Available online at www.sciencedirect.com
ScienceDirect
Journal of Biotechnology 133 (2008) 386–394
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Functional modification of Sendai virus by siRNA

Kotaro Saga, Katsuto Tamai, Masako Kawachi, Takashi Shimbo, Hiroshi Fujita, Takehiko Yamazaki, Yasufumi Kaneda *

Division of Gene Therapy Science, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

Received 26 March 2007; received in revised form 10 October 2007; accepted 23 October 2007

Abstract

Sendai virus (hemagglutinating virus of Japan; HVJ) is a negative-strand RNA virus with robust fusion activity, and has been utilized for gene transfer and drug delivery. Hemagglutinin-neuraminidase (HN) protein on the viral membrane is important for cell fusion, but causes agglutination of red blood cells. HN-depleted HVJ has been desired for *in vivo* transfection in order to improve safety. Here, we succeeded in producing HN-depleted HVJ using HN-specific short interfering RNA (siRNA). Viral production was not affected by the siRNA. HN protein was markedly decreased in the new HVJ, while other viral proteins were retained. Consequently, the hemagglutinating activity was substantially reduced and infection activity was suppressed. When the HN-depleted HVJ was mixed with cultured cells and the mixture was centrifuged for 10 min at $2000 \times g$, the modified HVJ recovered its infectivity to approximately 80% of wild HVJ. However, infectivity was abolished in the presence of anti-F antibody. Moreover, transfection of FITC-labeled oligodeoxynucleotides using the modified HVJ was also recovered by centrifugation. Thus, the HN-depleted HVJ produced using siRNA technology will be applicable to a delivery vector.

Keywords: Sendai virus; HVJ; siRNA; HN; Knock-down; Modification

1. Introduction

Sendai virus (hemagglutinating virus of Japan; HVJ) belongs to the paramyxovirus family, which has a negative-sense single-strand RNA genome (Curran and Kolakofsky, 1999; Lamb and Kolakofsky, 2001). The genome encodes six viral proteins: nucleocapsid (N), phospho (P), large (L), matrix (M), fusion (F) and hemagglutinin-neuraminidase (HN). Of these, HN and F are membrane glycoproteins on the HVJ envelope (Okada, 1993; Yeagle, 1993), and M binds to the cytoplasmic domain of these glycoproteins (Ali and Nayak, 2000; Mottet et al., 1999; Takimoto et al., 2001).

The F protein is translated as F_0 in host cells, and in principle, F_0 is activated in the trans Golgi, ensuring that F_1/F_2 is present in infectious viral progeny. In tissue culture cells lacking the furin-like protease; however, F_0 is not cleaved and the produced virion is not infectious (Tashiro et al., 1999). HN binds to sialic acid, its cell-surface receptor, and neuraminidase to degrade the receptor (Takimoto et al., 2002). Infection starts with the binding of HN to sialic acid and subsequent receptor degradation. F then induces membrane fusion by inserting its putative fusion peptide into the lipid bilayer of the cell membrane, facilitating viral genome introduction into the target cell (Asano and Asano, 1988).

The fusion activity of HVJ has been used to construct novel drug-delivery vectors. We recently succeeded in incorporating therapeutic molecules directly into inactivated HVJ particles without liposomes (Kaneda et al., 2002). The resulting HVJ-envelope vector (HVJ-E) enables delivery of proteins, synthetic oligonucleotides and drugs, as well as plasmid DNA, to target cells both *in vitro* and *in vivo* (Kaneda et al., 2005).

However, one of the limitations of these HVJ-derived vectors is hemagglutination and hemolysis of red blood cells (Inoue et al., 1985). Hemagglutination occurs when HN protein binds to sialic acid on the surface of red blood cells (Portner et al., 1987), while hemolysis is induced by both F and HN (Dallocchio et al., 1995; Hoekstra and Klappe, 1986). Therefore, regulation of HN expression on HVJ is necessary to develop less invasive vectors.

In order to address this issue, we report here successful regulation of HN activity on HVJ using HN-specific short interfering RNA (siRNA).

* Corresponding author. Tel.: +81 6 6879 3900; fax: +81 6 6879 3909.
E-mail address: kaneday@gts.med.osaka-u.ac.jp (Y. Kaneda).

0168-1656/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2007.10.003

2. Materials and methods

2.1. Virus

HVJ (VR-105 parainfluenza1 Sendai/52, Z strain) was purchased from American Type Culture Collection (Manassas, VA, USA), amplified in the chorioallantoic fluid of 10–14-day-old chick eggs, and purified by centrifugation, as described previously (Kaneda et al., 2002).

2.2. Antibodies

Mouse monoclonal antibody (IgG) against F protein, f236 (Tozawa et al., 1986) was kindly provided by H. Taira (Department of Bioscience and Technology, Faculty of Agriculture, Iwate University, Morioka, Japan).

