Chapter 15

Vectors for Highly Efficient and Neuron-Specific Retrograde Gene Transfer for Gene Therapy of Neurological Diseases

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/52611

1. Introduction

Viral vectors have been widely used to deliver several therapeutic genes in the clinical approach of gene therapy. The lentiviral vector permits stable and efficient gene transfer into non-dividing cells in the central nervous system of neurological and neurodegenerative diseases (Deeks, et al., 2002; Mavilo, et al., 2006; Rossi et al., 2007; Ciceri, et al., 2009; Naldini, 2011). Moreover, long-term expression of delivered gene attributed to genome integration has an advantage not only for clinical application, but also for gene therapy trials in animal models (Naldini et al., 1996; Reiser et al., 1996; Mochizuki et al., 1998; Mitrophanous et al., 1999; Wong et al., 2006; Lundberg et al., 2008). Among many lentiviral vector systems, the most familiar is the human immunodeficiency virus type-1 (HIV-1)-based vector of which molecular biological property has been extensively studied (Rabson and Martin, 1985; Joshi and Joshi, 1996; Nielsen et al., 2005; Pluta and Kacprzak, 2009).

Axonal transport in the retrograde direction, as observed in the case of some viral vectors, has a considerable advantage for transferring genes into neuronal cell bodies situated in regions remote from the injection sites of the vectors (see Fig.1). These viral vectors, for example, injected into the striatum, transfer the genes via retrograde transport into nigrostriatal dopaminergic neurons, which are the major target for gene therapy of Parkinson's disease (Zheng et al., 2005; Barkats et al., 2006). Intramuscular injection of the vectors also delivers retrogradely the genes into motor neurons that are the target for gene therapy of motor neuron diseases (Baumgartner & Shine, 1998; Perrelet et al., 2000; Mazarakis et al., 2001; Sakamoto et al., 2003; Azzouz et al., 2004).

In our previous study, we generated an HIV-1-based vector pseudotyped with a variant of rabies virus glycoprotein (RV-G) gene and tested gene transfer through retrograde axonal transport into several brain regions (Kato et al., 2007). Although this pseudotyped vector showed gene transfer through retrograde transport in the rodent and nonhuman primate brains, higher titer stocks of the vector was required for the application of gene therapy trials. To enhance the efficiency of retrograde gene transfer, we subsequently developed a novel type of lentiviral vector that shows highly efficient retrograde gene transfer (HiRet) by pseudotyping an HIV-1-based vector with fusion glycoprotein B type (FuG-B) composed of parts of RV-G and vesicular stomatitis virus glycoprotein (VSV-G) (Kato et al., 2011a,b).

More recently, we developed another vector system for neuron-specific retrograde gene transfer (NeuRet) by pseudotyping the HIV-1-based vector with fusion glycoprotein C type (FuG-C) composed of a different set of parts of RV-G and VSV-G (Kato et al., 2011c). Interestingly, the NeuRet vector shows high efficiency of retrograde gene transfer into various neuronal populations, whereas it remarkably reduces gene transduction into dividing cells including glial and nerural stem/progenitor cells around the vector injection sites. One significant issue on the therapeutic use of lentiviral vectors is transgene integration into the host genome in dividing cells, which may lead to tumorigenesis by altering the expression of proto-oncogenes adjacent to the integration sites (De Palma et al., 2005; Themis et al., 2005; Montini et al., 2006). In this context, the NeuRet vector can reduce the risk of vector transduction into dividing cells in the brain and improve the safety of future gene therapy trials for neurological and neurodegenerative disorders.

