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The use of an optimized chimeric envelope glycoprotein enhances the efficiency of retrograde gene transfer of a pseudotyped lentiviral vector in the primate brain

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ABSTRACT

Lentiviral vectors have been used not only for various basic research experiments, but also for a wide range of gene therapy trials in animal models. The development of a pseudotyped lentiviral vector with the property of retrograde infection allows us to introduce foreign genes into neurons that are localized in regions innervating the site of vector injection. Here, we report the efficiency of retrograde gene transfer of a recently developed FuG-E pseudotyped lentiviral vector in the primate brain by comparing its transduction pattern with that of the parental FuG-C pseudotyped vector. After injection of the FuG-E vector encoding green fluorescent protein (GFP) into the striatum of macaque monkeys, many GFP-immunoreactive neurons were found in regions projecting to the striatum, such as the cerebral cortex, thalamus, and substantia nigra. Quantitative analysis revealed that in all regions, the number of neurons retrogradely transduced with the FuG-E vector was larger than in the FuG-C vector injection case. It was also confirmed that the FuG-E vector displayed explicit neuronal specificity to the same extent as the FuG-C vector. This vector might promote approaches to pathway-selective gene manipulation and provide a powerful tool for effective gene therapy trials against neurological disorders through enhanced retrograde delivery.

1. Introduction

Replication-defective lentiviral vectors allow us to introduce foreign genes into non-dividing neuronal cells in the central nervous system and to achieve sustained long-term expression of the genes (Blömer et al., 1998; Cockrell and Kafri, 2007; Naldini et al., 1996). This property of the lentiviral vector makes it a useful tool not only for various basic research experiments, but also for a wide range of gene therapy trials in animal models (Björklund et al., 2000; Lundberg et al., 2008). Pseudotyping of lentiviral vectors with different envelope glycoproteins alters the pattern of viral entry, resulting in the difference in the host range of the vectors (Cronin et al., 2005). It has been revealed in our previous study that the use of the glycoprotein of rabies virus (RV-G) for preparation of a pseudotyped lentiviral vector based on human immunodeficiency virus type 1 (HIV-1) can enhance the efficiency of gene transfer through retrograde transport of the vector (Kato et al., 2007). Such a lentiviral vector largely allows for gene transfer into cell bodies of neurons that are located remote from the injection site of the vector.

Later, we developed four types of lentiviral vectors each of which is pseudotyped with fusion envelope glycoprotein type A, B, C, or D that is composed of a distinct combination of RV-G and vesicular stomatitis virus glycoprotein (VSV-G), and found that only the FuG-B and FuG-C types exhibit high efficiency of retrograde gene delivery (Kato et al., 2011a,b). One is the so-called NeuRet vector pseudotyped with the fusion glycoprotein type C (FuG-C), and the other is the so-called HiRet vector pseudotyped with the fusion glycoprotein type B (FuG-B). While both vectors display high efficiency of retrograde gene transfer in rodent and nonhuman primate brains, the NeuRet vector possesses higher neuronal specificity than the HiRet vector. These newly-developed vectors have been applied for investigating the functional roles of given pathways through their selective elimination or suppression (Ishida et al., 2016; Kato et al., 2011c). Particularly, the vectors provide a pow-

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erful tool for pathway-selective gene manipulation in the brain of nonhuman primates in which genetic modification has so far been largely limited (Inoue et al., 2012; Kinoshita et al., 2012). In order to increase the availability and applicability of this methodology, further improvement of the vector system is still needed.

We have recently demonstrated in mice that the use of a novel type of fusion glycoprotein (FuG-E) of which segmental junction was optimized results in increased retrograde gene transfer relative to the parental FuG-C fusion glycoprotein in the thalamostriatal and corticostriatal projections (Kato et al., 2014). In the present study, we examined the pattern and efficiency of transgene expression of the FuG-E pseudotyped lentiviral vector in the striatal input system of macaque monkeys by comparing with those of the FuG-C pseudotyped vector. We found the superiority of the newly developed FuG-E vector to the FuG-C vector.

2. Materials and methods

2.1. Animals

Four adult Japanese monkeys (*Macaca fuscata*) of either sex weighing 5.6-6.2 kg were used. The monkeys were fed regularly with diet pellets and had free access to water. The experimental protocol was approved by the Animal Welfare and Animal Care Committee of the Primate Research Institute, Kyoto University (Permission Number: 2015-033), and all experiments were conducted according to the Guidelines for Care and Use of Nonhuman Primates by the Primate Research Institute, Kyoto University (2010). The entire experiments were carried out in a special laboratory (biosafety level 2) designated for in vivo primate infectious experiments that had been installed at the Primate Research Institute, Kyoto University. Throughout the experiments, the monkeys were maintained in individual cages that were placed inside a special safety cabinet with temperature (23–26 °C) and light (12 h on/off cycle) controlled. Every effort was made to minimize monkeys' sufferings.

