Review

DOI: 10.5582/ddt.2016.01058

The role of growth factors in nerve regeneration

Mehmet Emin Önger*, Burcu Delibaş, Aysın Pınar Türkmen, Erkan Erener, Berrin Zuhal Altunkaynak, Süleyman Kaplan

Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey.

Summary **Nerve injuries result in functional loss in the innervated organ or body parts, and recovery is difficult unless surgical treatment has been done. Different surgical treatments have been suggested for nerve repair. Tissue engineering related to growth factors has arisen as an alternative approach for triggering and improving nerve regeneration. Therefore, the aim of this review is to provide a comprehensive analysis related to growth factors as tools for optimizing the regeneration process. Studies and reviews on the use of growth factors for nerve regeneration were compiled over the course of the review. According to literature review, it may be concluded that growth factors from different sources present promising treatment related to nerve regeneration involved in neuronal differentiation, greater myelination and axonal growth and proliferation of specific cells for nerve repair.**

Keywords: Nerve regeneration, growth factors, neurotrophins, glial cell-lined derived neurotrophic factors, neuropoeitic cytokines

1. Introduction

The main logic is that molecules can stimulate and assure neurons to act in new approaches, which lead to recovery of nerve fibers. Trophic factors are molecules, which behave on specific cell receptors to trigger some pathways such as protein synthesis and outgrowth. Nerve growth factor (NGF) is the main molecule of the growth factors family known as "neurotrophins". Neurotrophin is a protein molecule which provides essential functions to neurons including survival, growth, and morphologic plasticity of them (*1*). NGF was discovered in 1951 by Rita Levi-Montalcini and since then many neurotrophic factors have been described which have effects on the outgrowth of nerve fibers. The explanation about the neurotrophic factors especially functions and roles of them in the nervous system has still been increasing including embryonic and postnatal development after injury. There are many studies mention main aims of neurotrophic factor researches involved in developmental roles, plasticity in the central nervous system, and mechanisms of injury and signal transduction (*1-8*). The objective of this review is to give an overview of neurotrophic factors and emphasize importance of what we know about the functional mechanisms by which neurotrophic factors reveal their effects from an injury response window, including axonal growth and regeneration.

2. Growth factors

The neurotrophic growth factors family is a classical growth factors family of peptides. These peptides contribute surviving and differentiating of nerve fibers in both central and peripheral nervous system by having structural and functional relation to each other (*9,10*). NGF was purified and identified as a diffusible factor that enhances the axonal sprouting and neurite outgrowth of neurons both *in vitro* and *in vivo* by Viktor Hamburger and Rita Levi-Montalcini, as a member of the neurotrophin family in the twentieth century (*1,9*). After that, brain-derived neurotrophic factor (BDNF) was purified and cloned from mammalian brain as a second member of neurotrophin family. As a result of investigations in molecular biology, we know that NGF, BDNF, neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) constitute the neurotrophin family in mammals (*11*) (Figure 1).

Released online in J-STAGE as advance publication October 17, 2016.

**Address correspondence to:*
Dr. Mehmet Emin Önger, Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey.
E-mail: mehmetemin.onger@gmail.com

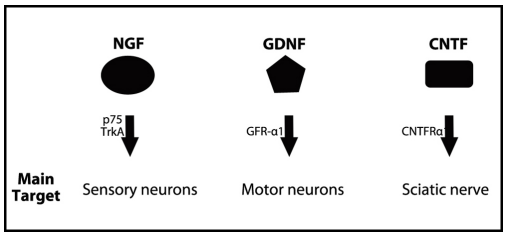

Figure 1. Main neurotrophic factors and their receptors used in nerve repair. Schematic illustration of 3 neurotrophic factor families. NGF: Neurotrophin family, GDNF: GDNF family, CNTF: Neuropoeitic cytokines.

