Journal of Biochemistry and Molecular Biology, Vol. 35, No. 1, January 2002, pp. 87-93

JOURNAL OF
Biochemistry and Molecular Biology

Review

Effect of Neurotrophic Factors on Neuronal Stem Cell Death

Yunhee Kim Kwon*
Department of Biology, Kyunghee University, Seoul 130-701, Korea

Received 8 November 2001

Neural cell survival is an essential concern in the aging brain and many diseases of the central nervous system. Neural transplantation of the stem cells are already applied to clinical trials for many degenerative neurological diseases, including Huntington's disease, Parkinson's disease, and strokes. A critical problem of the neural transplantation is how to reduce their apoptosis and improve cell survival. Neurotrophic factors generally contribute as extrinsic cues to promote cell survival of specific neurons in the developing mammalian brains, but the survival factor for neural stem cell is poorly defined. To understand the mechanism controlling stem cell death and improve cell survival of the transplanted stem cells, we investigated the effect of plausible neurotrophic factors on stem cell survival. The neural stem cell, HiB5, when treated with PDGF prior to transplantation, survived better than cells without PDGF. The resulting survival rate was two fold for four weeks and up to three fold for twelve weeks. When transplanted into dorsal hippocampus, they migrated along hippocampal alveus and integrated into pyramidal cell layers and dentate granule cell layers in an inside out sequence, which is perhaps the endogenous pathway that is similar to that in embryonic neurogenesis. Promotion of the long term-survival and differentiation of the transplanted neural precursors by PDGF may facilitate regeneration in the aging adult brain and probably in the injury sites of the brain.

Keywords: Regeneration, Stem cell, Survival

Introduction

Neural cell survival is an essential concern in aging brains as well as many diseases of the central nervous system. Although neuronal cells are among the longest living cells, neural cells die in large numbers every day, even in a normal brain. During development, about half of the generated cells in a mammalian brain undergoes cell death. Aging and many diseases of the central nervous system are characterized by neural degeneration (Yuan *et al*., 2000; Choi, 2001). It was recently discovered that brain stem cells divide and differentiate in the embryonic and adult brain. They are found in adult rodent brains and even in some regions of the adult human brain, such as the hippocampal dentate zyrus, olfactory bulb, and subventricular zones of the cerebral cortex (Johansson *et al*., 1999; Park *et al*., 2001). The discovery of the neuronal stem cells has led to development of the embryonic stem cell and neuronal stem cell for research and clinical applications. These include the transplantation of the stem cells and ex vivo gene therapy into the brains of rodents and patients that suffer degenerating nervous system diseases (Snyder *et al*., 1998; Renfranz *et al*., 1991; Reynolds and Weiss, 1992; McDonald *et al*., 1999). Neural transplantation of stem cells and gene therapy are already applied to clinical trials for many degenerative neurological diseases, including Huntingtons disease, Parkinsons disease, and strokes.

Apoptosis in neuronal stem cells and protective action of neurotrophic factors

Multipotent stem cells that are capable of self-renewal and differentiation into multiple cell types are thought to arise during the development of organs. A clonal analysis revealed that single progenitor cells that are cultured from embryonic rat hippocampus can give rise to three major cell types of the mature brain-neurons, astroytes, and oligodendrocyte. Multipotent stem cells that are isolated from the embryonic and adult rodent CNS have been propagated in vitro by effective and safe methods using mitogens, such as EGF and bFGF or transfecting v-myc or large T antigen (Ryder *et al*., 1990; Renfranz *et al*., 1991). The stem cell lines undergo neuronal differentiation and are capable of migration within the brain parenchyma. Following implantation into the rodent brain, the stem cells migrate and differentiate into multiple cell types in a temporally and regionally appropriate manner. They interact with host cells, differentiate by responding in a

*To whom correspondence should be addressed.
Tel: 822-961-0605; Fax: 822-966-4497
E-mail: kimyh@khu.ac.kr

manner that is similar to endogenous cells for local cues for their phenotyphic determination, and form synaptic connections to participate in normal development. The increase of the neuronal stem cell survival may also be important in the degenerating regions of the damaged brain where endogenous stem cells reinitiate self-renewal and commit differentiation, because neuronal cells undergo apoptosis mostly at the time when they stop the cell cycle and make decisions on its fate (Chung *et al*., 2000). This would be the specific factor that reduces stem cell death and may trigger stem cell differentiation in both the developing embryonic and adult brain.