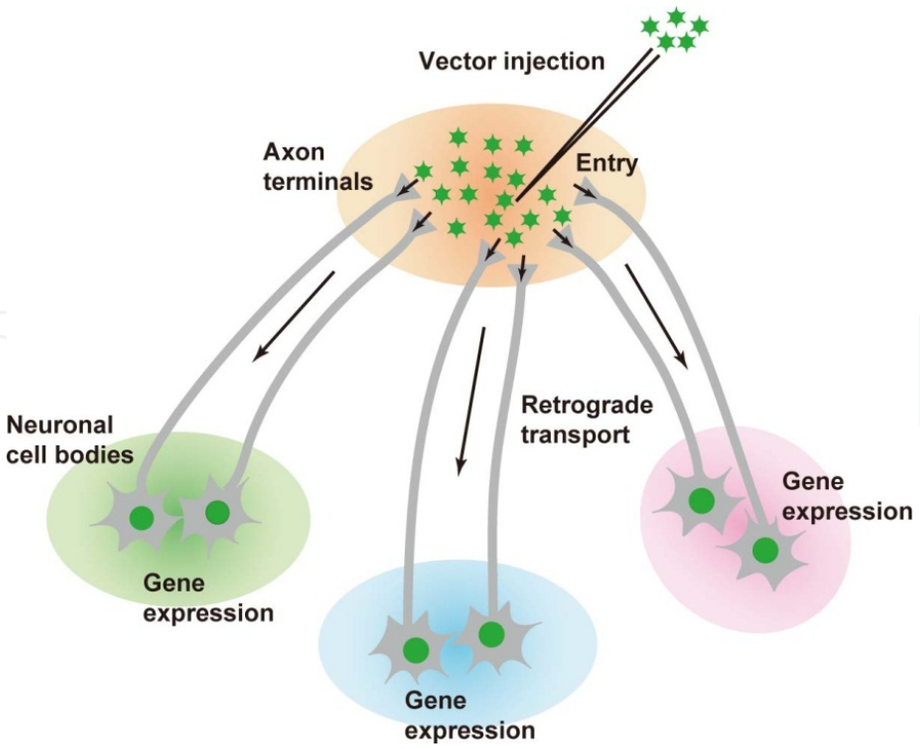

Figure 1. Gene transfer process through retrograde axonal transport.

http://dx.doi.org/10.5772/52611

The viral vectors enter nerve terminals and are retrogradely transported through axons into neuronal cell bodies, resulting in the induction of transgene expression.

In this chapter, we recapitulate gene transduction property of the HiRet and NeuRet vectors, and then describe the application of the NeuRet vector for retrograde gene transfer into the nigrostriatal dopamine system in nonhuman primates.

2. Gene transduction property of HiRet and NeuRet vectors

2.1. HiRet vector

The HiRet vector is a pseudotype of the HIV-1 lentiviral vector with FuG-B, which is composed of the extracellular and transmembrane domains of RV-G (challenged virus standard strain) and the cytoplasmic domain of VSV-G (Fig. 2A) (Kato et al., 2011a). When the HiRet vector encoding green fluorescent protein (GFP) was injected into the dorsal striatum of mice, we observed high efficiency of retrograde gene transfer into the brain regions innervating the striatum, including the primary motor cortex (M1), primary somatosensory cortex (S1), parafascicular nucleus (PF) in the thalamus, and substantia nigra pars compacta (SNc) in the ventral midbrain (Fig. 2B). The extent of gene transfer efficiency increased compared with that of the RV-G pseudotype, ranging from 8- to 14-folds dependent on the neural pathways. The high efficiency of gene transfer was also detected in the brain regions that project to the nucleus accumbens or medial prefrontal cortex in mice. In addition, we observed gene transduction of the HiRet vector into glial cells (~75%) and a small number of neuronal cells (~20%) in the striatum around the injection sites (Fig. 2C). Recently, we created a variant of FuG-B (termed FuG-B2), in which the extracellular and transmembrane domains of RV-G derived from the challenged virus standard strain was exchanged with the counterparts of Pasteur virus strain, and the vector pseudotyped with FuG-B2 exhibited a further increase in the retrograde gene transfer efficiency in the rodent brain (Kato et al., 2011b). More recently, Carpentier et al. (2012) reported the increased psudotyping efficiency of an HIV-1 vector by a chimeric envelope glycoprotein composed of RV-G and VSV-G domains, which corresponds to our FuG-B.

