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Distinct Effect of Neurotrophins Delivered Simultaneously by an Adenoviral Vector on Neurite Outgrowth of Neural Precursor Cells from Different Regions of the Brain

YOO, MINJOO[1,2], INSIL JOUNG[3], AH MI HAN[1,2], HYE HYUN YOON[4], AND YUNHEE KIM KWON[1,2]*

[1]*Department of Biology and* [2]*Department of Life and Nanopharmaceutical Science, Research Institute of Basic Sciences, Kyunghee University, Seoul 130-701, Korea*
[3]*Department of Biological Sciences, Hanseo University, Chungnam 352-820, Korea*
[4]*Department of Culinary Science and Arts, Kyunghee University, Seoul 130-701, Korea*

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Abstract For many years, it has been demonstrated that neurotrophins regulate the adult nervous system, implicating their potential as therapeutic agents for the treatment of neurodegenerative diseases. We generated adenoviral vectors encoding brain-derived neutotrophin factor (BDNF) and neurotrophin-3 (NT3) and tested either separately or together for the ability to induce differentiation of neuronal precursor cells with two different origins. Separate transduction of adenovirus delivering BDNF (BDNF-Ad) or NT3 (NT3-Ad) induced the neuronal differentiation in hippocampal and cortical precursor cells. NT3-Ad infected cells extended short neurites, whereas BDNF-Ad infected cells had longer neurites. In the early differentiation of hippocampal precursor cells, simultaneous infection of BDNF-Ad and NT3-Ad promoted further differentiation and neurite elongation compared with the separate infection of each virus. In contrast, simultaneous infection did not show the synergistic effect in the cortical precursor cells, suggesting that the neurotrophins play distinct roles in different regions of the brain. However, the numbers of neurites and spines per differentiated cells were markedly increased in cortical as well as hippocampal precursor cells, indicating the promotion of efficient neurite elongation and formation of dendritic spine, when BDNF-Ad and NT3-Ad were co-infected. These results suggest more studies in the effect of a combinatorial use of neurotrophins on different sites of brain need to be carried out to develop gene therapy protocols for neurodegenerative diseases.

Keywords: Adenovirus, neurite growth, neuronal precursor cells, neurotrophin

The neurotrophins are a family of growth factors involved in several phases of neuronal development including cell survival, migration, differentiation, neurite outgrowth, branching, and synaptic functions during embryogenesis [2, 3, 11, 13, 14, 23]. In addition, the neurotrophins are also involved in activity-dependent neuronal plasticity in the developing CNS [14, 23]. Among neurotrophins, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3) mRNAs are highly expressed in the hippocampus and neocortex of the brain [4, 15, 26].

BDNF is expressed in the hippocampus and cerebellar granule cells, and its expression is tightly regulated by the neuronal activity [13]. It has been reported that the hippocampus and cerebellar granule cells responded to BDNF with increased survival and neurite outgrowth *in vitro* [20]. These effects are mediated by the high-affinity receptor tyrosine kinase, TrkB. BDNF also increases survival and axonal outgrowth, but promotes decrease of neurite bundle size in pontocerebellar mossy fiber neurons *in vitro* [19]. NT3 is the neurotrophin that acts through the TrkC receptor and induces axonal growth and plasticity in the spinal cord and PNS. NT3 affects the survival and differentiation of neurons in the hippocampus and cortex. Both BDNF and NT3 play important roles in the activity-dependent maturation of cortical systems [17].

Since neuronal loss and synaptic loss are the main causes of the most neurodegenerative disorder, many efforts for treating diseases by the gene delivery of neurotrophic factors have been made. Neurotrophins can be delivered exogenously, by using viral vectors or cells, into the nervous system to support regeneration [5].

When NT3 was delivered to the spinal motoneurons by retrograde transport through the sciatic nerve using an adenovirus, the local expression of NT3 by motoneurons induced and supported the sprouting of axons from the contralateral CST (corticospinal tract) [27]. A transplant of BDNF and NT3 producing fibroblasts can stimulate limited growth from some host axons and significantly

*Corresponding author
Phone: 82-2-961-0605; Fax: 82-2-966-4497;
E-mail: kimyh@khu.ac.kr

decrease atrophy of red nucleus neurons after the initial spinal cord injury [24].

Despite the many efforts using individual neurotrophins, there are few studies investigating the effect of a combinatorial use of different neurotrophins on a distinct site of the brain. In the present study, we used a delivery system of adenoviral vectors carrying the gene for NT3 or BDNF to express the neurotrophic factors locally in neural precursor cells such as hippocampal cells and cortical cells. The effects of separate or simultaneous gene delivery of each neurotrophin on neuronal differentiation were investigated.

