Growth Factors and Astrocytes Metabolism: Possible Roles for Platelet Derived Growth Factor

Short running title: Growth factors and astrocytic metabolism

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Abstract

Astrocytes exert multiple functions in the brain such as the development of blood–brain barrier characteristics, the promotion of neurovascular coupling, attraction of cells through the release of chemokines, clearance of toxic substances and generation of antioxidant molecules and growth factors. In this aspect, astrocytes secrete several growth factors (BDNF, GDNF, NGF, and others) that are fundamental for cell viability, oxidant protection, genetic expression and modulation of metabolic functions. The platelet derived growth factor (PDGF), which is expressed by many SNC cells including astrocytes, is an important molecule that has shown neuroprotective potential, improvement of wound healing, regulation of calcium metabolism and mitochondrial function. Here we explore some of these astrocyte-driven functions of growth factors and their possible therapeutic uses during neurodegeneration.

Keywords: Astrocyte, Growth Factors, metabolism, mitochondria, neuroprotection, Platelet derived growth factor

Introduction

Numerous growth factors have a major impact in the maintenance of brain functions both during normal and in pathological conditions. Growth factor functions are mainly mediated through growth factor receptor activation and they include cell differentiation, neurotransmitter regulation, Central Nervous System (CNS) development, cognitive functions, neuroprotection and also the metabolic regulation of the brain [1-4]. In this aspect, previous studies have shown the importance of growth factors in the regulation of important metabolic functions such as oxidative damage, maintenance of mitochondrial membrane potential, glucose and calcium influx in the brain [1,5-7].

Although growth factors in the brain are produced by neurons, oligodendrocytes or microglia [4,8], their release by astrocytes is of primordial importance for the maintenance of neuronal functions [9,10]. Astrocytes are an important source of BDNF (Brain derived neurotrophic factor), GDNF (Glial cell line derived neurotrophic factor), NGF (nerve growth factor), PDGF and others with prospective neuroprotective functions in the brain [4,10,11]. In the present review, we explore the importance of growth factors in astrocytic metabolic regulation, and highlight the effect of PDGF as a potential therapeutic approach.

Astrocyte functions

Astrocytes are part of the glial cells along with oligodendrocytes and microglia [12]. These cells have a great range of functions that include Blood Brain Barrier (BBB) maintenance, uptake of glutamate and g-aminobutyric acid (GABA) by specific transporters, production of antioxidant compounds like glutathione (GSH) and superoxide dismutases (SOD) and growth factors that enhance neuronal viability, both in normal and in pathological conditions [13-17]. This cell type is characterized by a stellate morphology with various processes and ramifications, and by the expression of the intermediate filaments vimentin (Vim) and glial fibrillary acidic protein (GFAP)[13,15].

There are two main types of astrocytes depending on their location and metabolic functions: protoplasmic astrocytes of the grey matter, which envelope neuronal bodies and synapses, and fibrous astrocytes from the white matter that interact with the nodes of

Ranvier and oligodendroglia [18,19]. Protoplasmic astrocytes have been associated with increased accumulation of a-synuclein during Parkinson Disease (PD), Alzheimer disease (AD), and Epilepsy, and these cells the main targets during neurodegeneration [19]. Astrocytic terminal processes, known as endfeet, contact the brain vasculature surface facing ECs (endothelial cells) and pericytes and enwrap neuronal synapses, thus enabling the modulation of both neuronal activity and cerebral blood flow following an elevation in intracellular Ca^{2+} levels in the endfeet [20,21]. Importantly, astrocytic endfeet express specialized molecules such as Kir4.1 K^+ channels and aquaporin 4 that regulate ionic concentrations in BBB, and protein transporters like glucose transporter-1 and P-glycoprotein, suggesting the importance of the endfeet in astrocyte polarization [22,23].

Astrocytes respond to all types of cerebral insults (infection, trauma, ischemia, oxidative stress, neurodegenerative diseases) by a process called reactive astrogliosis, which involves both molecular and morphological changes including hypertrophy of cell bodies and processes, increased expression of GFAP, vimentin, nestin and chondroitin sulfate proteoglycans (CSPGs; 23-25). Additional features of astrogliosis include changes in glutamate uptake rate, protection against oxidative stress by the production of glutathione, neuroprotection by the release of adenosine, degradation of beta-amyloid peptides (Aβ), glial scarring, and, in some cases, release of inflammatory cytokines, including tumor necrosis factor (TNF) and ROS production [24,25].

Current research has shown that reactive astrogliosis has both positive and negative effects during CNS recovery for CNS, including the BBB remodeling, modulation of blood flow through the release of vasoconstrictors and neuronal protection via regulation of glutathione after production of oxidative stress [15]. On the other hand, during severe astrogliosis and glial scar formation, astrocytes produce chondroitin sulfate proteoglycans (CSPG), tenascin, Sema3 and ephrin, which act as inhibitors of axonal growth [26,27]. In addition, energetic metabolism, lipid and aminosugar metabolism, calcium signaling and oxidative balance are also somehow affected during astrogliosis [12, 28,29]. Altogether, these evidences suggest that the homeostatic regulation of astrocytes is important for neuronal metabolism through the regulation of neuronal glutamate or GABA by specific transporters and the release of gliotransmitters [23].

