EG 427

PINPOINT GENE THERAPY

Efficient retrograde transport of a non-replicative HSV-1 vector after stereotactic injection in the striatum

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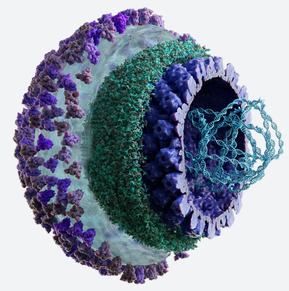

Background

Projection neurons send axons to distant targets. Efficient retrograde access to projection neurons for the delivery of a specific transgene represents a major advantage for gene therapies targeting neurodegenerative disorders. Recombinant adeno-associated viruses (AAVs) have become the reference for in vivo gene delivery in the central nervous system (CNS). However, their natural retrograde transport ability is low, thus limiting the efficient targeting of projecting neurons. Moreover, AAVs have limited packaging capacity and typically require high intraparenchymal doses (10^9 to 10^{12} particles).

Herpes simplex virus type 1 (HSV-1) is a human neurotropic virus that establishes productive infections in epithelial cells and latent infections in neurons. Non replicative (nr) HSV-1 based vectors have been developed to exploit their therapeutic potential by leveraging their biodistribution, safety, and long-term transgene expression abilities. In contrast to AAVs, nrHSV-1 vectors have large packaging capacity, can efficiently transduce neurons of the central nervous system at low doses, and can be transported retrogradely to connected brain regions. However, results obtained by different teams with nrHSV-1 are not always consistent, even when inoculated in the same region, probably reflecting that subtle vector differences, such as the viral strain used or its precise genetic composition can significantly affect biodistribution or expression within the brain.

Here we investigated the biodistribution pattern in mouse brain of two novel non replicative HSV1-derived vectors expressing the fluorescent protein mGreenLantern under the control of EF1α (EG133A) or CAG (EG143A) promoters.

Methods

We constructed two nrHSV-1 vectors (EG133A and EG143A) with deletions in the essential ICP4 and ICP27 genes and several other non-essential genes (UL55, UL56, as well as one copy of ICP34.5 and LAT). In addition, the promoters of the genes encoding ICP22 and ICP47 behave as early genes, instead of immediate-early genes. This vector conserves a single copy of ICP0, an HSV-1 regulatory gene, and expresses the mGreenLantern reporter gene under the control of EF1α (EG133A) or CAG (EG143A) promoter. The transgene is located in the LAT (Latency Associated Transcript) region (Figure 1). These vectors were injected in the striatum of mice (4E+06 PFU). Biodistribution pattern of mGreenLantern was analyzed by fluorescence imaging at 3 days after administration.

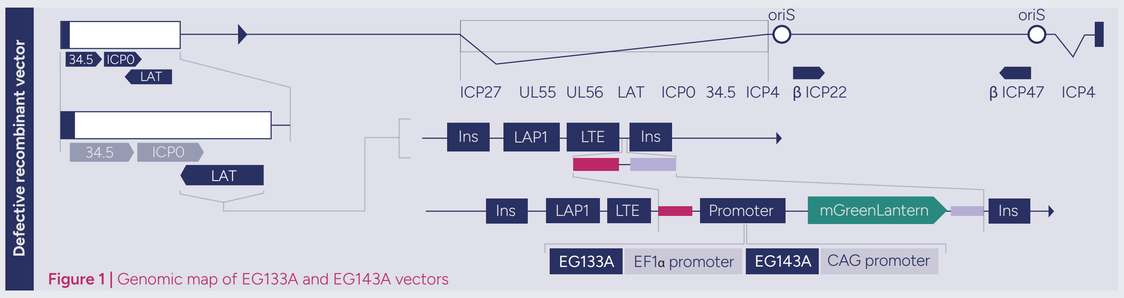

Figure 1 | Genomic map of EG133A and EG143A vectors

Results

1 Efficient transduction of neurons at site of administration and projecting neurons by nrHSV-1 vectors

Intrastriatal injection of EG133A or EG143A vectors (4E+06 PFU) was performed in mouse brain. Brains were collected after 3 days for immunofluorescence imaging. mGreenLantern transgene signal shows positive transduction of cells in the striatum and is transported retrogradely in projecting neurons in the cortex and substantia nigra (Figures 2 and 3).

