Dorsal root ganglion progenitors differentiate to gamma-aminobutyric acid- and choline acetyltransferase-positive neurons

Lingli Yu1,2, Yindi Ding1,2, Ambre Spencer1,2, Ji Ma1, Ruisheng Lu1, Brian B. Rudkin1,2, Chonggang Yuan1

1Laboratory of Molecular and Cellular Neurophysiology, School of Life Science, East China Normal University, Shanghai 200062, China
2Differentiation & Cell Cycle Group, Laboratory of Molecular and Cellular Science, UMR 5239 CNRS/ENS Lyon/University of Lyon 1, Ecole Normale Supérieure de Lyon, IFR128 “Biosciences Lyon-Gerland” Lyon, 69007, France

Abstract
This study examined the isolation and differentiation of dorsal root ganglion progenitor cells for therapeutic use in neurodegenerative diseases. Rat embryonic dorsal root ganglia progenitors were isolated and purified using the differential adhesion method combined with cytosine arabinoside treatment. After culture in serum-free medium supplemented with B27, basic fibroblast growth factor and epidermal growth factor, these cells remained viable and survived for more than 18 months in vitro. Most cells differentiated to neurons that were immunoreactive for gamma-aminobutyric acid and choline acetyltransferase as detected by immunohistochemical staining. In addition, nerve growth factor and neurotrophic tyrosine kinase receptor expression were also observed in dorsal root ganglion progenitors and differentiated cells. K252a, an inhibitor that blocks nerve growth factor-induced signaling, inhibited cell survival, suggesting the possible existence of a nerve growth factor autocrine loop in these proliferating cells.

Key Words: dorsal root ganglion; neural progenitor; differentiation characterization; nerve growth factor; tyrosine kinase receptor type 1

INTRODUCTION

Neural stem cells or progenitors exist in the nervous system610 and various non-neural peripheral tissues such as gut, skin, connective tissue and even heart of embryonic or adult mammals. Studies to isolate and characterize such progenitors have attempted to purify particular phenotypes with which to generate pluripotent cell lines, with the objective of increasing the efficacy of therapy with grafts of neuronal precursors or mature neurons into injured neurological systems to replace degenerated neurons such as acetylcholine neurons in Alzheimer’s disease and dopaminergic neurons in Parkinson’s disease6. Many sensory organ diseases resulting from aging or wounding are usually accompanied by sensory nerve fiber loss or sensory neuron degeneration. Thus, it is important to isolate sensory neural progenitors or stem cells for cell replacement therapy of sensory organ diseases5. An early study demonstrated that allotransplant and xenotransplant of fetal dorsal root ganglion (DRG) neurons could survive and reinnervate the denervated host peripheral targets in a variety of locations in a gangliectomized adult rat8. In addition, xenografts of human fetal DRGs could extend axons into the central nervous system to form functional connections in the deafferented rat spinal cord8-9. Although neural stem/progenitor cells have been isolated from postnatal and adult DRG10-12, adult otic placode-derived spiral ganglion13 and inner ear14, the differentiation status and neurotransmitter phenotype have not been characterized. In addition, embryonic stem cell-derived neuronal precursors can survive and differentiate to glia and neurons after central nervous system transplantation15-16. In the present study, DRG progenitors were isolated and purified from embryonic day 17 rats to investigate their proliferation and differentiation capacities. We hope that our study will be helpful for understanding neurogenesis in the peripheral nervous system and for harnessing the potential application of peripheral neural stem cells for the treatment of sensory organ diseases.
RESULTS

Purification of neuronal progenitors from embryonic day 17 DRG
To obtain a high yield of neurons from DRG neuronal progenitors, cells from embryonic day 17 rat DRGs were purified using the differential adhesion method combined with 0.5 μM cytosine arabinoside treatment. Cultures comprising approximately 90% neurons were obtained after purification (Figure 1).

Progenitors derived from embryonic day 17 DRG can be expanded and passaged long term (Figures 2 and 3)

To assess the ability of progenitor cells to proliferate, primary spheres were dissociated to a single-cell suspension, and new or secondary spheres were obtained between 1 to 2 weeks (Figure 2). Bromodeoxyuridine (BrdU) incorporation and subsequent immunostaining for BrdU and nestin revealed that neuronal progenitors existed in the embryonic DRG neurons (Figure 2). Cells maintained in culture for periods of more than 1 year retained their dependency on epidermal growth factor and basic fibroblast growth factor. In the early stage of culture (1–5 passages), there was a moderate increase in cell numbers. After passage 5, the growth rate increased, followed by stabilization at passage 15. The growth curve was measured at passage 10, and demonstrated that a logarithmic growth phase occurred 4 to 6 days after sub-culturing, and that the saturated phase occurred on day 6, after which cells aged and died (Figure 3).