Growth factors in astrocytic metabolism

Astrocytes secrete several growth factors that increase neuronal survival both in normal and in pathological conditions through the activation of MAPK and PI3K/AKT [4,10,11]. These astrocytic growth factors include BDNF, NGF, neurturin (NRTN), GDNF and FGFb (Basic fibroblast growth factor) [30-32]. Other growth factors with potential interest include VEGF-B (vascular endothelial growth factor), insulin-like growth factors (IGFs), Hepatocyte growth factor (HGF), and PDGF [2,4,11,30,32]. Although the main effect of growth factors is to improve the survival and proliferation of neurons and other CNS cell types, growth factors may also exert additional functions such as angiogenesis and wound healing, increased expression of NMDA receptors and antioxidant enzymes like SODs or GSH reductase, and preservation of BBB endothelial *adherens junctions* [*Figure 1*, 3,32,35,36].

Previous studies have pointed out the importance of growth factors in metabolic activities of the brain, both in physiological and neurodegenerative processes [1,5,6,37]. For example, BDNF from the neurotrophin family has been shown to be critical in the survival of cortical, hippocampal and serotonergic neurons. Reduction in BDNF levels is associated with PD, AD, Huntington Disease (HD), amyotrophic lateral sclerosis (ALS), depression and schizophrenia [38]. Furthermore, BDNF signaling through Trk (Track) receptors has been shown to protect neurons against excitotoxicity via the activation of MAPK, PI3K, and PLCγ, which are important regulators of cell survival. BDNF-induced downstream activation induces expression of Mn-SOD Bcl-2 (anti-apoptotic protein) and inhibitor of apoptosis proteins (IAPs) [3, 39]. Similarly, gene therapy with BDNF protects neurons following 6-OHDA (2.7 mg/ml) and MPTP (methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 1 mg/kg) toxicity in animals [11]. Also, BDNF has been shown to increase mitochondrial respiratory control index through the activation of MEK-Bcl-2 pathway in mice, suggesting an important effect in mitochondrial metabolism [37]. Additionally, BDNF at 5 ng/ml) has favors glucose utilization, and induces the increase of GLUT3 transporter and Na+/K--ATPase in mouse cortical neurons [1]. These findings suggest that BDNF may exert its

protective functions by preserving mitochondrial properties and regulating glucose transport.

Glial derived neurotrophic factor (GDNF) comprises a family of ligands that includes GDNF, NRTN, artemin (ARTN) and persephin that activate receptors GFR (GDNF family receptor) and RET (rearranged during transfection) proto-oncogene. GDNF, which is secreted by astrocytes and pericytes, is essential for the survival of dopaminergic neurons, peripheral motor neurons and neurons from the locus coeruleus [4, 38]. Previous studies have shown the importance of GDNF in dopaminergic neuronal resistance against 6-OHDA (5 mM) induced toxicity, and the maintenance of BBB through the increased expression of claudin-5 [40,41]. Recently, overexpression of GDNF RET receptor was shown to improve mitochondrial electron transport in a drosophila Parkinson model, demonstrating the importance of GDNF for mitochondrial impairment during the development and maturation of PD [42].

Nerve growth factor (NGF) is an important regulator for survival and differentiation of sensory and sympathetic neurons through [43]. NGF, which is structurally similar to BDNF, activates the tropomyosin-receptor-kinase (TrkA) receptor, and downstream pathways including PLC, PI3K (phosphatidylinositol 3-kinase), and ERK/MAPK [44].

Previous study indicated that NGF at 100 ng/ml stimulated mitochondrial biogenesis in PC12 cells (rat adrenal pheochromocytoma) through the activation of peroxisome proliferator-activated receptor gamma as a co-activator 1-a (PGC-1a) and preservation of mitochondrial membrane potential in a Huntington disease (HD) model [5]. This growth factor has also been shown to induce CREB phosphorylation, and increased mitochondrial respiration [5,43], demonstrating the importance of NGF for mitochondrial protection during neurodegenerative diseases.

Insulin like growth factor family protein (IGF) consists of insulin and insulin like growth factors type I and II (IGF-I and IGF-II), which are important mitogenes affecting growth, metabolism, cell differentiation and gene expression [45]. Both peptides mainly exert their functions through binding to IGF type I receptor (IGF-IR), in which activates the mitogenic PI3K/AKT signaling pathway and downstream effectors [45]. In the brain, both IGF-I and

II are expressed by neurons, microglia and astrocytes, making this growth factor an important regulator of brain functions [46]. Additionally, It has been shown that IGF-I and insulin (5µg/ml) increase the expression of GLT1 receptor in astrocytes, which is important for glutamate clearance from the extra-synaptic space [7]. In a recent study, it was demonstrated that 10^{-7} M IGF-I reduced oxidative damage in astrocytes by decreasing pro-oxidant thioredoxin-interacting protein 1 and stabilization of reactive oxidative levels, suggesting the importance of IGF-I in mediating neuroprotective actions upon oxidative stress [6]. Finally, VEGF-B was shown to improve cerebral blood flow by pericytes in a murine ischemic model [47]. Interestingly, VEGF-B at 20 ng/ml increased mitochondrial biogenesis and decreased astrocytic reactivity in a murine model by activation of PI3K/AKT and ERK pathways, demonstrating a novel role of VEGF in regulating astrocyte activation and mitochondrial function [48, 49].

PDGF: role in astrocyte metabolism

PDGFs are dimeric proteins of about 30 kDa belonging to the family of the PDGF/VEGF (vascular endothelial growth) and to date there are five identified dimeric isoforms or compositions: PDGF-AA, -BB, -AB, - CC and -DD [8, 50]. PDGF ligands and receptors are widely expressed in different CNS tissues including neurons and astrocytes [8, 51]. Additionally, PDGFs have been shown to be important for neuronal development, neuronal-astrocytic interaction in retina, wound healing and maintenance of BBB [52-55].

