at the low concentration. Immunoblotting with a phosphor-specific antibody against GSK3β, a substrate protein of Akt, indicated that AURKA expression was affected in correlation with Akt activity. While these results indicated that Akt is critical for AURKA expression, immunoblotting revealed different effects of the two inhibitors on Akt. In contrast to the blockage of Akt phosphorylation by LY294002, we found a surge in phosphorylated Akt by A-443654 treatment, which was attributed to a unique feature of the latter drug that inhibits the kinase and thus attenuates the feedback inhibition as well (Han et al., 2007). A decrease in the viral core protein by A-443654 confirmed the effect of Akt-mediated AURKA expression in promoting viral expression. In contrast, antiviral activity was not observed by LY294002, despite the specific inhibition of Akt1 phosphorylation by the drug. This is because LY294002 interferes with the activation of AKT, whereas A-443654 directly blocks the catalytic action of AKT. LY294002 inhibits phosphatidylinositol 3-kinase that catalyzes the synthesis of phosphatidylinositol triphosphate, a ligand critical for AKT activation. In this regard, the drug's effect can be more indirect compared to A-443654. Our data showing a significant reduction of P-Akt1 but relatively lesser reductions of P-GSK3β and AURKA (and HBV) indicates that the drug's effect gets attenuated while it diverges on targets further downstream of the pathway. The AURKA inhibitor MLN8237 also showed no significant effect, despite the specific blockage of histone H3 phosphorylation. Some inhibition of GSK3β phosphorylation observed by MLN8237 might be because AURKA can phosphorylate GSK3β, independently to Akt (Dar et al., 2009). These results demonstrated that Akt activity is critical for AURKA expression, and in combination with AURKA expression data, the results indicated that the virus promoting function of AURKA depends on its expression but does not require its kinase activity.

## 4. Discussion

In this study, we showed that AURKA, a kinase that is essential in the mitotic process but has not been demonstrated to be involved in HBV life cycle, promotes HBV replication. Our attention to AURKA was initially drawn by the antiviral effect observed for the Akt inhibitor A-443654. We found that the antiviral mechanism of the drug depends mainly on the inhibition of AURKA expression. Our data demonstrated that AURKA enhances viral gene expression in HBx-independent manner and that the virus promoting role of AURKA is apparently independent of the mitotic function of the kinase.

As a DNA virus, the HBV life cycle is closely associated with the cell cycle. Akt and PI3K constitute a central signaling pathway for promoting cell proliferation and preventing apoptosis. Several studies have shown that the PI3K/Akt pathway can be activated by the viral

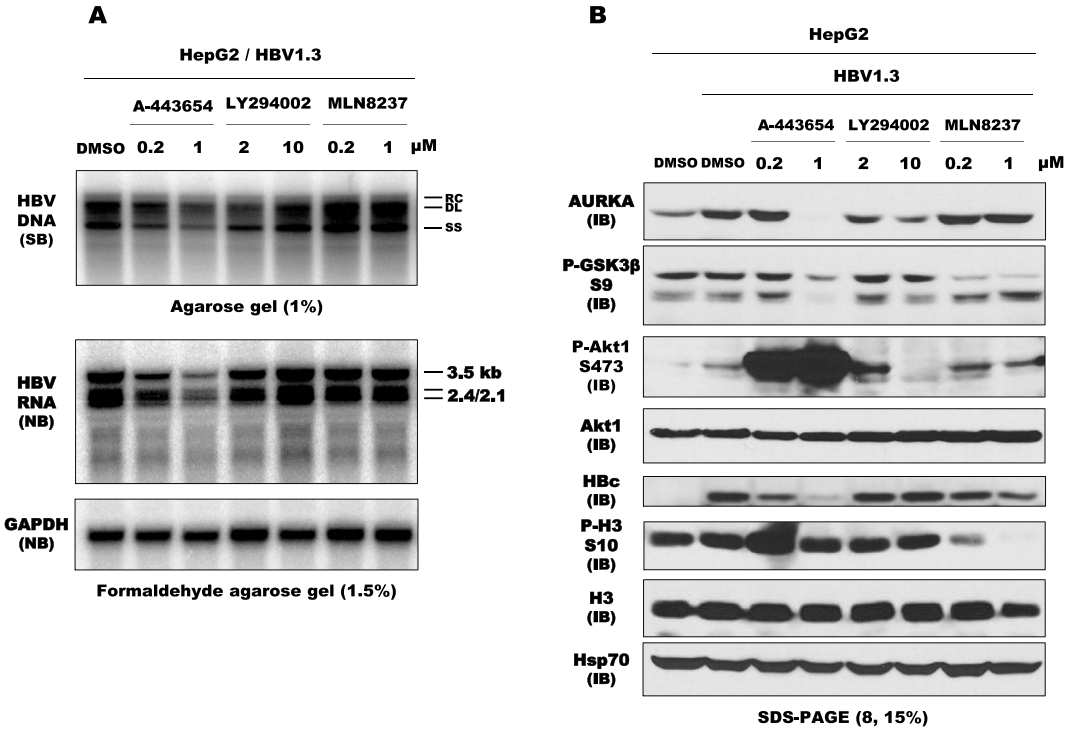


**Fig. 8. Comparison of A-443654 to other specific inhibitors**. (A) HepG2 cells were transfected with HBV1.3 (2 μg/well) followed by treatment with the indicated inhibitors for 24 h. HBV DNA extracted from the cells as described above was analyzed by southern blotting (Upper). Northern blot analysis of viral RNA and GAPDH mRNA was performed (Lower). (B) The cell lysate was immunoblotted with antibodies to Aurora kinase A, Akt1, HBc, Histone H3, and specific to the phosphorylation of GSK3β (S9), Akt1 (S473) and Histone H3 (S10) proteins, respectively. Hsp70 served as a loading control.

proteins: HBx (Shih et al., 2000; Lee et al., 2001; Chung et al., 2004; Wang et al., 2019); HBc (Liu et al., 2018); large surface antigen LS (Liu et al., 2011); and spliced RNA-derived protein HBSP (Wu et al., 2018). While these studies suggested that the activation of the PI3K/AKT pathway contributes to viral replication and tumorigenesis, other studies showed that the pathway works to suppress viral replication (Guo et al., 2007; Ondracek and McLachlan, 2011; Rawat and Bouchard, 2015; Xiang and Wang, 2018). Our finding of the antiviral effect of Akt inhibitor A-443654 led us to reveal an alternative, but supportive role of the pathway for the virus that depends on AURKA. Our data indicated that AURKA enhances viral gene expression in an HBx-independent manner and that the virus promoting role of AURKA does not require its kinase activity.

Consistent with the essential mitotic function of AURKA, this kinase is frequently overexpressed in multiple tumors, providing a popular target for the development of antitumor agents. Several inhibitors for the kinase are undergoing clinical trials (reviewed in Borisa and Bhatt, 2017). Although these approaches target the kinase function of AURKA, data have also emerged to suggest kinase-independent functions of AURKA in promoting cancer development, such as the enhancement of the expression and/or stability of cellular oncoproteins (Zheng et al., 2016; Otto et al., 2009). Our data supporting the virus-promoting role of the kinase might provide a novel target for antiviral therapy.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2019.104572.

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