We used a hippocampal stem cell line, HiB5, which was generated to be implanted and differentiated to many cell types. This cell line was isolated from a primary culture of embryonic day 16 rat hippocampus where the progenitor cells of pyramidal cells begin to proliferate (Altman and Bayer, 1990; Altman and Das, 1965). Because these cells are immortalized by infecting temperature sensitive SV40 large T antigen, they grow at the permissive temperature, 33°C. These cells have a flat morphology that is similar to that of cells in the neuroepithelium and express the stem cell marker nestin. At 39°C (body temperature of rodents), however, they stop growing and many cells die. Some of the remaining cells change their morphology, but do not express markers such as neurofilament or the glial fibrillary scidic protein (Renfranz *et al*., 1991). After longer days of incubation in a chemically defined N2 medium, some of the cell bodies become reduced in size and their processes get longer. When implanted into a rat brain, such as the hippocampus, cerebellum, and stiatum, the HiB5 stem cells integrated into the host tissue and differentiated into cells that show morphologies, which are characteristic of the neurons, glias, and oligodendrocytes that are found at the implant site**.** When we incubated HiB5 cells for several days in a chemically defined N2 medium at 39°C, some of the cells formed longer processes, but some gradually died. About 30% were left by the third day (Fig. 1A). (*We called this a differentiation condition.*)

Neurotrophic factors generally contribute as extrinsic cues to promote cell survival of specific neurons in the developing mammalian brains (Segal and Greenberg, 1996; Kwon *et al*., 1997), but the survival factor for the neural stem cell is poorly defined. In order to understand the mechanism that controls stem cell death and improve cell survival, as well as the differentiation of the transplanted stem cells, we investigated the effect of plausible neurotrophic factors on stem cell survival**.** When neurotrophic factors were added into a N2 medium, the platelet-derived growth factor BB form (PDGF-BB) among many factors promoted the stem cell survival about two fold. FGF, the mitogen for the stem cell, also promoted the survival under a nonproliferating condition, but less than PDGF. EGF, another mitogen for stem cell, however, had little effect. Also, there was little effect from neurotrophins, such as the brain-derived neurotrophic factor (BDNF) and Neurotrophic factor-3 (NT-3), the well-known survival factor for CNS neurons. On the third day, however, BDNF and EGF were enhanced 150% or less. NGF (survival factor for most PNS neurons) and neuregulin (survival factor for the Schwann cell) also showed little effect. We examined whether or not the addition of PDGF and other factors together have a synergistic effect on the stem cell survival. The addition of other factors, together with PDGF, however, did not increase the survival. These data indicate that PDGF-BB promotes the survival of neuronal stem cells. We also observed the similar induction of cell survival on a hippocampal stem cell line, HiJ1, and the human neural crest cell line, HNC (Renfranz *et al*., 1991).

While the addition of PDGF stopped the proliferation of the stem cell, HiB5, in the permissive temperature, PDGF appeared to facilitate neuronal differentiation as well as cell survival**.** When the HiB5 cells were incubated in a N2 medium that was supplemented with PDGF, neurites grew out of many of the cell bodies that were immunostained with neuronal markers, such as neurofilament and beta-tubulin isotype III (TuJ1). We previously reported that the expression of BDNF is stimulated by PDGF in the hippocampal stem cell lines, HiB5 and HiJ1, during PDGF enhanced differentiation (Kwon, 1997).

Some growth factors, such as fibroblast growth factors (FGFs) and platelet-derived growth factors (PDGFs), show trophic effects in the nervous system (so-called neurotrophic factors). Although they serve as mitogens in fibroblast, FGF facilitates the neuronal survival and neurite outgrowth in embryonic primary cell cultures of rat hippocampus, the developing Xenopus retina, and retinal regeneration (Mattson *et al.*, 1989; Creuzet *et al.*, 1995; Gao *et al.*, 1995; Park *et al.*, 1989; Vicario-Abejon *et al*., 1995). PDGF A and B chains are expressed in hippocampal neurons of newborn and adult rats and in the developing retina (Sasahara *et al.*, 1991; Hutchins and Jefferson, 1992). PDGF receptors are expressed in hippocampal neurons (Smits *et al.*, 1991). PDGF receptors are found in the hippocampal glial cells (Yeh *et al.*, 1993). PDGF receptors and FGF receptors are also members of the receptor tyrosine kinase. The neurotrophic factors activate tyrosine phosphorylation of the receptor, which then stimulates the activation of signal generators such as raf, phosphatidylinositol-3-kinase, phospholipase D, and Src (Barone and Courtneidge, 1996; Sung *et al*., 2001). Through the raf-ras-mitogen activated protein (MAP) kinase pathway the signal transduces to nuclei and induces the expression of the immediate early gene, such as c-fos and c-jun, and then the late genes. Although there are pioneering studies on the signal transduction pathway of PDGF in proliferating cells, the biological role and signal transduction pathways in the neuronal cells have yet to be established. In primary cultured embryonic hippocampal cells, PDGF supports neuronal differentiation, while CNTF and thyroid hormone T3 act to generate astrocytes and oligodendrocytes, respectively (Johe *et al.*, 1996). PDGF is also reported to initiate neuronal differentiation as an instructive signal in retrovirus-infected