The five dimeric PDGF isoforms show different ability to bind and activate the two tyrosine kinase receptors PDGF type: PDGFRα and PDGFRβ [56-58]. For most tyrosine kinase receptors, the ligand binding and subsequent dimerization of the subunits induce phosphorylation of tyrosine residues in the intracellular domain [8]. PDGFRα and PDGFRβ receptor tyrosine kinases have a common structure with five extracellular domains of immunoglobulin (IG) and an intracellular tyrosine kinase domain. This structure is shared with other receptors such as c-Kit, c-Fms and Flt [8, 59]. The phosphorylated receptor may activate several signaling pathways such as the mitogen activated protein kinase (MAPK), PI3K, the Wnt pathway, and phospolipase C (PLC). Additionally, it has been reported that activation of transcription factors such as STAT, ELK-1, c-jun/c-fos, HIF-1α, NF-κβ are

important for inflammation, response to hypoxia and survival [8, 56, 60-66], and these signaling pathways are dependent on calcium influx into the cell, which affects phosphorylation of GSK3 and β-catenin [65, 67; Figure 1].

Additional evidence has suggested that PDGF-BB can activate mechanisms related with the modification of the cytoskeleton and cell migration. The cellular responses seem to mediated via activation of phosphatidylinositol 3,4,5, which in turn phosphorylates Rac GTPase, a protein of the Rho family that is involved in modifying the actin cytoskeleton and inducing morphological changes [68-70]. In this regard, it has been demonstrated that PDGF-BB induces formation of lamellipodia and increased motility in fibroblast mediated rearrangements actin fibers, and these processes are dependent on PI3K signaling [71-72]. Furthermore, PDGF and VEGF were shown to induce RhoA activation protein in ECs during angiogenesis, suggesting the importance of PDGF-BB in regulating morphological changes during injury as well as recovery processes [68,73].

PDGF-BB also activates STATs (Signal transduction and activators of transcription), which are important in processes such as proliferation, differentiation, cell survival and transformation [61,74]. Activation of STATs 1, 3 and 6 by PDGF-BB has been demonstrated in vascular muscle cells during processes related with airway remodeling in asthma patients [74]. This activation appears to be dependent on the production of reactive oxygen species (ROS) as a result of the activation of the transmembrane NOX enzyme (NADPH oxidase / Dual oxidase enzyme), which is independent of ROS production by mitochondria [61-74]. Therefore, it is most likely that the production of hydrogen peroxide (H_2O_2) by PDGF has beneficial effects, as this is an intermediary in the second messenger signaling pathways activated by STATs, and regulation of protein phosphatases downstream [61, 74, 75]. On the other hand, previous studies in neuronal models have also shown that 24 hours pretreatment with PDGF-BB (50 ng/ml) exerts a significant protection against different insults such as H_2O_2, glucose deprivation and excitotoxic damage in cultured neurons [66, 76]. PDGF-BB preconditioning was reported to induce PDGFRB phosphorylation followed by an activation of downstream effectors like PI3K/AKT and MAPK, and increase in the antioxidant enzymes catalase and glutathione peroxidase and augmented expression of antiapoptotic genes like Bcl-xL and Bax families [66, 76].

Moreover, the activation of PI3K/AKT leads to inactivation of the kinase GSK3β, which in turn prevents β-catenin degradation, and results in its accumulation in cytoplasm. Subsequently, this event leads to β-catenin translocation to nucleus, and induces the activation of genes associated with cell survival such as Bcl-2 [67]. Additionally β-catenin is related to mitochondrial homeostasis, regulation of ATP production and lipid oxidation, and these functions are dependent on the nutritional status, severity and duration of the oxidative stress [77].

PDGF-BB is also an essential factor for BBB maintenance. For example, it has been shown that both astrocytes and pericytes are essential for brain angiogenesis and BBB regulation through the activation of PDGFRB signaling [54, 78]. Additionally, PDGF-BB impairment in mice has been associated with a reduced number of pericytes, edema formation and murine embryonic lethality, suggesting its importance for BBB development [53, 54]. Furthermore, different PDGF isoforms have been shown to exert a neuroprotective effect in neurons against H_2O_2, VIH TAT toxin and 6-OHDA, mainly through the activation of PI3-K/Akt and downstream effectors [65-67,79]. PDGF is likely an important regulator of mitochondrial functions, including mitochondrial volume, surface area of cristae, enhanced glycolysis and changes in mitochondrial metabolism [80-83]. An initial study by Gosslau and colleagues [80] reported that 10 ng/ml PDGF-AB increased mitochondrial volume by 57% and surface area of mitochondrial cristae in injured fibroblasts determined by electron microscopy, suggesting that low doses of PDGF-AB are important for increasing mitochondrial energetic processes [80]. More recently, Salabei and Hill [81] showed that PDGF-BB elicited mitochondrial fission accompanied by an increase in fatty acid oxidation in a vascular smooth muscle cell model. These findings suggest that PDGF-BB may increase metabolic performance of injured cells through regulating mitochondrial functions and morphology. Similarly, in a recent study from our research group we found that PDGF-BB at 200 ng/ml protected astrocytic cell line T98G against rotenone insult (50 uM) by providing an increased maintenance in mitochondrial membrane potential and mitochondrial volume [84]. These combined results, suggest that PDGFs are important regulators of mitochondrial function during neurodegeneration.