Materials and Methods

Cell Culture

Human embryonic kidney cell line 293 was obtained from the American Type Culture Collection and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Hyclone), penicillin, and streptomycin at 37°C [8]. Human bone marrow mesenchymal stem cells (MSCs), kindly gifted from Dr. J. W. Jang at Younsei University, were grown in α-MEM supplemented with 10% FBS, 2.2 g/l $NaHCO_3$, and antibiotics at 37°C. The medium was replaced every 2–3 days as cells were grown to 80% confluence [21]. A rat hippocampal stem cell line, HiB5, was maintained in DMEM containing 10% FBS and antibiotics at 33°C [10]. HiB5 cells were changed with a chemically defined medium N2, and incubated at 39°C, the normal rat body temperature, in order to induce cell differentiation. Embryonic day-14 (E14) cortical cells were isolated from SD rat embryonic brain and cultured in N2 serum-free medium supplemented with 10 ng/ml bFGF at 37°C in 5% CO_2. N_2 medium is the mixture of F12 and DMEM at 1:1 ratio containing 100 μg/ml transferrin, 20 nM progesterone, 30 nM selenium salt, 100 μM putrescine, 500 nM glutamine, 25 μg/ml insulin, 1.55 mg/ml D(+)glucose, 1.69 μg/ml sodium bicarbonate, and 100 U/ml penicillin/streptomycin.

Construction and Amplification of NT3-Ad and BDNF-Ad

The coding sequence of the NT3 was amplified from the rat brain tissue by RT-PCR using primers 5'gctggtaccatg-tccacatcaacatc3' and 5'tcggatccttaatgcttgtagaagct3'. The cDNA of rat NT3 was subcloned into pShuttle-IRES-hrGFP2 between the CMV early promoter and the SV40 polyadenylation site using the NotI and SalI sites. Subsequent clone was transformed into *E. coli* BJ-5183 along with pAdeasy1 in order to recombine NT3 cDNA with adenoviral genome according to the manufacturer's instruction (Stratagen). Recombinant DNA was subsequently transfected into 293 cells for the generation of adenovirus. The cDNA of human BDNF coding sequences were cut out from the cDNA sequences in a pBluescript plasmid provided by Dr. Louis F. Reichardt (Howard Hughes Medical Institute, University of California-San Francisco, U.S.A.). The coding sequences were inserted into pCA14 (Microbix) between the CMV early promoter and the SV40 polyadenylation site using the EcoRI sites. This plasmid was transfected into 293 cells along with pJM17 (Microbix) in order to generate the recombinant adenovirus. Viral DNA from each plaque was purified and analyzed by PCR to verify the presence of the coding sequences and for the absence of the adenovirus E1 sequence. A control recombinant adenovirus expressing *E. coli* β-galactosidase targeting nucleus (LacZ-Ad) was used as previously described [6].

Virus Preparation and Infection

Viruses from a single plaque were amplified in 293 cells and the viral stocks were made by repeated freezing and thawing of cells prior to the ultrasonication. The plaque forming unit (PFU) of virus particles was determined by a plaque assay in 293 cells. Viral stocks were kept as aliquots at −80°C until use. Virus infections in all cells were carried out in the appropriate growth medium containing 2% FBS at a multiplicity of infection (moi) of 20 for 2 h, unless otherwise stated. HiB5 cells were infected with adenovirus at 33°C and cultured in N2 medium for 2 days at 39°C. E14 cortical precursor cells infected with adenovirus were cultured in FGF free N2 medium for 5 days at 37°C.

Measurement of NT3 and BDNF Secreted into Culture Media

The measurement of NT3 or BDNF in the supernatant of virus-infected MSC cultures was performed in duplicate using a commercial ELISA system according to the manufacturer's instructions (DuoSet ELISA Development Kit; R&D Systems). The MSCs in a 35-mm dish were infected with BNDF-Ad or NT3-Ad and subsequently incubated in α-MEM containing 10% FBS at 37°C. After 12 h, the infected cells were incubated in 1 ml of serum-free α-MEM for one more day and the supernatant was harvested and stored at −70°C until use.

Immunoprecipitation Analysis of Trk Receptors and Immunoblotting

A total of 1.2×10^6 HiB5 cells were infected with LacZ-Ad, BDNF-Ad, and/or NT3-Ad at a moi of 100°C at 33°C for 24 h, and the serum-containing media were replaced with serum-free N2 media and left at 39°C for varying time periods. The conditioned media collected from the aforementioned cultures were treated for 10 min with 3T3-TrkB or 3T3-TrkC cells, which were deprived for 24 h prior to use. The cells used as positive controls were

treated with 100 ng/ml of recombinant BDNF or NT3 (R&D Systems). The cell lysates were prepared as previously described [10, 12]. Five hundred mg of proteins from cell lysate were immunoprecipitated with 4G10, a monoclonal antibody against phosphotyrosine (Upstate Biotechnology). The precipitated proteins were fractionized on 7% SDS polyacrylamide gel and subjected to immunoblot analysis using anti-Trk antibodies (Santacruz). The whole-cell lysates and cell culture supernatant were also fractionated and probed for NT3 using enhanced chemiluminescence (Amersham).

Immunocytochemistry

HiB5 cells and E14 cortex primary cells grown in coverslips were infected with adenoviruses and fixed in 4% formaldehyde in PBS for 15 min at 4°C [18]. Cells were permeabilized with 0.5% triton X-100 in PBS for 5 min and blocked with a mixture of 5% serums of normal horse, goat, and donkey for 1 h at 37°C. Cells were then incubated with primary antibodies overnight at 4°C, washed, and treated with FITC, TRICT, cy2, cy3-conjugated secondary antibody (Jackson Lab) for 1 h at room temperature. Nucleus was stained with 1 μg/ml DAPI (4,6-diamidino-2-phenylindole) for 5 min. Images were captured on Confocal Laser Scanning Microscopy LSM 510 (Carl Zeiss). The primary antibodies used were NF-200 (1:150, Sigma), pan-neurofilament (1:1,000, Covance), β-galactosidase (1:500, Promega), and BDNF (1:250, Santa kruz).