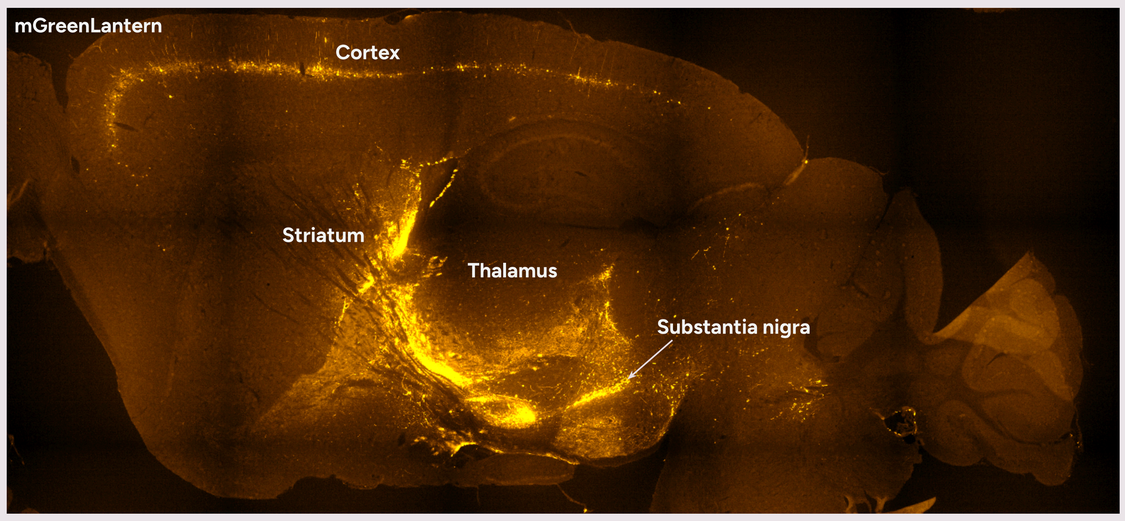

Figure 2 | Sagittal brain section, 3 days after administration of EG133A. Yellow color shows immunostaining for the mGreenLantern transgene.