Identification of DRG progenitors after differentiation
Co-localization of microtubule-associated protein 2 (MAP2) and cyclic nucleotide phosphodiesterase (CNP)
To investigate the subtype of cells derived from the DRG progenitors, cells were stained for MAP2, glial fibrillary acidic protein and CNP. We observed that most cells were immunoreactive for MAP2, with a high proportion being CNP positive cells, whereas no glial fibrillary acidic protein positive cells were observed. CNP is highly expressed in myelin-forming glial cells and is widely used as an immunohistological marker for oligodendrocytes. However, in the present study, CNP was present in patches throughout the soma and dendrites of cells, and significantly co-localized with MAP2. This expression pattern suggested an important role for CNP in the neuronal progenitors (Figure 4). Western blot analysis confirmed the immunocytochemical findings (Figure 5).

DRG progenitors differentiate to cells co-expressing choline acetyltransferase (ChAT) and gamma-aminobutyric acid (GABA)
To assess the progenitor cell neuronal subtypes, we screened for the presence of neurotransmitters using...
antibodies specific for tyrosine hydroxylase (TH), GABA, ChAT and 5-hydroxytryptamine/serotonin (5-HT). We observed both ChAT and GABA positive reactivity in the majority of the cells examined. However, 5-HT and TH were undetectable (Figure 4). Immuno-labeling analysis implied the co-localization of GABA and ChAT \[4\].

Tyrosine kinase (TrkA) and nerve growth factor (NGF) expression in DRG progenitors and differentiated neural cells

NGF is essential for the survival, differentiation, and maintenance of many sensory neurons. NGF induces biological effects through binding with receptor tyrosine kinase, TrkA, or the death-like domain containing receptor, p75\textsuperscript{NTR}, or both depending on their presence on a given cell and their surface localization. NGF activates TrkA and/or p75\textsuperscript{NTR}, triggering signal transduction cascades. Most DRG neurons require NGF for survival in early development \[18\]. We maintained and analyzed DRG progenitors in vitro for more than 1 year without adding exogenous NGF to the culture medium. As DRG cells can express NGF following injury \textit{in vivo} \[14\], NGF and TrkA may also be involved in the proliferation and differentiation of the progenitor cells. The expression of TrkA and NGF was evaluated in progenitors, and differentiated cells by immunofluorescence. We observed that the majority of the cells were stained positive for TrkA and NGF (Figure 5). Western blot analysis confirmed the expression of TrkA protein, but not NGF protein in those cells (Figure 6), possibly indicating that NGF was either expressed at a very low level or was being rapidly degraded.
As abundant TrkA and NGF were detected in differentiated DRG neurons by immunofluorescence, we investigated whether endogenous NGF produced by the cells was required for their maintenance. K252a, an inhibitor which blocks NGF-induced signaling in PC12 cells [19-21] was added to the culture medium. Survival rates of the cells were diminished with increasing concentrations of K252a (Figure 7). This result implied that NGF might have a key role in supporting the survival and function of DRG progenitors.

**DISCUSSION**

Previously, several in vitro systems have been described for the derivation of neural stem/progenitor cells from the central nervous system or peripheral nervous system [22]. The entire peripheral nervous system is derived from a migratory cell population termed neural crest cells. These cells generate a wide variety of cell and tissue types during embryonic and adult development including cartilage and bone, connective tissue, pigment and endocrine cells as well as neurons and glia amongst many others. Due to these specific properties they have been studied for their potential application in cell-based tissue and disease-specific repair [23]. DRGs are derived from precursors in the neural crest, suggesting that early postnatal DRGs may contain a population of neuronal progenitors that retain their capacity for neurogenesis. In the present study, we report the purification of a DRG neuronal stem/progenitor cell, and the further characterization of proliferation and differentiation of these cells.