Several studies have demonstrated the importance of PDGF in calcium regulation [67,79,82,83] through the activation of phospholipase C-y (PLC-y) and subsequent formation of inositol triphosphate (IP,) and diacylglycerol (DAG), leading to Ca^{2+} cytosolic mobilization from intracellular pools [82]. Additionally, PDGF may increase the influx of extracellular calcium through the activation of ionic channels such as TRPC (transient receptor potential channel) and increasing the neuroprotective activities of PDGF [67]. This increase in intracellular calcium was shown to promote the expression of Arc/Arg3.1 gene that has been implicated in long term potentiation and synaptic plasticity [83]. Taken together, these results demonstrate the crucial importance of PDGF in calcium modulation in the CNS. Additional research is needed in order to understand the possible roles of PDGF in astrocytic calcium metabolism, especially potential regulation of gliotransmitters release and mitochondrial functions.

Growth factor delivery in astrocytes: current developments

Currently, the main problem for the use of growth factors as therapeutic approach during neurodegenerative processes is their inability to cross the BBB thoroughly [85,86]. The BBB exerts a tightly regulation in the movement of ions, drugs and peptides molecules between the neural cells and the blood flux, thus maintaining the ionic homeostasis, hormonal and transmitter levels and transport of nutrients into the brain [86]. Various strategies have been suggested to increase growth factors delivery through the BBB, including injections into the lumbar or ventricular CSF, viral vectors with growth factor genes, the use of linked peptides or *"peptidomimetic"* monoclonal antibodies, nanoparticles or the transitorily (e.g. temporal) disruption of BBB [38]. For example, different types of nanomolecules have been used for peptide delivery including, liposomes, solid lipid nanoparticles, polymeric molecules between 60 and 200 nm such as polylactides, and branched polymers dendrimers [87]. In addition, the transient disruption of BBB for drug delivery has been successfully used in different approaches, including the use of ultrasound or electrical pulses without causing necrotic or apoptotic damage to the tissues, and this is a promising methodology for a successful drug delivery into the brain [85, 88, 89].

Additional approaches for the delivery of growth factors into the brain are the transplantation of dopaminergic neurons or glial precursor cells into the injured regions of the brain, which increases the expression of growth factors like BDNF, GDNF, and IGF [10, 90, 91]. In this aspect, a recent report by Proschel and coworkers [10] have demonstrated that the transplantation of *in vitro* generated glial precursor cells in 6-OHDA (10 uM) hemilesioned rats causes the recovery of DA neurons in striatum by an increase in the levels of GSH, GDNF, and BDNF. The developments of novel technologies will allow the transport of growth factors through BBB in the near future for the treatment of neuropathologies and related disorders.

Conclusions and future perspectives

Based on this review, it is clear that astrocytic dysfunction is greatly involved during the development and maturation of the brain pathologies that manifest as a neurodegenerative and cerebrovascular diseases**.** These astrocytic alterations may in turn affect neurons during reactive astrogliosis process, and loss of astrocytic metabolic support of neurons [23]. In this regard, the accumulating body of the evidence strongly suggest the importance of energetic homeostasis during neurodegeneration and cerebrovascular diseases. Alterations in mitochondrial bioenergetic balance and morphology coexist or are tightly associated to other chronic diseases such as type 2 diabetes Alzheimer, Parkinson and Huntington diseases. The protective effects of growth factors are not limited to the enhancement in growth and survival of the tissue, as they have also shown to improve mitochondrial morphology and metabolism both in astrocytes and neurons. However, further research is needed in order to understand the complete downstream signaling mechanism of growth factors in mitochondrial and metabolic protection, and its possible use in the context of the prophylactics and treatment strategies against these devastating diseases.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Burkhalter, J.; Fiumelli, H.; Allaman, I.; Chatton, J.Y, Martin, J.L. Brain-derived neurotrophic factor stimulates energy metabolism in developing cortical neurons. *J Neurosci. **2003**, 23,8212–8220.*

2. Aberg, N.D.; Brywe, K.G.; Isgaard, J. Aspects of growth hormone and insulin-like growth factor-I related to neuroprotection, regeneration, and functional plasticity in the adult brain. *Scient. World J., **2006,** 6, 53–80.*

3. Mattson, M.P. Glutamate and neurotrophic factors in neuronal plasticity and disease. Annals of the New York Academy of Sciences, **2008**, 1144, 97-112.

4. Yasuda,T.; Mochizuki, H. Use of growth factors for the treatment of Parkinson's disease. *Expert Rev. Neurother., 2010, 10, 915–924.doi:10.1586/ern.10.55.*

5. Chen, L.W.; Horng, L.Y.; Wu, C.L.; Sung, H.C.; Wu, R.T. Activating mitochondrial regulator PGC-1a expression by astrocytic NGF is a therapeutic strategy for Huntington's disease. *Neuropharmacology. **2012**, 63:719e732*

6. Genis, L.; Dávila, D.; Fernandez, S.; Pozo-Rodrigálvarez, A.; Martínez-Murillo, R.; Torres-Aleman, I. Astrocytes require insulin-like growth factor I to protect neurons against oxidative injury. *F1000Res. 2014, 3, 28. doi: 10.12688/f1000research.3-28.v1. eCollection 2014.*

7. Yin, F.; Jiang, T.; Cadenas, E. Metabolic triad in brain aging: Mitochondria, insulin/IGF-1 signalling and JNK signalling. *Biochem. Soc. Trans., **2013**,41, 101–105. doi: 10.1042/BST20120260.*

8. Andrae, J.; Gallini, R.; Betsholtz, C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev.*, ***2008,*** *22, 1276-1312*doi:22/10/127610.1101/gad.1653708.