Measurement of Neurite Length and in Differentiated Neuronal Cells

Neurite length of each differentiated cell was measured in immunostained cells with anti-pan neurofilament antibodies from the hillock sites to the neurite growing sites using a Confocal Laser Scanning Microscope LSM 510. Image analysis program (Carl Zeiss) was used to measure the length and number of spines of the individual neurite.

Statistical Analysis

The results were subjected to one-way analysis of variance (ANOVA) for repeated measurements across testing sessions. Differences of means between the groups were investigated using Student's *t*-tests. All data are presented as the mean±SEM.

RESULTS

Characterization of Recombinant Adenoviruses, NT3-Ad and BDNF-Ad

Among the recombinant adenoviruses used in this study, NT3-Ad was designed to produce the NT3 along with GFP for the ease of detection in transduced cells (Fig. 1A). In contrast, BDNF-Ad was generated by inserting human BDNF cDNA sequences into the E1 region of the adenoviral genome without the GFP coding sequence (Fig. 1B). PCR was used to verify the presence of the coding sequence of

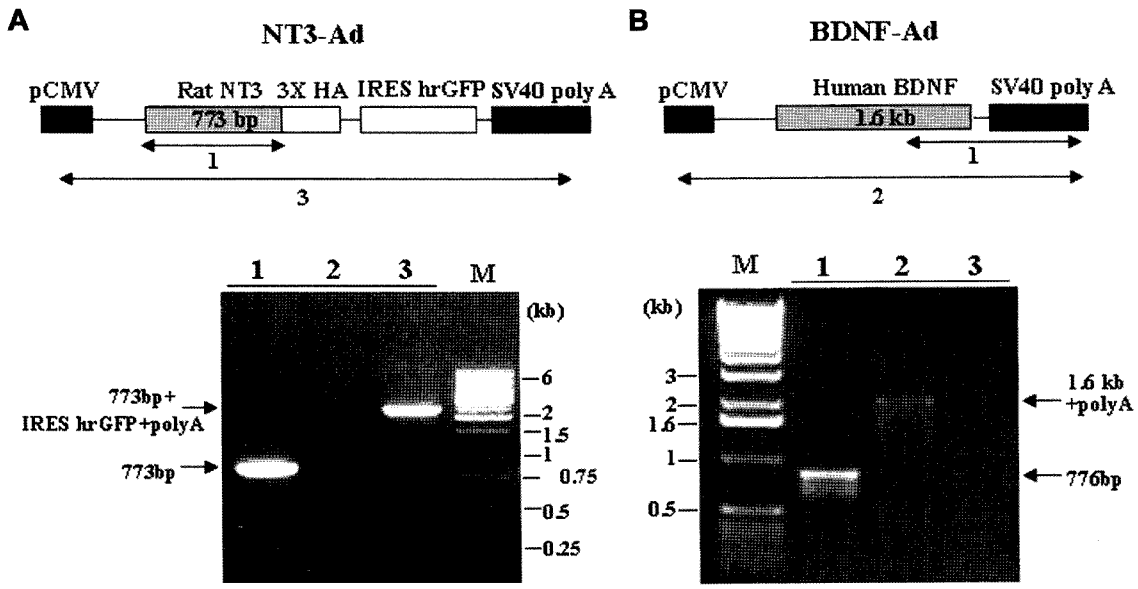

Fig. 1. Diagram and PCR analysis of adenoviral vector containing recombinant neurotrophins.
A. The schematic diagram of viral DNA designed to express the NT3 in which whole NT3 cDNA was inserted between the CMV early promoter and the SV40 polyadenylation site. PCR products of recombinant adenovirus DNA confirmed the presence of rat NT3 sequence (1), the absence of wild-type adenoviral sequences E1 (2), and insertion of NT3 sequence between the CMV promoter and SV40 poly(A) signal (3) in viral stocks. **B.** The diagram of recombinant adenoviral vector for BDNF is shown above. PCR analysis for the presence of BDNF coding sequence (1), and insertion of BDNF sequence between the CMV promoter and SV40 poly(A) signal (2), and the absence of wild-type adenoviral sequences E1 (3) is shown. The primers used for PCR are numbered in the upper diagram and the size markers (M) are also shown.

NT3 or BDNF, and the absence of the adenoviral E1 sequence demonstrating the successful insertion of fused DNA in the E1 region of the viral genome (Fig. 1; hereafter NT3-Ad and BDNF-Ad). The integrity of the inserted sequence was also confirmed by double-stranded DNA sequencing of viral DNA (data not shown).

