



SRPKIN-1 as an inhibitor against hepatitis B virus blocking the viral particle formation and the early step of the viral infection

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論文題目

SRPKIN-1 as an inhibitor against hepatitis B virus blocking the viral particle formation and the early step of the viral infection

(SRPKIN-1はB型肝炎ウイルス粒子形成と感染早期過程を阻害する)

論文の内容の要旨

[Introduction]

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV) and HBV-positive individuals are at higher risk of developing hepatocellular carcinoma (HCC). New antiviral agents are needed for the treatment of HBV infection because currently available drugs do not completely eradicate chronic HBV in patients. Phosphorylation dynamics of the HBV core protein (HBc) are considered to be involved in regulation of several processes in the HBV life cycle, including nucleocapsid formation, cell trafficking, and virus uncoating after entry. While serine/threonine protein kinases that phosphorylate HBc protein have been reported, such as Polo-like kinase 1 (PLK1), cyclin-dependent kinase 2 (CDK2), protein kinase C alpha (PKC α), and serine-arginine protein kinase (SRPK), the anti-HBV effects of serine/threonine kinase inhibitors and their mechanisms of action are not fully elucidated to date. The aim of this study was to elucidate anti-HBV activity of SRPK inhibitor and the underlying molecular mechanisms.

[Materials and Methods]

Human hepatoma HuH-7 cells which were transfected with pHBV1.05 containing a 1.05-fold unit length of the HBV genome were used for analyzing the inhibitory mechanism of SRPK inhibitor on the infectious HBV particle formation. Viral proteins expression level was examined by Western blotting with anti-HBs and anti-HBc antibodies. HBV DNA/RNA copies were quantified by RT-PCR. Nucleocapsid formation and envelopment was assessed by native agarose gel electrophoresis. Phosphorylation status of HBc protein was analyzed by PhosTag-SDS-PAGE and liquid chromatography-mass spectrometry (LC-MS/MS). To evaluate early steps of HBV infection, HBV was inoculated to human hepatoma HepG2-NTCP cells and PXB cells, which are fresh human hepatocytes isolated from chimeric mice, with humanized livers with various concentrations of SRPK inhibitor. This study was approved by Safety Committee for Recombinant DNA Experiments of Hamamatsu University School of Medicine (4-05, 4-09, 4-11).

[Results]

The SRPK inhibitors SPHINX31, SRPIN340, and SRPKIN-1 showed

concentration-dependent anti-HBV activity. Detailed analysis of the effects of SRPKIN-1, which exhibited the strongest inhibitory activity, on the HBV replication process showed that it inhibits the formation of infectious viral particles by inhibiting pregenomic RNA packaging into capsids and nucleocapsid envelopment. LC-MS/MS analysis combined with cell-free translation experiments revealed that four serine residues in the C-terminal region of HBc are phosphorylated by SRPK and its phosphorylation is inhibited by SRPKIN-1. Further, SRPKIN-1 exhibited concentration-dependent inhibition of HBV infection not only in HepG2-hNTCP-C4 cells but also in PXB cells and in the single-round infection system. Treatment with SRPKIN-1 at the time of infection didn't affect HBV attachment to cells. After HBV entry into cells, uncoating of the particles makes HBV DNA sensitive to nuclease activity in the nuclear fraction, a decrease in HBV DNA of approximately 50% upon MNase treatment, but such decrease was suppressed by SRPKIN-1 treatment.

[Discussion]

A detailed analysis of the inhibition mechanism of SRPKIN-1, which showed the highest anti-HBV activity among the SRPK inhibitors tested, revealed that SRPKIN-1 inhibits not only the process of HBV particle formation but also the early stages of the viral infection process by intervening in the post-translational modification of HBc. Several compounds that inhibit HBc multimerization have been developed as anti-HBV drugs targeting the HBc protein to date. This study shows that the mechanism of action of the anti-HBV activity of SRPK inhibitors differs from that of existing HBc-targeting drugs. For the first time the amino acid residues in HBc whose phosphorylation is actually inhibited by the protein kinase inhibitor have been identified.

SRPK phosphorylates SR proteins that contain an arginine/serine-rich (RS) domain, and the RS domain is highly conserved in the capsid proteins of several coronaviruses, including SARS-CoV-2. Indeed, it has been reported that phosphorylation by SRPK may be involved in formation and uncoating of coronavirus capsids. SRPK inhibitors have the potential to become versatile antiviral agents, targeting for example the particle formation process of various viruses.

SRPK1 activity has been reported to promote several characteristics of cancer cells, including proliferation, resistance to apoptosis, migration and angiogenesis, and SRPK inhibitors have been studied for development as anticancer agents. Effective chemotherapy for HBV-associated HCC has not yet been established, and SRPK inhibitors may be useful not only for their antiviral effects in chronic hepatitis B, but also as a treatment option in patients with HBV-positive liver cancer.

[Conclusion]

SRPK inhibitors, such as SRPKIN-1, differ from conventional HBc inhibitors in their

mechanism of action and are thus novel agents that can inhibit both particle formation and nucleocapsid uncoating, which depend upon HBc phosphorylation. SRPK inhibitors are not only a promising new option for the treatment of hepatitis B, but may also have potential for development as inhibitors of other viruses in which phosphorylation by SRPK is involved in particle formation.