The HIV reactivation test using HIV p24 antigen measurement demonstrated that CGMC0005 treated J1.1 cells and CGMC0006 treated ACH2 cells showed higher reactivation capacity (ED50 $\leq$ 0.1 μM) than SAHA or PXD-101 treated control cells, and other cases showed similar level of reactivation. Cell cycle changes after HIV reservoir reactivation in J1.1 and ACH2 cells were monitored by flow cytometry using propidium iodide. Both of CGMC0005 and CGMC0006 treatments at 0.1 μM elevated S phase cell population while untreated cells were arrested in G1 phase. p24 antigen production was reduced compared to the first reactivation when 0.1 μM HDAC inhibitors were treated again at the sixth day after the first treatment of CGMC0006 with 250 nM AZT. Cell viability was not severely reduced compared to untreated controls. In summary, our research demonstrated that new synthetic HDAC inhibitor, CGMC0005 and CGMC0006, potentiate to break virus reservoir in latently HIV-infected cells selectively. Our findings suggested that both of new inhibitors can be candidates to promote reduction or eradication of the latent HIV reservoir when they treated repeatedly with reverse transcriptase inhibitors like AZT.

doi:10.1016/j.antiviral.2008.01.077

64

Optimization of shRNA Features for Targeting Hepatitis C Virus

Brian Johnston [1,*], Alexander Vlassov [3], Brent Korba [2], Kristine Farrar [2], Sampa Mukerjee [2], Attila Seyhan [4], Heini ILves [1], Roger Kaspar [5], Devin Leake [6], Sergei Kazakov [1], Qing Ge [1]

[1] Somagenics Inc., Santa Cruz, USA; [2] Georgetown University Medical Center, Rockville, USA; [3] Ambion, Austin, USA; [4] Open Biosystems, Huntsville, USA; [5] Transderm Inc., Santa Cruz, USA; [6] Thermo Fisher Scientific, Lafayette, USA

Hepatitis C virus (HCV) is a leading cause of liver cirrhosis and hepatocellular carcinoma worldwide. Currently available treatment options are of limited efficacy, and there is an urgent need for development of alternative therapies. We screened in vitro-transcribed, 25-bp short hairpin RNAs (shRNAs) targeting the highly conserved internal ribosome entry site (IRES) of HCV for the ability to silence gene expression. We used a reporter plasmid in which firefly luciferase (fLuc) expression is dependent on the HCV IRES. A 44-nt region of domain IV of the IRES was identified, within which all tested shRNAs efficiently blocked IRES-mediated fLuc expression in transfected human 293 FT cells. Subsequent scans within this "accessible" site with 19 bp shRNAs identified even more potent molecules, providing effective inhibition at concentrations of 0.1 nM. Experiments varying features of the shRNA design showed that, for 25 bp shRNAs, neither the size of the loop (4, 5, 6 or 10 nt) nor the sequence or pairing status of the ends affects activity, whereas in the case of 19-bp shRNAs, larger loops and the presence of a 3′-UU overhang increase efficacy. Comparison of shRNA and siRNA of the same sequence revealed that shRNAs are of similar or greater potency than the corresponding siRNAs in a human hepatocyte cell line chronically infected with HCV subgenomic replicons, and also in mice transfected with the luciferase reporter. The results indicate that shRNAs, delivered as RNA transcripts or chemically synthesized, may be effective agents for the control of HCV.

doi:10.1016/j.antiviral.2008.01.078

65

Anti-Influenza A Virus Inhibitory Effect of (−)-Epigallocatechin-3-*O*-Gallate Fatty Acid Monoester Derivatives

Kunihiro Kaihatsu *, Shuichi Mori, Nobuo Kato

Osaka University, Osaka, Japan

Seasonal influenza epidemics and pandemic outbreaks of influenza cause significant disease burdens and mortality in humans. Surprisingly, there are only a few prescribed antiviral drugs for the treatment and prophylaxis of influenza. A neuraminidase inhibitor, oseltamivir phosphate, is the most commonly used antiviral drug, and acts by preventing the release of viral particles from infected cells. However, it has been reported that a highly pathogenic avian influenza (H5N1) possesses resistance to oseltamivir. Moreover, the limited availability of the drug's starting material, shikimic acid, leads to shortages in the drug's supply. Therefore, there is an urgent need to develop a novel anti-influenza virus agent. Here, we describe an approach utilizing Epigallocatechin-3-*O*-gallate (EGCG), a major green tea component, as a novel anti-influenza virus agent. We prepared a series of fatty acid monoesters of EGCG by one-pot lipase-catalyzed transesterification. Our lipase-catalyzed method affords EGCG-monoesters in nearly twice the yield compared to a conventional acid chloride method. Pretreatment of MDCK cells with EGCG-monoesters effectively prevented influenza A/PR8/34 (H1N1) virus infection, and the EC_{50} decreased in an alkyl length-dependent manner. EGCG-monopalmitate exhibited the most potent antiviral activity among the EGCG-monoesters, approximately 24-fold relative to natural EGCG. Further, virus infectivity was drastically reduced when directly incubated with EGCG-monopalmitate. These results suggest that the moderate cell membrane and viral membrane permeability of EGCG-monopalmitate enhanced its accessibility to viral particles and prevented infection at much lower concentrations. Our simple and robust methodology should expand the use of EGCG as a novel antiviral agent. EGCG-monopalmitate is useful for the treatment and prophylaxis of influenza, since it directly interferes with the infectivity of budding viruses. We are studying the antiviral mechanism of EGCG-monoesters to investigate their potential use against oseltamivir-resistant viruses.

doi:10.1016/j.antiviral.2008.01.079