Conditioned media from virus-transduced cell cultures were analyzed for the expression and secretion of NT3 and BDNF using standard ELISA. MSCs transduced with NT3-Ad or BDNF-Ad alone produced NT3 peptides (about 14 kDa) of 27.3±5.03 ng per 5×10^6 virus particles (moi of 100 for 5×10^4 cells) or BDNF of 11.5±0.42 ng per 10^7 virus particles (moi of 100 for 1×10^5 cells), respectively (Fig. 2A). NT3 peptides were hardly detected in non-transduced, LacZ-Ad, and BDNF-Ad infected MSC cultures. In contrast, small amounts of endogenous BDNF peptides (less than 0.6 ng per 10^{6-7} virus particles) were released in non-transduced, LacZ-Ad, and NT3-Ad infected MSC cultures without the infection of BDNF-Ad. Co-infection of BDNF-Ad and NT3-Ad (moi of 100 for 1×10^5 cells) did not affect the yield of the other neurotrophin significantly (24.7 ng for NT3, and 11.2 ng for BDNF), suggesting that the numbers of the adenovirus receptor on MSCs are enough for the entry of both viruses. These results demonstrate that BDNF-Ad and NT3-Ad produce and release the peptides outside of the cell efficiently, and their simultaneous transduction did not interfere with producing the other neurotrophin significantly.

Biologically Active Neurotrophins were Expressed and Released by Transduction of Recombinant Adenovirus NT3-Ad and BDNF-Ad

The biological activity of the Ad-vector-derived BNDF and NT3 were confirmed by analyzing the stimulation of the tyrosine kinase activity of their cognate receptors TrkB and TrkC, respectively. When the supernatant of conditioned medium and lysates of cells transduced with NT3-Ad were analyzed in the immunoblot, the N2 conditioned medium collected from HiB5 cell cultures contained predominantly the mature form of NT3, but the cell lysates had more precursor forms. In contrast, either supernatant or cell lysates from uninfected cells did not contain either form of NT3. Thus, the conditioned medium was used to test for the ability of stimulating its receptor. The phoshorylation of TrkC was markedly increased within 10 min in 3T3-TrkC cells when treated with N2 media collected from NT3-Ad-infected HiB5 cell cultures.

The conditioned media collected from BDNF-Ad-infected HiB5 cultures were also used to treat HiB5 and 3T3-TrkB cells. The phoshorylation of TrkB, in differentiating HiB5 cells and 3T3-TrkB cells, was stimulated in the presence of the conditioned media collected from BNDF-Ad-

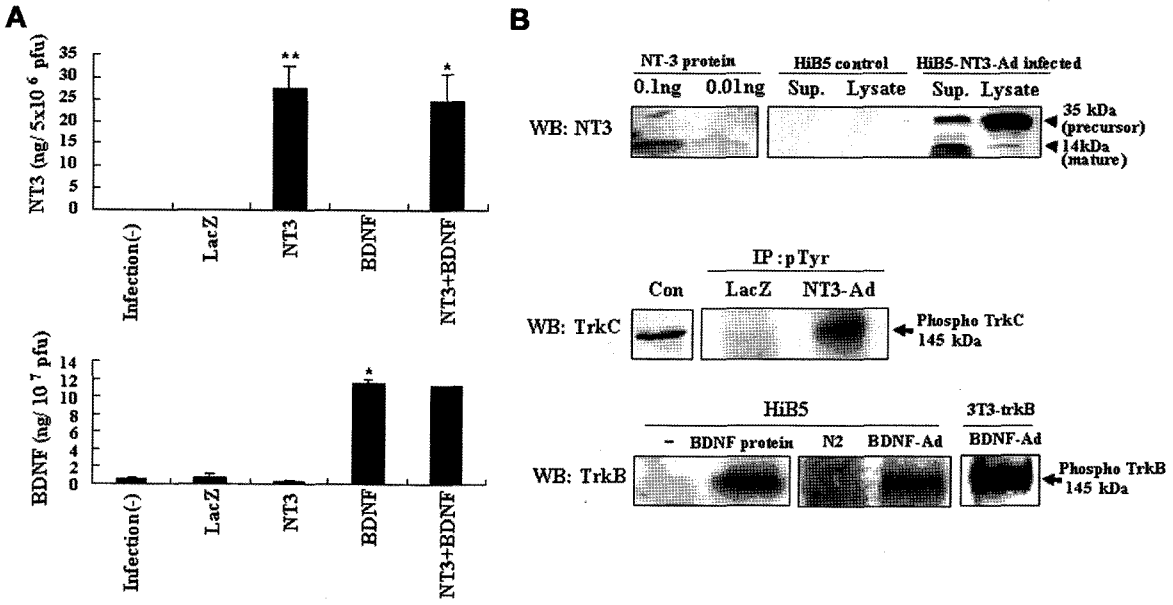

Fig. 2. Biological activity of NT3 and BDNF produced by adenovirus vectors.

A. Secretion of neurotrophin peptides by adenovirus vector. ELISA showed the amount of NT3 or BDNF released from human MSCs (5×10^4–1×10^5 cells) infected with recombinant adenovirus at a moi of 100. *p<0.05, **p<0.01. **B.** Biological activity of NT3 or BDNF secreted from infected cells. Supernatants of the conditioned media or lysates of HiB5 cells either uninfected (HiB5 control) or infected with NT3-Ad or BNDF-Ad were analyzed by Western blot analysis. For the receptor activation assay, the conditioned medium was added into 3T3-Trk or HiB5 cells for 10 min. As the positive control, recombinant NT3 or BDNF was treated (NT3 protein or BDNF protein). The cell lysates were immunoprecipitated by anti-pTyr antibodies and then immunoblotted by anti-Trk antibodies. Phosphorylated TrkB or TrkC is indicated by arrows.

infected HiB5 cultures (Fig. 2B). These data demonstrate that NT3-Ad and BDNF-Ad produced and secreted biologically active neurotrophins.

