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Inhibition of Hepatitis B Virus Replication by a Set of Small Interferon-stimulated Genes

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Hepatitis B virus (HBV) infection is a serious public health problem affecting as many as 400 million individuals worldwide. Alpha interferon (IFN-α) has been approved for use in the management of chronic hepatitis B. It is known that interferon's antiviral effects are mediated by induction of interferon-stimulated genes (ISGs) following activation of the Jak-Stat pathway. Therefore, screening the antiviral activity of specific ISGs can provide important information about interferon's mechanisms of action. Among hundreds of ISGs, small ISGs (sISGs) are classified based on their polypeptide gene products have relatively low molecular weights between 10 and 20 kDa, which are further separated into three subsets according to the gene homology. With the exception of ISG15 group, the functions of other two sISG groups, namely the 1–8 group (containing 1–8D, 1–8U, and 9–27), and the ISG12 group are unknown. By co-transfecting the 1.3mer HBV replication-competent construct and plasmid expressing individual sISG into hepatocyte-derived cell cultures (HepG2 and Huh7), we found that, with the exception of 1–8U, all other sISGs tested significantly reduced the steady state level of viral RNAs in both cell lines. As expected, the reduction in viral RNAs led to a subsequent reduction in viral protein expression, capsid assembly and DNA replication, in a dose-dependent manner. Interestingly, these sISGs could not reduce HBV RNA levels when HBV transcription was under the control of a CMV-IE promoter. This suggests that sISGs selectively inhibit HBV transcription. This is consistent with observations that HBV transcription is the primary antiviral target of IFN-α on hepatocyte-derived cell lines. The detailed mechanism of the inhibition of HBV replication by small ISGs is currently under the investigation. Understanding of the molecular mechanism of the antiviral effect of ISGs will lead to the new insight of the crosstalk between HBV and IFN signaling and the development of novel therapeutics to cure hepatitis B.

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Administration of a HepDirect™ Prodrug of 2′-*C*-methylcytidine to Hepatitis C Virus Infected Chimpanzees

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Hepatitis C virus (HCV) infects an estimated 170 million individuals worldwide and the current standard of care, a combination of pegylated interferon alpha and ribavirin, is efficacious in achieving sustained viral response in ∼50% of treated patients. Novel therapies under investigation include the use of nucleoside analog inhibitors of the viral RNA dependent RNA polymerase. Several nucleoside analogs incorporating a 2′-*C*-methyl modification of the ribose are potent inhibitors of the cell-based HCV replicon assay in vitro and have shown antiviral efficacy in vivo in short-term clinical studies or in the chimpanzee model of HCV infection. 2′-*C*-methylcytidine (2′-MeC) inhibits the genotype 1b HCV replicon assay with an EC_{50} of 1.1 micromolar and NM283, a 3′-valyl ester prodrug of 2′-MeC, has demonstrated antiviral efficacy in HCV infected patients (Afdhal, AASLD, 2004). One approach to increasing the antiviral efficacy of 2′-MeC is to increase the concentration of the active inhibitory species, the 5'-triphosphate, in infected hepatocytes. HepDirect™ prodrug technology can increase intracellular concentrations of a nucleoside triphosphate in hepatocytes by introducing the nucleoside monophosphate into the cell, bypassing the initial kinase step that is often rate-limiting. To determine antiviral efficacy *in vivo*, a 1-aryl-1,3-propanol HepDirect™ prodrug of the 5′-monophosphate of 2′-MeC was administered via oral dosing at 10 milligrams per kilogram of body weight (mpk) to two HCV infected chimpanzees once daily for 7 days. Circulating viral loads declined by 1.3 and 1.5 $\log_{10}$ IU/mL in the two chimpanzees. The compound was also administered via intravenous infusion at 4 mpk once daily for 5 days. One chimpanzee experienced a maximal viral load decline of 4.6 $\log_{10}$ IU/mL. Viral load declined by 3.6 $\log_{10}$ IU/mL in the other chimpanzee and reached the lower limit of quantitation of the Taqman assay after 2 days of dosing. Viral loads rebounded after the end of dosing to pre-dose levels. The results indicate that a robust antiviral response can be achieved upon administration of the compound.

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Poster Session 1: Retroviruses, Hepatitis Viruses, Respiratory Viruses, Emerging Viruses, and Antiviral Methods

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Antiviral Activity of Carbohydrate Binding Agents (CBAS) and the Role of DC-SIGN in Dengue Virus and HIV Infection

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Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is an important binding receptor for dengue virus (DENV) and human immunodeficiency virus (HIV) that recognizes glycan configurations on the viral envelope. Dendritic cells (DC) (DC-SIGN$^+$) in the skin and the peripheral mucosal tissues are the first targets of DENV and HIV, respectively. After virus capture, DC migrate to the secondary lymphoid organs and activate the immune system. Human B-lymphoblast Raji cells expressing DC-SIGN (Raji/DC-SIGN$^+$) is able to capture HIV particles and transmit HIV to CD4$^+$ T-cells in contrast to wild type Raji/0 cells. Several carbohydrate-binding agents (CBAs), such as the plant lectins HHA and GNA (mannose-specific) and UDA (N-acetylglucosamine-specific), inhibited dose-dependently the binding of HIV to Raji/DC-SIGN$^+$ cells with EC_{50s} ranging from 0.2 to 2.1 μM. Raji/DC-SIGN$^+$ cells that have captured HIV particles and then were co-cultured with CD4$^+$ T-cells will induce a profound CPE, resulting in abundant giant cell formation. This giant cell formation can also be efficiently inhibited by the CBAs. We could demonstrate that by flow cytometric and q-RT-PCR analysis DENV productively infects Raji/DC-SIGN$^+$ cells and the EC_{50}s of the CBAs to inhibit DENV infection varied from 0.1 to 2.2 μM. HIV and DENV can not bind, nor productively infect wild type Raji/0 cells. Our results demonstrate the importance of DC-SIGN as attachment receptor for HIV and as a receptor for DENV infection and replication. This interaction is inhibited by CBAs that exhibit a unique mechanism of antiviral action in inhibiting the virus entry process.

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