**Progenitors derived from embryonic DRGs can be expanded long term**

DRG cells from embryonic day 17 rats were purified using the differential adhesion method followed by treatment with cytosine arabinoside that causes the selective removal of glial cells. After purification, cells were cultured in serum-free medium DMEM/F12 (1:1) supplemented with B27, basic fibroblast growth factor and epidermal growth factor. Cells proliferated slowly in the first 2 to 3 weeks. After this time point, neurospheres were observed and new spheres were generated after each passage. These cells were routinely passaged once every 1 to 2 weeks depending on the density.
seeded. The growth curve of the 15th passage cells demonstrated that progenitors from embryonic DRGs could proliferate efficiently. Cells were maintained in culture for more than 1 year and retained their potential for proliferation and differentiation as specialized subtypes. Such long-term proliferation was unexpected and to our knowledge has not been reported previously. The incorporation of BrdU, together with positive nestin immunofluorescence, suggested that the purified cells from embryonic DRG were proliferating[24]. Thus, we termed these cells DRG progenitors. DRG progenitors exhibit characteristics similar to neural precursors

To investigate the differentiation characteristics of embryonic DRG progenitors, cells were incubated in culture medium with serum and without exogenous basic fibroblast growth factor and epidermal growth factor. Most DRG progenitors expressed MAP2, 40% of which were positive for both MAP2 and CNP. CNP is also present in various cell types in addition to myelinating cells, such as lymphocytes, retinal, liver, muscle, and Purkinje cells and hippocampal neurons[25-27], indicating that CNP is also expressed in some subpopulations of neuronal cells. CNP is a regulator of tubulin polymerization, where it associates with the cytoskeleton and has microtubule-associated protein-like characteristics[28]. Taken together and combined with our findings, these results suggest that CNP may be important in the modulation of the cytoskeleton in the differentiating DRG progenitors.

In addition to glial cells, mature DRGs are composed of many neurons with different morphologies and distinct biochemical properties. How distinct cell fates are generated from an initially homogeneous cell population in the embryonic DRG is a compelling question in developmental biology. Moreover, once DRG precursors aggregate to their final positions, there are still a number of “fate choices” that can occur[29]. The sensory neurons present in mature DRG receive sensory information including pain, temperature, touch and proprioception. Cells in DRG produce multiple neurotransmitters, such as GABA, acetylcholine, and glutamate catecholamine. Tyrosine hydroxylase is expressed in a subpopulation of small DRG neurons in the adult mouse[30]. Since therapeutic applications may require considerable in vitro expansion of neural precursors, we next investigated whether the expanded, multi-passage DRG progenitors retained key neural precursor properties. The purified DRG cells formed a subpopulation of neuronal progenitors rather than glial precursors. These cells could proliferate, but also expressed GABA and ChAT simultaneously while undergoing differentiation. Cholinergic neurons have been reported to be immunoreactive for either GABA or its synthesizing enzyme[31]. Furthermore, in the rat cerebral cortex, 88% co-localization of ChAT with GABA was observed in interneurons[32]. Thus, we assumed that the subpopulations of DRG progenitors mainly differentiated to neurons coexpressing acetylcholine and GABA. However, no TH and 5-HT containing cells were detected.

**DRG cells express NGF and its receptor TrkA**

*In vivo*, NGF signaling is required for both survival and the differentiation of DRG neural phenotypes[33]. Embryonic DRG neurons initially require NGF for survival *in vitro*. Withdrawal of NGF from DRG neurons isolated at embryonic day 15 within the first 10 days of culture, resulted in apoptotic death. However, by 21 days of culture, the majority of these neurons survived for long periods without exogenous NGF support[34-35]. Recent studies reported that NGF mRNA is expressed in a variety of cell types in the injured spinal cord. NGF mRNA is also up-regulated in DRG neurons after spinal cord injury and the percentage of sensory neurons expressing NGF mRNA correlates with proximity to the lesion epicenter. This suggests that NGF expression in DRG may be up-regulated by damage to the central processes of sensory neurons[36]. In addition, it is likely that DRG neurons will express NGF when stimulated or under special conditions. In this study, the progenitors from embryonic day 17 DRG that were either proliferating or had differentiated were independent of exogenous NGF *in vitro*. We observed NGF and TrkA expression in both proliferating and differentiated cells. However, p75NTR positive cells were not detected (data not shown). In addition, incubation with K252a attenuated cell survival and implied that DRG progenitors expressed NGF for their survival and function. To conclude, we isolated a subpopulation of DRG neural progenitors, which could be induced to differentiate to neurons co-expressing ChAT and GABA. These cells also expressed TrkA and NGF. These novel DRG progenitor cells represent a useful tool for studying the mechanisms of cell proliferation and differentiation, and have potential application as peripheral neural stem cells for the treatment of sensory organ diseases.