clones of uncommitted neuroepithelial cells that are isolated from the ventricular zone of the embryonic cerebral cortex (Williams *et al.*, 1997). This indicates that it has to be enough to incubate with PDGF for less than 2 h prior to implantation.

The studies presented here show that PDGF not only directs neuronal differentiation, but also supports neuronal cell survival and prevents cell death. To define whether or not the HiB5 cells die by apoptosis and PDGF prevents the apoptosis during HiB5 stem cell differentiation, we stained HiB5 cells with AnnexinV, which stains phosphatidylserine. Phosphatidylserine (almost exclusively located on the inner side of the plasma membrane) relocates to the outer surface of the cells at the beginning of apoptosis. The proliferating HiB5 stem cells were not stained with AnnexinV, while all the nuclei of the stem cells were stained with DAPI. Many of the differentiating HiB5 cells were double stained with AnnexinV and DAPI, whereas the addition of PDGF almost abolished the staining of both. To confirm whether or not the HiB5 stem cells undergo apoptosis, DNA fragmentation was investigated under differentiation conditions. DNA ladders were observed from 18 and 24 h in differentiation conditions, but were prevented in the cells that were maintained with PDGF. Cleavage of PARP in HiB5 stem cells was also protected 24 and 48 h under differentiation conditions in the stem cells that were maintained with PDGF (Shim *et al*., 2001). Prevention of apoptosis was further confirmed by the observation of DNA fragmentation with a terminal deoxynucleotiyltransferase assay and chromatin condensation in the stem cells that were stained with propidium iodide. These observations imply that HiB5 stem cells undergo apoptosis under differentiation conditions and PDGF protects the stem cells from apoptosis.

Cell survival pathways in neuronal stem cells

PDGF is known to promote cell survival in fibroblasts through the PI3kinase dependent pathway. Other factors, such as BDNF, promote the survival of cerebellar granule cells though the ras-MAPK-RSK dependent pathway in addition to the PI3kinase pathway, while IGF or NGF in PC12 cells does not (Bonni *et al.*, 1999;Yao and Cooper, 1995). The neurotrophic factor and signaling pathway that prevent apoptosis in neuronal stem cells, however, has not yet been identified. To determine whether PDGF prevents apoptosis of stem cells through the PI3kinase and MEK pathways, the activation of signaling proteins in the PI3kinase and MEK pathway was investigated. Both the PI3 kinase and downstream AKT were phosphorylated 3 mins after the addition of PDGF. However, the phosphorylation of PI3kinase disappeared within 15 min, and that of AKT lasted 15 mins. The downstream of AKT, BAD, was phosphorylated by AKT at the site of Ser 136, which was phosphorylated 3 mins after the PDGF addition and lasted for 6 h. The phosphorylation of the Ser112 site of BAD, which was phosphorylated by RSK, the downstream of MAPK, increased gradually by 15 mins, peaked at 2 h, then quickly disappeared. The addition of wortmannin, together with PDGF, inhibited the activation of AKT and BAD at Ser136, but much less at Ser 112. Since sodium phenylacetate, the inhibitor of Ras, reduced the PDGF induced HiB5 cell survival, prevention of the HiB5 cell apoptosis by PDGF may also require a Ras dependent pathway. We also observed the phosphorylation of the Ser112 site of BAD, which increased gradually by 15 mins, peaked at 2 h, then was slowly reduced, but not to the control level, even after the addition of wortmannin. Since the phosphorylation of the Ser112 site of BAD is mediated by RSK, the downstream of MAPK, this result suggests that PDGF also activated the MAPK pathway in the HiB5 cells. This is consistent with the observation that PD098059, the inhibitor of MEK, reduced the promotion effect of PDGF on the survival of HiB5 cells, and the phosphorylation of ERK 1 and ERK 2. This suggests that the MAPK pathway contributes to some extent to the action of PDGF. The phosphorylated BAD (at both Ser 112 and Ser 136) forms a complex with 14-3-3, which is known to lead to cell survival in many cell types. Therefore, we examined whether or not the binding of 14-3-3 with BAD can be promoted by PDGF in HiB5 cells. A co-immunoprecipitation analysis of the BAD that expresses HiB5 cells showed that the treatment of PDGF increased the formation of the BAD-14-3-3 complex. This increase was reduced by wortmannin.