2.1. *Neurotrophin family*

Neurotrophins are biological molecules that are composed of noncovalent homodimers containing cysteine and their cysteine parts play a very important role in the interaction of homodimer molecules with each other (*9,12*). Homodimers are basically composed of two pairs of beta chains. Beta chains are bound to each other with extremely flexible 3 short bonds and these binding sites are the places where amino acid difference, which separates neurotrophins from each other, occurs (*9*). Neurotrophins have a very important place among neurotrophic factors. In general, they show their effect by interacting with two different receptor families. These are p75, member of the tumor necrosis factor alpha family, and tropomyosin receptor kinase (trk), a member of the tyrosine kinase receptor family (*13*). P75 receptors bind to all neurotrophins with similar affinity and members of tyrosine kinase receptor family are more selective in binding. For example, BDNF binds to trkb receptor while NGF binds to trka (*14*). Two different extracellular domain sites have been identified for neurotrophins in these interactions. One of these domains, immunoglobulin (Ig)-like domain, has been reported to have an active role in both maintaining specificity between neurotrophin ligands and in regulating the binding and activation of neurotrophins (*15*).

Neurotrophins are very important among neurotrophic factors in terms of their ability to guide the axons in growth cone during regeneration. The role of neurotrophins in the chemotaxis of the growth cone has recently been revealed. NT-3, NT-4/5, NGF and BDNF have been reported to be capable of inducing chemotaxis in sensory neurons. In addition, responses are inhibited in both motor and sensory neurons when antibodies that functionally block neurotrophins are used (*9,10*).

2.2. *Glial cell-lined derived neurotrophic factor (GDNF) family*

The GDNF, persephin (PSP), neurturin (NTN) and artemin (ART) constitute the GDNF family of neurotrophic factors. GDNF has been defined by the effect of increasing the survival of motor neurons, while NTN has been defined by the effect of increasing the survival of sympathetic neurons. Similarly, PSP and ART's beneficial effects of neuronal survival have been shown *in vitro*. Although there are some differences in their biochemical structure, neurotrophic factors of GNDF family are structurally like neurotrophins (*10,16-18*).

The members of GDNF family show their effects through receptor complexes formed by high affinity ligand binding subunits (GFR-alpha). These subunits provide specificity at the same time. For example, while GDNF interacts with GFR alpha 1, NTN interacts with GFR alpha 2. In general, GFR alpha-receptors bind to cell membrane *via* glycosyl-phosphatidylinositol (GPI) (*12,16*).

2.3. *Neuropoeitic cytokine family*

Neuropoeitic cytokine family generally known as interleukin 6 (IL-6) family and it has functions, which contain different neural responses such as neural survival and differentiation (*19,20*). This cytokine family generally has a long chain alpha helix structure. Unlike both neurotrophins and the GDNF family, neuropoeitic cytokines are not biologically active homodimers, they are secretory proteins in the form of alpha helix (*19,21*).

3. Interactıons between neurotrophıc factors

Due to the general similarity between receptor activation and adaptor proteins related with signal mechanisms, it has been suggested that there may be significant interactions and similarities between biological responses caused by the members of aforementioned three neurotrophic factor families in the related cell. This state is supported by the results of studies conducted with the combinations of GDNF and neuropoeitic cytokines, which argue that they either increase each other's present effects or show a synergistic effect (*9,22*). It has been reported that in the differentiation of embryonic motor neurons, neurotrophic factors of each of the three families improve neurite outgrowth, even if in different degrees, and that different combinations show synergistic effect (*23*).

Neurotrophins, members of the GDNF family and neuropoeitic cytokines have many similar and different characteristics in terms of both receptor systems and related signal transduction pathways and also their biochemical components and biological responses. For example, with a biochemical approach, while neurotrophins and members of the GDNF family are homodimeric and biologically active molecules, neuropoeitic cytokines are long chain α-helix bundle proteins. Although neurotrophins bind to trk and p75, which are two different receptor classes, trk-p75 interaction is also important for neurotrophins in the

formation of high affinity binding sites. In addition, it is also known that in receptor systems, which use GDNF and neuropoeitic cytokines, biological effects such as neural survival, differentiation and neurite formation affect α-subunits and signal transduction and thus may cause more positive effects (*24,25*).