The host range of lentiviral vectors is altered by pseudotyping with different envelope glycoproteins (Cronin et al., 2005). Therefore, the possibility arises that some mutations in RV-G shift the efficiency of gene transduction or host cell specificity of the pseudotyped vector. Indeed, substitution of the cytoplasmic domain of RV-G with the corresponding part of the VSV-G enhanced the efficiency of retrograde gene transfer. The cytoplasmic domain differs in length between RV-G (44 amino acids) and VSV-G (29 amino acids), but their amino acid sequences do not show any particular homology (Rose et al., 1982). It appears that the cytoplasmic domain is involved in the mechanism underlying vector entry into synaptic terminals or the transduction level of the vector, resulting in enhanced retrograde gene transfer.

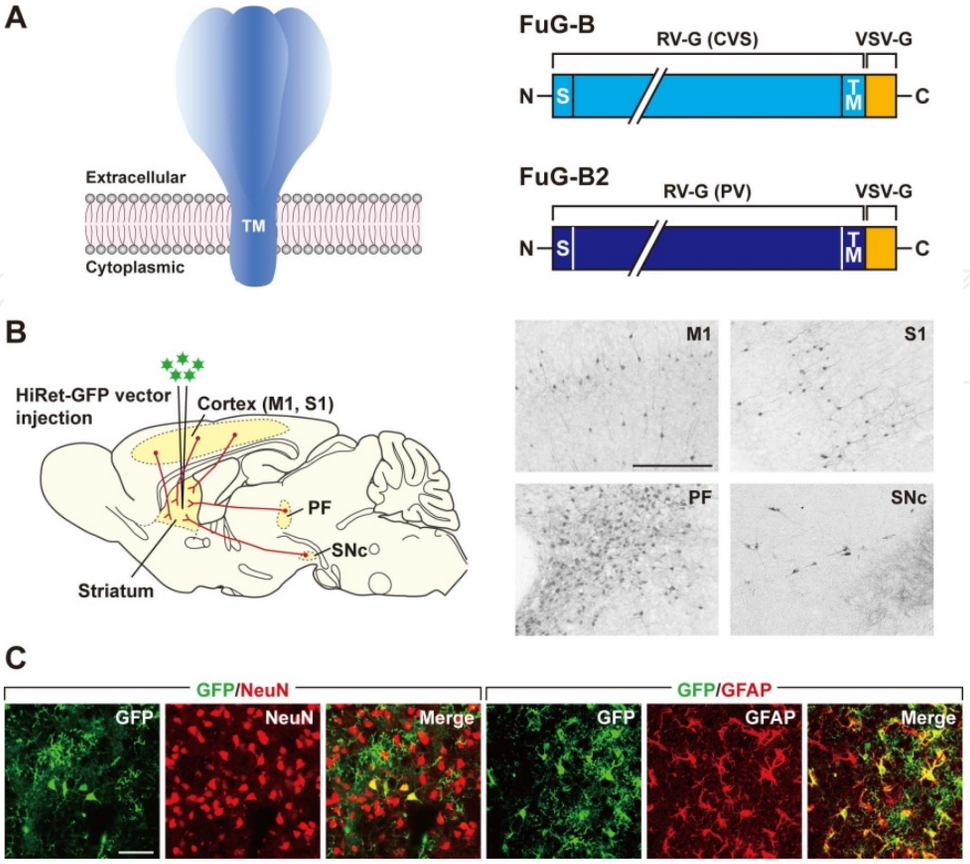

Figure 2. Gene trasnfer by HiRet vector. (**A**)Fusion envelope glycoprotein. The structure of viral envelope glycoprotein is schematically illustrated in the left panel. FuG-B is composed of the extracellular and transmembrane (TM) domains of RV-G derived from the challenge virus standard (CVS) strain fused to the cytoplasmic domain of VSV-G. In FuG-B2, the RV-G domains are exchanged by the counterparts of RV-G derived from Pasteur virus (PV) strain. S, signal peptide. (**B**) Gene transfer through retrograde transport. The HiRet vector pseudotyped with FuG-B, encoding GFP transgene was injected into the mouse striatum. Four weeks later, sections were processed for GFP immunostaining (right panel). GFP expression can be seen in the brain regions innervating the striatum, including the M1, S1, PF, and SNc. (**C**) Gene transduction around the injection sites. Sections through the striatum were stained by double immunofluorescence histochemistry for GFP/NeuN or for GFP/glial fibrillary acidic protein (GFAP). Scale bars: 50 μm. (Data from Kato et al., 2011a)

