Progress in transduction of cerebellar Purkinje cells in vivo using viral vectors

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Abstract
Expression of a foreign gene in cerebellar Purkinje cells in vivo is a powerful method for exploring the pathophysiology of the cerebellum. Although using developmental engineering many gene-modified mice have been generated, this approach is time-consuming and requires a lot of effort for crossing different lines of mice, genotyping and maintenance of animals. If a gene of interest can be transferred to and efficiently expressed in Purkinje cells of developing and mature animals, it saves much time, effort and money. Recent advances in viral vectors have markedly contributed to selective and efficient gene transfer to Purkinje cells in vivo. There are two approaches for selective gene expression in Purkinje cells: one is to take advantage of the viral tropism for Purkinje cells, which includes the tropism of adeno-associated virus and the vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped lentivirus. Another method, which might be used in combination with the first one, is utilization of a Purkinje-cell-specific promoter. Focusing mainly on these points, recent progress in viral-vector-mediated transduction of Purkinje cells in vivo is reviewed.

Key words: Purkinje cell, viral vector, lentivirus, adeno-associated virus, gene therapy

Introduction
The cerebellum plays important roles in coordinated movement, motor learning and vestibular function, and cerebellar damage results in impaired body balance and disturbance in gait and posture. A major input to the cerebellar cortex is mossy fibers that originate from thalamus, brainstem and spinal cord, and make excitatory synapses with cerebellar granule cells. The activity of granule cells is transferred through their axons, called parallel fibers, to Purkinje cells. A Purkinje cell has ramified dendrites studded with hundreds of thousands of spines, on which parallel fibers make excitatory synapses. Parallel fiber–Purkinje cell spine synapses are tightly covered with processes of Bergmann glia so as to quickly take up glutamate released from parallel fiber terminals and thereby to prevent the glutamate from spilling over and activating the adjacent synapses. Another input to the cerebellar cortex are the climbing fibers that originate from neurons in the inferior olivary nucleus and make excitatory synapses with Purkinje cells as well as neurons in the cerebellar nuclei. Purkinje cells, the sole source of output from the cerebellar cortex, exert inhibitory actions on the neurons of the cerebellar nuclei. The excitatory activity of granule cells and Purkinje cells is modulated by three types of inhibitory interneurons, Golgi cells, stellate cells and basket cells.

It is well known that long-term depression of synaptic transmission (LTD) at parallel fiber–Purkinje cell synapses can be induced upon conjunctive stimulation of parallel fibers and climbing fibers, which is thought to provide a cellular basis for motor learning. Purkinje cells are thus key elements of higher brain function in the cerebellum as well as in maintaining the regulation of coordinated movement and body balance. These important cells are easily damaged by toxins, ischemia, infection or inherited disorders (1). Accordingly, efficient and targeted expression of foreign genes in Purkinje cells is a powerful method for basic and translational research, such as studies of synaptic plasticity or gene therapy for diseases affecting Purkinje cells, including several major types of spinocerebellar ataxia. Gene transfer to Purkinje cells might not appear to be a big challenge, because the surface area of Purkinje cells is much larger than that of other cortical cells. However, it is indeed a challenge, partly because the well-developed dendrites occupying most of the surface area of Purkinje cells are

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Transduction of Purkinje cells using adenoviral vectors