4. Postinjury effects of neurotrophic factors

Possible changes that occur in neurotrophic factors and receptors are very important in terms of regenerative evaluation. Regulation of neurotrophic factors in motor neurons and distal regions especially after damage is important in appreciating their role in regeneration.

4.1. *Changes in motor neurons*

Following axonal damage, BDNF, which is underexpressed in healthy neurons, is induced quickly and BDNF mRNA (messenger ribonucleic acid) significantly increases within 8 hours after axotomy (*26*). The increase in BDNF level returns to normal levels within the 7th day following the damage. The BDNF mRNA increases in neurons after axotomy overlaps BDNF protein expression. This protein level reaches peak within 7 days after damage and stays high until the 14th day when compared with intact neurons (*26,27*). Following peripheral nerve injury, neurons neither increase nor express trkA. Following axotomy, trkB mRNA has been reported to start increasing on the second day, reach the highest level on the seventh day and maintain its increased level until the 21st day. On the contrary, trkC MRNA has been reported to stay relatively unchanged following axotomy while it has been reported to decrease significantly after sciatic nerve damage (*26,28,29*). Although what we know about the regulation of neuropoeitic cytokines after peripheral nerve damage is not as much as what we know about neurotrophins, it is known for example that IL-6 mRNA increases rapidly after axotomy and returns to the basal level 24 hours later. Here, the regulation of neuropoeitic cytokine receptors depends on both the content and localization of the damage (*21*).

4.2. *Changes in distal part*

Cellular and molecular changes in the distal part after damage are first degenerative and they are characterized by phagocytic processes, which are initiated by Schwann cells and maintained by macrophages. In this series of process called Wallerian degeneration, myelin sheath and axonal injuries are suppressed by the aforementioned cells. The transformation of Schwann cells from stabile cell form, which provide myelination into rapid proliferating cell forms, which do not cause myelination, is effective. With this transformation, a great number of growth proteins, neurotrophic factors, cell adhesion molecules and molecules such as basal membrane components also increase. Proliferated Schwann cells form linear bands called Bungner Bands, which guide regenerating axonal sprouts to reach the distal part (*30-32*). Meanwhile, the changes in the temporal expressions of the members of 3 neurotrophic factor families and receptors in the distal part have been reported to be much more dynamic when compared with those in axotomized motor neurons. After damage, the expression of NGF and BNDF, which are from neurotrophin family, increase in the distal part, while the expression of NT-3 and NT-4/5 decreases. Increase in the expression of GNDF from the GNDF family and IL-6 from the neuropoeitic cytokine and decrease in the expression of CNTF (ciliary neurotrophic factor) have also been reported. While NGF mRNA can hardly be detected in intact nerve normally, it has been reported to increase 10 times in the distal part within the first 12 hours following damage and to decrease to 5 times of the normal level on the 72nd hour and stay at this level for about three weeks (*25,33-35*). The BDNF mRNA increase, which occurs in the distal part following the damage, is quite slow when compared with NGF. However, the increase in BDNF mRNA expression reaches a detectable level on the 7th day following the damage and continues to increase until the 28th day. The maximum level of BDNF mRNA is about ten times more than that of NGF mRNA (*25,35,36*). The expression of NT-3, one of the members of neurotrophin family, can be easily detected in healthy nerves with NT-3 mRNA and its expression within 12 hours following the damage has been reported to decrease rapidly and return to basal level within 2 weeks. Similarly, NT-4/5 mRNA expression has also been reported to decrease within 6-12 hours following nerve transection (*29,33*). GDNF, one of the members of GDNF family, has been detected in healthy nerve and it has been reported to peak in distal part on the seventh day following the damage and maintain its high level at least for two weeks. While the expression of IL-6, one of the members of neuropoeitic cytokine family, is not in sufficient levels for detection, the expression of CTNF is in very high levels. However, after damage, the level of CTNF mRNA begins to drop within 24 hours, and goes about 5 times below the levels detected in healthy nerves. Unlike CTNF, the expression of IL-6 mRNA increases 35 times in the distal part after damage and it returns to basal level again 24 hours later (*35,37*).