9. Cabezas, R.; Avila, M.; Gonzalez, J.; El-Bachá, R.S.; Báez, E.; García-Segura, LM.; Jurado Coronel, J.C.; Capani, F.; Cardona-Gomez, G.P.; Barreto, G.E. Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. *Front Cell Neurosci.*, ***2014****, 8:211.*

10. Proschel, C.; Stripay, J.L.; Shih, C.H.; Munger, J.C.; Noble, M.D. Delayed transplantation of precursor cell-derived astrocytes provides multiple benefits in a rat model of Parkinsons. *EMBO Mol.Med.*, ***2014,*** *6, 504–518.doi:10. 1002/emmm.201302878*

11. Ramaswamy, S.; Kordower, J. Are growth factors the answer? Parkinsonism and Related Disorders. *15(Suppl 3),* ***2009****, S176-S180.*

12. Chen, Y.; Swanson, R.A. 2003. Astrocytes and brain injury. *J. Cereb. Blood Flow Metab.* ***2003****, 23, 137–149.*

13. Volterra, A.; Meldolesi, J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev.*, ***2005****, 6, 626–640.*

14. Chinta, S.; Andersen, J. Redox imbalance in Parkinson. *Biochim. Biophys. Acta* . 2008, 1780, 1362–1367 10.1016/j.bbagen.2008.02.005

15. Hamby, M.E.; Sofroniew, M. Reactive Astrocytes as therapeutic targets for CNS disorders. *Neurotherapeutics,* ***2010****, 7, 494-506.*

16. Kimelberg, H.K.; Nedergaard, M. Functions of astrocytes and their potential as therapeutic targets. *Neurotherapeutics,* ***2010,*** *7, 338–353 10.1016/j.nurt.2010.07.00*

17. Parpura, V.; Grubisic, V.; Verkhratsky, A. Ca(2 +) sources for the exocytotic release of glutamate from astrocytes. *Biochim.Biophys. Acta.*, ***2011****, 1813, 984–1018.*

18. Halliday, G.M.; Stevens, C.H. Glia: initiators and progressors of pathology in Parkinson's disease. *Mov. Disord.*, ***2011****, 26, 6–17 10.1002/mds.23455*

19. Oberheim, N.A.; Goldman, SA.; Nedergaard, M. Heterogeneity of astrocytic form and function. *Methods Mol. Biol.*, ***2012****, 814, 23–45.doi:10.1007/978- 1-61779-452-0_3.*

20. Zonta, M.; Angulo, M.C.; Gobbo, S.; Rosengarten, B.; Hossmann, K.A.; Pozzan, T. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat. Neurosci.*, ***2003****, 6, 43–50, 10.1038/nn980*

21. Maragakis N.J.; Rothstein, J.D. Mechanisms of disease: astrocytes in neurode generative disease. Nat. Clin. Pract. Neurol., **2006,** 2, 679–689 10.1038/ncpneuro0355

22. Abbott N.J.; Rönnbäck L.; Hansson, E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat. Rev. Neurosci., **2006,** 7, 41–53 10.1038/nrn1824

23. Barreto, G.; Veiga, S.; Azcoitia, I.; Garcia-Segura, L..M.; Garcia-Ovejero, D. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihidrotestosterone. *Eur J Neurosci., **2007**, 25, 3039-3046.*

24. Barreto, G.E.; Gonzalez, J.; Torres, Y.; Morales, L . Astrocytic-neuronal crosstalk: implications for neuroprotection from brain injury. *Neurosci. Res. **2011**, 71, 107– 113.*

25. Kang W.; Hebert, J.M. Signaling pathways in reactive astrocytes, a genetic perspective. *Mol. Neurobiol., **2011**, 43, 147–154 10.1007/s12035-*

26. Fitch, M.T.; Silver, J. CNS injury, glial scars and inflammation: inhibitory extracellular matrices and regeneration failure. *Exp. Neurol., **2008**, 209, 294–301* 10.1016/j.expneurol.2007.05.014.

27. Duffy, P.; Schmandke, A.; Schmandke, A.C.; Sigworth, J.; Shuh, N.; Cafferty, W. B. Rho-associated kinase II (ROCKII) limits axonal growth after trauma within the adult mouse spinal cord. *J. Neurosci., **2009**, 29, 15266–15276* 10.1523/JNEUROSCI.4650-09.2009

28. Zamanian, J.L.; Xu, L.; Foo, L.C.; Nouri, N.; Zhou, L. Genomic analysis of reactive astrogliosis. *J Neurosci.**,2012**, 32, 6391–6410.*

29. Kanemaru, K.; Kubota, J.; Sekiya, H.; Hirose, K.; Okubo, Y.; Iino, M. Calcium-dependent N-cadherin up-regulation mediates reactive astrogliosis and neuroprotection after brain injury. *Proc Natl Acad Sci U S A. **2013,** 110, 11612–11617.*

30. Itoh, N.; Ornitz, D.M. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. *J. Biochem., **2011**, 149, 121–130. doi:10.1093/jb/mvQ181.*

31. Pang, Y.; Zheng, B.; Campbell, LR.; Fan, LW.; Cai, Z.; Rhodes, PG. IGF-1 can either protect against or increase LPS-induced damage in the developing rat brain. *Pediatr. Res, **2010**, 67, 579–*584.

