84
Influence of Hsp70 on HIV-1 Infection

Ryuichi Sugiyama [1,*], Yuichiro Habu [2], Haruki Naganuma [1], Hiroshi Takaku [1,3]

[1] Department of Life and Environmental Science, Chiba Institute of Technology, Chiba, Japan; [2] Japanese Foundation for AIDS Prevention, Tokyo, Japan; [3] High Technology Research Center, Chiba Institute of Technology, Chiba, Japan

Introduction: HIV-1 virions are reported to be formed by assembly of Gag protein multimers which come into contact with cell membrane and undergo budding and release from the cell. During this process, it has been reported that APOBEC3G (A3G) and Heat shock protein 70 (Hsp70) are incorporated into HIV-1 virions. A3G is a host protein with anti-HIV-1 activity and Hsp70 is one of molecular chaperones which assist protein folding. Nowadays, molecular chaperones are said to be related with control of cell functions such as protein synthesis, transport and quality control. In addition, they are widely involved in signal transduction and cellular immunity. In this study, we investigated the interaction of A3G, Hsp70 and HIV-1 Gag, and influence upon HIV-1 virion formation and infectivity.
Methods: In order to investigate the interaction among A3G, Hsp70 and HIV-1 Gag, we co-transfected 293T cells with HIV-1 infectious molecular clone and the A3G expression vector, and then performed I.P and I.F. Next, the quantity of A3G incorporation to HIV-1 virions and infectivity after interaction of A3G and Hsp70 were investigated by co-transfection of 293T cells with Hsp70-specific siRNA or Hsp70 expression vector, A3G expression vector and the HIV-1 infectious molecular clone. After that, Western blot analysis and infection assay were performed.
Results and conclusion: According to the results of I.P and I.F, it was verified that A3G, Hsp70 and HIV-1 Gag interact each other within cytoplasm. The amount of A3G incorporation into HIV-1 virions decreased when Hsp70 was knock downed, whereas it increased when Hsp70 was over expressed. Moreover, HIV-1 virion yield also decreased when Hsp70 was knock downed and virion yield increased when Hsp70 was over expressed. However, HIV-1 infectivity increased when Hsp70 was knock downed and when it was over expressed, HIV-1 infectivity declined, showing possibility that over expression of Hsp70 could lead to inhibition of HIV-1 and we can suggest the possibility of HIV-1 therapy.

doi:10.1016/j.antiviral.2008.01.098

85
Suppression of HCV RNA Replication by Baculovirus-mediated shRNA Expression Vectors

Hitoshi Suzuki [1,*], Nobushige Tamai [1], Kunitada Shimotohno [3], Yoshiharu Matsuura [4], Hiroshi Takaku [1,2]

[1] Department of Life and Environmental Sciences, Chiba Institute of Technology, Narashino, Japan; [2] High Technology Research Center, Chiba Institute of Technology, Narashino, Japan; [3] Resaerch Institute, Chiba Institute of Technology, Narashino, Japan; [4] Research Center for Emerging Infectious Diseases, Research Institute for Microbial Diseases, Osaka University, Suita, Japan

Hepatitis C virus (HCV) core protein has been reported to interact with a variety of cellular protein and to influence numerous host cell functions. Recently, RNAi is a highly specific mechanism of posttranscriptional gene silencing mediated by double-stranded siRNAs ranging in size from 21–27 nt, which can be targeted to any gene of interest. The HCV core gene is a potential target for RNAi technology. On the other hand, the baculovirus autographa californica multiple nucleopolyhedrovirus (AcMNPV) can infect a variety of mammalian cells, facilitating its use as a virus vector for gene delivery in viral entry into cells. In this study, we describe the suppression of HCV replication by baculovirus-mediated shRNA expression vectors. We identified an effective site on the core region for suppression of the HCV core protein. This was carried out by core protein-shRNA expression Baculovirus vectors (core-shRNA-452, -479, and -523). Especially, the core-shRNA-452 containing sequence of 452–472 nt, as the target of the HCV core gene dramatically inhibited the expression of HCV core protein in replicon cells. Our results support the feasibility of using shRNA-based gene therapy to inhibit HCV core protein production.

doi:10.1016/j.antiviral.2008.01.099

86
Synthesis and Evaluation of Octadecyloxyethyl Esters of Five 3-Hydroxy-2-(Phosphonomethoxy)Propyl Nucleoside Phosphonates in HIV-1 Infected Cells

Nadejda Valiaeva [1,2,*], Kathy A. Aldern [1,2], Julissa Trahan [1,2], James R. Beadle [1,2], Karl Y. Hostetler [1,2]

[1] Division of Infectious Disease, University of California, San Diego, La Jolla, USA; [2] The Veterans Medical Research Foundation, San Diego, USA

Acyclic nucleoside phosphonates (ANPs) having the (*S*)-3-hydroxy-2-(phosphonomethoxy)-propyl (HPMP) side chain are an important group of potent and selective anti-DNA virus agents that includes cidofovir and (*S*)-HPMPA. Addition of a phosphonate-masking alkoxyalkyl group containing about 20 atoms (e.g. hexadecyloxypropyl, HDP) enhances the antiviral activity, oral absorption and pharmacokinetics of cidofovir, (*S*)-HPMPA and all other ANPs that our group has studied. Octadecyloxyethyl (ODE) esters are generally more active than HDP esters. We have shown previously that the