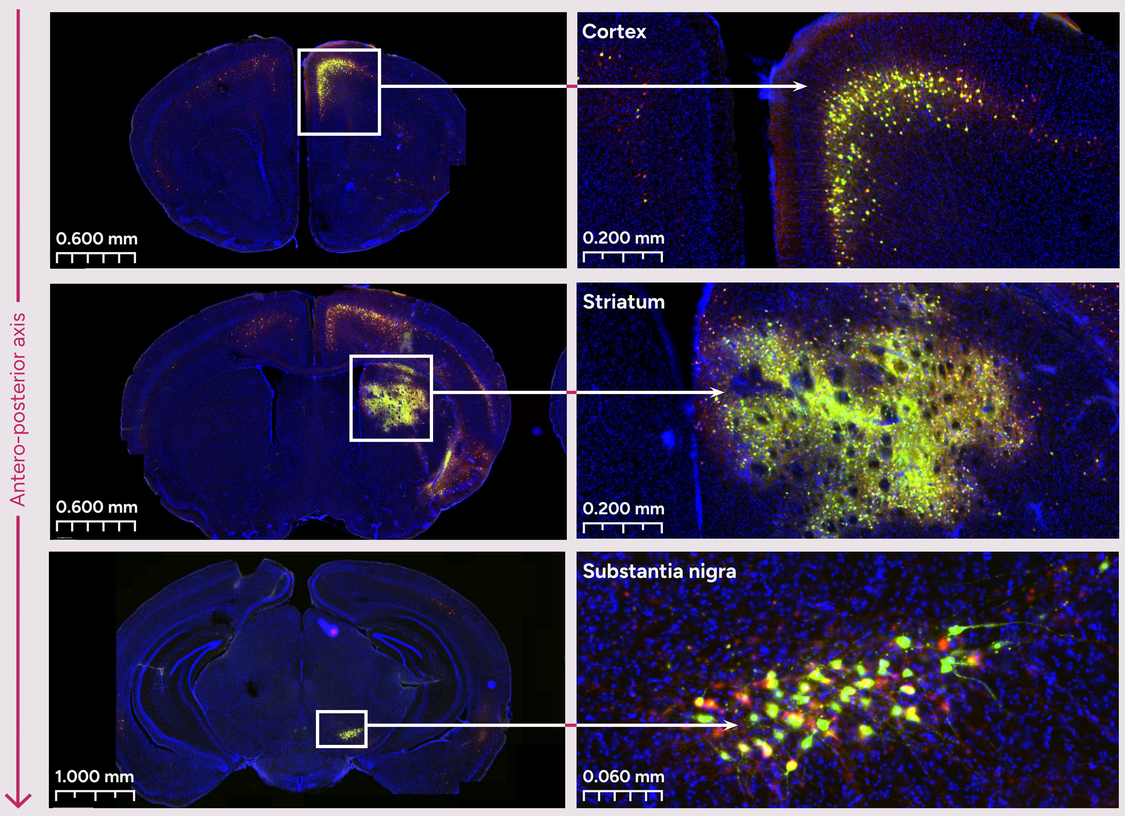

Figure 3 | Coronal brain sections, 3 days after administration of EG143A. Green color shows the direct mGreenLantern fluorescence. Red shows the immunostaining for mGreenLantern with anti-GFP antibody. Blue shows cell nuclei (Hoechst)

2 The majority of transduced cells are neurons

Brain sections were stained for mGreenLantern and the neuronal marker NeuN (Figure 4). In the striatum the majority of mGreenLantern positive cells were neurons. Astrocytes and microglia were also stained with GFAP and Iba1 markers, respectively (Figure 5). Only a low proportion of mGreenLantern-positive cells were astrocytes or microglia.

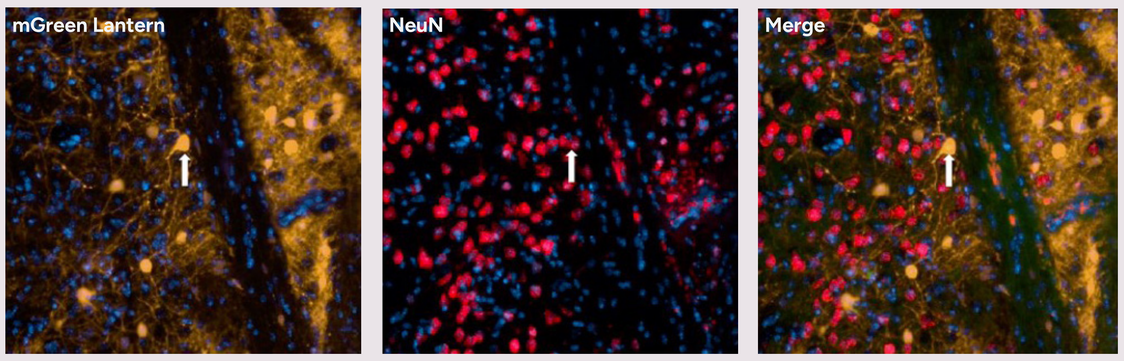

Figure 4 | Brain section, 3 days after administration of EG133A. Orange color shows the mGreenLantern transgene. Red color shows the immunostaining for the neuronal marker NeuN. Arrows show colocalization of mGreenLantern with the neuronal marker.