2.2. NeuRet vector

The NeuRet vector is another pseudotype of the HIV-1 lentiviral vector with FuG-C, which is composed of the N-terminal segment of the extracellular domain (439 amino acids) of RV-G and the C-terminal segment of the extracellular domain (16 amino acids) and transmembrane/cytoplasmic domains of VSV-G (Fig. 3A) (Kato et al., 2011c). After injection of the NeuRet vector encoding GFP transgene into the mouse striatum, we found enhanced retrograde gene transfer into the brain regions innervating the striatum, such as the M1, S1, PF, and SNc (Fig. 3B). The efficiency of gene transfer of the NeuRet vector was slightly different with that of the HiRet vector (FuG-B2 pseudo type), depending on the neural pathways (see a review by Kato et al. 2012). In addition, we tested gene transduction of the NeuRet vector surrounding the injection sites. Although the NeuRet vector transduced only a small num-

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ber of striatal neuronal cells (~6%), its transduction level into striatal glial cells was quite low (~0.3%) (Fig. 3C). The property of gene transduction of the NeuRet vector around the injection sites was quite different from that of the HiRet vector, and in particular, the transduction of glial cells was largely declined in the NeuRet vector. Furthermore, when the NeuRet vector was injected into the subventricular zone, gene transduction of the vector into neural stem/progenitor cells was also inefficient.

FuG-C pseudotyping of the NeuRet vector enhanced the efficiency of retrograde gene transfer into various neuronal populations, whereas it caused less efficiency of gene transduction into glial and neural stem/progenitor cells. The N-terminal segment of the RV-G extracellular domain of 439 amino acids appears to be involved in the retrograde gene transfer, probably by promoting the interaction with synaptic terminals required for retrograde transport. Actually, amino acid residues essential for rabies virus virulence are reported to exist in the RV-G-derived extracellular domain used for FuG-C construction (Prehaud et al., 1988; Coulon et al., 1998). In contrast, pseudotyping with FuG-B (FuG-B2) and FuG-C generates a marked difference in gene transduction into glial and neural stem/progenitor cells around the injection areas. This difference suggests that the C-terminal part of 16 amino acids in the extracellular domain of envelope glycoproteins may be implicated in determining the host cell specificity of vector transduction, and that this C-terminal part may contribute to the interaction with glial and neural stem/progenitor cells.

For gene therapy trials with lentiviral vectors, there is a significant issue that vector insertion into the host genome may lead to tumorigenesis by altering the expression of cellular oncogenes surrounding the integration sites (De Palma et al., 2005; Themis et al., 2005; Montini et al., 2006). One useful approach to protect this issue is to restrict vector transduction to neuronal cells. The NeuRet vector system provides a useful approach for gene therapy trials for neurological diseases through enhanced retrograde gene transfer and improves the safety of gene therapy by profoundly suppressing the efficacy of gene transduction into dividing cells in the brain.

3. Retrograde gene delivery into monkey nigrostriatal pathway by NeuRet vector

The nigrostriatal dopamine system is a major target for gene therapy of Parkinson's disease. The availability of the HiRet vector for gene transfer via retrograde transport into the nigrostriatal dopamine system in nonhuman primates was described in our previous review (Kato et al., 2011d). To verify the capability of the NeuRet vector for efficient retrograde gene transfer into the nigrostriatal pathway, we injected the NeuRetvector encoding the GFP transgene into the striatum (caudate nucleus and putamen) of crab-eating monkeys (Fig. 4A). Intrastriatal injection of the NeuRet vector produced a larger number of GFP-positive neurons in the SNc (Fig. 4B). These positive signals were in register with immunostaining for tyrosine hydroxylase, a marker of dopaminergic neurons (Fig. 4C), indicating the transgene expression in the nigrostriatal dopaminergic neurons. In addition, we assessed the

property of gene transduction with the NeuRet vector around the injection sites in the monkey striatum. The vector displayed a low level of gene transfer into neuronal cell bodies (~13%), and the level of vector transduction into glial cells was also quite low in the monkey striatum (~0.6%) (Fig. 4D).The pattern of gene transduction around the injection sites was similar to that obtained from the analysis of the mouse brain sections. Therefore, the NeuRet vector mediates enhanced retrograde gene transfer, whereas it reduces the gene transfer into glial cells around the injection areas in both rodent and monkey brains.