**MATERIALS AND METHODS**

**Design**
A parallel controlled *in vitro* experiment.

**Time and setting**
The experiments were performed at the Laboratory of Molecular and Cellular Neurophysiology, East China Normal University from September 2007 to April 2011.

**Materials**
Healthy adult Sprague-Dawley rats, weighing 150–200 g, were provided by the Experimental Animal Research Center of Shanghai, China. All rats were maintained in air-conditioned quarters under regulated light and dark periods. For mating, two to three females and two vigorous males were caged together. The vaginal canal was examined in the morning for the presence of sperm or vaginal plug on subsequent days. The day sperm or vaginal plug was found in the vagina was considered as...
embryonic day 0.5. At embryonic day 17.5, the mother was sacrificed, and the embryos were removed for dissection. All experimental procedures were carried out in accordance with the guidelines of the National Institutes of Health on animal care.

Methods

Cell culture and growth curve analysis

DRG neurons were isolated and purified using the differential adhesion method combined with treatment with 0.5 µM cytosine arabinoside. Briefly, DRG from all spinal levels were removed, and incubated in pre-warmed Hank’s buffered salt solution containing 0.25% trypsin (Invitrogen, Grand Island, NY, USA) for 20 minutes at 37°C. After enzymatic treatment, a single cell suspension was prepared by passing cells through a fire polished Pasteur pipette approximately 15 times, and then cells were plated in non-coated 6-well plates for 50 minutes to remove non-neuronal cells. The unattached cells were collected and cultured in serum-free Dulbecco’s modified eagle’s medium/F12, supplemented with 2% B27 (Invitrogen), and 0.5 µM cytosine arabinoside (Sigma, St. Louis, MO, USA) was added after 12 hours. Cells were then cultured in proliferation medium Dulbecco’s modified Eagle’s medium/F12 supplemented with 2% B27, 20 ng/mL epidermal growth factor, 10 ng/mL basic fibroblast growth factor-2 (all from Gibco, Grand Island, NY, USA). Half of the medium volume was replaced every 3 days. The primary spheres were dissociated into a single-cell suspension, and new or secondary spheres could be obtained between 1 to 2 weeks. Expanded cultures were passaged routinely when new spheres formed and near confluence (70–80% confluence). To evaluate the proliferation capacity, 10 000 cells were seeded per well in triplicate in 24-well plates. The number of the cells was counted every 24 hours for 8 days. To induce cell differentiation, spheres were mechanically dissociated into single cell suspensions before being placed on poly-L-lysine and laminin coated coverslips in 24-well plates, and differentiation medium supplemented with 2% fetal calf serum was added and refreshed every 3 days. Immunohistochemistry for MAP2, ChAT, 5-HT, GABA, glial fibrillary acidic protein, CNP, TH, NGF, TrkA, and BrdU

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, and then incubated with antibodies specific for MAP2 (Shanghai Branch of Sigma, Shanghai, China), ChAT (Shanghai Branch of Chemicon, Shanghai, China), 5-HT (Shanghai Branch of Chemicon), GABA (Shanghai Branch of Chemicon), glial fibrillary acidic protein (Shanghai Branch of Sigma), CNP (Shanghai Branch of Sigma), TH (Shanghai Branch of Sigma), NGF (Shanghai Branch of Santa Cruz Biotechnology, Shanghai, China), TrkA (Shanghai Branch of Chemicon) and BrdU (Shanghai Branch of Sigma) overnight at 4°C, followed by incubation with an appropriate fluorescein isothiocyanate-conjugated secondary antibody (Shanghai Branch of Chemicon) for 1 hour at room temperature. Cell nuclei were stained with 4’,6-diamidino-2-phenylinodole (Shanghai Branch of Chemicon). The staining for nestin (Shanghai Brance of Millipore, Shanghai, China) was performed using the streptavidin-biotin-alkaline phosphatase (ABC) method using the ABC kit (Shanghai Branch of Vectastain, Shanghai, China) according to the manufacturer’s protocol. Stained samples were visualized via fluorescent microscopy (Leica DMi4000B). Images were analyzed using Leica Imager, and brightness and contrast were adjusted using ImageJ software (version 1.38x, China).