Simultaneous Transduction with Neurotrophin-Ad Promotes Neuronal Differentiation in Hippocampal Precursor Cells but Not in Cortical Precursor Cells

We examined the ability of neurotrophin-Ad to induce neuronal differentiation. The neuronal precursor cell line HiB5, originated from the rat embryonic hippocampus, was infected with LacZ-Ad or neurotrophin-Ad, and then induced for the differentiation. Cells were subsequently immunostained with anti-pan neurofilament antibodies to visualize differentiated cells (red in Fig. 3A). Compared with the separate infection, co-infection with BDNF-Ad and NT3-Ad showed more cells immunostained with neurofilament antibodies, a larger bundle size, and more neurites and spine-like structures per differentiated cells.

To evaluate the extent of neurotrophin-Ad-induced differentiation in HiB5 cells, the number of cells immunostained with anti-neurofilament antibodies, whose neurites were longer than double of a cell body width, were counted from 10 fields. These numbers were divided by total cell numbers and expressed as the percentage of differentiation. The induction of differentiation was evident in the cells infected with BDNF-Ad (19.1%) or NT3-Ad (23.9%) compared with the cells infected with LacZ-Ad (4.5%; Fig. 3B). In our previous study, the expressions of *trkB* and *trkC*, the receptor for BNDF and NT3, respectively, were stimulated during differentiation of HiB5 cells. However, *trkC* but not

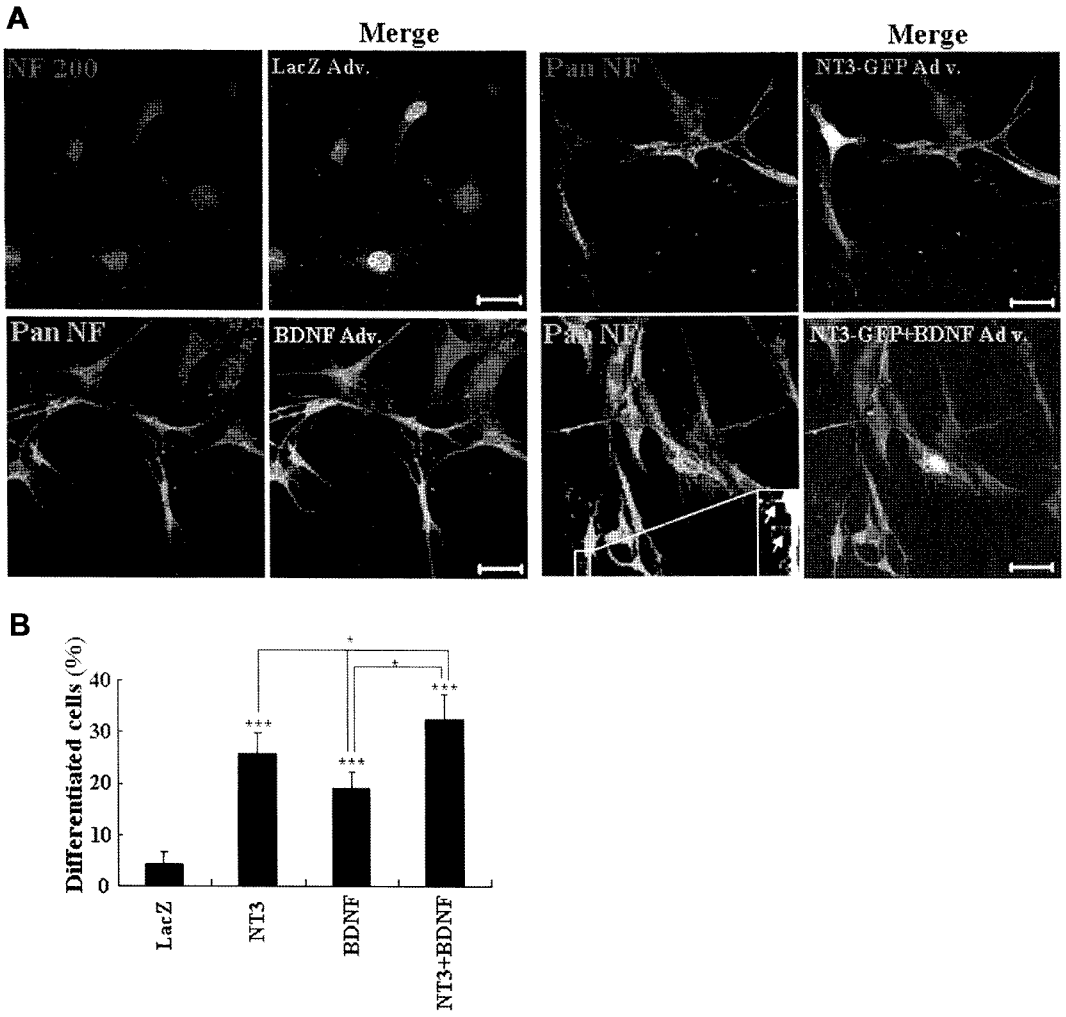

Fig. 3. A. Expression of neurofilament in the hippocampal neuronal precursor cell, HiB5, infected with NT3-Ad (visualized as green by GFP) and/or BDNF-Ad (20 moi for each virus). LacZ-Ad was used for the virus control. The HiB5 cells were induced for differentiation after infection and immunostained 2 days after differentiation by anti-neurofilament antibodies (red) and the nucleus was counterstained with DAPI (blue). The inset show the enlarged spine-like structures. **B.** The percentage of differentiated cells containing neurites longer than 2-fold of cell body width per total cells.
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Scale Bar: 50 μm.