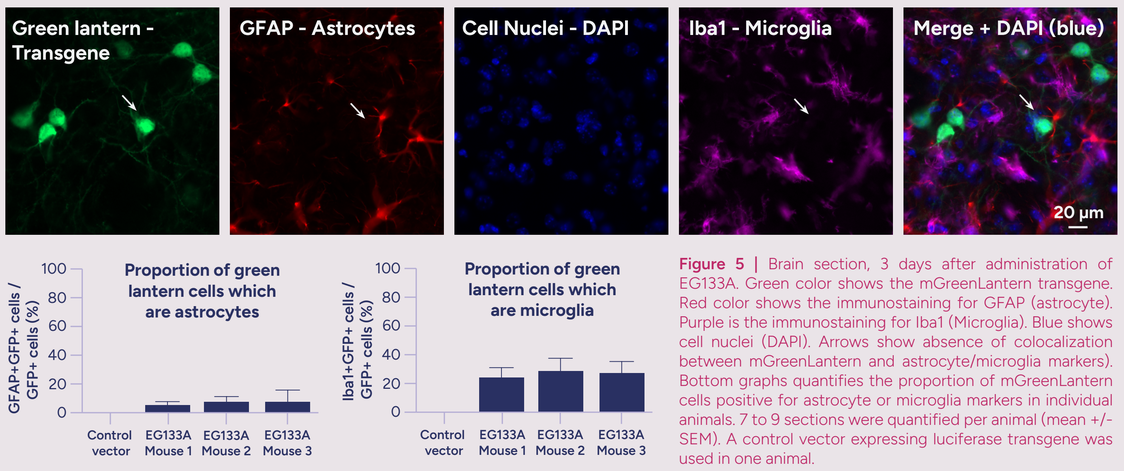

Figure 5 | Brain section, 3 days after administration of EG133A. Green color shows the mGreenLantern transgene. Red color shows the immunostaining for GFAP (astrocyte). Purple is the immunostaining for Iba1 (Microglia). Blue shows cell nuclei (DAPI). Arrows show absence of colocalization between mGreenLantern and astrocyte/microglia markers). Bottom graphs quantifies the proportion of mGreenLantern cells positive for astrocyte or microglia markers in individual animals. 7 to 9 sections were quantified per animal (mean +/- SEM). A control vector expressing luciferase transgene was used in one animal.

3 Efficient targeting of dopaminergic neurons of substantia nigra after intra-striatal administration

Brain sections were stained for mGreenLantern and the marker of dopaminergic neurons TH (Figure 6). A significant proportion of dopaminergic neurons of the substantia nigra were transduced following intra-striatal administration.

