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Short Communication

Transfection of tyrosine kinase deleted FGF receptor-1 into rat brain substantia nigra reduces the number of tyrosine hydroxylase expressing neurons and decreases concentration levels of striatal dopamine

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Abstract

The effects of HSV-1 amplicon and polyethyleneimine (PEI)-mediated transfection of dominant negative FGF receptor-1 mutant FGFR1(TK−) into the rat brain substantia nigra (SN) were examined in vivo to model the reduced FGF signaling documented to occur in Parkinson's disease. The number of SN neurons that expressed tyrosine hydroxylase (TH) was significantly reduced following HSV-1 FGFR1(TK−) intranigral delivery and similar changes were observed after PEI-mediated FGFR1(TK−) transfections. Further, we also observed a significantly lower striatal dopamine content following the PEI transfection of FGFR1(TK−). Thus, we conclude that reduced FGF signaling in the SN of Parkinsonian patients could play a role in the impaired dopaminergic transmission associated with the degenerative disease.

Theme: Development and regeneration
Topic: Neurotrophic factors: receptors and cellular mechanisms

Keywords: Parkinson's disease; Fibroblast growth factor receptor; Substantia nigra; Gene transfection; Herpes simplex virus-1; Polyethyleneimine; β-galactosidase

In Parkinson's disease (PD), prior to the observed cell degeneration, Toyama and co-workers noted that the content of Fibroblast Growth Factor-2 (FGF-2) in the substantia nigra (SN) dopamine (DA) neurons becomes depleted [23]. In this report, we tested the hypothesis that diminished FGF signaling in the SN could affect DA neurons and may be a contributing factor in the etiology of PD. To model the proposed inhibition of FGF signaling in rats, we expressed fibroblast growth factor receptor-1 mutant with a deleted tyrosine kinase domain, FGFR1(TK−) in the SN in vivo. The FGFR1(TK−) forms nonfunctional heterodimers with any of the first three types of FGF receptors (FGFR1, FGFR2, or FGFR3), thus eliminating the native signaling by wild-type

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FGFRs [25]. In vitro, FGFR1(TK−) transfected with polyethyleneimine (PEI) inhibits bone morphogenetic protein-7 (BMP-7) and induces dendritic growth in rat sympathetic neurons [9]. Further, FGFR1(TK−) inhibits cAMP-induced axonal outgrowth in human neuronal precursor cells [20,21] thus suggesting that the FGFR1(TK−) complex can modify considerably cell growth dynamics.

To deliver the dominant negative FGFR1(TK−) gene into the rat brain, we used a chemical and a viral method. The use of PEI as an effective gene transfection agent in mammalian brain has already been described [3] and effective gene transfers into the brain have also been accomplished with different viral vectors including HSV-1 amplicon [4,11].

In this study, we first confirmed the efficacy of HSV-1-derived amplicon vector and PEI-mediated plasmid transfection by expressing recombinant β-galactosidase (*lacZ*) gene and then testing the effects of FGFR1(TK−) on DA neurons. Plasmids pcDNA3.1-FGFR1(TK−) and pCMV-β-gal (expressing β-galactosidase) cDNA from the cytomegalovirus (CMV) late promoter have been previously described [16,17,21]. In brief, the plasmids were isolated and purified using QIAGEN (Valencia, CA) endotoxin-free kit. PEI/DNA complexes were prepared using ExGen 500 (MBI Fermentas Inc.), a cationic linear PEI polymer. Right before surgery, 25 μl of the 0.6 mg/ml DNA solutions was mixed with 2.7 μl and 3.6 μl of ExGen500 PEI solutions to obtain PEI–DNA complexes of 6 and 8 equivalents, respectively (one equivalence = amount of PEI required to neutralize the negative charges of DNA phosphate groups). The dextrose concentration in the final solutions was 5%. After brief vortexing and centrifugation, the solutions were kept at room temperature for 10 min prior to injection into the rat brain.

