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Research Report

Targeted gene transfer to nigrostriatal neurons in the rat brain by helper virus-free HSV-1 vector particles that contain either a chimeric HSV-1 glycoprotein C-GDNF or a gC-BDNF protein

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Abstract

Direct gene transfer into neurons has potential for both studying neuronal physiology and for developing gene therapy treatments for specific neurological conditions. Due to the heterogeneous cellular composition of the brain, cell-type-specific recombinant gene expression is required for many potential applications of neuronal gene transfer. The two prevalent approaches for achieving cell-type-specific expression are to use a cell-type-specific promoter to control recombinant gene expression or to modify a virus vector particle to target gene transfer to a specific cell type. Targeted gene transfer to multiple peripheral cell types has been described, but targeted gene transfer to a specific type of neuron in the brain has yet to be reported. Targeted gene transfer approaches with Herpes Simplex Virus (HSV-1) vectors have focused on modifying glycoprotein C (gC) to remove the heparin binding domain and add a binding activity for a specific protein on the cell surface. This study was designed to develop HSV-1 vectors that target gene transfer to cells that contain receptors for either glial-cell-line-derived neurotrophic factor (GDNF) or brain-derived neurotrophic factor (BDNF), such as nigrostriatal neurons. We isolated chimeric gC-GDNF or chimeric gC-BDNF constructs, and the resulting proteins were incorporated into HSV-1 virus particles. We performed helper virus-free HSV-1 vector packaging in the presence of each chimeric protein. The resulting vector stocks supported 2.2- to 5.0-fold targeted gene transfer to nigrostriatal neurons in the rat brain, compared to vector particles that contained wild-type (wt) gC. Gene transfer to nigrostriatal neurons by vector particles that contained chimeric gC-BDNF was reduced by preincubation with an anti-BDNF antibody. Targeted gene transfer to neurons that contain specific neurotrophic factor receptors may benefit specific physiological and gene therapy studies.

Theme: Cellular and molecular biology
Topic: Gene structure and function: general

Keywords: Targeted gene transfer; Glycoprotein C; Herpes Simplex Virus vector; Glial-cell-line-derived neurotrophic factor; Brain-derived neurotrophic factor; Nigrostriatal neuron; Heparin sulfate

1. Introduction

Direct gene transfer into neurons using virus vectors has potential for both studying neuronal physiology and for developing gene therapy treatments for specific neurological conditions. However, because of the heterogeneous cellular composition of the brain, cell-type-specific recombinant gene expression is required for many potential applications of neuronal gene transfer. The two prevalent approaches for achieving cell-type-specific expression are to use a cell-type-specific promoter to control recombinant gene expression or to modify a virus vector particle to target gene transfer to a specific cell type [17,28,37,46,47,51]. These two approaches are complementary, and a higher level of cell-type-specific expression may be achieved by using these two approaches in combination. In the brain, neuronal-specific or neuronal subtype-specific promoters have been

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used to restrict recombinant gene expression to neurons or a specific type of neuron, respectively [37,51]. However, in virus vectors, cell-type-specific promoters usually support a low level of ectopic expression in inappropriate cell types. Thus, modifying a vector particle to target gene transfer to a specific type of neuron may be a useful approach for restricting recombinant gene expression to a chosen type of neuron. Modified vector particles have been used to target gene transfer to multiple peripheral cell types and organs, but targeted gene transfer to a specific type of neuron in the brain has yet to be reported.

Targeted gene transfer with modified vector particles has been developed using classical retrovirus, lentivirus, adeno-associated virus (AAV), adenovirus, and Herpes Simplex Virus (HSV-1) vectors [2,6,13,17,22,32,46–48]. The targeting strategies modify the surface of a virus vector particle to add a new cell tropism to the vector particle, reduce the normal cell tropism, and preserve efficient vector particle assembly. The most direct targeting strategy is to genetically modify a vector particle protein to add a specific binding capability; one of the first reports used retrovirus vector particles that contained a chimeric erythropoietin (EPO)-virus envelope (*env*) protein to target gene transfer to cells that contained EPO receptors [17]. Because addition of a large polypeptide to a vector particle protein may disrupt vector particle assembly, another approach has been to add a bridging molecule that binds to both the vector particle and a cell surface ligand [46,48]. As an indication of the potential utility and specificity of vector targeting, AAV particles with a desired targeting specificity have been genetically selected by expressing a library of binding sites at a specific site in the AAV capsid protein and subjecting the resulting virus particles to repeated rounds of infection of the chosen cell type [28].

