Neuroscience Letters 524 (2012) 10–15

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The delivery of tyrosine hydroxylase accelerates the neurorestoration of Macaca Rhesus model of Parkinson's disease provided by Neurturin

Wenju Wang, Maosheng Sun, Hongjun Li*, Wanpu Wang, Min Yan

Institute of Medical Biology, Peking Union Medical College, Chinese Academy of Medical Science, Kunming 650118, People's Republic of China

HIGHLIGHTS

- We applied Neurturin and TH to provide an enhanced gene therapy to Monkey model of PD.
- We constructed a bicistronic adenovirus expressing NTN and TH simultaneously.
- Bicistronic treated group displayed instant and faster behavior recovery.
- The delivery of TH could accelerate the behavior recovery mediated by NTN.

ARTICLE INFO

Article history:
Received 11 April 2012
Received in revised form 20 June 2012
Accepted 24 June 2012

Key words:
Parkinson's disease
Bicistronic
Neurturin
Tyrosine hydroxylase
Rhesus Macaque

ABSTRACT

Neurturin (NTN) is a desired candidate therapeutic gene for PD treatment, however, only neuroprotective effect may not work for PD clinical remission due to nearly 50% of the dopaminergic neurons have died way when symptoms appear. In this study, we constructed a bicistronic adenovirus expressing both Neurturin and tyrosine hydroxylase (TH). We hypothesized that the expression of NTN could provide neuroprotection to dopaminergic neurons and stop progressive neurodegeneration, while TH enhanced the synthesis of dopamine and accelerated the recovery of Parkinsonism. The chimeric adenovirus have been prepared and assayed in vitro and delivered into the target nucleuses of PD Macaca Rhesus models by MRI based stereotaxic injection. The observation assessments and physiological results indicated that compared to the group treated with NTN only, instant behavior recovery was seen after bicistronic adenovirus infusion. Although both groups displayed neuroprotection of dopaminergic neurons finally, the addition of TH genuinely accelerated animal behavior recovery, which showed great potential for clinical application.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder due to aging, drugs abuse and genetic risks [1,15,19]. The pathologic site of PD is localized within the substantia nigra, which is an ideal for gene therapy. Neurturin (NTN) and glial cell-derived neurotrophic factor (GDNF) have been found to promote the survival of dorsal root ganglia, striatal neurons, and substantia nigra DA neurons in vitro, and therefore, are excellent candidate genes for providing a neuroprotective effect to DA neurons in a PD model [12]. Additionally, tyrosine hydroxylase (TH) is a rate-limiting enzyme in dopamine biosynthesis, catalyzing the conversion of L-dopa from L-tyrosine [16,17]. Abnormalities of TH function in DA neurons, which may be caused by genetic or iron metabolic factors, can give rise to severe PD symptoms [3,6]. Thus, the correction of the function of TH in vivo by molecular methods is another approach to alleviating the symptoms of PD.

Currently, different viral delivery systems are employed in the gene therapy of PD. Recombinant viruses delivering GDNF and NTN have been successful in different experimental stages [2,4,8,10,11]. In this study, we have constructed a bicistronic adenovirus named HJ326 which could express modified NTN and TH simultaneously in biologically active form. In this manner, two physiological approaches to cure PD were employed by HJ326. On the one hand, NTN expressed by the bicistronic adenovirus could protect the dopamine producing units by promoting the survival of DA neurons; on the other hand, delivery of TH could enhance the capability of dopamine biosynthesis through increasing the expression of TH in target tissues. We hypothesized that targeting administration of HJ326 could accelerate the behavior recovery of the animal model and providing neuroprotection to DA neurons but also arrest the progress of the disease in PD model.

* Corresponding author at: No. 935 JiaoLing Road, Kunming City, Yunnan Province, China. Tel.: +86 0871 8334401; fax: +86 0871 8335391.
E-mail address: lihj6912@hotmail.com (H. Li).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.06.063

2. Materials and methods

The AdEasy XL System was purchased from Stratagene (CA, USA). The Rhesus Macaque monkeys were obtained from Institute of Medical Biology, Chinese Academy of Medical Science. All experimental procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The animal studies and protocols were approved by the Experimental Animal Ethics Committee of Institute of Medical Biology Chinese Academy of Medical Science.

2.1. Construction of the bicistronic adenovirus HJ326

The NTN gene we used was referred to CERE120 which has been reported as having an excellent expression capability and biological function in vivo [5]. The NTN and TH bicistronic expression cassette triggered by two independent promotors was cloned into the AdEasy plasmid DNA (Fig. 1A). The bicistronic adenoviruses HJ326 were amplified and harvested from 293 cell in large scale. Then the product was purified and tittered which was ready for injection [7].

2.2. The immunofluorescence and Western blot assay of the expression of NTN and TH in Vero cells

After infection of Vero cells, the expression of target genes were detected by immunofluorescence. NTN and TH were labeled by anti-human NTN antibody (R&D MAB3871) and anti-TH antibody (R&D MAB1423) respectively, and then corresponding secondary antibody were applied. In order to confirm that HJ326 could co-express NTN and TH in the same cell, double labeled immunofluorescence was employed. The testing cells were observed under a laser confocal microscope. To investigate whether modified NTN undergoes proper posttranslational processing, immunoblotting was used to detect the expression and secretion of NTN in the cells' culture medium. The conditioning medium and cells were harvested 48 h after HJ326 infection. The samples were labeled with antibody and HRP linked antibody as secondary antibody. A chemiluminescence-based method was used to visualize the band.