Helper virus-free amplicon stocks were also prepared using a modified helper virus-free packaging method [5]. The packaging system utilizes a bacterial artificial chromosome (BAC, kindly provided by C. Strathdee) that contains the HSV genome without its cognate packaging (pac) signals as a co-transfection reagent with amplicon DNA. BHK cells (2×10^7) were transfected with amplicon vector DNA using Lipofectamine Plus Reagent (Gibco-BRL). The packaging flask was incubated 3 days before the virus was harvested and stored at −80 °C until purification. Expression titers were determined by enumeration of X-gal-positive NIH 3T3 cells transduced with serial dilutions of amplicon stocks. Wild-type reversion frequency was less than 1 in 10^7 expressing amplicon particles as determined by plaque assay on transduced Vero cells [10]. FGFR1(TK−) cDNA was cloned into pHSVPrPUC parent amplicon plasmid. Helper virus-free HSV FGFR1(TK−) amplicon stocks were prepared as described above. Control HSV1 KD6 vector not expressing β-galactosidase [6,12,18] was prepared as previously described [22]. All stocks used had <1 revertant per 1×10^6 PFU of ICP4(−) plaques (as determined on E5 cells).

Adult Male Fischer 344 rats (160–250 g; Harlan Sprague–Dawley Inc., NIA colony) were used according to the Institutional Animal Care and Use Committee guidelines of The State University of New York at Buffalo. Rats were anesthetized with a mixture of ketamine (100 mg/ml), xylazine (20 mg/ml), and ace-promazine (10 mg/ml) in 0.9% NaCl (0.2 ml/100 g body weight, i.p.) and placed in a stereotaxic apparatus. HSV1 amplicon, PEI/DNA, control HSV1 KD6, or 5% dextrose solution (3–5 μl) was injected unilaterally or bilaterally into the SN region of the brain (−5.6 mm posterior, 1.5 mm lateral, and −8.2 mm ventral from bregma [15]) at 0.5 μl/min via a 30-G stainless steel needle. At 7 or 28 days after surgery, the rats were deeply anesthetized and were then perfused transcardially with saline followed by 4% paraformaldehyde [22]. The brains were cut into 2-mm-thick coronal sections using a stainless steel brain mold. These sections were post-fixed and subjected to X-Gal staining as described previously and photographed [22]. All solutions were prepared fresh and their pH was adjusted to 7.3 in order to prevent the detection of endogenous (acidic) β-galactosidase. Following photography, the 2-mm tissue sections were cryoprotected in 20% sucrose at 4 °C and further cut into 50-μm sections using a freezing stage microtome. Brain sections were mounted onto glass slides, counterstained with Eosin Y, dehydrated, and coverslipped. Sections were photographed on an Edge 400 microscope.

Figs. 1A–F shows the results of β-galactosidase activity analysis in 2-mm-thick brain sections at the midbrain/pons levels and also shows the photomicrographs of the 50-μm brain sections through the SN. No staining was detected in 2-mm or 50-μm brain sections from control rats injected with β-galactosidase-nonexpressing HSV1 KD6 or 5% dextrose either 1 week or 1 month after injections. Examples of HSV1 KD6 and 5% dextrose-injected tissues are shown on panels A, A′ and D, D′, respectively. The amplicon virus (panels B′ and E′) showed transfection at the 1-week and 1-month time points, respectively. The β-galactosidase activity staining was observed both on 2-mm and 50-μm brain sections. One month or 1 week following PEI/pCMV-β-gal injections, we observed evident cell staining in the 50-μm brain sections. However, at 1 week β-galactosidase activity in 2-mm brain sections was less visible.

Because it was previously observed that a decrease in FGF-2 in SN-DA neurons occurs in Parkinson's patients [23], we hypothesized that decreased signaling by the FGF-2's main receptor in SNc, the FGFR1, might play a role in the PD. To test this hypothesis, we chose to deliver into the SN cells of the dominant negative FGFR1 mutant, FGFR1(TK−), which lacks the tyrosine kinase domain [16,17]. Toward this goal, we constructed an HSV1 amplicon that expresses FGFR1(TK−) from a strong viral promoter. The FGFR1(TK−) expressing HSV-1 amplicon virus (experimental group, $n = 6$) or β-gal-expressing (control group, $n = 6$) HSV-1 amplicon was injected unilaterally into the SN. For each group, half of the animals were injected on the right side and half on the left side. The rats were perfused 1 month later and every 5th 50-μm brain section was stained with anti-TH specific