trkB and *trkA*, the NGF receptor, was expressed in HiB5 cells at the proliferation condition (data not shown), explaining the more differentiation rate in NT3-Ad-infected cells. Simultaneous transduction with BDNF-Ad and NT3-Ad increased the differentiation rate (34.8%), judged by the number of cells with neurites longer than two-fold of cell body width (Fig. 3B).

To investigate whether neurotrophins have differential effects on neural precursor cells originated from the different regions of the brain, we cultured cortical cells primarily from the rat embryonic brain (E14) and then transduced with neurotrophin-Ads. In the cortical precursor cells, transduction of BDNF-Ad or NT3-Ad promoted neuronal differentiation up to 44.8% or 50.1% respectively (Fig. 4B). Compared with HiB5 cells, cortical cells had longer neurites without any infection or when infected with LacZ-Ad (26.7%; Fig. 4). Interestingly, simultaneous transduction of cortical cells with neurotrophin-Ad did not enhance neuronal differentiation (Fig. 4B). Furthermore, co-infecting NGF-Ad to cortical cells along with BDNF-Ad and NT3-Ad decreased the neurites extention. In contrast, such infection increased the differentiation and neurites length in HiB5 cells (data not shown). The simultaneous transduction of neurotrophin-Ads, however, showed the larger bundle size and more neurites and spines per differentiated cells in cortical cells as in the HiB5 cells. These results suggest that the effect of neurotrophin-Ad may be different according to the regions of the brain.

To determine the neurites elongation induced by neurotrophin-Ads, differentiated cells were sorted by the length of neurite. Fig. 5A shows the number of neurites with given length expressed as the percentage of total cell