32. Sullivan, A.M.; Toulouse, A. Neurotrophic factors for the treatment of Parkinson's disease. *Cytokine Growth Factor Rev., **2011**, 22, 157–165.doi:10.1016/j. cytogfr.2011.05.001*

33. Timmer, M.; Muller-Ostermeyer, F.; Kloth,V.; Winkler,C.; Grothe, C.; Nikkhah,G. Enhanced survival, reinnervation, and functional recovery of intrastriatal dopamine grafts co-transplanted with Schwann cells overexpressing high molecular weight FGF-2 isoforms. *Exp.Neurol.*, ***2004***, *187, 118–136.doi:10. 1016/j.expneurol.2004.01.013.*

34. Huang, B.; Krafft, P.R.; Ma, Q.; Rolland, W.B.; Caner, B.; Lekic. Fibroblast growth factors preserve blood-brain barrier integrity through RhoA inhibition after intracerebral hemorrhage in mice. *Neurobiol.Dis.*, ***2012***, *46, 204–214. doi:10.1016/j.nbd.2012.01.008*

35. Peterson, A.L.; Nutt, J.G. Treatment of Parkinson's disease with trophic factors. *Neurotherapeutics*, ***2008***, *5, 270–280 10.1016/j.nurt.2008.02.003*

36. Gill, S.S.; Patel, N.K.; Hotton, G.R Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med*. **2003,** 9, 589 –595.

37. Markham, A.; Cameron, I.; Bains, R.; Franklin, P.; Kiss, J.P.; Schwendimann, L.; Gressens, P.; Spedding, M. Brain-derived neurotrophic factor-mediated effects on mitochondrial respiratory coupling and neuroprotection share the same molecular signalling pathways. *Eur J Neurosci.* ***2012***, *35,366–374.*

38. Allen, S.J.; Watson, J.J.; Shoemark, D.K.; Barua, N.U.; Patel, N.K. GDNF, NGF, and BDNF as therapeutic options for neurodegeneration. *Pharmacol. Ther.*, ***2013***, *138, 155–175 10.1016/j.pharmthera.2013.01.004*

39. Lee, H.; Pienaar, I.S. Disruption of the blood-brain barrier in Parkinson's disease: curse or route to a cure? *Front. Biosci. (Landmark Ed.).* ***2014***, *19, 272–280 10.2741/4206*

40.Sano, Y.; Shimizu, F.; Nakayama, H.; Abe, M.; Maeda, T.; Ohtsuki, S. Endothelial cells constituting blood nerve barrier have highly specialized characteristics as barrier-forming cells. *Cell Struct. Funct.*, ***2007***, *32, 139–147 10.1247/csf.07015*

41. Safi, R.; Gardaneh, M.; Panahi, Y.; Maghsoudi, N.; Zaefizadeh, M.; Gharib, E. Optimized quantities of GDNF overexpressed by engineered astrocytes are critical for protection of neuroblastoma cells against 6-OHDA toxicity. *J. Mol. Neurosci.*, ***2012***, *46, 654–665 10.1007/s12031-011-9654-8*

42. Klein, P.; Müller-Rischart, A.K.; Motori, E.; Schönbauer, C.; Schnorrer, F.; Winklhofer, K.F.; Klein, R. Ret rescues mitochondrial morphology and muscle degeneration of Drosophila Pink1 mutants. *EMBO J.*, ***2014***, *33,341-355. doi: 10.1002/embj.201284290.*

43. Sofroniew, M.V.; Howe, C.L.; Mobley, W.C. Nerve growth factor signaling, neuroprotection, and neural repair. *Annu. Rev. Neurosci.*, ***2001,*** *24, 1217e1281.*

44. Geetha. T.; Rege, S.D.; Mathews, S.E.; Meakin, S.O.; White, M.F.; Babu, J.R. Nerve growth factor receptor TrkA, a new receptor in insulin signaling pathway in PC12 cells. *J Biol Chem.*, ***2013,*** *288, 23807-23813. doi: 10.1074/jbc.M112.436279.*

45. Clemmons, D.R. Metabolic actions of insulin-like growth factor-I in normal physiology and diabetes. *Endocrinol Metab Clin North Am*, **2012**, 41,425–443, vii-viii.

46. McDonald, T.J.; Nijland, M.J.; Nathanielsz, P.W.; The insulin-like growth factor system and the fetal brain: effects of poor maternal nutrition. *Rev Endocr Metab Disord.*, ***2007****,8,71-84.*

47. Zechariah, A.; Elali, A.; Doeppner, T.R.; Jin, F.; Hasan, M.R.; Helfrich, I. Vascular endothelial growth factor promotes pericyte coverage of brain capillaries, improves cerebral blood flow during subsequent focal cerebral ischemia and preserves the metabolic penumbra. *Stroke, **2012**, 44, 1690–1697 10.1161/STROKEAHA.111.000240*

48. Wright, G.L.; Maroulakou, I.G.; Eldridge, J.; Liby, T.L.; Sridharan, V.; Tsichlis, P.N.; Muise-Helmericks, R.C. VEGF stimulation of mitochondrial biogenesis: requirement of AKT3 kinase. *FASEB J.*, ***2008;****22,3264–3275*.

49. Lladó, J.; Tolosa, L.; Olmos,G. Cellular and molecular mechanisms involved in the neuroprotective effects of VEGF on motoneurons. *Front Cell Neurosci.*, ***2013****, **7**,181.*

50. Yu, J.; Ustach, C.; Kim, H.R. Platelet-derived growth factor signaling and human cancer. J Biochem Mol Biol., **2003**,36,49-59.