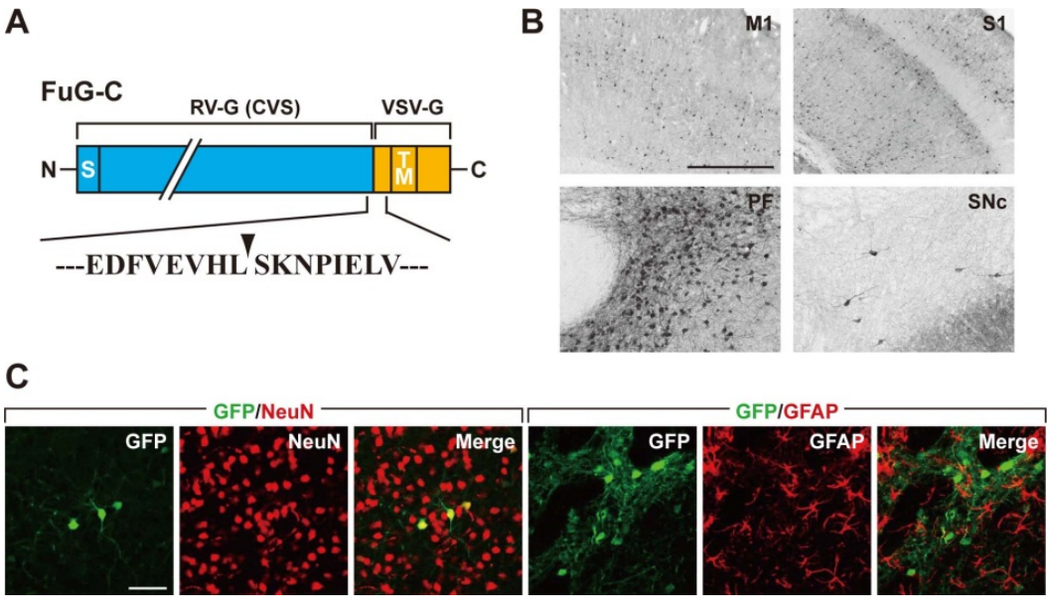

Figure 3. Gene delivery by NeuRet vector. (**A**) Structure of fusion envelope glycoprotein. FuG-C is composed of the N-terminal segment of the extracellular domain of RV-G and the C-terminal segment of the extracellular domain and the transmembrane(TM)/cytoplasmic domains of VSV-G. Amino acid sequences around the junction between the RV-G and VSV-G segments are shown. S, signal peptide. (**B**) Gene transfer through retrograde transport. The NeuRet vector encoding GFP transgene was injected into the mouse striatum, and four weeks later sections were processed for GFP immunostaining. GFP expression can be visualized in the M1, S1, PF, and SNc. (**C**) Gene transduction around the injection sites. Sections through the striatum were stained by double immunofluorescence histochemistry for GFP/NeuN or for GFP/glial fibrillary acidic protein (GFAP). Scale bars: 50 µm. (Data from Kato et al., 2011c)

The NeuRet vector system successfully achieved efficient gene transfer through retrograde transport into the nigrostriatal dopaminergic neurons in nonhuman primates. Our vector system will provide a powerful strategy for gene therapy of Parkinson's disease with enhanced retrograde gene transfer in the near future. This system will improve the safety of gene therapy by reducing the risk of gene transduction into proliferating cells (glial and neural stem/progenitor cells) in the brain.

