available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

In vivo transduction of murine cerebellar Purkinje cells by HIV-derived lentiviral vectors

Takashi Torashima[a,b,d], *Shigeo Okoyama*[c], *Tomoyuki Nishizaki*[d], *Hirokazu Hirai*[a,b,*]

[a]*Innovative Brain Science Project, Advanced Science Research Center, Kanazawa University, Kanazawa 920-8640, Japan*
[b]*PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho Kawaguchi, Saitama 332-0012, Japan*
[c]*Laboratory of Neuroanatomy, Center for Biomedical Research and Education, Graduate School of Medical Science, Kanazawa University, Kanazawa 920-8640, Japan*
[d]*Department of Physiology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya 663-8501, Japan*

ARTICLE INFO

Article history:
Accepted 22 January 2006
Available online 6 March 2006

Keywords:
Lentivirus
HIV
Purkinje cell
Cerebellum
Bergmann glia
Stellate cell

ABSTRACT

Cerebellar Purkinje cells are key elements in motor learning and motor coordination, and therefore, it is important to clarify the mechanisms by which Purkinje cells integrate information and control cerebellar function. Gene transfer into neurons, followed by the assessment of the effects on neural function, is an effective approach for examining gene function. However, this method has not been used fully in the study of the cerebellum because adenovirus vectors, the vectors most commonly used for in vivo gene transfer, have very low affinity for Purkinje cells. In this study, we used a human immunodeficiency virus (HIV)-derived lentiviral vector and examined the transduction profile of the vector in the cerebellum. A lentiviral vector carrying the GFP gene was injected into the cerebellar cortex. Seven days after the injection, Purkinje cells were efficiently transduced without significant influence on the cell viability and synaptic functions. GFP was also expressed, though less efficiently, in other cortical interneurons and Bergmann glias. In contrast to reported findings with other viral vectors, no transduced cells were observed outside of the cerebellar cortex. Thus, when HIV-derived lentiviral vectors were injected into the cerebellar cortex, transduction was limited to the cells in the cerebellar cortex, with the highest tropism for Purkinje cells. These results suggest that HIV-derived lentiviral vectors are useful for the study of gene function in Purkinje cells as well as for application as a gene therapy tool for the treatment of diseases that affect Purkinje cells.

* *Corresponding author*. Innovative Brain Science Project, Advanced Science Research Center, Kanazawa University, Kanazawa 920-8640, Japan. Fax: +81 76 265 2489.
E-mail address: hirai@med.kanazawa-u.ac.jp (H. Hirai).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.01.104

Abbreviations:
AAV, adeno-associated virus
CF, climbing fiber
EPSC, excitatory postsynaptic current
FIV, feline immunodeficiency virus
GFAP, glial fibrillary acidic protein
HEK, human embryonic kidney
HIV, human immunodeficiency virus
HSV1, herpes simplex virus type 1
MSCV, murine embryonic stem cell virus
NeuN, neuron-specific nuclear protein
PBS, phosphate-buffered saline
PF, parallel fiber
SCA, spinocerebellar ataxia
TU, transducing units
VSV-G, vesicular stomatitis virus G protein

1. Introduction

The cerebellum plays substantial roles in motor coordination and motor learning (Ito et al., 2002). Damage of the cerebellum causes numerous motor coordination problems that include oculomotor disturbances, postural instability, and gait and limb ataxia. Cerebellar Purkinje cells, the sole outputs from the cerebellar cortex, are key elements regulating the cerebellar function. Purkinje cells receive two excitatory inputs from parallel fibers (PFs), axons of granule cells and climbing fibers (CFs), axons of inferior olivary neurons, whose excitatory activity is modulated by two sorts of inhibitory interneurons, stellate cells and basket cells (Figs. 1a, b). Transfer of exogenous genes into cerebellar Purkinje cells would allow us to dissect the molecular mechanisms regulating various physiological events in Purkinje cells such as dendritic development, synapse formation, and synaptic plasticity; however, gene transfer into Purkinje cells has remained a big challenge. In the late 1990s, two reports demonstrated LacZ gene expression in Purkinje cells using adenovirus vectors, but the transduction efficiency was very low: only a few Purkinje cells were β-galactosidase-positive despite the transduction of thousands of glial cells (Hashimoto et al., 1996; Terashima et al., 1997), suggesting that adenovirus vectors are not efficiently incorporated into Purkinje cells.

Since 2000, neurotrophic vectors derived from adeno-associated virus (AAV), herpes virus and immunodeficiency virus were used for gene delivery into Purkinje cells. Recent in vivo gene expression experiments using those viral vectors revealed that the efficiency of transduction of Purkinje cells by each virus depends on the injection site. With herpes simplex virus type 1 (HSV1) vectors, injection into the inferior olive, but not into the cerebellar cortex, causes efficient transduction of Purkinje cells (Agudo et al., 2002): the vectors are delivered from the inferior olive to the deep cerebellar nuclei and then to Purkinje cells by retrograde axonal transport. On the other hand, AAV vectors injected into the cerebellar cortex are directly taken up by Purkinje cells and transduce them efficiently (Alisky et al., 2000; Kaemmerer et al., 2000). These results suggest that both HSV1 and AAV vectors appear to be effective means for transferring genes into Purkinje cells; however, these vectors have several disadvantages. For example, herpes vectors could cause an inflammatory response that would affect the viability of transduced cells, and the insert size in AAV vectors is limited to only ~4 kb.

Alisky et al. (2000) tested a lentiviral vector derived from feline immunodeficiency virus (FIV), whose insert capacity (~8 kb) is two times as large as that of AAV vectors, to transfer a reporter gene into Purkinje cells and showed that the FIV-derived vectors injected into the cerebellar cortex transduced Purkinje cells, like HSV1 and AAV vectors. Furthermore, unlike herpes virus-based vectors, lentiviral vectors are known to elicit no or minimal inflammatory response. Thus, lentiviral vectors seem to be a promising tool for gene transfer into Purkinje cells. Recently, a human immunodeficiency virus (HIV)-derived lentiviral vector has been increasingly used for gene transfer into neurons (de Almeida et al., 2001; Desmaris et al., 2001; Dittgen et al., 2004; Naldini et al., 1996). Despite this trend, there has been no report yet on gene delivery into cerebellar neurons using HIV-derived vectors. Therefore, in the present study, we tested the potential of HIV-derived lentiviral vector to transduce cerebellar Purkinje cells and found a unique feature of this viral vector in transducing neurons in the cerebellar cortex.

2. Results

2.1. *Cerebellar cells transduced by HIV-derived lentiviral vectors*

Lentiviral vectors carrying the GFP gene (Fig. 1c) were generated in HEK 293 T cells, and the virus-containing medium was concentrated by ultracentrifugation. We used the viral vectors