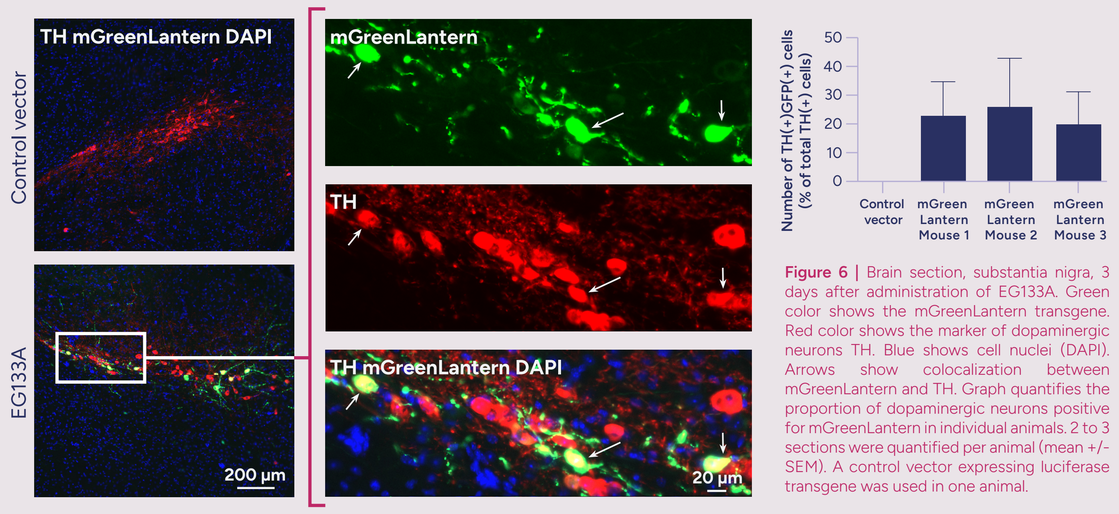

Figure 6 | Brain section, substantia nigra, 3 days after administration of EG133A. Green color shows the mGreenLantern transgene. Red color shows the marker of dopaminergic neurons TH. Blue shows cell nuclei (DAPI). Arrows show colocalization between mGreenLantern and TH. Graph quantifies the proportion of dopaminergic neurons positive for mGreenLantern in individual animals. 2 to 3 sections were quantified per animal (mean +/- SEM). A control vector expressing luciferase transgene was used in one animal.

4 Safety of nrHSV-1 vectors

Brain sections were stained for mGreen lantern, the astrocyte marker GFAP and the microglial marker Iba1, 3 days after administration of EG133A (Figure 7). Quantification of the number of astrocytes and microglia in the striatum showed no difference between EG133A-injected animals and controls suggesting minimal gliosis in response to the vector. Histopathology analysis showed presence of striatal perivascular cuffs (inclusive of lymphocytes) and variable forms of neural cavitation (local loss of neuropil/neurons, with scattered karyorrhectic debris and local gliosis; Figure 8). These findings were generally discreet and within the vicinity of the administration area.

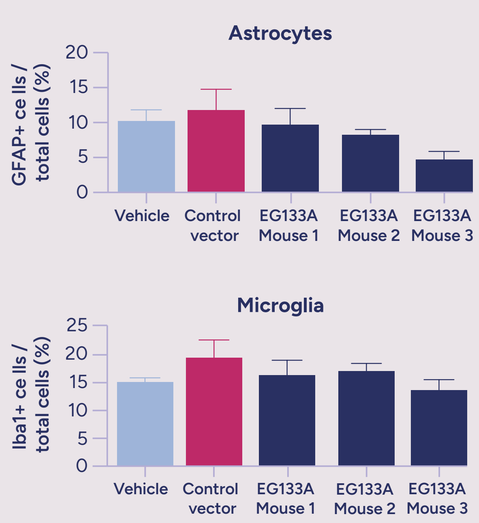

Figure 7 | Quantification of the number of astrocytes and microglia in the striatum, 3 days after administration of EG133A, in individual animals. 2 to 3 sections were quantified per animal (mean +/- SEM). A control vector expressing luciferase transgene or vehicle were used as controls.

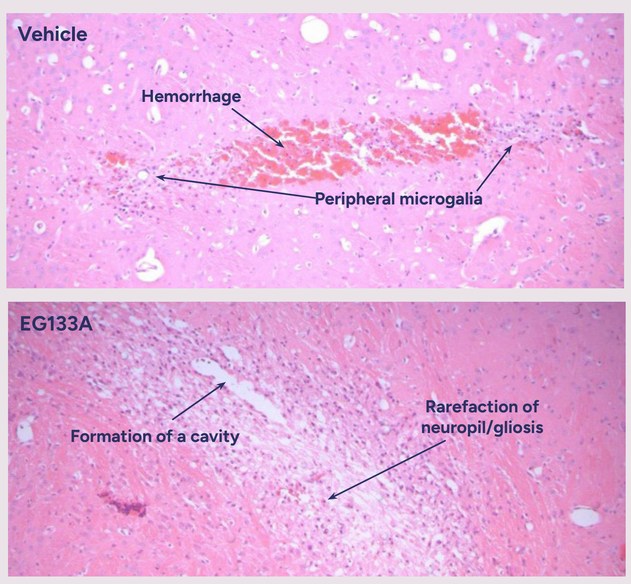

Figure 8 | Hematoxylin and eosin staining of brain section (striatum), 3 days after injection of EG133A or vehicle.

Conclusions

Our results suggest that intra-striatal administration of low amount of EG133A and EG143A efficiently targets neurons at the administration site and projecting neurons by retrograde transport, especially in cortex and substantia nigra, with a favorable safety profile. These vectors are a promising tool for gene therapies targeting both dopaminergic and cortical neurons.