A great number of experimental studies have shown that neurotrophic factors increase the survival rates of axotomized or injured nerves. Methods, which provide long-term neurotrophic factor release to increase survival, include many different strategies ranging from exogen neurotrophic factor to adenoviral transfer. In the following sections, we will review the basic effects of neurotrophic factors one by one.

4.3. *Basic effects of neurotrophins*

It is well acknowledged that BDNF plays a role as a survival factor for damaged neurons (*38*). This survival increasing effect of exogen BDNF is temporary and dependent on functional trkB receptors (*39,40*). For example, within 4-5 weeks following the damage, 95% of the neurons had died although the treatment continues. The neural survival effect of BDNF after axotomy is dose-dependent (*41,42*). The survival increasing effect of NT-3 and NT-4/5 in damaged neurons is still disputed and controversial. Some studies claim NT-3 and NT-4/5 are as effective as BDNF in increasing neural survival whereas other studies have reported NT-3 and NT-4/5 to increase survival in axotomized neurons, but this effect was less than the effect of BDNF. In addition, it has been reported that NT-3 does not increase survival in damaged neurons when compared with the control group (*9*).

4.4. *Basic effects of GDNF family*

The survival enhancing effect of GDNF in motor neurons following damage has been reported. In adult animals, exogenous GDNF application has been reported to save axotomized motor neurons and in addition to injured nerves. However, similar to BDNF, one dose GNDF has been reported to have a temporary effect (*17,18,34,35*). Thus, different methods have been examined for long-term survival increasing effect. Although there are *in vitro* studies about the motoneuronal survival increasing effect of NTN and PSP, it has not been completely found out whether the other members of the GDNF family prevent cell death induced by axotomy (*43,44*).

4.5. *Basic effects of neuropoeitic cytokines*

The beneficial effects of CTNF, and IL-6 in nerve regeneration have been presented (*20,45,46*). Similar to the members of neurotrophic factor family such as BDNF and GDNF, neural survival effect of CTNF is also transient. In response to nerve damage, the changes in neuronal form have been expressed as the transformation of phenotype from "transmitter" to "regenerative" (*47*). *In vitro* studies show that, apart from the supporting effect of survival for both peripheral and central nervous system neurons, CNTF also initiates axonal sprouting and rescues the neurons from axotomy-induced cell death (*48*).

5. Regenerative events following damage

In addition to the survival increasing effects of three neurotrophic factor families on injured nerves, the similarity of their signal transduction mechanisms shows that they have similar abilities to reverse the effects of axotomy.

It has been reported that neurotrophic factors provide neural survival following damage and that there is a direct association between exogenous neurotrophic factor application and axonal regeneration (*22,49*). In the evaluation of peripheral nerve regeneration, total number of axons in the distal part of nerve and functional tests are used (*50-52*). However, axonal assessment only in the distal part means ignoring the axon outgrowths, which originate from the proximal part. Thus, axon outgrowths, which develop as a response to exogenous neurotrophic factor treatment, are insufficient to estimate direct information about the accurate number of axotomized neurons (*51,53,54*). Briefly, combined quantitative evaluations will give more precise information about regeneration.

5.1. *Neurotrophins*

In axonal regeneration, BDNF can improve axonal sprouting. It has been shown that, following nerve injury, BDNF does not increase functional recovery in tests such as sciatic function index, however, it has triggering effects. Although exogenous BDNF has inefficacies in postinjury functional recovering, endogenous BDNF is known to play an important role in peripheral nerve regeneration (*38,55*). Anti BDNF antibody application after damage has been reported both to diminish axonal elongation and to decrease the axonal density and number (*56*). One of the most important factors of poor recovery in motor functions following peripheral nerve injury is the decrease in the axonal regeneration abilities of motor neurons. During regeneration, a time-dependent decrease is seen in the total number of regenerated axons and reinnervation of muscle fibers (*25,47*). However, it is suggested that low dose and long term BDNF application increases both axonal regeneration and neural cell repair and thus it is very effective in reversing the negative effects caused by chronic axotomy (*38*). Moreover, similarly, low dose BDNF application has been shown to increase the number of reinnervated muscle fibers significantly (*9*). The use of BDNF by axotomized neurons can be suitable for the start of axonal growth following damage; however, temporary expression is not sufficient in the long-term support of axonal regeneration. During the first week after damage, NGF upregulation occurs in distal nerve root. NGF plays an important role of support in increasing Schwann cell organization. This support is realized through Bungner band. In addition, this temporary upregulation of NGF starts the slow developing regeneration after damage. Although the axonal regeneration initiating effect of exogenous BDNF is limited, the same is not true for NT-3. Especially in injury models, which are formed through sciatic nerve resection, NT-3 has been shown to increase both the number of regenerated axons and