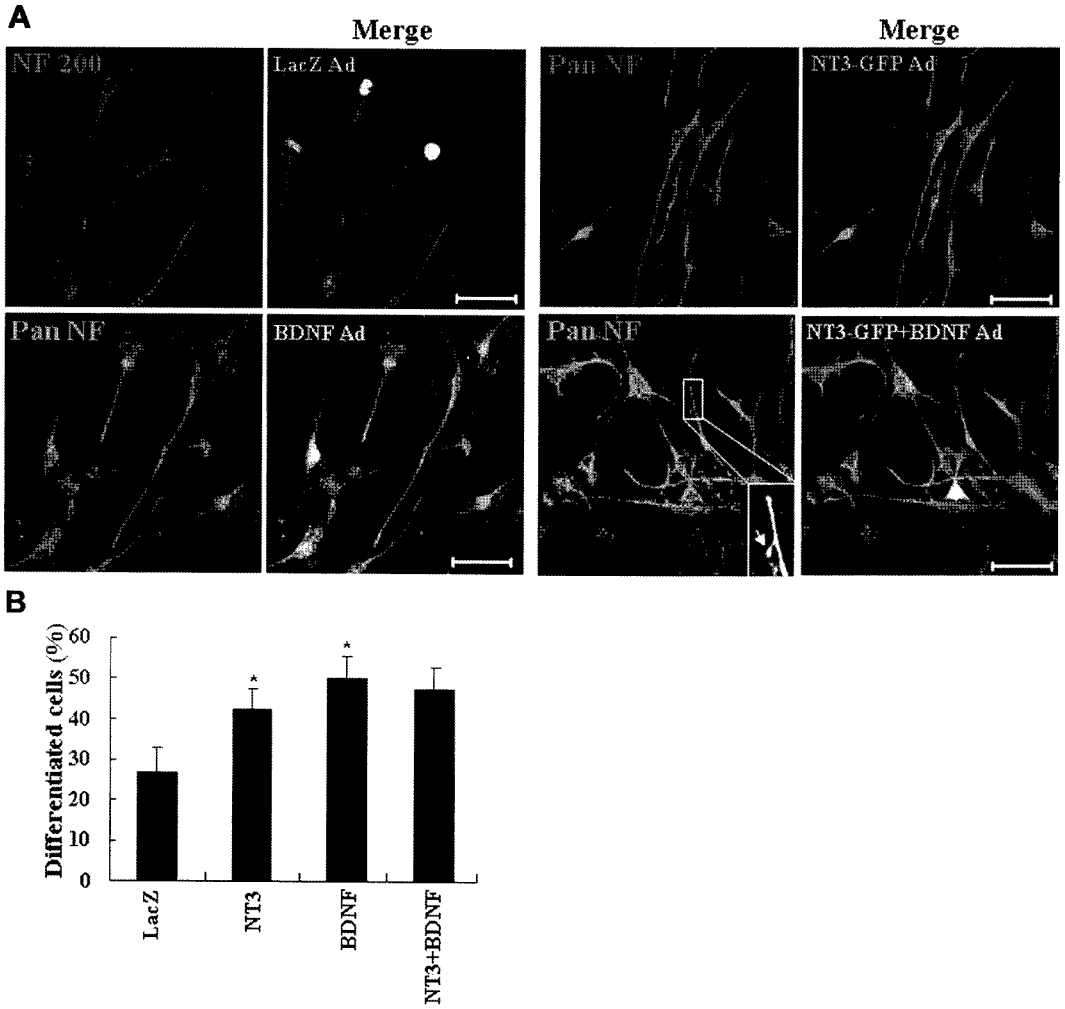

Fig. 4. A. Expression of neurofilament in cortical precursor cells primarily cultured from the rat E14 embryonic brain, infected with NT3-Ad and/or BDNF-Ad (20 moi for each virus) 5 days after culture. LacZ-Ad was used for the virus control. Cells were immunostained as in Fig. 3. The inset shows the enlarged spine-like structures. **B.** The percentage of differentiated cells containing neurites longer than 2-fold of cell body width per total cells.
*p<0.05, **p<0.01, ***p<0.001. Scale Bar: 50 μm.

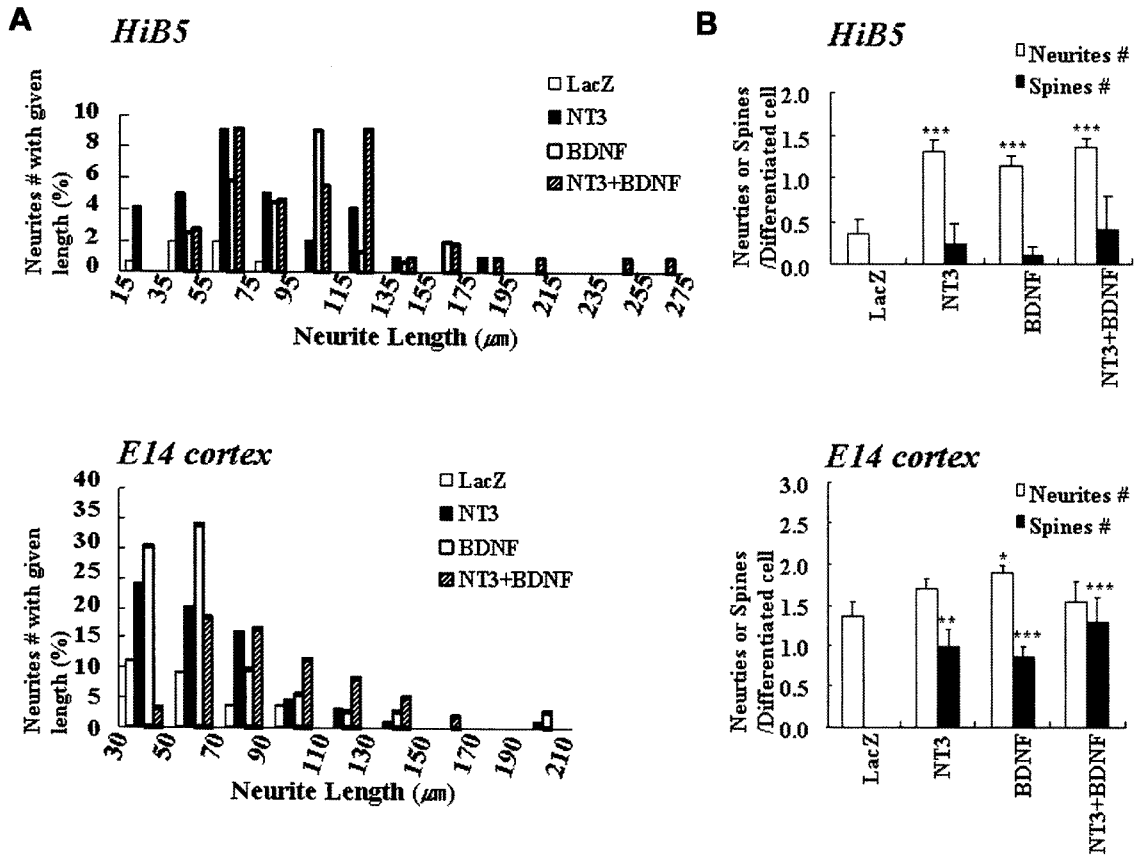

Fig. 5. **A**. Neurite length of differentiating cells were measured by confocal laser microscopy and an image analyzer and classified by percentage of the given length of neurite numbers per total cell numbers. **B**. Numbers of neurites and spine-like structures. $*p<0.05$, $**p<0.01$, $***p<0.001$ *vs.* LacZ-Ad.

numbers. In HiB5 cells, NT3-Ad generated differentiated cells with shorter ranges of neurite length (15–135 μm, average of 76.2 μm), whereas BDNF-Ad generated longer neurites (35–155 μm, average of 88.1 μm). Co-infection with BDNF-Ad and NT3-Ad generated the longest neurite elongation (35–255 μm, average length of 108.1 μm). LacZ-Ad barely produced neurite extending cells (15–75 μm, average of 50.8 μm).