HSV-1 vectors are attractive because they have a large capacity and can efficiently transduce neurons [9,11]. The HSV-1 particle contains four components: (i) The ~152 kb genome is contained within (ii) an icosahedral protein capsid, which is surrounded by (iii) a layer of proteins termed the tegument, which is enclosed within (iv) the envelope, a lipid bilayer that contains 10 glycoproteins encoded by the virus [33]. HSV-1 infection proceeds in two stages [39]. Initial binding to cells is mediated by specific domains on glycoprotein C (gC) and gB that bind to glycosaminoglycans, principally heparin sulfate, on cell surface proteoglycans [23,26,35,42]. Subsequent entry requires the binding of gD to a receptor. Receptors for gD include the herpesvirus entry mediator (HVEM), a member of the tumor necrosis receptor family; nectin-1 or nectin-2, members of the immunoglobulin superfamily; and specific sites in heparin sulfate produced by particular isoforms of 3-*O*-sulfotransferases [39]. Entry occurs by fusion of the envelope to the cell membrane and requires gB, gD, gH, and gL.

Targeted gene transfer approaches with HSV-1 vectors have focused on modifying gC to remove the heparin binding domain and add a ligand for a specific protein on the cell surface. The first study [22] isolated a recombinant HSV-1 virus that contained a chimeric gC-EPO protein and deleted the heparin sulfate binding domains on both gC and gB. This virus showed enhanced binding to cells that contained EPO receptors, but targeted infection was not reported. In the other study [13], an HSV-1 plasmid (amplicon) vector that expressed a chimeric gC-His tag was packaged using a helper virus that contained a deletion in gC, and the vector stocks targeted infection to a cultured cell line that contained an artificial pseudo-His-tag receptor. Both of these reports [13,22] used specific HSV-1 viruses that grow productively and kill infected cells, confounding potential gene transfer studies.

In this study, we performed helper virus-free HSV-1 vector packaging in the presence of either a chimeric gC-glial-cell-line-derived neurotrophic factor (GDNF) protein or a chimeric gC-brain-derived neurotrophic factor (BDNF) protein. Microinjection of either vector stock into the midbrain resulted in 2.2- to 5.0-fold targeted gene transfer to nigrostriatal neurons, which contain GDNF receptor α-1 (GFRα-1 [34,43]) and the high-affinity BDNF receptor, *TrkB* [29,49]. Preincubation of a vector stock that contained the chimeric gC-BDNF protein with an anti-bdnf antibody blocked the targeted gene transfer.

2. Materials and methods

2.1. Materials

Restriction endonucleases and T4 DNA ligase were obtained from New England Biolabs and Roche. Dulbecco's modified minimal essential medium, fetal bovine serum, G418, lipofectamine, and OPTI-MEM I were obtained from Invitrogen. 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal) was obtained from Sigma. Rabbit anti-GDNF and rabbit anti-BDNF antibodies were obtained from Santa Cruz Biotechnology, mouse anti-HSV-1 gC was obtained from QED Bioscience Inc., rabbit and mouse anti-*E. coli* β-galactosidase (β-gal) and mouse monoclonal anti-flag were obtained from Sigma, rabbit and mouse anti-tyrosine hydroxylase (TH) were obtained from Chemicon, rabbit anti-glutamic acid decarboxylase (GAD) was obtained from Chemicon, and rabbit anti-glial fibrillary acidic protein (GFAP) was obtained from Santa Cruz Biotechnology. Fluorescein or rhodamine isothiocyanate-conjugated goat anti-rabbit immunoglobulin (Ig) G and fluorescein or rhodamine isothiocyanate-conjugated goat anti-mouse IgG were obtained from Jackson ImmunoResearch Laboratories. Horseradish-peroxidase (HRP)-conjugated-goat anti-mouse IgG was obtained from Dakocytomation. SDS-polyacrylamide gels and Immuno-Blot PVDF membranes were from BIO-RAD, and ECL Western Blotting Detection Reagents were from Amersham.