Western blot method for MAP2, ChAT, GABA, CNP, and TrkA expression

Briefly, protein samples extracted from DRG cells were separated by 7.5–12% sodium dodecyl sulfate polyacrylamide gel electrophoresis, electrotransferred onto nitrocellulose membranes (Millipore, China), membranes incubated with 5% bovine serum albumin in Tris-buffer saline at room temperature for 1 hour to inhibit non-specific binding, and then incubated with antibodies specific for MAP2 (Sigma, China), ChAT (Chemicon, China), GABA (Chemicon, China), CNP (Sigma, China), TrkA (Chemicon, China), β-tubulin (Santa Cruz Biotechnology) overnight at 4°C. The primary antibodies were probed with IR-Dye-880 or IR-Dye -680-conjugated secondary antibodies (LI-COR Biosciences, USA). Specific protein bands were detected and measured by Odyssey Infrared Imaging System (LI-COR Biosciences).

Cell counting kit-8 assay

DRG cell viability was detected using a cell counting kit-8 assay (Dojido Laboratories, Japan) according to the manufacturer’s protocol. Cells were seeded at 5 000 cells/well in 96-well plates. K252a at various concentrations (0, 10, 50, 100, 500, 1 000 nM; Shanghai Branch of Sigma) was added to the culture for 24 hours before cell counting kit-8 detection.

Statistical analysis

Results were expressed as mean ± SEM. Analyses were performed using SigmaPlot version 10.0 (Systat Software, Chicago, IL, USA). Fisher’s least significant difference was used to analyze the statistical significance of the differences. Results were considered statistically significant when P < 0.05.

Funding: This work was supported by the Rhone Alps Region of France, MIRA grants for the collaboration, the Agence National de la Recherche (ANR) RIB and the CNRS (International program for Scientific Cooperation, “PICS”).

Author contributions: The work presented here was carried out in collaboration between all authors. Lingli Yu and Yindi Ding defined the research theme, designed methods and experiments, performed the laboratory experiments, analyzed the data, interpreted the results and wrote the paper. All authors have contributed to and approved the manuscript. Conflicts of interest: None declared.
REFERENCES