It is well-known that Bcl-2 family proteins have either pro-apoptotic or anti-apoptotic functions, although they consist of more than a dozen proteins, including BAD and BclxL. We examined the expression of anti-apoptotic proteins, Bcl-2 and BclxL, as well as the pro-apoptotic protein, Bax. The expression of anti-apoptotic Bcl-2 and BclxL was increased by PDGF, while the expression of pro-apoptotic Bax was unchanged. Therefore, these results indicate that PDGF prevents the apoptosis of HiB5 cells under differentiation conditions. Also, the protection effect of PDGF is mediated by the multiple signaling pathways, including PI3kinase and MAPK, and eventually by the formation of the BAD-14-3-3 complex and the expression of anti-apoptotic proteins, such as Bcl-2 and BclxL.

Cell survival in neruonal cells transplanted into the rat brain

A critical problem of the neural transplantation is cell survival. In the case of Parkinson's disease patients, brain cells from three to five fetuses are implanted into one side of the patients brain, but in many cases they had to be reimplanted several months later, because of the low survival rate of the transplanted cells (Olanow, 1996). Thus, a lot of effort is underway to develop stem cell lines, as well as to find survival factors for stem cells that block cell death when implanted into brains.

To investigate whether PDGF enhances the survival of the stem cell *in vivo* during differentiation, HiB5 cells were implanted into the adult and postnatal day-7 rat brain. To mark the HiB5 cells, the cells were labeled by a fluorescence

dye, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbo cyanine perchlorate (DiI-C18-(3) and by infecting recombinant LacZ adenovirus. The HiB5 cells were exposed to PDGF and implanted into the medial aspect of the dorsal hippocampal formation (above the CA1 subfield). After varying post-surgery times, the brain sections were stained with X-gal to show the cell distribution. We previously reported that LacZ adenovirus can infect HiB5 cells efficiently with no cytotoxic effect, and β-galactosidase expressed from the virus are localized in nucleus even 6 weeks after infection of the rat sciatic nerves (Jung *et al.*, 2000).

Three days after the implantation, clusters of the cells were found at the injection sites and some cells were dispersed. The implanted cells were dispersed more and started to migrate laterally in the rats that were implanted with the cells that were treated with PDGF, more so then the cells that were treated without PDGF prior to implantation one week after implantation. After two weeks in vivo, the cells just dorsal to the CA1 region were migrating laterally towards dorsal to the CA2 region. However, greater cell numbers and migration lengths were observed in the cells that were treated with PDGF. At a higher magnification, projections that came out of their cell bodies were shown in DiI-C18-(3). Four weeks later, the implanted cells were found distributed across the hippocampal CA1, CA2, CA3, CA4 regions, and dentate gyrus regions. Some of the cells were localized in the hippocampal fissure, just dorsal to the dentate gyrus with projections. Others were seen above CA regions between the dorsal hippocampus and cerebral cortex. By eight and twelve weeks the HiB5 cells that were treated with PDGF were present across the entire hippocampus, while the untreated cells almost disappeared. Despite the inherent variability in the precise placement of the cells in a large number of the different experimental animals, the HiB5 cells integrated into the lateral and medial horns of the dentate gyrus (the hippocampal subfields CA1CA3 and the hilar region). Comparatively few cells were observed in the cortex dorsal to the hippocampus. Many of the cell nuclei that integrated into the host tissue, however, were found in the pyramidal cell layer of the CA subfields and granule cell layer of the dentate gyrus. At a higher magnification, the labeled cells appeared to be intimately associated with the endogenous neurons. These cells looked remarkably like endogenous hippocampal pyramidal cells and deTate granule neurons. These data corroborate the Lacz staining data, further indicating that implanted HiB5 cells differentiate into granule neurons.