myelination significantly (*10,29,38*).

5.2. *GDNF family*

Overexpression of GDNF in muscle fibers may result in hyperinnervation at the neuromuscular junctions. Thus, it is argued that GDNF eases synapse formation during regeneration or acts as "synaptotrophin" for developing neuromuscular connections. Since a similar hyperinnervation does not occur in the overexpression of NT-3 or NT-4/5, this effect of GDNF has been reported to be specific (*9*). Similarly, GDNF application has been shown to increase regenerated axonal outgrowth significantly in spinal cord and peripheral nerve injury (*57,58*). In addition, there are studies, which show that GDNF not only advances the formation of neuromuscular connections, but also induces muscle innervation plasticity and/or remodeling (*59*). It is argued that it plays a facilitative role in triggering axonal regeneration of axotomized motor neurons. The role of other members of the GDNF family in axonal regeneration has not been specified yet.

5.3. *Neuropoietic cytokines*

CNTF and neurotrophins are known to play an important role separately or in combination in axonal outgrowth and functional survival after damage (*25*). Studies have reported that the combinations of CNTF / BDNF treatment has neuroprotective role in the retina (*60*). IL-6 has been shown to be significant in axonal regeneration. Antibody application, which prevents IL-6 from binding with its receptor, has been shown to decrease axonal regeneration significantly (*9*). The role of neurotrophic cytokines in peripheral nerve regeneration has been examined in adult rat sensory neurons and spinal cord injury models. It has been shown that neurotrophic cytokines promote axonal regeneration and functional recovery (*20,61*). However, it is not known whether neurotrophic cytokines affect by increasing the rate of axonal regeneration or by increasing regenerative or terminal outgrowth in axons.

6. Conclusion

Although it is clear that neurotrophic factors support neural survival after damage and intracellular pathways are clearly determined, quantitative assessments in especially axonal regeneration are very recent. The literature shows that regeneration and functional recovery depend on the positive and negative signal balance between growth factors. Further studies of *in vitro* and *in vivo* nerve injury and repair models are required to make our information about neurotrophic factors more comprehensible. Results can cause the design of more specific treatment methods in the treatment of low functional recovery, which develops after damage.