[1] McKay R. Stem cells in the central nervous system. Science.1997; 276:66-71.
[2] Kruger GM, Mosher JT, Bixby S, et al. Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. Neuron. 2002;35:657-669.
[3] Fernando JK, McKenzie IA, Mill P, et al. A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol. 2004; 6:1082-1093.
[4] Young HE, Duplaa C, Romero-Ramos M, et al. Adult reserve stem cells and their potential for tissue engineering. Cell Biochem Biophys. 2004;40:1-80.
[5] Tomita Y, Matsumura K, Wakamatsu Y, et al. Cardiac neural crest cells contribute to the dormant multipotent stem cell in the mammalian heart. J Cell Biol. 2005;170:1135-1146.
[6] Zhu Q, Ma J, Yu L, et al. Grafted neural stem cells migrate to substantia nigra and improve behavior in Parkinsonian rats. Neurosci Lett. 2009;462:213-218.
[7] Gobel S, Gubernator M, Minger SL. Progress and prospects: stem cells and neurological diseases. Gene Ther. 2011;18:1-6.
[8] Kozlova EN, Rosario CM, Stromberg I, et al. Peripherally grafted human foetal dorsal root ganglion cells extend axons into the spinal cord of adult host rats by circumventing dorsal root entry zone astrocytes. Neuroreport. 1995;6:269-272.
[9] Levinson A, Holmberg H, Schouenborg J, et al. Functional connections are established in the deafferented rat spinal cord by peripherally transplanted human embryonic sensory neurons. Eur J Neurosci. 2000;12:3589-3595.
[10] Namaka MP, Sawchuk M, MacDonald SC, et al. Neurogenesis in postnatal mouse dorsal root ganglia. Exp Neurol. 2001;172:60-69.
[11] Li HY, Say EH, Zou XF. Isolation and characterization of neural crest progenitors from adult dorsal root ganglia. Stem Cells. 2007; 25:2053-2065.
[12] Singh RP, Cheng YH, Nelson P, et al. Retentive multipotency of adult dorsal root ganglia stem cells. Cell Transplant. 2009;18: 55-68.
[13] Hughes SE, Silverman MS. Explorations of otxc transplantation. Exp Neurol. 1992;115:37-43.
[14] Lou X, Zhang Y, Yuan C. Multipotent stem cells from the young rat inner ear. Neurosci Lett. 2007;416:28-33.
[15] Arnhold S, Lenartz D, Kruttwig K, et al. Differentiation of green fluorescent protein-labeled embryonic stem cell-derived neural precursor cells into Thy-1-positive neurons and glia after transplantation into adult rat striatum. J Neurosci. 2000;93: 1026-1032.
[16] Petrova ES. Studies of histogenetic and neurodegenerative processes in the nervous system using heterotopic neurotransplantation. Neurosci Behav Physiol. 2010;40:823-832.
[17] Weissbarth S, Maker HS, Raes I, et al. The activity of 2',3'-cyclic nucleotide 3'-phosphodiesterase in rat tissues. J Neurochem. 1981;37:677-680.
[18] Ruit KG, Elliott JL, Osborne PA, et al. Selective dependence of mammalian dorsal root ganglion neurons on nerve growth factor during embryonic development. Neuron. 1992;8:573-587.
[19] Kaplan DR, Martin-Zanca D, Parada LF. Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. Nature. 1991;350:158-160.
[20] Tapley P, Lamballe F, Barbacid M. K252a is a selective inhibitor of the tyrosine protein kinase activity of the trk family of oncogenes and neurotrophin receptors. Oncogene. 1992;7:371-381.
[21] Buck H, Winter J. K252a modulates the expression of nerve growth factor-dependent capsaicin sensitivity and substance P levels in cultured adult rat dorsal root ganglion neurons. J Neurochem. 1996;67:345-351.
[22] Colombo E, Giannelli SG, Galli R, et al. Embryonic stem-derived versus somatic neural stem cells: a comparative analysis of their developmental potential and molecular phenotype. Stem Cells. 2006;24:825-834.
[23] Achilles A, Trainar PA. Neural crest stem cells: discovery, properties and potential for therapy. Cell Res. in press.
[24] Shi H, Cui H, Alam G, et al. Nestin expression defines both glial and neuronal progenitors in postnatal sympathetic ganglia. J Comp Neurol. 2008;508:867-878.
[25] Vogel US, Thompson RJ. Molecular structure, localization, and possible functions of the myelin-associated enzyme 2',3'-cyclic nucleotide 3'-phosphodiesterase. J Neurochem. 1988;50: 1667-1677.
[26] Dreiling CE, Schilling RJ, Reitz RC. 2',3'-cyclic nucleotide 3'-phosphohydrolase in rat liver mitochondria. Biochim Biophys Acta. 1981;640:114-120.
[27] Cho SJ, Jung JS, Jin I, et al. 2',3'-cyclic nucleotide 3'-phosphodiesterase is expressed in dissociated rat cerebellar cells and included in the postsynaptic density fraction. Mol Cells. 2003;16:128-135.
[28] Bifulco M, Laezza C, Stingo S, et al. 2',3'-Cyclic nucleotide 3'-phosphodiesterase: a membrane-bound, microtubule-associated protein and membrane anchor for tubulin. Proc Natl Acad Sci U S A. 2002;99:1807-1812.
[29] Raible DW, Ungers JM. Specification of sensory neuron cell fate from the neural crest. Adv Exp Med Biol. 2006;589:170-180.
[30] Brumovsky P, Villar MJ, Hokfelt T. Tyrosine hydroxylase is expressed in a subpopulation of small dorsal root ganglion neurons in the adult mouse. Exp Neurol. 2006;200:153-165.
[31] Kosaka T, Tauchi M, Dahl JL. Cholinergic neurons containing GABA-like and/or glutamic acid decarboxylase-like immunoreactivities in various brain regions of the rat. Exp Brain Res. 1988;70:605-617.
[32] Bayraktar T, Staijer JF, Acasy L, et al. Co-localization of vasoactive intestinal polypeptide, gamma-aminobutyric acid and choline acetyltransferase in neocortical interneurons of the adult rat. Brain Res. 1997;757:209-217.
[33] Ernsberger U. Role of neurotrophin signalling in the differentiation of neurons from dorsal root ganglia and sympathetic ganglia. Cell Tissue Res. 2009;336:349-384.
[34] Tong JX, Eichler ME, Rich KM. Intracellular calcium levels influence apoptosis in mature sensory neurons after trophic factor deprivation. Exp Neurol. 1996;138:170-180.
[35] Memberg SP, Hall AK. Proliferation, differentiation, and survival of rat sensory neuron precursors in vitro require specific trophic factors. Mol Cell Neurosci. 1995;6:323-335.
[36] Brown A, Ricci MJ, Weaver LC. NGF mRNA is expressed in the dorsal root ganglia after spinal cord injury in the rat. Exp Neurol. 2007;205:283-286.