The number of HiB5 cells that localized specifically to the hippocampal formation was estimated as 1 to 12 weeks after implantation by counting the LacZ stained nuclei, and by counting DiI-C18-(3) labeled cells within 36 h after the sacrifice in all of the serial sections. Although there was no large difference in cell survival between PDGF treated and untreated cells one and two weeks after transplantation, PDGF treated cells were about two fold higher by four weeks and about three fold higher by twelve weeks following transplantation. Thus, between 4 and 12 weeks after transplantation, the survival rate of cells with the PDGF treatment did not significantly decline, while the untreated cells were continuously reduced. These results, therefore, indicate that PDGF promotes migration into the neuronal cell layer, and the long term-survival of differentiating neuronal precursor cells that are implanted into the adult rat brain *in vivo.*

Regeneration of neuronal cells by gene transfer using recombinant adenovirus

Replication defective adenoviral vectors have been demonstrated as an effective method for delivering genes into a variety of cell types and tissues in vivo and in vitro. Transfecting genes into neuronal cells is difficult, because of their lack of cell division. Since the major problem in neurological disease is the degeneration of the terminally differentiated neuronal cells, the adenoviral vectors ability to transfer genes into differentiated post-mitotic cells make them advantageous for a gene delivery system for the nervous system. We have shown that the replication defective recombinant adenovirus that carries the *lacZ* gene could infect the neuronal stem cells and even the differentiated neuronal cells that are derived from the central nervous system. The *lacZ* gene that is delivered into neuronal cells was efficiently expressed. The recombinant virus infected intact and injured nerves in vivo, and the expression of the *lacZ* gene lasted for 5 weeks (Jung *et al*., 2000).

In Alzheimers disease patients, the neuronal degeneration is very serious in the CA1 subfield of the hippocampus and entorhinal cortex, and BDNF is a survival factor for pyramidal cells in the hippocampus and cholinergic neurons in the basal forebrain. In pyramidal neurons that are cultured from the rat embryonic hippocampus, BDNF is a major survival factor among many neurotrophic factors. The mRNA of BDNF is expressed in neurons throughout the adult rat brain, and the maximal expression is observed in the developing and adult hippocampus (Hofer *et al.*, 1990). BDNF transduces the signals through the receptor tyrosine kinase, Trk B. Other members of the neurotrophin family, such as the nerve growth factor (NGF), neurotrophin 3 (NT3), and neurotrophin 4/5 (NT4/5), bind with high affinity to Trk A, Trk C, and Trk B respectively. NT4/5 is not expressed in the brain, while BDNF is expressed in the central nervous system and muscles (Klein *et al.*, 1991; Segal *et al.*, 1994; Lewin and Barde, 1996). Neurotrophins can also bind to p75, a low affinity NGF receptor that facilitates the neuronal cell death (Frade *et al.*, 1996). Our study showed that the neurotrophic factor BDNF facilitated neuronal survival and differentiation by the expression and phosphorylation of the receptor TrkB during neuronal stem cell differentation. We generated recombinant BDNF-adenovirus and injected it into the AD animal model, which was generated by injecting ibotenic acid into the entorhinal cortex. The results showed that it improved

neuronal survival in pyramidal cells in the CA1 region and dentate zyrus in addition to entorhinal cortex. Our findings suggest that the promotion of the long tem-survival and differentiation of the transplanted neural precursors by PDGF, and recombinant BDNF adenovirus that is injected into the AD animal model, facilitate the efficient replacement of the degenerating cells. It also ultimately leads to regeneration in the aging adult brain and probably in the injury sites of the adult brain.

Conclusion

In previous studies, implanted cells settled preferentially in dentate zyrus, the region known to undergo neurogenesis in adult brains (Brustle *et al.*, 1995), but HiB5 cells were integrated into the CA1 and CA3 pyramidal regions of the hippocampus, and appeared to acquire a morphological phenotype that is appropriate for pyramidal cells. These results indicate that HiB5 progenitor cells that are differentiated by PDGF can migrate long distances by responding to extracellular guidance cues that are present in the hippocampus, and are distributed similarly to endogenous neurons, even in the adult brain.