References

1. Zochodne DW. Neurobiology of peripheral nerve regeneration. Cambridge University Press; Cambridge, UK, 2008.
2. Rask CA. Biological actions of nerve growth factor in the peripheral nervous system. Eur Neurol. 1999; 41 (Suppl 1):14-19.
3. Thoenen H. Neurotrophins and activity-dependent plasticity. Prog Brain Res. 2000; 128:183-191.
4. Costales J, Kolevzon A. The therapeutic potential of insulin-like growth factor-1 in central nervous system disorders. Neurosci Biobehav Rev. 2016; 63:207-222.
5. Keskin I, Kaplan S, Kalkan S, Sutcu M, Ulkay MB, Esener OB. Evaluation of neuroprotection by melatonin against adverse effects of prenatal exposure to a nonsteroidal anti-inflammatory drug during peripheral nerve development. Int J Dev Neurosci. 2015; 41:1-7.
6. Geuna S, Raimondo S, Fregnan F, Haastert-Talini K, Grothe C. *In vitro* models for peripheral nerve regeneration. Eur J Neurosci. 2016; 43:287-296.
7. Geuna S. The sciatic nerve injury model in pre-clinical research. J Neurosci Methods. 2015; 243:39-46.
8. Turgut M, Kaplan S. Effects of melatonin on peripheral nerve regeneration. Recent Pat Endocr Metab Immune Drug Discov. 2011; 5:100-108.
9. Boyd JG, Gordon T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Mol Neurobiol. 2003; 27:277-324.
10. Sebben AD, Lichtenfels M, da Silva JLB. Perıpheral nerve regeneratıon: Cell therapy and neurotrophıc factors. Revista Brasileira de Ortopedia (English Edition). 2011; 46:643-649.
11. Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J. 1982; 1:549-553.
12. Yan H, Zhang F, Chen MB, Lineaweaver WC. Chapter 10: Conduit luminal additives for peripheral nerve repair. Int Rev Neurobiol. 2009; 87:199-225.
13. Yano H, Chao MV. Neurotrophin receptor structure and interactions. Pharm Acta Helv. 2000; 74:253-260.
14. Barbacid M. The Trk family of neurotrophin receptors. J Neurobiol. 1994; 25:1386-1403.
15. Schneider R, Schweiger M. A novel modular mosaic of cell adhesion motifs in the extracellular domains of the neurogenic trk and trkB tyrosine kinase receptors. Oncogene. 1991; 6:1807-1811.
16. Saarma M, Sariola H. Other neurotrophic factors: glial cell line-derived neurotrophic factor (GDNF). Microsc Res Tech. 1999; 45:292-302.
17. Henderson CE, Phillips HS, Pollock RA, *et al*. GDNF: A potent survival factor for motoneurons present in peripheral nerve and muscle. Science. 1994; 266:1062-1064.
18. Patel M, Mao L, Wu B, VandeVord P. GDNF blended chitosan nerve guides: An *in vivo* study. J Biomed Mater Res A. 2009; 90:154-165.
19. Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J. 1998; 334:297-314.
20. Yang P, Wen H, Ou S, Cui J, Fan D. IL-6 promotes regeneration and functional recovery after cortical spinal