2.3. The bioassay of the neurons survival promoting activity of NTN and L-Dopa conversion activity of TH

The dorsal root ganglia were separated from the nine day old chick embryos under aseptic conditions. The cultural medium was a mixture of conditioning medium of HJ326 and Neural Basal plus B27 serum free supplements, and conditioning medium infected by control adenovirus was tested as control [14]. The mesencephalon DA neurons were isolated from the embryos of pregnant Sprague Dawley rats, and the cultural medium was the conditioning medium and Neural Basal plus B27 serum free supplements [21]. The DA neurons were incubated and the medium was replaced by half every three days. After 21 days' observation, a double staining immunofluorescence was performed to assay the DA neurons. The cytoplasm of the sample was labeled by mouse-anti-human TH antibody and the nucleus was stained with propidium iodide. The assays of the L-Dopa conversion capability of TH were conducted by using enzyme-catalyzed reactions followed by capillary electrophoresis. The reactions were composed of L-tyrosine, Fe^{2+}, (6R)-L-erythro-tetrahydrobiopterin (RBPH4) as cofactor and HJ326 infected cell lysate, and incubated at 37 °C for 2 h. Capillary electrophoresis was performed to analyze the presence of L-Dopa after the incubation.

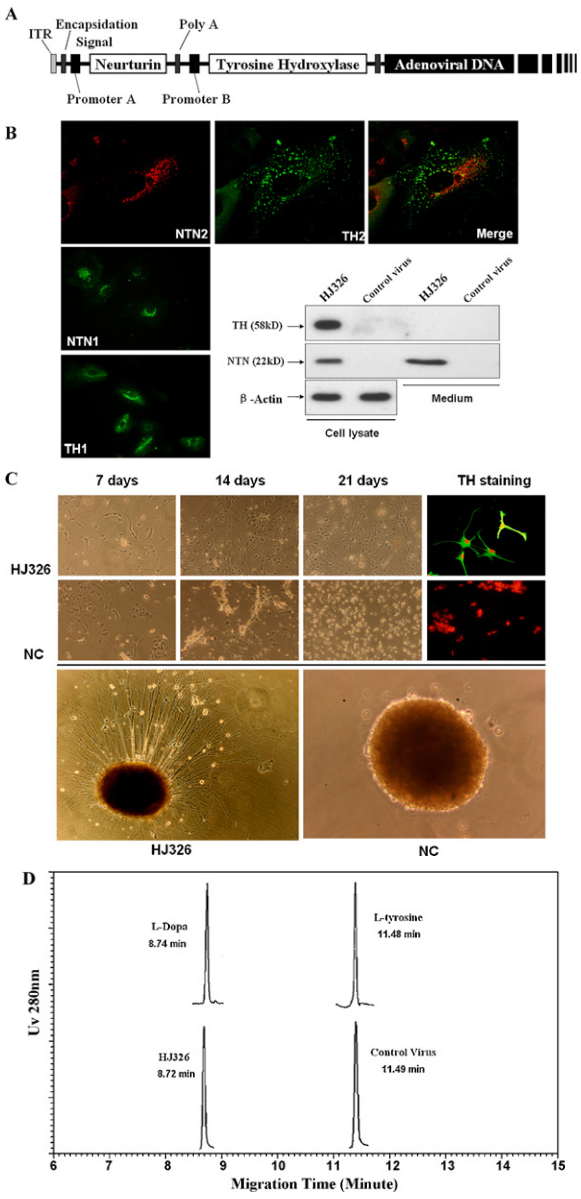

Fig. 1. (A) Schematic representation of the HJ326 genome. (B) Immunofluorescence and Western blot detection of the expression and secretion of NTN and TH both respectively and simultaneously. NTN1 and TH1 were immunostaining of NTN and TH respectively in HJ326 infected Vero cells; NTN2 and TH2 were co-staining of NTN and TH in HJ326 infected cells examined by cofocal microscopy. (C) Up panel: the mesencephalon embryo DA neurons survival promoting test cultured with HJ326 and control conditioning medium. Down panel: dorsal root ganglia culture by HJ326 and control conditioning medium and neural basal plus B27 serum free supplement for 48 h. (D) The assay of the biological activity of tyrosine hydroxylase by analyzing the conversion of L-tyrosine to L-Dopa with capillary electrophoresis. The L-tyrosine and L-Dopa were characterized by its own migration time in the fiber.

2.4. Animal model study

Rhesus monkeys aged from 4 to 5 years were selected as the animal model study subjects. Each was raised individually in a single cage and supplied with sufficient food and water. The study proceeded in three steps, namely animal model establishment, stereotaxic surgery, and evaluation of the therapy.

2.5. Semi-Parkinsonian animal model establishment

Monkeys were anesthetized with hydrochloric acidulated ketamine for inducement and sodium pentobarbital for

Download English Version:

https://daneshyari.com/en/article/4344260

Download Persian Version:

https://daneshyari.com/article/4344260

Daneshyari.com