The studies presented here shows that PDGF BB not only directs neuronal differentiation, but also supports long-term survival of the differentiating neural stem cell and prevents apoptosis. HiB5 neural precursors and other clonal neural precursors previously were shown to undergo regional incorporation. However, when transplanted into the adult cortex or in regions of neuronal degeneration, only a few cells undergo neuronal differentiation. HiB5 cells (a relatively earlier stage precursor cell line) show highly immature characteristics in vitro. They express TrkC, but not TrkB (receptors for NT-3 and BDNF relatively), which are the later stage developmental signals and highly up-regulated in the brain injury sites. We demonstrated that PDGF primed HiB5 cells that express TrkB (the receptor for the later stage developmental signal), thus probably giving competence to survive and differentiate to the appropriate neuronal phenotype in the final location.

Acknowledgments The Authors wish to acknowledge financial support from the Biomedical Brain Research Center and KOSEF (1999-1-213-0025).

References

Alderson, R. F., Alterman, A. L., Barde, Y. A. and Lindsay, R. M. (1990) Brain-derived Neurotrophic Factor Increases Survival and Differentiated Functions of Rat Septal Cholinergic Neurons In Culture. *Neuron* **5**, 297-306.

Altman, J. and Bayer, S. A. (1990) Prolonged sojourn of developing pyramidal cells in the intermediate zone of the hippocampus and their settling in the stratum pyramidale. *J. Comp. Neurol.* **301**, 343-364.

Altman J. and Bayer. S. A. (1990) Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J. Comp. Neurol.* **301**, 365-381.

Altman J. and Das G. D. (1965) Post-natal origin of microneurones in the rat brain. *Nature* **28**, 953-956.

Benihoud, K., Yeh, P. and Perricaudet, M. (1999) Adenovirus vectors for gene delivery. *Curr. Opin. Biotech.* **10**, 440-447.

Bonni, A., Brunet, A., West A. E., Datta, S. R., Takasu, M. A. and Greenberg, M. E. (1999) Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* **286**, 1358-1362.

Brustle, O., Maskos, U. and McKay, R. D. (1995) Host-guided migration allows targeted introduction of neurons into the embryonic brain. *Neuron* **15**, 1275-1285

Cheng, B. and Mattson, M. P. (1994) NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. *Brain Res.* **640**, 56-67.

Choi, D. W. (2001) Excitotoxicity, apoptosis, and ischemic stroke. *J. Biochem. Mol. Biol.* **34**, 8-14.

Chung, J., Cho, S., Kwon, Y. K., Kim, D. H. and Kim K. (2000) Activation of retinoic acid receptor g induces proliferation of immortalized hippocampal progenitor cells. *Mol. Brain Res*. **83**, 52-62.

Collazo, D., Takahashi, H. and Mckay, R. D. G. (1992) Cellular Targets and Trophic Functions of neurotrophin-3 In the Developing Rat Hippocampus. *Cell* **9**, 643-656.

Creuzet, C., Loeb, J. and Barbin, G. (1995) Fibroblast growth factors stimulate protein tyrosine phosphorylation and mitogen-activated protein kinase activity in primary cultures of hippocampal neurons. *J. Neurochem.* **64**, 1541-1547.

Ernfors, P., Ibanez, C. F., Ebendal, T., Olson, L. and Persson, H. (1990) Molecular Cloning and Neurotrophic Activities of a Protein with Structural Similarities to Nerve Growth Factor: Developmental and Topographical Expression in the Brain. *Proc. Natl. Acad. Sci. USA* **87**, 5454-5458.

Frade, J. M., Rodriguez-Tebar, A. and Barde, Y-A. (1996) Induction of cell death by endogenous nerve growth factor through its p75 receptor, *Nature* **383**, 166-168.

Gall, C. M. and Isackson, P. J. (1989) Limbic seizures increase neuronal production of messenger RNA for nerve growth factor. *Science* **245**, 758-761.

Gao, H. and Hollyfield, Joe G. (1995) Basic Fibroblast Growth Factor in Retinal Development: Differential Levels of bFGF Expression and Content in Normal and Retinal Degeneration (*rd*) Mutant Mice. *Dev. Biol.* **169**, 168-184.

Giger, R., Ziegler, U., Hermens, W., Kunz, B., Kunz, S. and Sonderegger, P. (1997) Adenovirus-mediated gene transfer in neurons: construction and characterization of a vector for heterologous expression of the axonal cell adhesion molecule axonin-1. *J. Neurosci. Methods* **71**, 99-111.

Hofer, M., Pagliusi, S. R., Hohn, A., Leibrock, J. and Barde, Y. A. (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. *EMBO J.* **9**, 2459-2464.