tract injury by reactivating intrinsic growth program of neurons and enhancing synapse formation. Exp Neurol. 2012; 236:19-27.
21. Stolp HB. Neuropoietic cytokines in normal brain development and neurodevelopmental disorders. Mol Cell Neurosci. 2013; 53:63-68.
22. Harvey AR, Lovett SJ, Majda BT, Yoon JH, Wheeler LPG, Hodgetts SI. Neurotrophic factors for spinal cord repair: Which, where, how and when to apply, and for what period of time? Brain Res. 2015; 1619:36-71.
23. Zurn AD, Winkel L, Menoud A, Djabali K, Aebischer P. Combined effects of GDNF, BDNF, and CNTF on motoneuron differentiation *in vitro*. J Neurosci Res. 1996; 44:133-141.
24. Priestley JV, Ramer MS, King VR, McMahon SB, Brown RA. Stimulating regeneration in the damaged spinal cord. J Physiol Paris. 2002; 96:123-133.
25. Hoyng SA, De Winter F, Gnavi S, de Boer R, Boon LI, Korvers LM, Tannemaat MR, Malessy MJ, Verhaagen J. A comparative morphological, electrophysiological and functional analysis of axon regeneration through peripheral nerve autografts genetically modified to overexpress BDNF, CNTF, GDNF, NGF, NT3 or VEGF. Exp Neurol. 2014; 261:578-593.
26. Kobayashi NR, Bedard AM, Hincke MT, Tetzlaff W. Increased expression of BDNF and trkB mRNA in rat facial motoneurons after axotomy. Eur J Neurosci. 1996; 8:1018-1029.
27. Vögelin E, Baker JM, Gates J, Dixit V, Constantinescu MA, Jones NF. Effects of local continuous release of brain derived neurotrophic factor (BDNF) on peripheral nerve regeneration in a rat model. Exp Neurol. 2006; 199:348-353.
28. Tchetchelnitski V, van den Eijnden M, Schmidt F, Stoker AW. Developmental co-expression and functional redundancy of tyrosine phosphatases with neurotrophin receptors in developing sensory neurons. Int J Dev Neurosci. 2014; 34:48-59.
29. Gibbons AS, Bailey KA. BDNF and NT-3 regulation of trkB and trkC mRNA levels in the developing chick spinal cord. Neurosci Lett. 2005; 385:41-45.
30. Kaplan S, Odaci E, Unal B, Sahin B, Fornaro M. Chapter 2: Development of the peripheral nerve. Int Rev Neurobiol. 2009; 87:9-26.
31. Onger ME, Türkmen AP, Elibol E, *et al*. Chapter 3 − Embryology of the peripheral nerves A2 − Nerves and nerve injuries. Academic Press, San Diego, USA, 2015; pp. 37-40.
32. Türkmen AP, Altunkaynak BZ, Onger ME, *et al*. Chapter 4 − Development of the cranial nerves A2 − Nerves and nerve injuries. Academic Press, San Diego, USA, 2015; pp. 41-53.
33. Funakoshi H, Frisén J, Barbany G, Timmusk T, Zachrisson O, Verge VM, Persson H. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. J Cell Biol. 1993; 123:455-465.
34. Naveilhan P, ElShamy WM, Ernfors P. Differential regulation of mRNAs for GDNF and its receptors Ret and GDNFR alpha after sciatic nerve lesion in the mouse. Eur J Neurosci. 1997; 9:1450-1460.
35. Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol Ther. 2013; 138:155-175.
36. Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: Different mechanisms are responsible for the regulation of BDNF and NGF mRNA. J Cell Biol. 1992; 119:45-54.
37. Ito Y, Yamamoto M, Li M, Doyu M, Tanaka F, Mutch T, Mitsuma T, Sobue G. Differential temporal expression of mRNAs for ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), interleukin-6 (IL-6), and their receptors (CNTFR alpha, LIFR beta, IL-6R alpha and gp130) in injured peripheral nerves. Brain Res. 1998; 793:321-327.
38. Santos D, Giudetti G, Micera S, Navarro X, del Valle J. Focal release of neurotrophic factors by biodegradable microspheres enhance motor and sensory axonal regeneration *in vitro* and *in vivo*. Brain Res. 2016; 1636:93-106.
39. Yang JW, Ru J, Ma W, Gao Y, Liang Z, Liu J, Guo JH, Li LY. BDNF promotes the growth of human neurons through crosstalk with the Wnt/β-catenin signaling pathway *via* GSK-3β. Neuropeptides. 2015; 54:35-46.
40. Vermehren-Schmaedick A, Khanjian RA, Balkowiec A. Cellular mechanisms of activity-dependent BDNF expression in primary sensory neurons. Neuroscience. 2015; 310:665-673.
41. Andero R, Choi DC, Ressler KJ. Chapter Six – BDNF-TrkB receptor regulation of distributed adult neural plasticity, memory formation, and psychiatric disorders. In: Progress in Molecular Biology and Translational Science (Zafar UK, Muly EC, eds.). Academic Press, 2014; pp. 169-192.
42. Wei Z, Liao J, Qi F, Meng Z, Pan S. Evidence for the contribution of BDNF-TrkB signal strength in neurogenesis: An organotypic study. Neurosci Lett. 2015; 606:48-52.
43. Milbrandt J, de Sauvage FJ, Fahrner TJ, *et al*. Persephin, a novel neurotrophic factor related to GDNF and neurturin. Neuron. 1998; 20:245-253.
44. Quartu M, Serra MP, Boi M, Sestu N, Lai ML, Del Fiacco M. Tissue distribution of neurturin, persephin and artemin in the human brainstem at fetal, neonatal and adult age. Brain Res. 2007; 1143:102-115.
45. Pasquin S, Sharma M, Gauchat JF. Ciliary neurotrophic factor (CNTF): New facets of an old molecule for treating neurodegenerative and metabolic syndrome pathologies. Cytokine Growth Factor Rev. 2015; 26:507-515.
46. Villacampa N, Almolda B, Campbell IL, González B, Castellano B. CNS-targeted IL-6 production leads to higher recruitment of pro-inflammatory T-helper cells after facial nerve axotomy. J Neuroimmunol. 2014; 275:177.
47. Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, Fornaro M. Chapter 3: Histology of the peripheral nerve and changes occurring during nerve regeneration. Int Rev Neurobiol. 2009; 87:27-46.
48. Richardson PM. Ciliary neurotrophic factor: A review. Pharmacol Ther. 1994; 63:187-198.
49. Audisio C, Mantovani C, Raimondo S, Geuna S, Perroteau I, Terenghi G. Neuregulin1 administration increases axonal elongation in dissociated primary sensory neuron cultures. Exp Cell Res. 2012; 318:570-577.
50. Kaplan S, Eşrefoglu M, Aktaş A, Gül M, Onger ME, Altunkaynak ME, Ulkay MB, Ragbetli MÇ. The effect of prenatal exposure of a non-steroidal anti-inflammatory drug on the optic nerve of female rats: A stereological, histological, and electron microscopic study. J Matern Fetal Neonatal Med. 2013; 26:1860-1864.