2.2. Viral vector production

The constructions of envelope plasmids (pCAGGS-FuG-C and pCAGGS-FuG-E) were described elsewhere (Kato et al., 2011b, 2014). The transfer plasmid (pCL20c-MSCV-GFP) contained the cDNA encoding enhanced green fluorescent protein (GFP) downstream of the murine stem-cell virus (MSCV) promoter. DNA transfection and viral vector preparation were performed as previously described (Hanawa et al., 2002a,b; Hanawa et al., 2004; Kato et al., 2014). Briefly, HEK293T cells were transfected with transfer, envelope, and packaging plasmids (pCAG-kGP4.1R and pCAG4-RTR2) by the calcium-phosphate precipitation method. Eighteen hours after transfection, the medium was replaced with fresh medium and cells were subsequently incubated for 24 h. The medium was then harvested and filtered through a 0.45-μm Millex-HV filter unit (Millipore, USA). Viral vector particles were pelleted by centrifugation at 6000 × *g* for 16–18 h and resuspended in 0.01 M phosphate-buffered saline (PBS). The particles were then applied to a Sepharose Q FF ion-exchange column (GE Healthcare, UK) in PBS and eluted with a linear 0–1.5 M NaCl gradient. The fractions were monitored at 260/280 nm of absorbance wavelength. The peak fractions containing the particles were collected and concentrated by centrifugation through a Vivaspin filter (Vivascience, UK).

For measuring viral titer, viral RNA in a vector stock solution was isolated with a NucleoSpin RNA virus kit (Takara, Japan), and the copy number of the RNA genome was determined by quantitative PCR using Taq-Man technology (Thermo Fisher Scientific, USA). Purities of the vectors were assessed by visualizing viral proteins and other protein signals with 4–12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorescent staining (Oriole, BIO-RAD, USA).

2.3. Surgical procedures

The monkeys were first sedated with ketamine hydrochloride (5 mg/kg, i.m.) and xylazine hydrochloride (0.5 mg/kg, i.m.), and then anesthetized with sodium pentobarbital (20 mg/kg, i.v.). During the surgical operation, the monkeys were kept hydrated with a lactated Ringer's solution (i.v.). An antibiotic (Ceftazidime; 25 mg/kg, i.v.) and an analgesic (Meloxicam; 0.2 mg/kg, s.c.) were administered at the initial anesthesia. After partial removal of the skull, multiple injections of the vectors were made on one side of the striatum by the aid of a magnetic resonance imaging (MRI)-guided navigation system (Brainsight Primate, Rogue Research, Canada). A total of 60 μl of the FuG-C or FuG-E pseudotyped lentiviral vector (7.0×10^{10} genome copies per ml) was injected unilaterally into both the caudate nucleus and the putamen at six rostrocaudally different levels (3–5 μl/site, two sites per track, eight tracks per monkey; three tracks for the caudate nucleus and five tracks for the putamen) through a 10-μl Hamilton microsyringe. When the injections were complete, the scalp incision was closed. The monkeys were monitored until they fully recovered from the anesthesia.

2.4. Immunohistochemistry

Four weeks after the vector injection, the monkeys were deeply anesthetized with an overdose of sodium pentobarbital (50 mg/kg, i.v.) and transcardially perfused with 0.1 M PBS, followed by 10% formalin dissolved in 0.1 M phosphate buffer. The fixed brains were removed from the skull, postfixed in the same fresh fixative overnight at 4 °C, and equilibrated with 30% sucrose in 0.1 M PBS at 4 °C. Coronal sections were then cut serially at 50 μm thickness on a freezing microtome. Every tenth section was mounted onto gelatin-coated glass slides and Nissl-stained with 1% Cresyl violet. The remaining series of the sections 500 μm apart were processed for immunohistochemistry.

For immunoperoxidase staining, the sections were pretreated with 0.3% H_2O_2 for 30 min, washed three times in 0.1 M PBS, and immersed in 1% skim milk for 1 h. Subsequently, the sections were incubated for 2 days at 4 °C with monoclonal rabbit anti-GFP antibody (1:4000 dilution; Thermo Fisher Scientific) in 0.1 M PBS containing 2% normal donkey serum and 0.1% Triton X-100. The sections were then incubated with biotinylated donkey anti-rabbit IgG antibody (1:1000 dilution; Jackson laboratories, USA) in the same fresh medium for 2 h at room temperature, followed by the avidin-biotin-peroxidase complex kit (ABC Elite; 1:200 dilution; Vector laboratories, USA) in 0.1 M PBS for 2 h at room temperature. To visualize the antigen, the sections were reacted for 10–20 min in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.04% diaminobenzine tetrahydrochloride (Wako, Japan), 0.04% $NiCl_2$, and 0.002% H_2O_2. The reaction time was adjusted to make the density of background immunostaining almost identical throughout the cases. These sections were counterstained with 0.5% Neutral red, mounted onto gelatin-coated glass slides, dehydrated, and then coverslipped.

For double immunofluorescence histochemistry for GFP and one of neuronal nuclei (NeuN), glial fibrillary acidic protein (GFAP), and tyrosine hydroxylase (TH), the sections were rinsed three times in 0.1 M PBS, immersed in 1% skim milk for 1 h, and then incubated with sheep polyclonal anti-GFP antibody (1:20,000 dilution) and one of the following mouse monoclonal antibodies: anti-NeuN antibody (1:2000 dilution; Millipore, USA), anti-GFAP antibody (1:500 dilution; Sigma, USA), and anti-TH antibody (1:500 dilution; Millipore). The sections were then incubated for 2 h at room