Hutchins J. B. and Jefferson V. E. (1992) Developmental distribution of platelet-derived growth factor in the mouse central nervous system. *Dev. Brain. Res.* **67**, 121-135.

Hyman, C., Hofer, M., Barde, Y. A., Juhasz, M., Yancopoulos, G. D., Squinto, S. P. and Lindsay, R. M. (1991) BDNF is a

neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **350**, 230-232.

Johe, K. K., Hazel, T. G., Muller, T., Dugich-Djordjevic, M. M., and McKay, R. D. (1996) Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. *Genes & Dev.* **15**, 3129-3140.

Johansson, C. B., Momma, S., Clarke, D. L., Risling, M., Lendahl, U. and Frisen, J. (1999) Identification of a neural stem cell in the adult mammalian central nervous system. *Cell* **96**, 25-34.

Joung, I., Kim, H. S., Hong, J. S., Kwon, H. and Kwon, Y. K. (2000) Effective gene transfer into regenerating sciatic nerves by adenoviral vectors: potentials for gene therapy of peripheral nerve injury. *Mol. Cells.* 10, 540-545.

Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F. and Barbacid, M. (1991) The TrkB Tyrosine Protein Kinase Is a Receptor For Brain-derived Neurotrophic Factor and neurotrophin-3. *Cell* **66**, 395-403.

Kwon, H., Kim, K. S., Park, S. Y., Lee, D. K. and Yang, C. H. (2001) Inhibitory effect of paeoniflorin on Fos- Jun- DNA complex foemation and stimulation of apoptosis in HL-60 cells. *J. Biochem. Mol. Biol.* **34**, 28-32.

Kwon, Y. Kim (1997) Expression of brain-derived neutrophic factor mRNA stimulated by basic fibroblast growth factor and platelet-derived growth factor in rat hippocampal cell line. *Mol. Cells* **7**, 320-325.

Kwon, Y. Kim, Bhattacharyya, W. V., Cheon, K., Stiles, C. D. and Pomeroy, S. L. (1997) Activation of ErbB2 during wallerian degeneration of sciatic nerve. *J. Neurosci.* **17**, 8293-8299.

Lendahl, U. and McKay, R. D. G. (1990) The use of cell lines in neurobiology. *Trends Neurosci.* **13**, 132-137.

Lewin, G. R. and Barde, Y-A. (1996) Physiology of the neurotrophins. *Annu. Rev. Neurosci.* **19**, 289-317.

Lindvall, O., Ernfors, P., Bengzon, J., Kokaia, Z., Smith, M. L., Siesjo, B. K. and Persson, H. (1992) Differential regulation of mRNAs for nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 in the adult rat brain following cerebral ischemia and hypoglycemic coma. *Proc. Natl. Acad. Sci. USA* **89**, 648-652.

Marsh, H. N., Scholz, W. K., Lamballe, F., Klein, R., Nanduri, V., Barbacid, M. and Palfrey, H. C. (1993) Signal transduction events mediated by the BDNF receptor gp 145trkB in primary hippocampal pyramidal cell culture. *J. Neurosci.* **13**, 4281-4292.

Mattson, M. P., Murrain, M., Guthrie, P. B. and Kater, S. B. (1989) Fibroblast growth factor and glutamate: opposing roles in the generation and degeneration of hippocampal neuroarchitecture. *J. Neurosci.* **9**, 3728-3740.

McDonald, J. W., Liu, X. Z., Qu, Y., Liu, S., Mickey, S. K., Turetsky, D., Gottlieb, D. I. and Choi, D. W. (1999) Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nature Med.* **5**, 1410-1412.

Olanow, C. W., Kordower, J. H. and Freeman, T. B. (1996) Fetal nigral transplantation as a therapy for Parkinson's disease. *Trends Neurosci.* **19**, 102-109.

Park, C. M. and Hollenerg, M. J. (1989) Basic fibroblast growth factor induces retinal regeneration *in vivo*. *Dev. Biol.* **134**, 201-205.

Park, C., Kang, M., Kwon, Y. K., Chung, J., Ahn, H. and Huh, Y. (2001) Inhibition of neuronal nitric oxide synthase enhances cell proliferation in the dentate gyrus of the adrenalectomized rat. *Neurosci. Lett.* **309**, 9-12.

Renfranz, P., Cunningham, M. and Mckay, R. D. (1991) Region-specific differentiation of hippocampal stem cell line HiB5 upon implantation into the developing mammalian brain. *Cell* **66**, 713-729.