51. Çolakoğlu S, Aktaş A, Raimondo S, Türkmen AP, Altunkaynak BZ, Odacı E, Geuna S, Kaplan S. Effects of prenatal exposure to diclofenac sodium and saline on the optic nerve of 4- and 20-week-old male rats: a stereological and histological study. Biotech Histochem. 2014; 89:136-144.
52. Onger ME, Altun G, Aydın I, Kıvrak EG, Yurt KK Altunkaynak BZ, Kaplan S. A Stereological Investigation Technique for Peripheral Nerve. Turkiye Klinikleri J Neurol-Special Topics. 2014;7:56-60.
53. Kaplan S, Geuna S, Ronchi G, Ulkay MB, von Bartheld CS. Calibration of the stereological estimation of the number of myelinated axons in the rat sciatic nerve: A multicenter study. J Neurosci Methods. 2010; 187:90-99.
54. Piskin A, Kaplan S, Aktaş A, Ayyildiz M, Raimondo S, Aliç T, Bozkurt HH, Geuna S. Platelet gel does not improve peripheral nerve regeneratıon: An electrophysıological, stereologıcal, and electron microscopic study. Microsurgery. 2009; 29:144-153.
55. Kishino A, Ishige Y, Tatsuno T, Nakayama C, Noguchi H. BDNF prevents and reverses adult rat motor neuron degeneration and induces axonal outgrowth. Exp Neurol. 1997; 144:273-286.
56. Zhang JY, Luo XG, Xian CJ, Liu ZH, Zhou XF. Endogenous BDNF is required for myelination and regeneration of injured sciatic nerve in rodents. Eur J Neurosci. 2000; 12:4171-4180.
57. Deng LX, Hu J, Liu N, Wang X, Smith GM, Wen X, Xu XM. GDNF modifies reactive astrogliosis allowing robust axonal regeneration through Schwann cell-seeded guidance channels after spinal cord injury. Exp Neurol. 2011; 229:238-250.
58. Shakhbazau A, Mohanty C, Shcharbin D, Bryszewska M, Caminade AM, Majoral JP, Alant J, Midha R. Doxycycline-regulated GDNF expression promotes axonal regeneration and functional recovery in transected peripheral nerve. J Control Release. 2013; 172:841-851.
59. Gyorkos AM, McCullough MJ, Spitsbergen JM. Glial cell line-derived neurotrophic factor (GDNF) expression and NMJ plasticity in skeletal muscle following endurance exercise. Neuroscience. 2014; 257:111-118.
60. Azadi S, Johnson LE, Paquet-Durand F, Perez MT, Zhang Y, Ekström PA, van Veen T. CNTF + BDNF treatment and neuroprotective pathways in the rd1 mouse retina. Brain Res. 2007; 1129:116-129.
61. Thompson SWN, Priestley JV, Southall A. GP130 cytokines, leukemia inhibitory factor and interleukin-6, induce neuropeptide expression in intact adult rat sensory neurons *in vivo*: Time-course, specificity and comparison with sciatic nerve axotomy. Neuroscience. 1998; 84:1247-1255.

(Received September 7, 2016; Revised October 5, 2016; Accepted October 6, 2016)