Rabbit polyclonal antibodies (IgG) against M and HN were generated by Hokkaido System Science Co. Ltd. (Sapporo, Japan), using peptide antigens of HN and M. The peptide sequence of HN was 276-VDERTDYSSDGIED-289, and that of M was 23-LRTGPDKKAIPHIR-36.

2.3. Cell culture

Monkey kidney cells (LLCMK2) were maintained in minimum essential medium (MEM) (Gibco-BRL, Rockville, MD, USA), and Baby hamster kidney cells (BHK-21) and normal embryonic mouse liver cells (BNL-CL2) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Nacalai Tesque Inc., Tokyo, Japan). Both MEM and DMEM were supplemented with 10% fetal bovine serum (FBS) (Biowest, Nuaillé, France), 100 units/ml penicillin and 0.1 mg/ml streptomycin (Penicillin–Streptomycin Mixed Solution) (Nacalai Tesque Inc.).

2.4. siRNA synthesis for HN mRNA knock-down

Five siRNAs for HN mRNA knock-down were designed and synthesized using SMART siRNA Technology™ at Dharmacon Research (Dharmacon, Lafayette, CO, USA). Each siRNA (HN-223, -342, -899, -1142 and -1427) targeted a different sequence: GCAUUGAACAUGAGCAGCA (HN mRNA nucleotides 223–241), GAACAAAAACAGCAGGGAU (342–360), GAACUAAGUCUCACCGGUA (899–917), GCGUGAUCAUCCAGGUCAA (1142–1160), and GCGUAUACACUGAUGCUUA (1427–1447). Scramble siRNA for use as a control had a random sequence (GCGCGCUUUGUAGGAUUCG).

2.5. Transfection of culture cells with siRNA

Synthesized siRNAs were transferred to LLCMK2 cells at concentrations of 50, 100, 200 or 320 pmol/ml using Lipofectamin Reagent™ (Invitrogen, California, USA) and Plus Reagent (Invitrogen), according to the manufacturer's instructions.

2.6. Infection of culture cells with HVJ

After washing culture cells (LLCMK2, BHK and BNL-CL2) with Dulbecco's phosphate-buffered saline (PBS) (Nacalai Tesque Inc.), Opti-MEM I (GIBCO™, Invitrogen) containing different amounts of HVJ was added to the cells in order to determine the optimum particle numbers (0.06–3.0 particles/cell), and cells were infected with HVJ for 1 h under 5% CO_2 at 37 °C. The infection medium was then removed, and infected cells were washed with PBS, followed by incubation in culture medium for 24 h under 5% CO_2 at 37 °C.

2.7. Isolation of HN-depleted HVJ

LLCMK2 cells transfected with HN-899 or scramble siRNA at 100 pmol/ml by Lipofectamin Reagent and Plus Reagent for 24 h, were infected with HVJ (1.5 particles/cell) for 1 h. Untransfected control cells were subjected to the same procedure. Infected cells were then cultured in MEM containing 100 units/ml penicillin and 0.1 mg/ml streptomycin for 48 h, and culture medium was passed through a filter (pore size, 1.2 μm). The supernatant was centrifuged at 100,000 × g for 2 h at 4 °C to precipitate HVJ particles.

2.8. Quantification of viral genome by RT-PCR

HVJ RNA genomes were isolated using the PURESCRIPT® Cell and Tissue RNA Purification Trial Kit (Gentra Systems Inc., Minneapolis, MN, USA). cDNA was synthesized from the purified HVJ RNA genome (about 200 ng) using the SuperScript™ III First Strand Synthesis System (Invitrogen). HVJ genome (about 250 ng of cDNA) was quantified by real-time RT-PCR using the TaqMan® Universal PCR Master Mix (Applied Biosystems Japan Ltd., Tokyo, Japan) and TaqMan® MGB Probe (Applied Biosystems Japan Ltd.). The TaqMan MGB Probe (5′-FAM-ATCCACCTAGCAGCTGT-MGB-3′) recognized the L protein coding region of the HVJ genome.

2.9. Northern blotting

Total RNA was extracted from culture cells using the RNeasy Mini Kit (Qiagen K.K., Tokyo, Japan). Total RNA (30 μg) was electrophoresed on a 1% agarose gel (Cambrex Bio Science Rockland Inc., Rockland, MD, USA), and was transferred to a Hybond-N+ nylon transfer membrane (Amersham Biosciences UK Ltd., Buckinghamshire, UK). In order to detect HN, F, M and G3PDH mRNA in the membrane, hybridization was performed with ^{32}P-labeled cDNA probes using the Random Primers DNA Labeling System (Invitrogen) in PerfectHyb™ (Toyobo Co. Ltd., Osaka, Japan).

2.10. Western blotting

HVJ (about 6×10^7 particles) dissolved in sample buffer (125 mM Tris–HCl (pH 6.8), 10% 2-mercaptoethanol, 4% sodium dodecylsulfate, 10% sucrose, 0.004% bromophenol