Compared with the hippocampal HiB5 cells, which had more neurites in longer range in the BDNF-Ad-infected group (75–115 μm) than in NT3-Ad (15–75 μm), cortical neurons infected with NT3 (30–70 μm, average of 66.9 mm) or BDNF (30–70 μm, average of 65.5 μm) produced shorter ranges of neurites elongation. Co-infection of both NT3-Ad and BDNF-Ad (50–150 μm, average of 88.2 μm) produced the longer average length of neurite than separate infection, but shorter than the average length in HiB5 cells. Furthermore, the distribution of neurite length in cortical cells (50–150 mm) was much shorter than that in HiB5 cells (35–255 mm). In contrast to HiB5 cells, transduction of NGF-Ad into cortical cells in addition to BDNF-Ad and NT3-Ad did not increase the average length of neurites (data not shown).

Unlike the length of neurites, the numbers of neurites and dendritic spine-like structures were incresaed in differentiating cortical cells (Fig. 5B). Transduction of NT3-Ad or BDNF-Ad produced more numbers of neurites and spine-like structures compared with LacZ-Ad in both hippocampal cells and cortical cells. Simultaneous transduction with NT3-Ad and BDNF-Ad also increased the number of the structures but not significantly in both types of cells. The number of branching per cells had no significant difference between the infection groups.

DISCUSSION

Neurotrophins are proteins that regulate neuronal survival, axonal elongation, synaptic plasticity, and growth cone size. They are members of the neurotrophic factor family and include factors such as the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF), the neurotrophin-3 (NT3) and NT4/5. NT4/5 binds TrkB and acts in the peripheral nervous system as BDNF. In this paper, we generated the recombinant adenoviruses expressing BDNF or NT3 to test their ability to induce neuronal differentiation,

a prerequisite for the possible use in neuronal gene therapy. Adenovirus has been widely used in cancer gene therapy [16]. Recently, adenovirus has attracted the interest in gene therapy of neuronal disease owing to its plausibility of transferring foreign genes in neuronal cells. We observed the efficient gene transfer using adenovirus into neuronal precursor cells as well as neuroblastoma cells (unpublished result) [6]. Adenovirus is quite efficient in neuronal gene transfer compared with the retrovirus-based vector, which showed 40%–60% gene delivery efficiency at most [7]. The amount of produced BNDF using adenovirus in MSCs was even higher than that secreted from the neuroblastoma cell culture [5], demonstrating that the adenoviral system used in this study is quite efficient for high production of neurotrophins.

We next evaluated the effects of neurotrophins delivered by the adenoviral vector on neuronal differentiation, length of neurites, and the number of spine-like structures. We also investigated the effects on neuronal precursor cells originated from different regions of the brain, the cortex and hippocampaus. In both cell types, infection with BDNF-Ad or NT3-Ad promoted neuronal differentiation and neurite outgrowth. NT3-Ad infection generated more intermediate-length neurites, and BDNF-Ad produced longer ones. The thicker bundles and more spines were also observed by simultaneous transduction in both cell types. The results suggest the cooperative effect of the two neurotrophic factors BDNF and NT3.

Simultaneous transduction of BDNF-Ad and NT3-Ad generated more differentiated cells with longer neurite elongation in hippocampal cells. In E14 cortical neuron, however, simultaneous infection with NT3-Ad did not increase the differentiated cells, although BDNF-Ad alone promoted neuronal differentiation, neurite length, and number of spine-like structures. This could be supported in part by the finding that BDNF and NT3 oppose one another in regulating the dendritic growth in different layers of cortex [17]. NT3 inhibits the dendritic growth effect stimulated by BDNF in layer 4, whereas BDNF inhibits the effect promoted by NT3 in layer 6. These antagonizing actions of BDNF and NT3 may provide a mechanism controlling dendritic growth and retraction during development, in an opposing manner. This may explain why the synergistic effect of neurites elongation by co-infection of BDNF-Ad and NT3-Ad was not prominent compared with separate infection in cortical cells.

In this report, we generated BDNF-Ad and NT3-Ad and showed they can efficiently produce functionally active proteins and release outside the cells efficiently. This could provide a more effective gene delivery means into MSCs, which can be used in the treatment of stroke, since it produced about 10 times more BDNF in MSCs than the one used in a previous report [9]. The amount of produced BDNF by adenovirus in this experiment is much higher (11.5 ± 0.42 ng/1×10^5 MSCs at moi of 100) compared with the previously reported one (2.3 ng/5×10^5 MSCs at moi 1,000) [9]. We also showed that simultaneous delivery of BDNF and NT3 exert differential effects on neuronal differentiation depending on the cell origin. Endogenously produced BDNF or NT3 showed distinct effect in neuronal survival and differentiation in MSC [1], suggesting the usefulness of our system for studying distinct effect of neurotrophins in different sites of the brain. There have been many efforts to use various neurotrophins, including BDNF, NT3, GDNF, and NGF, in treating neuronal diseases such as Alzheimer's and Parkinson's diseases [22, 25]. More studies on delivering BNDF and NT3 using our system for gene and cell therapy may support the treatment of degenerating neuronal diseases.

Acknowledgments

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