Supplemental Data

Growth factor-expressing human neural progenitor cell grafts protect motor neurons but do not ameliorate motor performance and survival in ALS mice

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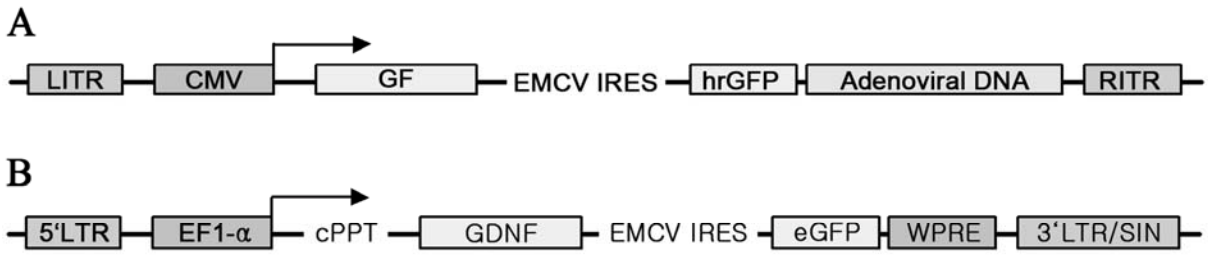
Methods

Viral vector construction

We constructed recombinant adenoviral vectors bearing human BDNF, IGF-1, VEGF, NT-3, or GDNF under the control of the constitutive cytomegalovirus (CMV) promoter, and placed these in tandem with a sequence of humanized version of the recombinant green fluorescent protein (hrGFP; Stratagene, La Jolla, CA) gene under the control of an internal ribosomal entry site (IRES) (Supplemental Data Figure S1A). Adenoviral vectors constructed in this fashion were designated as AdBDNF, AdIGF-1, AdVEGF, AdNT-3, AdGDNF, or AdGF as a whole, and produced in conformity with the AdEasy Adenoviral Vector System manual (Stratagene). Infectious recombinant viruses were purified by CsCl gradient centrifugation and titrated on 293 cells by Tissue Culture Infecting Dose 50 (TCID50; QBiogene). We constructed a lentiviral vector carrying the GDNF gene, which was designated as LtGDNF. LtGDNF was produced by calcium-precipitate-transfection (transient transfection) method using vectors that had been obtained from Trono lab (http://tronolab.epfl.ch/) in accordance with the method offered by the manufacturer. The backbone of transfer vector is pWPI (Supplemental Data Figure S1B). The packaging and envelope plasmids are psPAX2 and pMD2G, respectively. Enhanced green fluorescent protein (eGFP) is a reporter gene. The transfection was conducted to the 293FT cell line and produced viral vectors were concentrated by ultracentrifugation under the sucrose cushion. The viral titer was checked as transducing units (TU) by FACS analysis method offered by Trono lab.

Fig S1.


A
LITR
CMV
GF
EMCV IRES
hrGFP
Adenoviral DNA
RITR
B
5'LTR
EF1-α
cPPT
GDNF
EMCV IRES
eGFP
WPRE
3'LTR/SIN