51. Krupinski, J.; Issa, R.; Bujny, T.; Slevin, M.; Kumar, P.; Kumar, S.; Kaluza, J. A putative role for platelet-derived growth factor in angiogenesis and neuroprotection after ischemic stroke in humans. *Stroke, **1997**, 28,564-573.*

52. Fruttiger, M.; Calver, A.R.; Kruger, W.H.; Mudhar, H.S.; Michalovich, D.; Takakura, N.; Nishikawa, S.; Richardson, W.D. PDGF mediates a neuron-astrocyte interaction in the developing retina. *Neuron, **1996**, 17, 1117-1131.*

53. Bergers, G.; Song, S. The role of pericytes in blood-vessel formation and maintenance. *Neuro Oncol.*, ***2005***, 7, 452–464.doi:10.1215/s1152851705000232

54. Bonkowski, D.; Katyshev,V.; Balabanov, R.D.; Borisov, A.; Dore-Duffy, P. The CNS microvascular pericyte: pericyte-astrocyte crosstalk in the regulation of tissue survival. *Fluids Barriers CNS.*, ***2011***, 8,8. doi:10.1186/2045- 8118-8-8

55. Funa, K.; Sasahara, M. The roles of PDGF in development and during neurogenesis in the normal and diseased nervous system. *The Journal of Neuroimmune Pharmacology*. **2013**, 9, 168-181.

56.Heldin, C.H.; Ostman, A.; Rönnstrand, L. Signal transduction via platelet-derived growth factor receptors. *Biochim.Biophys.Acta.*, ***1998***, *1378, F79–F113.*

57. Heldin, C.H.;Westermark, B. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev.*, ***1999***, *79:1283-1316.*

58. Fredriksson, L.; Li, H.; Eriksson, U. The PDGF family: four gene products form five dimeric isoforms. *Cytokine Growth Factor Rev.*, ***2004***, *15,197-204.*

59. Kazlauskas, A.; Cooper, J.A. Autophosphorylation of the PDGF receptor in the kinase insert region regulates interactionswith cell proteins. *Cell*, ***1998***, *58,1121–1133*.

60**.** Romashkova, J.A.; Makarov, S.S. NF-kappa B is a target of AKT in anti-apoptotic PDGF signalling. *Nature.* **1999**, *401, 86-90.*

61. Shin, H.W.; Park, S.Y.; Lee, K.B.; Shin, E.; Nam, S.W.; Lee, J.Y.; Jang, J.J. Transcriptional profiling and Wnt signaling activation in proliferation of human hepatic stellate cells induced by PDGF-BB. *Korean J Hepatol.*, ***2009***, *15, 486-495.*

62. Kang, S.W. Two axes in platelet-derived growth factor signaling: tyrosine phosphorylation and reactive oxygen species. *Cell Mol Life Sci.*, ***2007***, *64,533-541.*

63. Iqbal, S.; Zhang, S.; Driss, A.; Liu, Z.R.; Kim, H.R.; Wang, Y.; Ritenour, C.; Zhau, HE.; Kucuk, O.; Chung, L.W.; Wu, D. PDGF upregulates Mcl-1 through activation of betacatenin and HIF-1alpha-dependent signaling in human prostate cancer cells. *PLoS One. 2012*, ***7(1):e30764***

64. Wang, Z.; Ahmad, A.; Li, Y.; Kong, D.; Azmi, A,S.; Banerjee, S.; Sarkar, F.H. Emerging roles of PDGF-D signaling pathway in tumor development and progression. *Biochim Biophys Acta.*, ***2010***, *1806, 122-130.*

65. Zhu, X.; Yao, H.; Peng, F.; Callen, S.; Buch, S. PDGF-mediated protection of SH-SY5Y cells against Tat toxin involves regulation of extracellular glutamate and intracellular calcium. *Toxicol Appl Pharmacol.*, ***2009***, *240, 286-291.*

66. Zheng, L.; Ishii, Y.; Tokunaga, A.; Hamashima, T.; Shen, J.; Zhao, Q.L. Neuroprotective effects of PDGF against oxidative stress and the signaling pathway involved. *J Neurosci Res.*, ***2010***, *88, 1273-1284.*

67. Peng, F.; Yao, H.; Kaan, H.; Buch, S. Platelet-Derived Growth Factor CC-Mediated Neuroprotection against HIV Tat Involves TRPC-Mediated Inactivation of GSK 3beta. *PLoS One*, ***2012***, *7, e47572.*

68. Nobes, C.D.; Hawkins, P.; Stephens, L.; Hall, A. Activation of the small GTP-binding proteins rho and rac by growth factor receptors. *J. of Cell Sci.*, ***1995***, *108: 225-233.*

69. Li, L.; Li, J.; Wang, J.Y.; Yang, C.Q.; Jia, M.L.; W.; Jiang,W. Role of RhoA in platelet-derived growth factor-BB-induced migration of rat hepatic stellate cells. *Chin Med J.*, ***2010***, *123, 2502-2509.*

70. Ball, S.G.; Shuttleworth, A.; York, C.M. Inhibition of platelet derived growth factor receptor signaling regulates Oct4 and Nanog expression, cell shape, and mesenchymal stem cell potency. *Stem Cells.* ***2012****, 30, 548-560.*

71. Hedberg, K.M.; Bengtsson, T.; Safiejko-Mroczka, B.; Bell, P.B.; Lindroth, M PDGF and neomycin induce similar changes in the actin cytoskeleton in human fibroblasts. *Cell Motil Cytoskeleton.*, ***1993****, 24, 139-149.*

72. Ruusala, A.; Sundberg, C.; Arvidsson, A.K.; Rupp-Thuresson, E.; Heldin, C.H.; Claesson-Welsh, L. Platelet-derived growth factor (PDGF)-induced actin rearrangement is deregulated in cells expressing a mutant Y778F PDGF beta-receptor. *J Cell Sci.*, ***1998****, 111(Pt 1),111-120.*