Reynolds, B. A. and Weiss, S. (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255**, 1707-1710.

Ross, R. and Collins, T. (1991) PDGF B-chain in neurons of the central nervous system, posterior pituitary, and in a transgenic model. *Cell* **64**, 217-227

Ryder, E. F., Snyder, E. Y. and Cepko, C. L. (1990) Establishment and characterization of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. *J. Neurobiol.* **21**, 356-375.

Sasahara, M., Fries, J. W., Raines, E. W., Gown, A. M., Westrum, L. E., Frosch, M. P., Bonthron, D. T., Sawai, H., Clarke, David B., Kittlerova, P., Bray, Garth M. and Aguayo, Albert J. (1996) Brain-Derived Neurotrophic Factor and Neurotrophin-4/5 Stimulate Growth of Axonal Branches from Regenerating Retinal Ganglion Cells. *J. Neurosci.* **16**, 3887-3894.

Segal, R. A., Goumnerova, L. C., Kwon, Yunhee Kim, Stiles, C. D. and Pomeroy, S. L. (1994) Expression of the neurotrophin receptor TrkC is linked to a favorable outcome in medulloblastoma,. *Proc. Natl. Acad. Sci. USA* **91**, 12867-12871.

Segal, R. A. and Greenberg, M. E. (1996) Intracellular signaling pathways activated by neurotrophic factors. *Annu. Rev. Neurosci.* **19**, 491-515.

Shim, J. S., Lee, H. J., Park, S. S., Cha, B. G. and Chang, H. R. (2001) Curumin-induced apoptosis of A-431 cells involves caspase-3 activation. *J. Biochem. Mol. Biol.* **34**, 189-193.

Slack, R. and Miller, F. (1996) Viral vectors for modulating gene expression in neurons. *Curr. Opin. Neurobiol.* **6**, 576-583.

Smits, A., Kato, M., Westermark, B., Nister, M., Heldin, C.-H. and Funa, K. (1991) Neurotrophic activity of platelet-derived growth factor (PDGF): Rat neuronal cells possess functional PDGF beta-type receptors and respond to PDGF. *Proc. Natl. Acad. Sci. USA* **88**, 8159-8163.

Sung, J. Y., Lee, S. Y., Min, D. S., Eom, T. Y., Ahn, Y. S., Choi, M., Kwon, Y. K. and Chung, K. C. (2001) Differential activation of phospholipases by mitogenic EGF and neurogenic PDGF in immortalized hippocampal stem cells lines. *J. Neurochem*. **78**, 1044-1053.

Timmusk, T., Lendahl, U., Funakoshi, H., Arenas, E., Persson, H. and Metsis, M. (1995) Identification of brain-derived neurotrophic factor promoter regions mediating tissue-specific, axotomy-, and neuronal activity-induced expression in transgenic mice. *J. Cell Biol.* **128**, 185-199.

Vicario-Abejon, C., Johe, Karl K., Hazel, Thomas G., Collazo, D. and McKay, D. G. (1995) Functions of basic fibroblast growth factor and neurotrophins in the differentiation of hippocampal neurons. *Neuron* **15**, 105-114.

Williams, B. P., Park, J. K., Alberta, J. A., Muhlebach, S.G., Hwang, G. Y., Roberts, T. M. and Stiles, C. D. (1997) A PDGF-regulated immediate early gene response initiates neuronal differentiation in ventricular zone progenitor cells.

Neuron **18**, 553-562.

Yao, R. and Cooper, G. M. (1995) Regulation of the Ras signaling pathway by GTPase-activating protein in PC12 cells. *Oncogene*. **11**, 1607-1614.

Yeh, H-J., Silo-Santiago, I., Wang, Y-X., George, R. J., Snider, W. D. and Deuel, T. F. (1993) Developmental expression of the platelet-derived growth factor alpha-receptor gene in mammalian central nervous system. *Proc. Natl. Acad. Sci. USA* **90**, 1952-1956.

Yuan, Junying and Yankner, Bruce A. (2000) Apoptosis in the nervous system. *Nature* **407**, 802-809.

Zafra, F., Hengerer, B., Leibrock, J., Toenen, T. and Lindholm, L. (1990) Activity dependent regulation of BDNF and NGF mRNAs in the rat hippocampus is mediated by non-NMDA glutamate receptors. *EMBO J.* **9**, 3545-3550.