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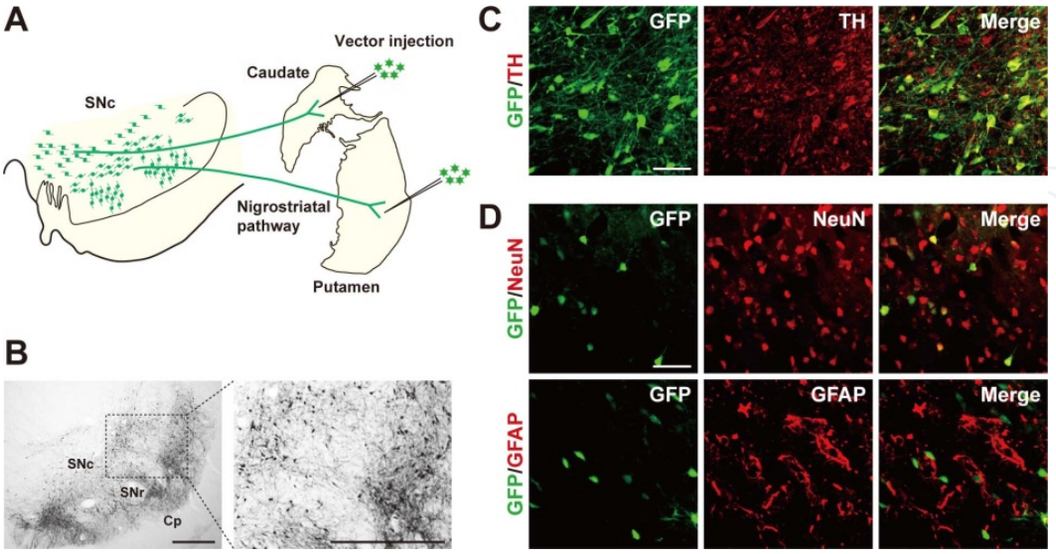

Figure 4. Transgene expression in the nigrostriatal dopamine system by NeuRet vector injection into the monkey striatum. (**A**) Gene transfer through retrograde transport after intrastriatal injection. The NeuRet vector encoding GFP transgene was stereotaxically injected into the caudate nucleus and the putamen, and histological analysis was performed on the brains fixed at the 4-week postinjection period. (**B**) GFP immunostaining in the SNc.Cp, cerebral peduncle; SNr, substantia nigra pars reticulata. (**C**) Double immunofluorescence staining for GFP and tyrosine hydroxylase (TH) in the SNc. (**D**) Double immunofluorescence staining for GFP/NeuN or GFP/glial fibrillary acidic protein (GFAP) in the striatum. Scale bars: 500 μm (**B**), and 50 μm (**C, D**). (Data from Kato et al., 2011c)

4. Conclusion

In this chapter, we mentioned the gene transduction property of the HiRet and NeuRet vectors pseudotyped with different fusion envelope glycoproteins. These two vectors showed the enhancement in gene transfer through retrograde axonal transport into various neuronal populations in both rodent and nonhuman primate brains. The HiRet vector transduced prominently glial cells around the injection sites, whereas gene transduction of the NeuRet vector into glial cells was much less efficient. The transduction level of the NeuRet vector into neural stem/progenitor cells was also low. The variation in the structure of envelope glycoproteins shifted the efficiency of retrograde gene transfer and the preference of host range. In addition, we described the application of the NeuRet vector for retrograde gene transfer into the nigrostriatal dopamine system of monkeys. The NeuRet vector, together with the HiRet vector, will offer a promising technology for gene therapy of neurological diseases through enhanced retrograde gene transfer. In particular, the NeuRet vector system will improve the safety of gene therapy by greatly suppressing the risk of gene transduction into dividing cells in the central nervous system.

Acknowledgements

This work was supported by grants-in aid from Core Research for Evolutional Science and Technology (CREST) of Japan Science and Technology Agency (JST). A part of this work was supported by "Highly Creative Animal Model Development for Brain Sciences" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan. We thank St. Jude Children's Research Hospital (Dr. A. Nienhuis) and the George Washington University for providing the HIV-1-based vector system. We also grateful to M. Kikuchi, N. Sato, M. Watanabe, and T. Kobayashi for their technical support in the animal experiments.

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