73.van Nieuw Amerongen, G.P.; Koolwijk, P.; Versteilen, A.; van Hinsbergh, V.V. Involvement of RhoA/Rho kinase signaling in VEGF-induced endothelial cell migration and angiogenesis in vitro. *Arterioscler Thromb Vasc Biol.*, ***2003****, 23, 211-217.*

74**.** Simon, A.R.; Takajashi, S.; Fanburg, B.L.; Cochran, B.H. Role of the JAK-STAT pathway in PDGF-stimulated proliferation of human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol., 2001, 282, L1296-L1304.*

75.Thannickal, V.J.; Fanbourg, B.L. Reactive oxygen species in cell signaling.¨ Am J *Physiol Lung Cell Mol Physiol.*, **2000**, *279, L1005-L1028.*

76. Cheng, B.; Mattson, M.P. PDGFs protect hippocampal neurons against energy deprivation and oxidative injury: evidence for induction of antioxidant pathways. *J Neurosci.*, ***1995****, 15,7095-7104.*

77. Lehwald, N.; Tao, G.Z.; Jang, K.Y.; Papandreou, I.; Liu,B. b-catenin regulates hepatic mitochondrial function and energy balance in livers of mice. Gastroenterology , **2012**, 143: 754–764.

78. Dejana, E. Endothelial cell-cell junctions: happy together. *Nat. Rev.Mol. Cell Biol*, ***2004***, 5, 261–270.doi:10.1038/nrm1357

79. Tang, Z.; Arjunan, P, Lee, C.; Li, Y.; Kumar, A.; Hou, X.; Wang,B.; Wardega, P.; Zhang, F.; Dong, L.; Zhang, Y.; Zhang, S.Z.; Ding, H.; Fariss, R.N.; Becker, K.G.; Lennartson, J . Survival effect of PDGF-CC rescues neurons from apoptosis in both brain and retina by regulating GSK3beta phosphorylation. *J Exp Med.*, ***2010****, 207,867-880 doi:jem.2009170410.1084/jem.20091704*

80. Gosslau, A.; Dittrich, W.; Willig, A.; Jaros, P.P. Cytological effects of platelet-derived growth factor on mitochondrial ultastructure in fibroblasts. *Comp Biochem Physiol A Mol Integr Physiol.*, ***2001,*** *128 (2): 241-249.*

81. Salabei, J.K.; Hill, B.G. Mitochondrial fission induced by platelet-derived growth factor regulates vascular smooth muscle cell bioenergetics and cell proliferation. *Redox Biology*. ***2013****;1,542–551.*

82. Ridefelt, P.; Yokote, K.; Claesson-Welsh, L.; Siegbahn, A. PDGF-BB triggered cytoplasmic calcium responses in cells with endogenous or stably transfected PDGF beta-receptors. *Growth Factors.* ***1995****, 12, 191-201.*

83. Peng, F.; Yao, H.; Bai, X.; Zhu, X.; Reiner, BC. Platelet-derived growth factor-mediated induction of the synaptic plasticity gene Arc/Arg3.1. *J Biol Chem.*, ***2010,*** *285, 21615–21624.*

84. Cabezas, R.; Avila, M.F.; González, J.; El-Bachá, R.S.; Barreto, GE. PDGF-BB Protects Mitochondria from Rotenone in T98G Cells. Neurotox Res. **2014,** Dec 17. DOI 10.1007/s12640-014-9509-5.

85. Aryal, M.; Arvanitis, C.D.; Alexander, P.M.; McDannold, N. Ultrasound-mediated blood-brain barrier disruption for targeted drug delivery in the central nervous system. *Adv Drug Deliv Rev.*, ***2014****, 72,94-109. doi: 10.1016/j.addr.2014.01.008.*

86. Daneman, R. The blood-brain barrier in health and disease. *Ann. Neurol.* ***2012,*** *72, 648–672 10.1002/ana.23648.*

87. Masserini, M. Nanoparticles for Brain Drug Delivery. *ISRN Biochemistry,* ***2013****, 2013, 1-18.*

88. McDannold, N.; Vykhodtseva, N.; Jolesz, F.A.; Hynynen, K. MRI investigation of the threshold for thermally induced blood–brain barrier disruption and brain tissue damage in the rabbit brain. *Magn. Reson. Med.*, ***2004****, 51, 913–923.*

89. Arena, C.B.; Garcia, P.A.; Sano, M.B.; Olson, J.D.; Rogers-Cotrone, T.; Rossmeisl, J.H.; Davalos, R.V. Focal blood-brain-barrier disruption with high frequency pulsed electric fields. *Technology,* ***2014****, 02, 206. DOI: 10.1142/S2339547814500186*

90. Hauser, R.A. Future treatments for Parkinsons disease: surfing the PD pipeline. *Int. J. Neurosci. ,* ***2011****, 121(Suppl. 2), 53–62 10.3109/00207454.2011.620195*

91. Jankovic, J.; Poewe, W. Therapies in Parkinson's disease. *Curr. Opin. Neurol.,* ***2012****, 25, 433–447 10.1097/WCO.0b013e3283542fc2*

Figure 1. ***Growth factors effects in astrocytes metabolism***. Astrocytes secrete growth factor molecules such as BDNF, GDNF, and NGF, thus activating various signaling pathways including PI3K/AKT and MAPK that are important for cell survival process, neuronal protection, antioxidant expression, BBB interruption and regulation, angiogenic effects, and protection to mitochondrion.