Early adenoviral vectors were associated with toxicity that led to an inability to achieve stable transgene expression. Thereafter, the vectors were substantially altered to improve the stability of transgene expression by eliminating the expression of viral proteins that cause the immune response. Consequently, many studies have succeeded in expressing foreign genes efficiently in neurons of various brain regions, including the striatum, hippocampus and cerebral cortex (2–4). In line with this, the potential of adenoviral vectors to transduce Purkinje cells was tested. Adenoviral vectors expressing LacZ under the control of the Purkinje-cell-specific L7 promoter demonstrated Purkinje-cell-specific transduction (5). However, the efficiency was very low: in that study, only two transduced Purkinje cells in one section (285 × 285 μm) were presented. The low transduction efficiency of Purkinje cells in vivo using adenoviral vectors was confirmed by a different group (6) in which virus-mediated LacZ expression was detected in very few Purkinje cells upon cortical injection, and the majority of cells transduced were Bergmann glia. Since we also observed essentially the same expression pattern, preferential transduction of Bergmann glia when adenoviral vectors were injected into the cerebellar cortex (unpublished data), we gave up using adenoviral vectors for gene transfer to Purkinje cells in vivo. Ozawa’s group took advantage of the fact that adenoviral vectors preferentially infect Bergmann glia (7). Native Bergmann glia express only GluR1 and GluR4 subunits of AMPA-type glutamate receptors (AMPA receptors), and lack GluR2, which confers Ca\(^{2+}\)-permeability on AMPA receptors of Bergmann glia. Using adenoviral vectors, Ozawa’s group succeeded in expressing exogenous GluR2 subunit selectively in Bergmann glia and thereby replacing the Ca\(^{2+}\)-permeable AMPA receptors with Ca\(^{2+}\)-impermeable ones. Thus, the adenoviral vectors currently used are effective for selective gene transfer to Bergmann glia, but have substantially limited potential for transducing Purkinje cells in vivo, although Purkinje cells in culture could be transduced efficiently using these vectors (5,8).

Transduction of Purkinje cells using herpes simplex viral vectors

A unique feature of herpes simplex virus 1 (HSV-1) vector is long neuronal transport through axons using microtubule motors of the infected neurons (9,10). Because HSV-1 is a neurotropic virus that causes encephalitis when introduced into the brain, much effort was made to modify HSV-1 so that the virus could be safely used as a gene transfer vector (11). The defective helper-dependent amplicon vector is a toxicity-attenuated mutant of HSV-1 that lacks all portions of the genome except for an origin of DNA replication and DNA packaging signal sequences (12). Agdo et al. (13) used HSV-1 amplicon vectors carrying the lacZ gene to transduce Purkinje cells, and showed that injection of HSV-1 vectors into the inferior olivary nucleus, rather than into the cerebellar cortex, led to efficient transduction of Purkinje cells. The vector transport from the inferior olivary complex to the cerebellar cortex is discussed in a later section. The HSV-1-vector-mediated expression of the lacZ gene within Purkinje cells was persistent and was maintained at the same level for at least 40 days with no significant signs of toxicity or inflammation. However, viral injection into the inferior olivary nucleus located in the deep medulla has potential to cause significant, and in the worst case, life-threatening damage to the medulla, which could be a big problem in clinical application. As an alternative way, it is intriguing to test whether the injection of HSV-1 vectors into the cerebellar nuclei could cause efficient transduction of Purkinje cells.

Transduction of Purkinje cells using adeno-associated viral vectors

Recombinant adeno-associated virus (rAAV) is a promising gene therapy vector because wild-type adeno-associated virus is nonpathogenic, and expression of transgenes is sustained without integration of the viral genome into the host genome: integration of proviruses into a host chromosome could cause insertional mutagenesis associated with severe side effects. One major limitation of rAAV is the insert capacity, which primarily depends on the genome size of wild-type AAV (14). Wild-type AAV genome with the size of 4.7 kb is replaced by a transgene cassette consisting of a promoter plus transgene, which should be theoretically less than 4.7 kb; otherwise, virus production is significantly affected due to impaired packaging. Thus, from the point of packaging capacity, the ideal size for rAAV-mediated-gene transfer is thought to be 4 kb or less, although it differs depending on the size of the promoter accommodated. Currently, at least 10 serotypes of AAV have been used to engineer recombinant viral vectors (15,16). As different AAV serotypes enter cells via distinct cell surface receptors, the tropism of different serotypes for neurons has been tested. rAAV2 is the serotype most widely used for gene transfer to neuronal cells because it transduces mostly neurons (16–19).